From: Saif, Linda
To: Liu, Shan-Lu; lishan_su@med.unc.edu
Subject: FW: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 1:28:39 PM
Attachments: EMI-2019-nCoV_Commentary_LJS_SLL_Refs-rsb.docx

Hi

Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!

Regards,

Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "rbaric@email.unc.edu" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 12:32 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: Linda Saif <saif.2@osu.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 10:11 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

From: "Baric, Ralph S" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 at 10:02 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>

Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

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From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 1:12 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

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[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

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[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song, H.D. et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A 102, 2430-2435 (2005)). Given that there are greater than 1000 nt differences between the human

Commented [BRS1]: Not a dna virus

SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

Commented [BRS2]: In Chinese social media

Commented [BRS3]: >5,000 nts

The recombinant mouse-adapted SARS virus (MA15) (Roberts, A. et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog 3, e5 (2007)) was generated by serial passage of SARS CoV in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations

Commented [BRS4]: No, wildtype was passaged

Commented [BRS5]: wildtype

associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age.....leading to severe pathogenesis in aged, but not young animals [6].

Commented [BRS6]: these six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice.

Commented [BRS7]: SARS-CoV, as well as its closely related SHC014 bat strain and the chimera all differ by over 6,000 nts as compared with SARS-CoV 2.
Genome identities

Differences between Genomes.xlsx

Commented [BRS8]: This is not correct.

But was fully attenuated and displayed reduced virus infection in the airway epithelium as compared to SARS-CoV MA15 which is lethal.

Did not produce lethal disease like wildtype sars, so its attenuated!

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were subject to pause, reviewed and later approved under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

Commented [BRS9]: reduced

Commented [BRS10]: as written, suggests experiments were done before review. May want to reformulate

Commented [BRS11]: PMC6954302 PMC5567817

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (a manuscript sharing site prior to any peer review ~~and not yet peer reviewed for accuracy~~) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.---And should not be present? in naturally isolated viruses such as RaTG13. Currently, there is no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

1. Wang, D., *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang, *et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N., *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N., *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D., *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y., *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W., *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F., *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

From: Saif, Linda
To: Liu, Shan-Lu; Su, Lishan
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 2:38:08 PM
Attachments: image001.png
EMI-2019-nCoV_Commentary_Final LJS 2020.docx

Thanks—a few minor last edits

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 2:05 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 2:00 PM
To: "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

From: "Saif, Linda" <saif.2@osu.edu>
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SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

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Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

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Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

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When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-~~

~~MA15 can replicate efficiently in the mouse lung, leading to severe pathogiogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 ~~nucleotident~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV

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Commented [LS1]: Lishan: see Ralph's comments to revise, as I am confused!

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Commented [BRS2]: PMC6954302 PMC5567817

sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
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5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Saif, Linda
To: Liu, Shan-Lu; Su, Lishan
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 2:54:23 PM
Attachments: image001.png
EMI-2019-nCoV_Commentary_Final LJS 2020.docx

Sorry just caught the error in the title!

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Linda Saif <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 2:38 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

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I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
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Phone: (614) 292-8690
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Tentative Title: Is 2019-nCoV laboratory origin?

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SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

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Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. These six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the

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When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathoglogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 nucleotide~~st~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is

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Commented [LS1]: Lishan: see Ralph's comments to revise, as I am confused!

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not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb

15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Saif, Linda
To: Liu, Shan-Lu; Su, Lishan
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 2:56:06 PM
Attachments: image001.png
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Also sent prior draft—here is latest one LJS2x

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Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were only later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 nucleotident differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical pre-clinicalIND data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In aA rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is

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Commented [LS1]: Lishan: see Ralph's comments to revise, as I am confused!

Commented [J2]: Ralph wants us to clarify that these exp were not restricted when he did them—only later!

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not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.

6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Liu, Shan-Lu
To: Saif, Linda
Cc: Su, Lishan
Subject: FW: [External] Commentary for EMI
Date: Wednesday, February 12, 2020 4:09:18 PM
Attachments: EMI-2019-nCoV_Commentary_Final for submission .docx
image001.png
image002.png

Hi Linda,

Susan Weiss has decided to join the authorship – see the final version attached.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
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From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Wednesday, February 12, 2020 at 4:00 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: [External] Commentary for EMI

Shan-LU
I am still in Spain, going home on Saturday.
Yes please add my name as a co-author. This is important!!
Is the new virus now names SARS-2; maybe not a good name – should be different from SARS

I hope I am not too late

susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>

Date: Wednesday, February 12, 2020 at 5:26 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: [External] Commentary for EMI

Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of "Emerging Microbes and Infections", Dr. Shan Lu (don't get confused, it's not me). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

THE OHIO STATE UNIVERSITY

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SARS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu[3, 5,6.7]

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Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
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5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
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12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From:	Emerging Microbes and Infections
To:	Liu, Shan-Lu
Cc:	Liu, Shan-Lu; Saif, Linda; weisssr@pennmedicine.upenn.edu; lishan_su@med.unc.edu
Subject:	Emerging Microbes & Infections - Manuscript ID TEMI-2020-0121 has been submitted online
Date:	Wednesday, February 12, 2020 9:55:21 PM

12-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections

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Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__!!KGKeukY!iZuBSqFeEfVqrLHbkUeoF7XsNXtARiE1kDnvBvwaKd9rBosvuwrxy7vjNjs58A$ and edit your user information as appropriate

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Thank you for submitting your manuscript to Emerging Microbes & Infections

Sincerely,
Emerging Microbes & Infections Editorial Office

From: Min Yang
To: Liu, Shan-Lu; Su, Lishan; Saif, Linda; Weiss, Susan
Cc: Lu, Shan
Subject: Re: EMI commentary
Date: Thursday, February 13, 2020 1:10:05 AM
Attachments: image001.png

Dear Dr Liu,

Thank you for your quick response.

This is to confirm that we have already received your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin". (TEMI-2020-0121)

Thanks and regards,

Min Yang

Emerging Microbes & Infections (EMI) Editorial Office
4F Fuxing Building
131 Dongan Road
Shanghai
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Tel: 86-21-54237992
E-mail: min.yang@emi2012.org

发件人: "Liu, Shan-Lu" <liu.6244@osu.edu>
日期: 2020年2月13日 星期四 上午11:25
收件人: Min Yang <min.yang@emi2012.org>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
抄送: "Lu, Shan" <Shan.Lu@umassmed.edu>
主题: Re: EMI commentary

Min:

It should have been successfully submitted. See below email:

12-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections.

Your manuscript ID is TEMI-2020-0121.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to

ScholarOne Manuscripts at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__;!!KGKeukY!klcqOriA-xIzLyrzwwKWtghNAQgvfbCh7pqavzMYm77fJJsm_iShbXJWIKEtRML7ExI$ and edit your user information as appropriate.

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Thank you for submitting your manuscript to Emerging Microbes & Infections.

Sincerely,
Emerging Microbes & Infections Editorial Office

From: Min Yang <min.yang@emi2012.org>
Date: Wednesday, February 12, 2020 at 10:17 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Dear Dr Liu,

Thank you for your support to EMI.

According to the attachment, it looks like your submission is a DRAFT still which has not been submitted successfully yet.

Could you please check and confirm?

Thanks and regards,

Min Yang

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发件人: "Liu, Shan-Lu" <liu.6244@osu.edu>
日期: 2020年2月13日 星期四 上午10:58
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抄送: Min Yang <min.yang@emi2012.org>, "Lu, Shan" <Shan.Lu@umassmed.edu>
主题: EMI commentary

Dear all,

I have just submitted a commentary to EMI. See attached the submitted version.

Thank you.

Shan-Lu

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Shan-Lu Liu, M.D., Ph.D.
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From: Liu, Shan-Lu
To: Su, Lishan; Saif, Linda
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 3:34:29 PM
Attachments: EMI-2019-nCoV_Commentary_Final for submission .docx
image001.png
image002.png

Hi Linda and Lishan,

I have finalized it, please take a look at it and let me know.

Not sure if abstract and acknowledgment are needed at this point. Will check with the editor.

Shan-Lu

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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 3:17 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Saif, Linda" <saif.2@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu and Linda:
I have incorporated all comments and added the two references (in both text and endnote file).
Please do a final proof read, and finalize it.
Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 3:03 PM

To: "Saif, Linda" <saif.2@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks Linda, all good!

Shan-Lu

From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 2:56 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Also sent prior draft—here is latest one LJS2x
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Linda Saif <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 2:54 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Sorry just caught the error in the title!
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
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Wooster, Oh 44691

From: Linda Saif <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 2:38 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks—a few minor last edits
Linda J. Saif, PhD
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From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 2:05 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 2:00 PM
To: "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 1:34 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
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1680 Madison Ave
Wooster, Oh 44691

From: "rbaric@email.unc.edu" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 12:32 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: Linda Saif <saif.2@osu.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 10:11 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss.

Thanks,

-Lishan

From: "Baric, Ralph S" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 at 10:02 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 1:12 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

SARS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

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[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

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[4] Center for Retrovirus Research, The Ohio State University,

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[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

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[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From: Liu, Shan-Lu
To: Saif, Linda
Cc: Su, Lishan
Subject: Re: [External] Commentary for EMI
Date: Wednesday, February 12, 2020 5:00:51 PM
Attachments: EMI-2019-nCoV_Commentary_Final for submission .docx
image001.png
image002.png
image003.png

Thank you, Linda. Susan is in Barcelona, with no comments.

I have made all your requested changes, but would like to check on your suggestion for Ralph's point. I thought the word "later" is sufficient. See attached updated version and let me know if there are still errors.

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research).

Thanks.

Shan-Lu

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 4:37 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Commentary for EMI

Hi All
There were a few minor edits on this prior draft. Did Susan provide any edits?
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
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1680 Madison Ave
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From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 4:09 PM
To: Linda Saif <saif.2@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: FW: [External] Commentary for EMI

Hi Linda,

Susan Weiss has decided to join the authorship – see the final version attached.

Shan-Lu

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Wednesday, February 12, 2020 at 4:00 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: [External] Commentary for EMI

Shan-LU

I am still in Spain, going home on Saturday.

Yes please add my name as a co-author. This is important!!

Is the new virus now names SARS-2; maybe not a good name – should be different from SARS

I hope I am not too late

susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>

Date: Wednesday, February 12, 2020 at 5:26 PM

To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>

Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>

Subject: [External] Commentary for EMI

Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of "Emerging Microbes and Infections", Dr. Shan Lu (don't get confused, it's not me). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

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SARS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu[3, 5,6.7]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

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University of Pennsylvania, Philadelphia, Pennsylvania, USA

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Columbus, OH 43210, USA

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Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From: Saif, Linda
To: Liu, Shan-Lu
Cc: Su, Lishan
Subject: Re: [External] Commentary for EMI
Date: Wednesday, February 12, 2020 4:37:32 PM
Attachments: image001.png
image002.png
EMI-2019-nCoV_Commentary_Final LJS2x 2020.docx

Hi All

There were a few minor edits on this prior draft. Did Susan provide any edits?

Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 4:09 PM
To: Linda Saif <saif.2@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: FW: [External] Commentary for EMI

Hi Linda,

Susan Weiss has decided to join the authorship – see the final version attached.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
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Center for Retrovirus Research
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Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Wednesday, February 12, 2020 at 4:00 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: [External] Commentary for EMI

Shan-LU
I am still in Spain, going home on Saturday.
Yes please add my name as a co-author. This is important!!
Is the new virus now names SARS-2; maybe not a good name – should be different from SARS

I hope I am not too late

susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 5:26 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: [External] Commentary for EMI

Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of "Emerging Microbes and Infections", Dr. Shan Lu (don't get confused, it's not me). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

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Shan-Lu Liu, M.D., Ph.D.
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SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

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Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry~~have~~ CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. These six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the

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severe disease phenotype in mice. It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was ~~fully~~ attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathoglogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were only later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 nucleotident differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical pre-clinicalIND data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In aA rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is

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Commented [LS1]: Lishan: see Ralph's comments to revise, as I am confused!

Commented [J2]: Ralph wants us to clarify that these exp were not restricted when he did them—only later!

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not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
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3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
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6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
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9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
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13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Saif, Linda
To: Liu, Shan-Lu
Subject: FW: Lancet Statement Posted!
Date: Tuesday, February 18, 2020 3:00:45 PM
Attachments: Lancet Statement 2020.pdf

Hi Shan-lu,
Here is the statement with the opportunity for others to sign. Please distribute to colleagues!
Thanks
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Tuesday, February 18, 2020 at 12:44 PM
To: Christian Drosten <drosten@virology-bonn.de>, John Mackenzie <J.Mackenzie@curtin.edu.au>, Jonna Mazet <jkmazet@ucdavis.edu>, "llmpoon@hku.hk" <llmpoon@hku.hk>, Larry Madoff <lmadoffpmm@gmail.com>, Prof Lam Sai Kit <sklam@nipahvirus.org>, "j.farrar@wellcome.ac.uk" <j.farrar@wellcome.ac.uk>, "peter.palese@mssm.edu" <peter.palese@mssm.edu>, Dennis Carroll <dcarroll008@gmail.com>, "bernard.roizman@bsd.uchicago.edu" <bernard.roizman@bsd.uchicago.edu>, "Stanley-Perlman@uiowa.edu" <Stanley-Perlman@uiowa.edu>, Charles H Calisher <calisher@cybersafe.net>, "a.e.gorbalenya@lumc.nl" <a.e.gorbalenya@lumc.nl>, "L.Enjuanes@cnb.csic.es" <L.Enjuanes@cnb.csic.es>, "b.haagmans@erasmusmc.nl" <b.haagmans@erasmusmc.nl>, "rcolwell@umiacs.umd.edu" <rcolwell@umiacs.umd.edu>, "JMHUGHE@emory.edu" <jmhughe@emory.edu>, Hume Field <hume.field@ecohealthalliance.org>, "jlubroth@gmail.com" <jlubroth@gmail.com>, Linda Saif <saif.2@osu.edu>, "William B. Karesh" <karesh@ecohealthalliance.org>, "rbcorley@bu.edu" <rbcorley@bu.edu>, "Keusch, Gerald T" <keusch@bu.edu>, "Subbarao, Kanta" <kanta.subbarao@influenzacentre.org>, "J.Golding@wellcome.ac.uk" <J.Golding@wellcome.ac.uk>, Mike Turner <M.Turner@wellcome.ac.uk>
Cc: Hongying Li <li@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>
Subject: Lancet Statement Posted!

Dear All,

Our statement is live as of just a few minutes ago!

https://www.thelancet.com/lancet/article/s0140-6736(20)30418-9

Please take time to send this out via twitter, email to your networks, post on your institution or other websites, and distribute as widely as possible to get the word out. Include the link too (http://chng.it/SDpTB9Kf), so other people can register their support of the statement.

I really want to thank all of you for rallying for this - especially with such a short timeline. This looks terrific and I know it will do a world of good towards buoying the spirits of our colleagues in China and gaining an ear from those in policy to support collaborative, open approaches to fighting this as well as future outbreaks.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance develops science-based solutions tp prevent pandemics and promote conservation.

From: Liu, Shan-Lu
To: Saif, Linda
Subject: Re: Revised commentary for EMI - final!
Date: Tuesday, February 18, 2020 11:05:34 AM
Attachments: image001.png

Thanks. Would love to see it in Lancet, so please chare.

Shan-Lu

From: "Saif, Linda" <saif.2@osu.edu>
Date: Tuesday, February 18, 2020 at 11:00 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Revised commentary for EMI - final!

Thanks—Good seminar this AM and so glad we could access it.
I will send you a copy of joint correspondence on SARS-CoV-2 initiated by Peter Daszak that will be published today in Lancet!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
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1680 Madison Ave
Wooster, Oh 44691

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 18, 2020 at 10:55 AM
To: Linda Saif <saif.2@osu.edu>
Subject: Re: Revised commentary for EMI - final!

Hi Linda,

I will be out for an NIH virology B study section Feb 20-21 so will miss your webinar. I am sure it will go well!

Shan-Lu

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From: Weiss, Susan
To: Liu, Shan-Lu; Saif, Linda; Su, Lishan; Lu, Shan
Subject: Re: [External] Re: Revised commentary for EMI - final!
Date: Monday, February 17, 2020 6:58:27 PM
Attachments: image001.png

Thanks, this is good

susan

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From: Liu, Shan-Lu
To: Saif, Linda
Subject: Re: Revised commentary for EMI - final!
Date: Tuesday, February 18, 2020 9:44:01 AM

Yes

Shan-Lu Liu sent from iPhone

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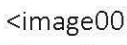
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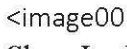
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No Credible evidence supporting claims of the laborary engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
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[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural

evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed

to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already

exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, such a

virus, and closely related, do pose great public health threats and must be handled properly in the laboratory and also properly regulated by governments and scientific community.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
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6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
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From:	Liu, Shan-Lu
To:	Su, Lishan; Saif, Linda; Weiss, Susan
Cc:	Lu, Shan
Subject:	Revised commentary for EMI
Date:	Sunday, February 16, 2020 1:38:44 PM
Attachments:	Liu et al_EMI Commentary_revision_Feb 16"2020.docx image001.png

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Let me know what you think.

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No credible evidence supporting claims of the laborary engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
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[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
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[3] Department of Microbial Infection and Immunity, The Ohio State University,
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[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
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[5] Food Animal Health Research Program,
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[6] Department of Microbiology, Perelman School of Medicine,
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[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

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wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

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References

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From: Saif, Linda
To: Su, Lishan; Lu, Shan; Liu, Shan-Lu; Weiss, Susan
Subject: Re: Revised commentary for EMI - final!
Date: Sunday, February 16, 2020 7:20:04 PM
Attachments: image001.png
Liu et al_EMI Commentary_Revision_Final-sls.docx

Attached

Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 16, 2020 3:14 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: Revised commentary for EMI - final!

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Thanks,

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From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 1:55 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: RE: Revised commentary for EMI - final!

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From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 1:45 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Revised commentary for EMI - final!

Please look at this new version, sorry!

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Date: Sunday, February 16, 2020 at 1:38 PM

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No ~~Credible~~ credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

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To: Lu, Shan; Liu, Shan-Lu; Saif, Linda; Weiss, Susan
Subject: Re: Revised commentary for EMI - final!
Date: Sunday, February 16, 2020 3:17:09 PM
Attachments: image001.png
Liu et al_EMI Commentary_Revision_Final-sls.docx

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From: Shan-Lu Liu <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 1:38 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

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No ~~Credible~~ credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

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The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural

evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed

to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already

exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses

with such great public health threats must be handled properly in the laboratory and also properly regulated by scientific community and governments. ~~We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, such a virus, and closely related, do pose great public health threats and must be handled properly in the laboratory and also properly regulated by governments and scientific community.~~

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

From: Emerging Microbes and Infections
To: Liu, Shan-Lu; Saif, Linda; weisssr@pennmedicine.upenn.edu; lishan_su@med.unc.edu
Subject: Emerging Microbes & Infections - Decision on Manuscript ID TEMI-2020-0121
Date: Thursday, February 13, 2020 9:49:29 AM

13-Feb-2020

Dear Professor Liu:

Ref: SARS-CoV-2: no evidence of a laboratory origin

Our reviewers have now considered your paper and have recommended publication in Emerging Microbes & Infections. We are pleased to accept your paper in its current form which will now be forwarded to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter, along with those of the editor who coordinated the review of your paper.

You will receive proofs for checking, and instructions for transfer of copyright in due course.

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Thank you for your contribution to Emerging Microbes & Infections and we look forward to receiving further submissions from you.

Sincerely,

Shan Lu
Editor-in-Chief
Emerging Microbes & Infections

Review Editor Comments to the Author:
EMI would like to thank the authors for providing a timely piece. It will have major impact to clear many people's confusion.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
This is a timely commentary. It is perfectly written. All four authors are well established virologists. I suggest to publish it right away.

From:	Emerging Microbes and Infections
To:	Liu, Shan-Lu
Cc:	Liu, Shan-Lu; Saif, Linda; weisssr@pennmedicine.upenn.edu; lishan_su@med.unc.edu
Subject:	Emerging Microbes & Infections - Manuscript ID TEMI-2020-0121 has been submitted online
Date:	Thursday, February 13, 2020 9:11:20 AM

13-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections.

Your manuscript ID is TEMI-2020-0121.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__;!!KGKeukY!g1on9C-9Z6nI-dSjtB5Ub-UfJstd8c_r64qQXOqSWkMHVLgs8YcAW0auDGDzEQ$ and edit your user information as appropriate.

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Thank you for submitting your manuscript to Emerging Microbes & Infections.

Sincerely,
Emerging Microbes & Infections Editorial Office

From:	Liu, Shan-Lu
To:	Su, Lishan; Saif, Linda; Weiss, Susan
Cc:	min.yang@emi2012.org; Lu, Shan
Subject:	EMI commentary
Date:	Wednesday, February 12, 2020 9:58:16 PM
Attachments:	EMI_Commentary_submitted.pdf image001.png

Dear all,

I have just submitted a commentary to EMI. See attached the submitted version.

Thank you.

Shan-Lu

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SARS-CoV-2: no evidence of a laboratory origin

Journal:	*Emerging Microbes & Infections*
Manuscript ID	Draft
Manuscript Type:	Commentary
Date Submitted by the Author:	n/a
Complete List of Authors:	Liu, Shan-Lu; The Ohio State University, Infectious Diseases Institute Saif, Linda J.; The Ohio State University Weiss, Susan; University of Pennsylvania Su, Lishan; University of North Carolina at Chapel Hill
Keywords:	SARS-CoV-2, COIVD-2019, origin, evolution
Abstract:	

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Manuscripts

SARS-CoV-2: no evidence of a laboratory origin

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Dr. Shan-Lu Liu, Liu.6244@osu.edu

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RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

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When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

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director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

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Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

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References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

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Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

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14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.

16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

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From: Liu, Shan-Lu
To: Lu, Shan
Cc: Su, Lishan; Saif, Linda; Weiss, Susan
Subject: EMI commentary
Date: Wednesday, February 12, 2020 5:12:43 PM
Attachments: EMI-2019-nCoV_Commentary_Final for submission .docx

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Hi Shan,

Attached please find the final version of the commentary for your consideration to be published at EMI.

Kindly advise.

Regards.

Shan-Lu

SARS-CoV-2: no evidence of a laboratory origin

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The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From: Su, Lishan
To: Liu, Shan-Lu; Saif, Linda
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 3:17:49 PM
Attachments: image001.png
EMI-2019-nCoV_Commentary_Final LJS 2020ls.docx
2019 CoV Copy.enl

Shan-Lu and Linda:
I have incorporated all comments and added the two references (in both text and endnote file).
Please do a final proof read, and finalize it.
Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 3:03 PM
To: "Saif, Linda" <saif.2@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks Linda, all good!

Shan-Lu

From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 2:56 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Also sent prior draft—here is latest one LJS2x
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Linda Saif <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 2:54 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Sorry just caught the error in the title!
Linda J. Saif, PhD

Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Linda Saif <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 2:38 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks—a few minor last edits
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 2:05 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 2:00 PM
To: "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 1:34 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
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1680 Madison Ave
Wooster, Oh 44691

From: "rbaric@email.unc.edu" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 12:32 PM

To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: Linda Saif <saif.2@osu.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 10:11 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

From: "Baric, Ralph S" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 at 10:02 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 1:12 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

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Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between

the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. ~~These six mutations were reintroduced into a SARS~~

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~~molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice~~ (Not necessary here, I had deleted these details from previous versions to shorten the description of the study). It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes ~~regardless of~~in aged mice ~~Importantly,~~

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~~SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogiogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (~~from Oct. 2014 to Dec. 2017;~~ https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >~~5~~5,000 nucleotide~~nt~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad ~~-based~~spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo (Sheahan, T.P. et al. Sci Transl Med 9 (2017), Sheahan, T.P. et al. Nat Commun 11, 222 (2020)), providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a

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Commented [LS1]: Lishan: see Ralph's comments to revise, as I am confused!

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manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In aA rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
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4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
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From: Liu, Shan-Lu
To: Su, Lishan; Saif, Linda
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 2:05:52 PM
Attachments: EMI-2019-nCoV_Commentary_LJS_SLL_Refs-rsb.docx
EMI-2019-nCoV_Commentary_Final LJS 2020.docx
image001.png

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 2:00 PM
To: "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 1:34 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "rbaric@email.unc.edu" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 12:32 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: Linda Saif <saif.2@osu.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

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From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 10:11 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss.
Thanks,

-Lishan

From: "Baric, Ralph S" <rbaric@email.unc.edu>

Date: Wednesday, February 12, 2020 at 10:02 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 1:12 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

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[4] Center for Retrovirus Research, The Ohio State University,

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[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song, H.D. et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A 102, 2430-2435 (2005)). Given that there are greater than 1000 nt differences between the human

Commented [BRS1]: Not a dna virus

SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

Commented [BRS2]: In Chinese social media

Commented [BRS3]: >5,000 nts

The recombinant mouse-adapted SARS virus (MA15) (Roberts, A. et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog 3, e5 (2007)) was generated by serial passage of SARS CoV in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations

Commented [BRS4]: No, wildtype was passaged

Commented [BRS5]: wildtype

associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

Commented [BRS6]: these six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age.....leading to severe pathogenesis in aged, but not young animals [6].

Commented [BRS7]: SARS-CoV, as well as its closely related SHC014 bat strain and the chimera all differ by over 6,000 nts as compared with SARS-CoV 2.
Genome identities

Differences between Genomes.xlsx

Commented [BRS8]: This is not correct.

But was fully attenuated and displayed reduced virus infection in the airway epithelium as compared to SARS-CoV MA15 which is lethal.

Did not produce lethal disease like wildtype sars, so its attenuated!

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were subject to pause, reviewed and later approved under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

Commented [BRS9]: reduced

Commented [BRS10]: as written, suggests experiments were done before review. May want to reformulate

Commented [BRS11]: PMC6954302 PMC5567817

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (a manuscript sharing site prior to any peer review ~~and not yet peer reviewed for accuracy~~) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.---And should not be present? in naturally isolated viruses such as RaTG13. Currently, there is no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

1. Wang, D., *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang, *et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N., *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
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6. Menachery, V.D., *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y., *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
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SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

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Contact:

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Dr. Shan-Lu Liu, Liu.6244@osu.edu

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According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

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Commented [LS1]: Lishan: see Ralph's comments to revise, as I am confused!

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References

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From: Baric, Ralph S
To: Su, Lishan
Cc: Saif, Linda
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses
Date: Wednesday, February 12, 2020 12:34:00 PM
Attachments: EMI-2019-nCoV_Commentary_LJS_SLL_Refs-rsb.docx

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 10:11 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

From: "Baric, Ralph S" <rbaric@email.unc.edu>
Date: Wednesday, February 12, 2020 at 10:02 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 1:12 AM
To: Baric, Ralph S <rbaric@email.unc.edu>
Subject: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

SARVS-CoV-2: no evidence of a laboratory origin

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Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song, H.D. et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A 102, 2430-2435 (2005)). Given that there are greater than 1000 nt differences between the human

Commented [BRS1]: Not a dna virus

SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

Commented [BRS2]: In Chinese social media

Commented [BRS3]: >5,000 nts

The recombinant mouse-adapted SARS virus (MA15) (Roberts, A. et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog 3, e5 (2007)) was generated by serial passage of SARS CoV in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations

Commented [BRS4]: No, wildtype was passaged

Commented [BRS5]: wildtype

associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age.....leading to severe pathogenesis in aged, but not young animals [6].

Commented [BRS6]: these six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice.

Commented [BRS7]: SARS-CoV, as well as its closely related SHC014 bat strain and the chimera all differ by over 6,000 nts as compared with SARS-CoV 2.
Genome identities

Differences between Genomes.xlsx

Commented [BRS8]: This is not correct.

But was fully attenuated and displayed reduced virus infection in the airway epithelium as compared to SARS-CoV MA15 which is lethal.

Did not produce lethal disease like wildtype sars, so its attenuated!

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were subject to pause, reviewed and later approved under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

Commented [BRS9]: reduced

Commented [BRS10]: as written, suggests experiments were done before review. May want to reformulate

Commented [BRS11]: PMC6954302 PMC5567817

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (a manuscript sharing site prior to any peer review ~~and not yet peer reviewed for accuracy~~) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.---And should not be present? in naturally isolated viruses such as RaTG13. Currently, there is no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

1. Wang, D., *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang, *et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N., *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N., *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D., *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y., *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W., *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F., *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

From: Saif, Linda
To: rbaric@email.unc.edu
Subject: EIM commentary
Date: Wednesday, February 12, 2020 11:49:11 AM
Attachments: EMI-2019-nCoV_Commentary_Final LJS 2020.docx

Hi Ralph,
My 2 Chinese colleagues and I have prepared this commentary to try to scientifically address some of the rumors and conspiracy theories on the internet about the origin of the 2019-nCoV, now designated SARS-2. Since we have tried to address concerns about some of your chimeric SARS constructs, it would be extremely helpful if you could review this and edit or add anything that might be useful. I realize from what Peter said you may not want to add your name but certainly your unacknowledged input and insights would be helpful to be certain we have provided the key evidence against such rumors and a false claims.
I recognize that it is essential for scientists to do whatever they can to counter fake news and false information and to support our esteemed colleagues and scientists like yourself which is what prompted this commentary!

In another matter Dr Wang and I want to try to get the SARS-2 CoV from BEI and attempt to infect pigs in our BSL 3 Ag facility. Do you know of any funds we could apply for to do these pilot studies, just to see if pigs are susceptible based on similar ACE2?

Hope you are well in spite of all the turmoil!
Regards,
Linda

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According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [12], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathology [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding

that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 13], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

13. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Liu, Shan-Lu
To: Saif, Linda
Cc: Su, Lishan; Lu, Shan
Subject: Re: Commentary for Emerging Microbes & Infections
Date: Wednesday, February 12, 2020 11:01:25 AM
Attachments: EMI-2019-nCoV_Commentary_Final.docx
image001.png

Dear Linda;

Attached please find almost the final version of the commentary for EMI, so please feel free to share it with Ralph. Let me know if you have additional suggestions – all your points are incorporated into the new version, please check.

Note that I was trying to find official website links for the new names of the virus (ICTV) and diseases (WHO), but failed; I therefore decided to use the following website, which contains both.

https://globalbiodefense.com/novel-coronavirus-covid-19-portal/

We will try to submit it today, but are considering to add a few more coronavirus experts – anyone that you would like to suggest? We will contact Stanley Perlman right now.

Shan-Lu

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From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 9:37 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Commentary for Emerging Microbes & Infections

Can you please send me the updated version first and then I will try to share with Ralph!

Thanks
Linda
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From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 12:47 AM
To: Linda Saif <saif.2@osu.edu>
Subject: Re: Commentary for Emerging Microbes & Infections

Hi Linda.
Thanks so much, and your comments are extremely helpful. Please feel free to share with Ralph to get his feedback if possible. We would like to publish this in the next few days.
I will work on reference tomorrow and send you a updated version.

Shan-Lu Liu sent from iPhone

> On Feb 11, 2020, at 11:54 PM, Saif, Linda <saif.2@osu.edu> wrote:
>
> Hi Shan-Lu,
> I edited this version and added my name as I too feel strongly about denouncing this.
> Here are more comments and some refs that I have made in replies to some reporters about this issue if you think any are useful to include. I also wonder if we might share this with Ralph Baric since he is a conspiracy target and maybe he could add additional points, but I know he would not want to be a co-author—not sure if he has time to answer.
>
> The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that 2019-nCoV evolved by natural evolution. Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.

The closest virus relative to 2019-nCoV is bat CoV RaTG13. There are 4% nt differences between 2019-nCoV and RaTG13, corresponding to >1000 nt based on a genome size of 29k. These changes (SNP) are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region.
(Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature doi:10.1038/s41586-020-2012-7.

Regarding differences between civet cat SARSr-CoV and SARS-CoV, here is the accurate data: . A total of 202 SNVs with multiple occurrences were identified, among which 200 were in the CDSs. Among the 128 nonsynonymous mutations, 89 led to a predicted radical amino acid changes
Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. Epub 2005 Feb 4.
Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human.
Song HD1, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L, Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP.

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From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 10:32 PM
To: Linda Saif <saif.2@osu.edu>
Subject: Commentary for Emerging Microbes & Infections

Hi Linda,

Invited by the editor in chief of EMI, Lushan Su from UNC and I have written a commentary on the possible origin of the 2019-nCoV or SARS-CoV-2 in order to dispute some rumors, and we would like to invite you as a coauthor. Attached please find an almost complete draft (references needed) of the commentary, so kindly let me know what you think. Your comments and suggestions are very much appreciated.

Thanks.

Shan-Lu

<image001.png>
Shan-Lu Liu, M.D., Ph.D.
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<image001.png>
<EMI-2019-nCoV_Commentary LJS.docx>

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Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [12], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs

already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 13], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

13. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Saif, Linda
To: Liu, Shan-Lu
Subject: Re: Commentary for Emerging Microbes & Infections
Date: Tuesday, February 11, 2020 11:54:35 PM
Attachments: image001.png
EMI-2019-nCoV_Commentary LJS.docx

Hi Shan-Lu,
I edited this version and added my name as I too feel strongly about denouncing this.
Here are more comments and some refs that I have made in replies to some reporters about this issue if you think any are useful to include. I also wonder if we might share this with Ralph Baric since he is a conspiracy target and maybe he could add additional points, but I know he would not want to be a co-author—not sure if he has time to answer.

The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that 2019-nCoV evolved by natural evolution. Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.

The closest virus relative to 2019-nCoV is bat CoV RaTG13. There are 4% nt differences between 2019-nCoV and RaTG13, corresponding to >1000 nt based on a genome size of 29k. These changes (SNP) are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region.
(Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature doi:10.1038/s41586-020-2012-7.

Regarding differences between civet cat SARSr-CoV and SARS-CoV, here is the accurate data: .
A total of 202 SNVs with multiple occurrences were identified, among which 200 were in the CDSs. Among the 128 nonsynonymous mutations, 89 led to a predicted radical amino acid changes
Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. Epub 2005 Feb 4.
Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human.
Song HD1, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L,

Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP.

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From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 10:32 PM
To: Linda Saif <saif.2@osu.edu>
Subject: Commentary for Emerging Microbes & Infections

Hi Linda,

Invited by the editor in chief of EMI, Lushan Su from UNC and I have written a commentary on the possible origin of the 2019-nCoV or SARS-CoV-2 in order to dispute some rumors, and we would like to invite you as a coauthor. Attached please find an almost complete draft (references needed) of the commentary, so kindly let me know what you think. Your comments and suggestions are very much appreciated.

Thanks.

Shan-Lu

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Evidence refuting~~Is SARS-CoV-2 a~~ laboratory origin of COVID-19 (2019-nCoV)~~?~~

Lishan Su[1], ~~and~~ Shan-Lu Liu[2, 3,4,5] and Linda J. Saif [5,6]

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[2] Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210, USA

[3] Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA

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Dr Linda J. Saif, Saif.2@osu.edu

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The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, ~~SARS-CoV-2~~COVID-19, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease discovered in 2019 (COVID-19 cite WHO ref here).

Commented [J1]: Not sure how widely used or accepted this is—please check or to avoid confusion use COVID-19?

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The ~~SARS-CoV-2~~COVID-19 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity (Nature 2020 refs).

Currently, there are speculations, ~~or~~ rumors and conspiracy theories that COVID-19 ~~the 2019-CoV~~ is of ~~a~~ laboratory origin. ~~Some people have Certain people suspected~~ alleged that the human ~~SARS-CoV-2~~COVID-19 was~~is directly~~ leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the ~~SARS-CoV-2~~COVID-19 (Nature, 2020). However, as we know, the human SARS-CoV and intermediate host palm civet~~s~~ SARS-like CoV shared 99.8% homology, which is only about 60 nt differences in the whole 29Kb (ck) genome sequence (refs). Given that there are greater than 1000 nt differences between the human ~~SARS-CoV-2~~COVID-19 and the bat RaTG13-CoV (refs), it is highly unlikely that RaTG13 CoV is the immediate source of ~~SARS-CoV-2~~COVID-19. ~~; this is particularly true in light of a low mutation rate of the coronaviruses (refs).~~ ~~A s~~Searching for an

Commented [J2]: CoVs have a high mutation rate like other RNA viruses!

intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human COVID-19. There is speculation that pangolins might have CoVs closely related to COVID-19, but the data to substantiate this is not yet published (ref).

Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells (refs). However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new COVID-19.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passage~~s~~ of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 ~~rounds of~~ passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was un~~not~~ able to use human ACE2 as a receptor for entry into human cells (refs). Civets were proposed to be an intermediate host of the bat-CoVs, capable of ~~before they~~ spreading SARS CoV

to humans (refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats ~~in 2013~~ and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV (JVI 2012), it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts (refs). To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could ~~can~~ indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis (Nat. Med. 2015).

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are now restricted ~~considered~~ as gain of function (GOF) studies under the US government-mandated pause policy (refs). The current NCP epidemic has restarted the debate over the risks of constructing such viruses that could have~~with~~ pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups (EMI, Nature…2020), the ~~SARS-CoV-2~~COVID-19 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible

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There are also rumors that the ~~SARS-CoV-2~~COVID-19 was~~is~~ artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (and not yet peer reviewed for accuracy) claiming that ~~SARS-CoV-2~~COVID-19 has HIV sequence in it and was~~is~~ thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the ~~SARS-CoV-2~~COVID-19 is not HIV-1 specific but random (EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have recently ~~decided to~~ withdrawn this report.

In summary, ~~we believe that~~ there is no credible evidence to support the claim that the ~~SARS-CoV-2~~COVID-19 ~~was~~ originated from a laboratory-engineered CoV. It is much more likely~~However, we cannot rule out the possibility~~ that ~~SARS-CoV-2~~COVID-19 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of ~~SARS-CoV-2~~COVID-19.

From: Liu, Shan-Lu
To: Saif, Linda
Subject: Commentary for Emerging Microbes & Infections
Date: Tuesday, February 11, 2020 10:32:03 PM
Attachments: EMI-2019-nCoV_Commentary.docx
image001.png

Hi Linda,

Invited by the editor in chief of EMI, Lushan Su from UNC and I have written a commentary on the possible origin of the 2019-nCoV or SARS-CoV-2 in order to dispute some rumors, and we would like to invite you as a coauthor. Attached please find an almost complete draft (references needed) of the commentary, so kindly let me know what you think. Your comments and suggestions are very much appreciated.

Thanks.

Shan-Lu

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Is SARS-CoV-2 a laboratory origin?

Lishan Su[1], and Shan-Lu Liu[2, 3,4.5]

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According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but is most similar to some bat beta-coronaviruses, with the highest being >96% identity (refs).

Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. Certain people suspected that the SARS-CoV-2 is directly leaked from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 (Nature, 2020). However, as we know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt differences in the whole genome sequence (refs). Given that there are greater than 1000 nt differences between the SARS-CoV-2 and the RaTG13-CoV (refs), it is highly unlikely RaTG13 is the immediate source of SARS-CoV-2; this is particularly true in light of a low mutation rate of the coronaviruses (refs). Searching for an intermediate host between bat and humans is needed.

Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells (refs). However, this claim lacks any scientific basis and must be discounted.

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of the many concerns raised by the international community, the authors who made the initial claim have recently decided to withdraw this report.

In summary, we believe that there is no credible evidence to support the claim that the SARS-CoV-2 was originated from a laboratory-engineered CoV. However, we cannot rule out the possibility that SARS-CoV-2 is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of SARS-CoV-2.

From: Liu, Shan-Lu
To: Saif, Linda
Subject: Re: EMI commentary
Date: Saturday, April 4, 2020 1:24:35 PM
Attachments: EMI_Published.pdf
image001.png

Thought I sent, but here it is again.

Be safe!

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
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From: "Saif, Linda" <saif.2@osu.edu>
Date: Saturday, April 4, 2020 at 1:21 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: EMI commentary

Please email me the final published pdf of this commentary
Thanks,
linda

Linda J. Saif, PhD
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From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 10:58 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan"

<weisssr@pennmedicine.upenn.edu>
Cc: "min.yang@emi2012.org" <min.yang@emi2012.org>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: EMI commentary

Dear all,

I have just submitted a commentary to EMI. See attached the submitted version.

Thank you.

Shan-Lu

THE OHIO STATE UNIVERSITY

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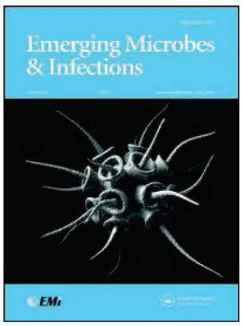

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Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su

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COMMENTARY

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

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The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1,100 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

It was proposed that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary

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evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to MA15 chimeric virus with the original human SARS S gene in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1585

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections invol ving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1623

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coro navirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30;395(10223):507 513.

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia out break associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2012 7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24;382(8):727 733.

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evol ution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characteriz ation of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS corona virus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin con verting enzyme 2 is a functional receptor for the

SARS coronavirus. Nature. 2003 Nov 27;426(6965): 450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2008 3
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

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Subject: Re: OSU COVID-19 working groups
Date: Friday, March 27, 2020 8:13:06 AM
Attachments: image001.png

The genome release time was Jan 11 in Chinese time but Jan 10 in the US. The email was based on my note.
Just a clarification.

Shan-Lu

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Subject: OSU COVID-19 working groups

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See below a link, and also attached, in yesterday's *Cell* regarding the origin and emergence of the SARS-CoV-2 that causes COVID-19. These authors released the first genome sequence of SARS-CoV-2 on January 10.

https://www.cell.com/cell/fulltext/S0092-8674(20)30328-7?dgcid=raven_jbs_aip_email

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Subject: OSU COVID-19 working groups

Date: Friday, March 27, 2020 8:02:38 AM

Attachments: SARS-CoV-2-Emergence_review.pdf
image001.png

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Leading Edge
Commentary

Cell

A Genomic Perspective on the Origin and Emergence of SARS-CoV-2

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https://doi.org/10.1016/j.cell.2020.03.035

The ongoing pandemic of a new human coronavirus, SARS-CoV-2, has generated enormous global concern. We and others in China were involved in the initial genome sequencing of the virus. Herein, we describe what genomic data reveal about the emergence SARS-CoV-2 and discuss the gaps in our understanding of its origins.

A New Human Coronavirus

The first reports of a novel pneumonia (COVID-19) in Wuhan city, Hubei province, China, occurred in late December 2019, although retrospective analyses have identified a patient with symptom onset as early as December 1st. Because the number of SARS-CoV-2 cases is growing rapidly and spreading globally, we will refrain from citing the number of confirmed infections. However, it is likely that the true number of cases will be substantially greater than reported because very mild or asymptomatic infections will often be excluded from counts. Any under-reporting of case numbers obviously means that the case fatality rate (CFR) associated with COVID-19 in the worst-hit regions will be lower than that currently cited. CFRs will also vary geographically, between age groups and temporally. Although these uncertainties will likely not be resolved without large-scale serological surveys, from current data it is clear that the CFR for COVID-19 is substantially higher than that of seasonal influenza but lower than that of two closely related coronaviruses that have similarly recently emerged in humans: SARS-CoV, responsible for the SARS outbreak of 2002–2003, and MERS-CoV that since 2015 has been responsible for the ongoing outbreak of MERS largely centered on the Arabian peninsula. However, it is also evident that SARS-CoV-2 is more infectious than both SARS-CoV and MERS-CoV and that individuals can transmit the virus when asymptomatic or presymptomatic, although how frequently remains uncertain.

An important early association was observed between the first reported cases of COVID-19 and the Huanan seafood and wildlife market in Wuhan city (which we both visited several years ago) where a variety of mammalian species were available for purchase at the time of the outbreak (Figure 1). Given that SARS-CoV-2 undoubtedly has a zoonotic origin, the link to such a "wet" market should come as no surprise. However, as not all of the early cases were market associated, it is possible that the emergence story is more complicated than first suspected. Genome sequences of "environmental samples"—likely surfaces—from the market have now been obtained, and phylogenetic analysis reveals that they are very closely related to viruses sampled from the earliest Wuhan patients. While this again suggests that the market played an important role in virus emergence, it is not clear whether the samples were derived from people who inadvertently deposited infectious material or from animals or animal matter present at that location. Unfortunately, the apparent lack of direct animal sampling in the market may mean that it will be difficult, perhaps even impossible, to accurately identify any animal reservoir at this location.

After clinical cases began to appear, our research team, along with a number of others, attempted to determine the genome sequence of the causative pathogen (Lu et al., 2020; Wu et al., 2020; Zhou et al., 2020; Zhu et al., 2020). We focused on a patient admitted to the Central Hospital of Wuhan on December 26, 2019, six days after the onset of symptoms (Wu et al., 2020). This patient was experiencing fever, chest tightness, cough, pain, and weakness, along with lung abnormalities indicative of pneumonia that appear to be commonplace in COVID-19 (Huang et al., 2020). Fortunately, next-generation meta-transcriptomic sequencing enabled us to obtain a complete viral genome from this patient on January 5, 2020. Initial analysis revealed that the virus was closely related to those of SARS-like viruses (family *Coronaviridae*). This result was immediately reported to the relevant authorities, and an annotated version of the genome sequence (strain Wuhan-Hu-1) was submitted to NCBI/GenBank on the same day. Although the GenBank sequence (GenBank: MN908947) was the first of SARS-CoV-2 available, it was subsequently corrected to ensure its accuracy. With the help of Dr. Andrew Rambaut (University of Edinburgh), we released the genome sequence of the virus on the open access Virological website (http://virological.org/) early on January 11, 2020. Afterwards, the China CDC similarly released SARS-CoV-2 genome sequences (with associated epidemiological data) on the public access GISAID database (https://www.gisaid.org/). At the time of writing, almost 200 SARS-CoV-2 genomes are publicly available, representing the genomic diversity of the virus in China and beyond and providing a freely accessible global resource. Importantly, the release of the SARS-CoV-2 genome sequence data facilitated the rapid development of diagnostic tests (Corman et al., 2020) and now an

Figure 1. The Huanan Seafood and Wildlife Market in Wuhan, China

The photographs (credit: E.C.H.) were taken when both authors visited the market together in October 2014 and highlight some of the wide variety of wildlife on sale, providing a potent mechanism for zoonotic transmission. Importantly, although many of the early COVID 19 cases were linked to this market, its role in the initial emergence of SARS CoV 2 remains uncertain.

infectious clone (Thao et al., 2020). The race to develop an effective vaccine and antivirals is ongoing, with trails of the latter underway (Wang et al., 2020).

Comparisons between SARS-CoV-2 and Other Coronaviruses

The earliest genomic genome sequence data made it clear that SARS-CoV-2 was a member of the genus *Betacoronavirus* and fell within a subgenus (*Sarbecovirus*) that includes SARS-CoV (MERS-CoV falls in a separate subgenus, *Merbecovirus*) (Lu et al., 2020; Wu et al., 2020; Zhou et al., 2020; Zhu et al., 2020). Indeed, initial comparisons revealed that SARS-CoV-2 was approximately 79% similar to SARS-CoV at the nucleotide level. Of course, patterns of similarity vary greatly between genes, and SARS-CoV and SARS-CoV-2 exhibit only ~72% nucleotide sequence similarity in the spike (S) protein, the key surface glycoprotein that interacts with host cell receptors.

Given these close evolutionary relationships, it is unsurprising that the genome structure of SARS-CoV-2 resembles those of other betacoronaviruses, with the gene order 5′-replicase ORF1ab-S-envelope(E)-membrane(M)-N-3′. The long replicase ORF1ab gene of SARS-CoV-2 is over 21 kb in length and contains 16 predicted non-structural proteins and a number of downstream open reading frames (ORFs) likely of similar function to those of SARS-CoV. Comparative genomic analysis has been greatly assisted by the availability of a related virus from a *Rhinolophus affinis* (i.e., horseshoe) bat sampled in Yunnan province, China, in 2013 (Zhou et al., 2020). This virus, denoted RaTG13, is ~96% similar to SARS-CoV-2 at the nucleotide sequence level. Despite this sequence similarity, SARS-CoV-2 and RaTG13 differ in a number of key genomic features, arguably the most important of which is that SARS-CoV-2 contains a polybasic (furin) cleavage site insertion (residues PRRA) at the junction of the S1 and S2 subunits of the S protein (Coutard et al., 2020). This insertion, which may increase the infectivity of the virus, is not present in related betacoronaviruses, although similar polybasic insertions are present in other human coronaviruses, including HCoV-HKU1, as well as in highly pathogenic strains of avian influenza virus. In addition, the receptor binding domain (RBD) of SARS-CoV-2 and RaTG13 are only ~85% similar and share just one of six critical amino acid residues. Both sequence and structural comparisons suggest that the SARS-CoV-2 RBD is well suited for binding to the human ACE2 receptor that was also utilized by SARS-CoV (Wrapp et al., 2020). Importantly, an independent insertion(s) of the amino acids PAA at the S1/S2 cleavage site was recently observed in a virus (RmYN02) sampled in mid-2019 from another *Rhinolophus* bat in Yunnan province, indicating that these insertion events reflect a natural part of ongoing coronavirus evolution (Zhou et al., 2020). While RmYN02 is relatively divergent from SARS-CoV-2 in the S protein (~72% sequence similarity), it is the closest relative (~97% nucleotide sequence similarity) of the human virus in the long replicase gene.

Although SARS-CoV and MERS-CoV are both closely related to SARS-CoV-2

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and have bat reservoirs, the biological differences between these viruses are striking. As noted above, SARS-CoV-2 is markedly more infectious, resulting in very different epidemiological dynamics to those of SARS-CoV and MERS-CoV. In these latter two viruses, there was a relatively slow rise in case numbers, and MERS-CoV has never been able to fully adapt to human transmission: the majority of the cases are due to spillover from camels on the Arabian peninsula with only sporadic human-to-human transmission (Sabir et al., 2016). In contrast, the remarkable local and global spread of SARS-CoV-2 caught most by surprise. Determining the virological characteristics that underpin such transmissibility is clearly a priority.

The Zoonotic Origins of SARS-CoV-2

The emergence and rapid spread of COVID-19 signifies a perfect epidemiological storm. A respiratory pathogen of relatively high virulence from a virus family that has an unusual knack of jumping species boundaries, that emerged in a major population center and travel hub shortly before the biggest travel period of the year: the Chinese Spring Festival. Indeed, it is no surprise that epidemiological modeling suggests that SARS-CoV-2 had already spread widely in China before the city of Wuhan was placed under strict quarantine (Chinazzi et al., 2020).

It was also no surprise that early genomic comparisons revealed that the most closely related viruses to SARS-CoV-2 came from bats (Zhou et al., 2020). Sampling in recent years has identified an impressive array of bat coronaviruses, including RaTG13 and RmYN02 (Hu et al., 2017; Yang et al., 2015). Hence, bats are undoubtedly important reservoir species for a diverse range of coronaviruses (Cui et al., 2019). Despite this, the exact role played by bats in the zoonotic origin of SARS-CoV-2 is not established. In particular, the bat viruses most closely related to SARS-CoV-2 were sampled from animals in Yunnan province, over 1,500 km from Wuhan. There are relatively few bat coronaviruses from Hubei province, and those that have been sequenced are relatively distant to SARS-CoV-2 in phylogenetic trees (Lin et al., 2017). The simple inference from this is that our sampling of bat viruses is strongly biased toward some geographical locations. This will need to be rectified in future studies. In addition, although sequence similarity values of 96%–97% make it sound like the available bat viruses are very closely related to SARS-CoV-2, in reality this likely represents more than 20 years of sequence evolution (although the underlying molecular clock may tick at an uncertain rate if there was strong adaptive evolution of the virus in humans). It is therefore almost a certainty that more sampling will identify additional bat viruses that are even closer relatives of SARS-CoV-2. A key issue is whether these viruses, or those from any other animal species, contain the key RBD mutations and the same furin-like cleavage site insertion as found in SARS-CoV-2.

Although bats are likely the reservoir hosts for this virus, their general ecological separation from humans makes it probable that other mammalian species act as "intermediate" or "amplifying" hosts, within which SARS-CoV-2 was able to acquire some or all of the mutations needed for efficient human transmission. In the case of SARS and MERS, civets and camels, respectively, played the role of intermediate hosts, although as MERS-CoV was likely present in camels for some decades before it emerged in humans during multiple cross-species events, these animals may be better thought of as true reservoir hosts (Sabir et al., 2016). To determine what these intermediate host species might be, it is imperative to perform a far wider sampling of animals from wet markets or that live close to human populations. This is highlighted by the recent discovery of viruses closely related to SARS-CoV-2 in Malayan pangolins (*Manis javanica*) illegally imported into southern China (Guangdong and Guangxi provinces). The Guangdong pangolin viruses are particularly closely related to SARS-CoV-2 in the RBD, containing all six of the six key mutations thought to shape binding to the ACE2 receptor and exhibiting 97% amino acid sequence similarity (although they are more divergent from SARS-CoV-2 in the remainder of the genome). Although pangolins are of great interest because of how frequently they are involved in illegal trafficking and their endangered status, that they carry a virus related to SARS-CoV-2 strongly suggests that a far greater diversity of related betacoronaviruses exists in a variety of mammalian species but has yet to be sampled.

While our past experience with coronaviruses suggests that evolution in animal hosts, both reservoirs and intermediates, is needed to explain the emergence of SARS-CoV-2 in humans, it cannot be excluded that the virus acquired some of its key mutations during a period of "cryptic" spread in humans prior to its first detection in December 2019. Specifically, it is possible that the virus emerged earlier in human populations than envisaged (perhaps not even in Wuhan) but was not detected because asymptomatic infections, those with mild respiratory symptoms, and even sporadic cases of pneumonia were not visible to the standard systems used for surveillance and pathogen identification. During this period of cryptic transmission, the virus could have gradually acquired the key mutations, perhaps including the RBD and furin cleavage site insertions, that enabled it to adapt fully to humans. It wasn't until a cluster of pneumonia cases occurred that we were able to detect COVID-19 via the routine surveillance system. Obviously, retrospective serological or metagenomic studies of respiratory infection will go a long way to determining whether this scenario is correct, although such early cases may never be detected.

Another issue that has received considerable attention is whether SARS-CoV-2 is a recombinant virus, and whether such recombination might have facilitated its emergence (Lu et al., 2020; Wu et al., 2020). The complicating factor here is that sarbeviruses, and coronaviruses more broadly, experience widespread recombination, so that distinguishing recombination that assisted virus emergence from "background" recombination events is not trivial. Recombination is visible at multiple locations across the sarbevirus genome, including in the S protein, and in bat viruses closely related to SARS-CoV-2. For example, there is some evidence for recombination among SARS-CoV-2, RaTG13, and the Guangdong pangolin CoVs (Lam et al., 2020), and the genome of RmYN02 has similarly been widely impacted by recombination (Zhou et al., 2020). However, trying to

determine the exact pattern and genomic ancestry of recombination events is difficult, particularly as many of the recombinant regions may be small and are likely to change as we sample more viruses related to SARS-CoV-2. To resolve these issues, it will again be necessary to perform a far wider sampling of viral diversity in animal populations.

Ongoing Genomic Evolution of SARS-CoV-2

As the COVID-19 epidemic has progressed, so more viral genomes have been sequenced. As expected given their recent common ancestry, the earliest samples from Wuhan contained relatively little genetic diversity. While this can prevent detailed phylogenetic and phylogeographic inferences, it does show that the public health authorities in Wuhan did a remarkable job in detecting the first cluster of pneumonia cases. However, this seemingly recent common ancestry does not exclude a pre-outbreak period of cryptic transmission in humans. Although accumulating genetic diversity means that it is now possible to detect distinct phylogenetic clusters of SARS-CoV-2 sequences, it is difficult to determine using genomic comparisons alone whether the virus is fixing phenotypically important mutations as it spreads through the global population, and any such claims require careful experimental verification.

Given the high mutation rates that characterize RNA viruses, it is obvious that many more mutations will appear in the viral genome and that these will help us to track the spread of SARS-CoV-2 (Grubaugh et al., 2019). However, as the epidemic grows, our sample size of sequences will likely be so small relative to the total number of cases that it will be very difficult, if not impossible, to detect individual transmission chains. Caution must therefore always be exercised when attempting to infer exact transmission events. As an aside, although coronaviruses likely have lower mutation rates than other RNA viruses because of an inherent capacity for some proof-reading activity due to a 3′-to-5′ exoribonuclease (Minskaia et al., 2006), their long-term rates of nucleotide substitution (i.e., of molecular evolution) fall within the distribution of those seen in other RNA viruses (Holmes et al., 2016). This suggests that lower mutation rates are to some extent compensated by high rates of virus replication within hosts. Although there is no evidence that this capacity to mutate (common to RNA viruses) will result in any radical changes in phenotype—such as in transmissibility and virulence—as these only rarely change at the scale of individual disease outbreaks (Grubaugh et al., 2020), it is obviously important to monitor any changes in phenotype as the virus spreads. In all likelihood, any drop in the number of cases and/or CFR of COVID-19 will likely be due to rising immunity in the human population and epidemiological context rather than mutational changes in the virus.

Conclusions

It seems inevitable that SARS-CoV-2 will become the fifth endemic coronavirus in the human population (along with HKU1, NL63, OC43, and 229E) and one that is currently spreading in a totally susceptible population. Coronaviruses clearly have the capacity to jump species boundaries and adapt to new hosts, making it straightforward to predict that more will emerge in the future, although quite why coronaviruses possess this capacity in comparison to some other RNA viruses is unclear. Critically, the surveillance of animal coronaviruses should include animals other than bats, as the role of intermediate hosts is likely of major importance, providing a more direct pathway for the virus to emerge in humans. Given the enormous diversity of viruses in wildlife and their ongoing evolution, arguably the simplest and most cost-effective way to reduce the risk of future outbreaks is to limit our exposure to animal pathogens as much as possible. While our intimate relationship with the animal world means we cannot build impregnable barriers, stronger action against the illegal wildlife trade and removing all mammalian (and perhaps avian) wildlife from wet markets will provide an important buffer.

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WEB RESOURCES

GISAID, https://www.gisaid.org/
Virological, http://virological.org/

REFERENCES

Chinazzi, M., Davis, J.T., Ajelli, M., Gioannini, C., Litvinova, M., Merler, S., Pastore Y Piontti, A., Mu, K., Rossi, L., Sun, K., et al. (2020). The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID 19) outbreak. Science. eaba9757. Published online March 6, 2020. https://doi.org/10.1126/science.aba9757.

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K.W., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M.L., et al. (2020). Detection of 2019 novel coronavirus (2019 nCoV) by real time RT PCR. Euro Surveill. *25*, 2000045.

Coutard, B., Valle, C., de Lamballerie, X., Canard, B., Seidah, N.G., and Decroly, E. (2020). The spike glycoprotein of the new coronavirus 2019 nCoV contains a furin like cleavage site absent in CoV of the same clade. Antiviral Res. *176*, 104742.

Cui, J., Li, F., and Shi, Z. L. (2019). Origin and evo lution of pathogenic coronaviruses. Nat. Rev. Mi crobiol. *17*, 181 192.

Grubaugh, N.D., Ladner, J.T., Lemey, P., Pybus, O.G., Rambaut, A., Holmes, E.C., and Andersen, K.G. (2019). Tracking virus outbreaks in the twenty first century. Nat. Microbiol. *4*, 10 19.

Grubaugh, N.D., Petrone, M.E., and Holmes, E.C. (2020). We shouldn't worry when a virus mutates during disease outbreaks. Nat Microbiol. Pub lished online February 18, 2020. https://doi.org/10.1038/s41564 020 0690 4.

Holmes, E.C., Dudas, G., Rambaut, A., and Ander sen, K.G. (2016). The evolution of Ebola virus: In sights from the 2013 2016 epidemic. Nature *538*, 193 200.

Hu, B., Zeng, L.P., Yang, X.L., Ge, X.Y., Zhang, W., Li, B., Xie, J.Z., Shen, X.R., Zhang, Y.Z., Wang, N., et al. (2017). Discovery of a rich gene pool of bat SARS related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS Pathog. *13*, e1006698.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet *395*, 497 506.

Lam, T.T. Y., Shum, M.H. H., Zhu, H. C., Tong, Y. G., Ni, X. B., Liao, Y. S., Wei, W., Cheung, W.Y. M., Li, W. J., Li, L. F., et al. (2020). Identifi cation of 2019 nCoV related coronaviruses in Malayan pangolins in southern China. bioRxiv. https://doi.org/10.1101/2020.02.13.945485.

Lin, X. D., Wang, W., Hao, Z. Y., Wang, Z. X., Guo, W. P., Guan, X. Q., Wang, M. R., Wang, H. W., Zhou, R. H., Li, M. H., et al. (2017). Extensive di versity of coronaviruses in bats from China. Virology *507*, 1 10.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet *395*, 565 574.

Minskaia, E., Hertzig, T., Gorbalenya, A.E., Campanacci, V., Cambillau, C., Canard, B., and Ziebuhr, J. (2006). Discovery of an RNA virus 3′ >5′ exoribonuclease that is critically involved in coronavirus RNA synthesis. Proc. Natl. Acad. Sci. USA *103*, 5108 5113.

Sabir, J.S.M., Lam, T.T. Y., Ahmed, M.M.A., Li, L., Shen, Y., Abo Aba, S.E.M., Qureshi, M.I., Abu Zeid, M., Zhang, Y., Khiyami, M.A., et al. (2016). Co circulation of three camel coronavirus species and recombination of MERS CoVs in Saudi Arabia. Science *351*, 81 84.

Thao, T.T.N., Labroussaa, F., Ebert, N., V'kovski, P., Stalder, H., Portmann, J., Kelly, J., Steiner, S., Holwerda, M., Kratzel, A., et al. (2020). Rapid reconstruction of SARS CoV 2 using a synthetic genomics platform. bioRxiv. https://doi.org/10.1101/2020.02.21.959817.

Wang, Y., Zhou, F., Zhang, D., Zhao, J., Du, R., Hu, Y., Cheng, Z., Gao, L., Jin, Y., Luo, G., et al. (2020). Evaluation of the Efficacy and Safety of Intravenous Remdesivir in Adult Patients with Severe Pneumonia caused by COVID 19 virus infection: study protocol for a Phase 3 Randomized, Double blind, Placebo controlled, Multicentre trial. BMC Trials. https://doi.org/10.21203/rs.2.24058/v1.

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C. L., Abiona, O., Graham, B.S., and McLellan, J.S. (2020). Cryo EM structure of the 2019 nCoV spike in the prefusion conformation. Science *367*, 1260 1263.

Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian, J.H., Pei, Y.Y., et al. (2020). A new coronavirus associated with human respiratory disease in China. Nature *579*, 265 269.

Yang, X.L., Hu, B., Wang, B., Wang, M.N., Zhang, Q., Zhang, W., Wu, L.J., Ge, X.Y., Zhang, Y.Z., Daszak, P., et al. (2015). Isolation and characterization of a novel bat coronavirus closely related to the direct progenitor of Severe Acute Respiratory Syndrome coronavirus. J. Virol. *90*, 3253 3256.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature *579*, 270 273.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., et al.; China Novel Coronavirus Investigating and Research Team (2020). A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. *382*, 727 733.

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Subject: Re: OSU COIVD-19 working groups
Date: Tuesday, March 24, 2020 10:34:25 PM
Attachments: image001.png
TrendsMolMed Sun 2020 preprint.pdf

The Trends in Molecular Med COVID review did not have canine ACE2 listed in Table 1. I did the alignment and added it to the table in the attached version, in case this is useful to anyone else. Overall homology was about 82%, and that of the proposed critical binding residues is 13/19.

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[WARNING: External Email - Use Caution]

Good morning everybody!

Thank you for your interest in joining the OSU COIVD-18 discussion and working groups. I believe I have included everyone who expressed an interest, but if not, please let me know.

Today, I would like to share a new paper just appearing on the BioRxiv website. I thought this is a cool study.

https://www.biorxiv.org/content/10.1101/2020.03.22.002386v1

Attached also please find a review article, which I thought is comprehensive.

Shan-Lu

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Trends in Molecular Medicine

CellPress REVIEWS

Review

COVID-19: Epidemiology, Evolution, and Cross-Disciplinary Perspectives

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The recent outbreak of COVID-19 in Wuhan turned into a public health emergency of international concern. With no antiviral drugs nor vaccines, and the presence of carriers without obvious symptoms, traditional public health intervention measures are significantly less effective. Here, we report the epidemiological and virological characteristics of the COVID-19 outbreak. Originated in bats, 2019-nCoV/ severe acute respiratory syndrome coronavirus (SARS-CoV)-2 likely experienced adaptive evolution in intermediate hosts before transfer to humans at a concentrated source of transmission. Similarities of receptor sequence binding to 2019-nCoV between humans and animals suggest a low species barrier for transmission of the virus to farm animals. We propose, based on the One Health model, that veterinarians and animal specialists should be involved in a cross-disciplinary collaboration in the fight against this epidemic.

Highlights

The basic reproductive number (R_0) of 2019 nCoV is higher than R_0 of severe acute respiratory syndrome coronavirus (SARS CoV) and Middle East respiratory syndrome coronavirus (MERS CoV). COVID 19 presents with asymptomatic infections, with potential to propagate and perpetuate this epidemic.

2019 nCoV isolated from patients shows limited sequence diversity, suggesting that the interspecies transmission event was very recent and that the source of the virus was focused, possibly a point source event.

The amino acid sequence in the ACE2 receptor responsible for 2019 nCoV binding in farm animals and cats has only a few exchanges compared with the human receptor, suggesting that the species barrier for virus transmission is small.

Emergence of COVID-19

In December 2019, a cluster of pneumonia with unknown etiology appeared in Wuhan City, Hubei Province of China. Several of the initial patients visited a wet seafood market where other wildlife species were also sold. Subsequent virus isolation from human patients and molecular analysis showed that the pathogen was a new coronavirus (CoV), first named 2019-nCoV, and subsequently this disease was renamed by WHO as COVID-19. A study group of the International Committee on Taxonomy of Viruses (ICTV) proposed the name SARS-CoV-2, but this name remains to be officially approved [1]. This new CoV is now the seventh member of the *Coronaviridae* known to infect humans. With the explosive increase of confirmed cases, the WHO declared this outbreak a public health emergency of international concern (PHEIC) on January 30, 2020.

CoVs are a class of genetic diverse viruses found in a wide range of host species, including birds and mammals. Many CoVs cause intestinal and respiratory infections in animals and in humans [2–5]. CoV came into the spotlight in 2002–2003, when clusters of 'atypical pneumonia' were first reported in Guangdong Province, subsequently spreading to Hong Kong. Researchers in Hong Kong isolated a novel CoV virus (SARS-CoV) and the disease was later renamed **severe acute respiratory syndrome (SARS)** (see Glossary). Because of international travel, the virus spread from Hong Kong to the rest of the world and more than 8000 people in 26 countries became infected, with a case fatality rate of approximately 10% (https://www.who.int/csr/sars/country/table2004_04_21/en/). SARS posed a serious public health threat to the world at that time, with a significant negative impact on the economy in affected areas. Subsequent studies found that SARS-CoV originated from bats and interspecies transmission to humans took place via an intermediate host: Himalayan palm civets (*Paguma larvata*) or raccoon dogs (*Nyctereutes procyonoides*) [5–7]. Another well-known CoV of animal origin is **Middle East respiratory syndrome coronavirus (MERS-CoV)**, which has an even higher case fatality rate, but it is rarely transmitted between humans.

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As major natural reservoir species of *Alphacoronavirus* and *Betacoronavirus*, bats carry highly diverse SARS-like-CoVs. These bats are distributed in many provinces of China. The genetic diversity of these SARS-like-CoVs and their molecular evolution within their natural host species have been studied intensively [2,8–11]. Here, we review the recent but still very limited facts about the current epidemiology of COVID-19 and discuss viral characteristics of 2019-nCoV on the backdrop of our knowledge about the previous epidemic of SARS and MERS.

Epidemiology of COVID-19

As of 24:00 February 20, 2020 (UTC+8), there are a total of 75 995 confirmed cases, including 2239 fatalities in China (mainland: 75 891; Hong Kong: 68; Macao: 10; and Taiwan: 26), and 1200 confirmed cases, including eight fatal ones outside China, in all five continents (Figure 1). The epidemiology curve can roughly be divided into three phases.

i. The local outbreak by exposure in the aforementioned food wholesale market marks the first phase. From the first case in December 2019 to the emergence of new cases outside Wuhan by January 13, 2020, a total of 41 cases were confirmed. Epidemiologic analysis showed that already in this initial phase, person-to-person transmission had occurred by close contact [12].
ii. The second phase started on January 13, marked by rapid expansion and spread of the virus within hospitals (nosocomial infection) and by family transmission (close-contact transmission). In this phase the epidemic spread from Wuhan to other areas [12–18]. The first case outside of China was reported in Thailand on January 13, caused by a Wuhan resident travelling to this country. On January 19 cases were reported from outside Wuhan, in Beijing City, and in the Guangdong Province, indicating that the virus had spread within China, and the total number of confirmed cases rose to 205. Already by January 23, 29 provinces, plus six foreign countries, had reported a total of 846 confirmed cases, an approximately 20-fold increase from the first phase. Meanwhile, Wuhan city implemented a 'lock-down' (i.e., shutting down all movement within and out of the city). Unfortunately, this period coincided with the traditional mass movement of people, a form of 'home-coming', before Chinese New Year and thus more than 5 million people had already left Wuhan.
iii. The third phase started on January 26, which is marked by the rapid increase of cluster cases. On February 10, retrospective analysis showed that the number of clustered cases accounted for 50–80% of all confirmed cases in Beijing, Shanghai, Jiangsu, and Shandong [19]. On January 30, the number increased 240-fold, reaching 9826 confirmed cases, and the WHO declared this epidemic a PHEIC. By February 11, 44 730 confirmed cases and 16 067 suspected cases were reported in about 1386 counties and districts in China [20]. However, there were only 441 confirmed cases in 24 countries outside of China. The fatality rate remained high in China, with a total of 1114 deaths, but with just one fatality outside China, in the Philippines. By February 12, due to adoption of a new clinical definition for diagnosis in Hubei province, newly confirmed cases jumped to 14 840, of which 13 332 cases were based only on clinical diagnosis. By that time, 25 countries had reported 60 329 infections, with 1471 times the initial number (Figure 1A). Of note, February 3 seems to be a tipping point of the epidemic, from which time the daily number of confirmed cases outside Hubei began to decline. Whether it reflects a success of the 'Wuhan lock-down' and other public health measures, or virus transmission reduced for other reasons, remains unclear.

Furthermore, 85.8% of 37 269 confirmed cases had either lived in or traveled to Wuhan, or had close contact with persons who had been to Wuhan [20,21]. Unfortunately, as of February 11, 1716 medical-related staff from 422 medical institutions were infected, of which 1688 confirmed

cases were analyzed. Among them, 64% were infected in Wuhan city and 23.3% in the rest of Hubei, excluding Wuhan [20]. The specific causes of the infection of medical staff and the failure of protection need further investigation.

Initial evaluation of COVID-19 transmission dynamics showed that the **basic reproductive number (R_0)** of 2019-nCoV is estimated to be 1.4–3.9 [12]. The R_0 of SARS-CoV in the absence of interventions was 2.3–3.7 [22,23]. Breban *et al.* estimated MERS-CoV R_0 to be 0.50–0.92 by analysis of 55 of the first 64 laboratory-confirmed cases [24]. With the implementation of rapid diagnosis, coupled with effective isolation of patients, the R_0 of SARS-CoV dropped to less than 1, explaining why the SARS-CoV outbreak could eventually be controlled [25–27]. However, it is worth noting that R_0 estimates may vary upon numerous biologic, socio-behavioral, and environmental factors, and must be interpreted with caution [28].

Clinical Phenotype of COVID-19

Major initial symptoms of COVID-19 include fever, cough, muscular soreness, and dyspnea. Some patients showed atypical symptoms, such as diarrhea and vomiting. However, the clinical phenotype is confounded by the fact that 25.2% patients had at least one other underlying medical condition [13,15,29–32]. The overall clinical characteristics of COVID-19 were also influenced by the different phases of this epidemic [12,13,21,29,33]. Patients in the first and second phase of the epidemic were older, more likely to be male, and likely to have exposure to the seafood market. Clinically, they had more bilateral patchy shadows, or ground glass opacity in the lungs [13,21,29,33–36]. In addition, the mortality rate of the first and second phases of the epidemic was 4.3–15% and thus significantly higher than the 1.36% determined for the later phase of the epidemic [13,21,29,33,34]. This higher mortality rate was either due to: (i) more people with underlying medical conditions, such as high blood pressure and diabetes [12,13,19,20,29,31,33]; (ii) during the early phase of this epidemic the virus was more pathogenic; or (iii) the lower mortality rate was skewed by a larger sample size at the later phase of this epidemic. Importantly, 889 asymptomatic or subclinically symptomatic infected cases were reported [20,37]. Asymptomatic infection was also documented in Germany: two asymptomatic patients' throat samples were tested positive by reverse transcription (RT)-PCR and by virus isolation, while both patients remained well and afebrile for 7 days [38]. Importantly, the asymptomatic manifestation jeopardizes the screening of infected people by temperature measurements or by overt signs and symptoms [12,13,19,20,29,31,33]. Virus infection is not selective in age, as it was reported even in a 1-month-old infant [20,21,37]. Of the 44 672 confirmed cases, 77.8% are between 30 and 69 years old and 51.4% are male [20]. Until now, there is no evidence for intrauterine infection by vertical transmission in women who developed COVID-19 during late pregnancy and no evidence that pregnant women are more susceptible compared with other adult patients [34,39]. Although currently the number of new infections is decreasing, the COVID-19 epidemic is still ongoing. The order to Chinese citizens to return to work, which is accompanied by massive population movement, will likely increase the risk of transmission again. Overall, the current mortality rate of COVID-19 in China is 2.9% and in foreign countries 0.7%. The overall mortality rate remains the highest in Hubei (3.4%), 4.9 times higher than in other provinces (0.7%). For comparison, SARS-CoV exhibited a case fatality rate of 9.6% (774/8096) and MERS-CoV had a fatality rate of 34.4% (858/2494) (https://www.who.int/csr/sars/country/table2004_04_21/en/; https://www.who.int/emergencies/mers-cov/en/). However, 2019-nCoV is more infectious than SARS-CoV or MERS-CoV [40,41].

Origin and Evolution of 2019-nCoV

As animal markets had been implicated in the SARS-CoV outbreak of 2002–2003, and initial 2019-nCoV infections are also related to the seafood market with wildlife trading, it was soon assumed that wild animals were also involved in the emergence of 2019-nCoV. Yet, from

Glossary

Avian influenza virus: influenza viruses that circulate in birds, mainly in water fowl, without causing clinical symptoms (low pathogenic influenza virus). Occasionally they are introduced into poultry, where they might acquire a polybasic cleavage site within their main glycoprotein hemagglutinin (HA). HA is then cleaved by the ubiquitous protease furin and the now highly pathogenic virus causes a systemic and hence deadly infection ('bird flu').

Basic reproductive number (R_0): an epidemiologic metric to describe the contagiousness or transmissibility of infectious agents. It refers to the expected number of secondary infections that one infected person generates on average in an entirely susceptible population. It allows estimation of the potential of an agent to cause an epidemic, the extent of transmission without control measures, and the efficiency of control measures to reduce transmission.

Enfuvirtide: antiviral drug (trade name Fuzeon), licensed for the treatment of HIV infection, that inhibits the membrane fusion activity of its glycoprotein and hence cell entry of the virus.

Middle East respiratory syndrome coronavirus (MERS-CoV): a highly lethal and zoonotic pathogen that was first identified in Saudi Arabia in 2012. Since 2012, MERS has been reported in 27 countries. Scientific evidence suggests that people are infected through direct or indirect contact with infected dromedary camels.

Plaque: a plaque is an area of dead cells within a cell monolayer. The plaque is caused by an infection of a single cell by one virus that then spreads to neighboring cells. Plaque assays are used to determine the number of infectious virus particles.

Severe acute respiratory syndrome (SARS): caused by SARS coronavirus (SARS CoV), which first occurred in Guangdong province, China, and became a global epidemic disease in 2002–2003. The disease was reported by 26 countries, with a case fatality rate of approximately 10%. Studies showed that SARS CoV originated from bats and was transmitted to humans via palm civets or raccoon dogs.

ZDHHC family: family of polytopic membrane proteins that are characterized by the amino acid motif DHHC, which is located within a cysteine rich domain in one of its cytoplasmic loops. Many of the family

which species and under what circumstance the virus crossed the species barrier to infect humans remains to be clarified. Early investigations about the origin of COVID-19 suggested that the 2019-nCoV may have jumped from bats to human [42,43]. This is not unprecedented since bat viruses have been shown to 'jump' the species barrier frequently to infect new species [44–50]. However, since bats were in hibernation when the outbreak occurred, and it was uncertain whether bats were sold at the market, the virus is more likely to have been transmitted via

members have been shown to transfer long chain fatty acids to cysteine residues of cellular and viral proteins.

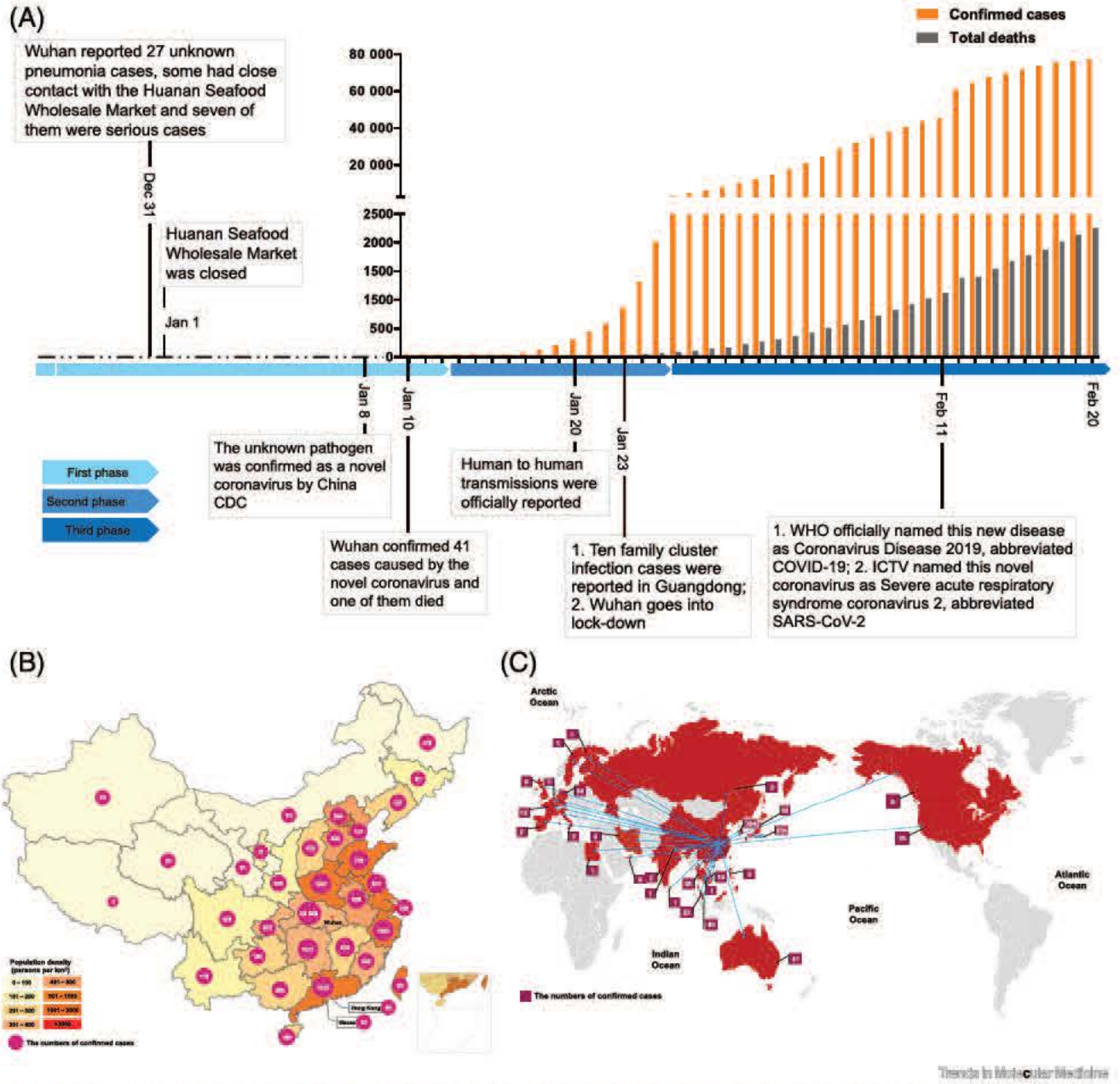

Figure 1. Spreading of the 2019 nCoV Epidemic. (A) Timeline of events during the 2019 nCoV epidemic. (B) Human confirmed cases of 2019 nCoV infection in China. (C) Human confirmed cases of 2019 nCoV infection in the world (Last update on 24:00 UTC+8, 20 February 2020). Abbreviations: CDC, Centers for Disease Control; ICTV, International Committee on Taxonomy of Viruses.

Box 1. Evolution Analysis Methods

Sequences analyzed: 18 betacoronavirus sequences and 95 full length 2019 nCoV genomes kindly made available from GISAID (https://www.gisaid.org/) and from the National Center for Biotechnology Information GenBank (https://www.ncbi.nlm.nih.gov/) platforms. Some sequences were omitted, as they were too short, contained sequencing artefacts, resulted from resequencing of the same sample, or had insufficient annotations.

Sequence alignment and potential recombination analysis: sequences were aligned using MAFFT [83] and manually adjusted in MEGA7 [84]. The breakpoints were detected using the phylogenetic incongruence among segments in sequence alignments using GARD and are shown by using the Simplot version 3.5.1 and Kimura model. Slide windows were set as 1000 bp, with each step 500 bp.

Phylogenetic analysis: all ML trees were reconstructed using the general time reversible substitution model with gamma distributed rate heterogeneity and 1000 bootstraps by RAxML (v4.8.10) [85].

other species on the market. Genomic analyses of 2019-nCoV demonstrate a 96% nucleotide identity with a CoV isolated from a bat: BetaCoV/RaTG13/2013 [42]. Previous reports showed that species from the bat genera *Rhinolophus* in southern China are a rich pool of SARS-like-CoVs, which belong to the subgenera *Sarbecovirus*. These viruses exhibit rich genetic diversity and frequent recombination events, which may increase the potential for cross-species transmission [7,42,51–55]. Here, we reconstructed the evolutionary history of the 2019-nCoV cluster (Box

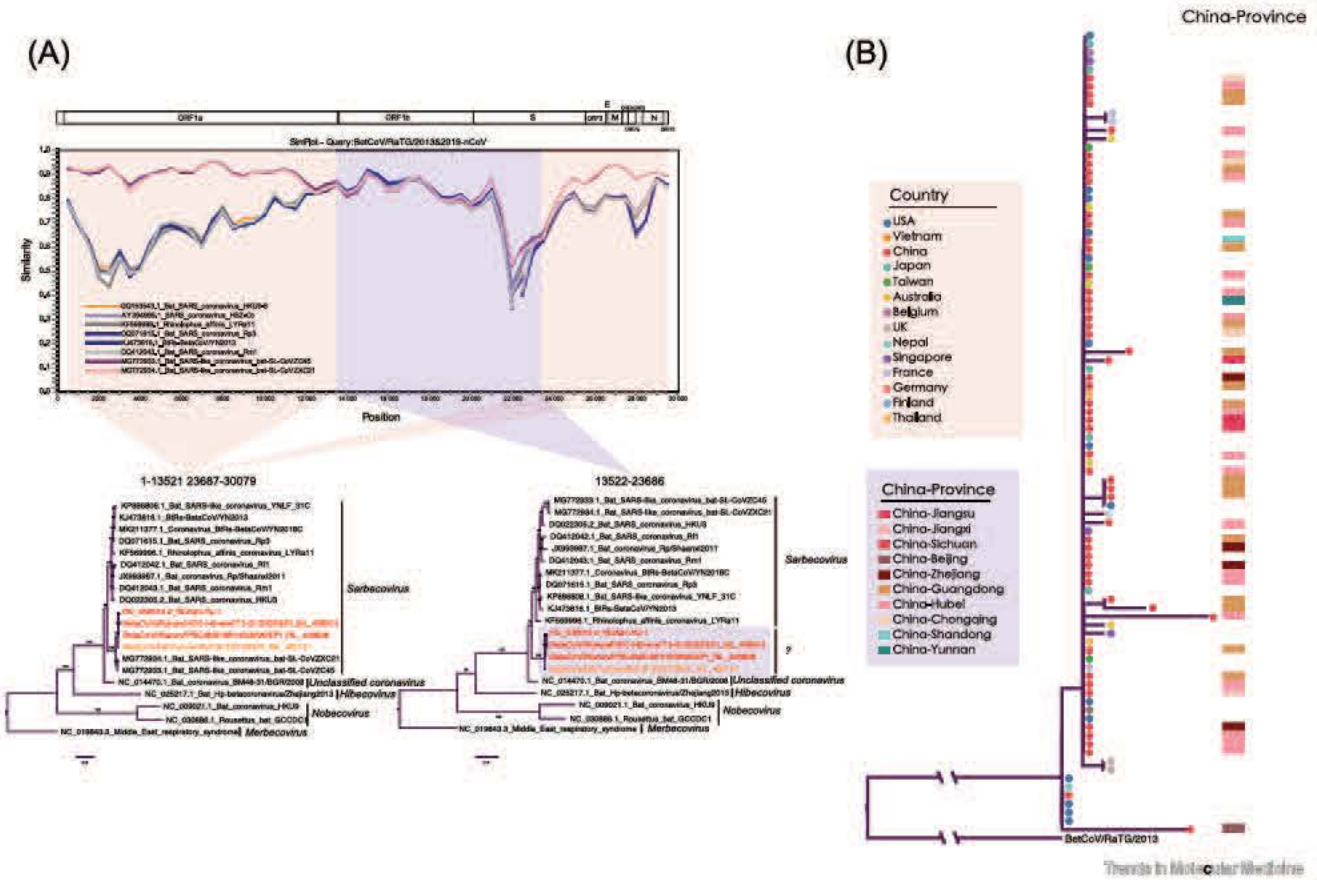

Figure 2. Structure of the 2019 nCoV Genome. (A) Recombination analysis of 2019 nCoV. A rescaled structure of the 2019 nCoV genome (top) and similarity recombination analysis with reference sequences using Simplot v3.5.1 (accession number BetaCoV/Wuhan/WIV02/2019|EPI_ISL_402127 EPI_ISL_402131, KJ473816, DQ071615, DQ412043, GQ153543, AY394995, KF569996, MG772933, MG772934). Sequences were separated based on potential recombination breakpoint on nucleotides 13 522 and 23 686. Maximum likelihood (ML) phylogenetic trees inferred for the pink and purple regions confirm different topologies and recombination. (B) ML tree of 2019 nCoV spike protein gene. The ML tree was reconstructed using the general time reversible substitution model with gamma distributed rate heterogeneity and 1000 bootstraps using RAxML (v4.8.10).

1). Based on recombination analysis and phylogenetic trees (Figure 2A), we found that 2019-nCoV shares a most recent common ancestor with BetaCoV/RaTG13/2013 (EPI ISL 402131), because both viruses are in the same cluster. However, our results indicate that this cluster may be the result of convergent evolution or complex recombination events involving at least two virus species with differing evolutionary histories (Figure 2A). The two external segments of this clustered viral genome, encompassing nucleotide (nt) 1 to nt 13 521, and nt 23 687 to nt 30 079, are similar to bat CoVs ZC45 and ZXC21. The first segment includes ORF1a and the second segment includes the C terminus of the S protein, ORF3, E, M, ORF6, ORF7a, ORF8, N, and ORF10 (Figure 2A). This finding is also supported by reconstructing maximum likelihood (ML) phylogenetic trees, which reveal that segments from nt 1 to nt 13 521 and from nt 23 687 to nt 30 079 are clustered with *Sarbecovirus*. However, based on the ML tree result, the middle segment from nt 13 522 to nt 23 686 of 2019-nCoV genome and RaTG13 does not cluster with *Sarbecovirus*. It forms a new branch in the phylogenetic tree, located between *Sarbecovirus* and an Unclassified CoV. In addition, a recent preliminary report showed that the receptor-binding motif (RBM) of these two genomes shares a very low sequence similarity [56]. This divergence indicates a possible alternative source for the RBM encoding sequence in 2019-nCoV, as suggested by other preliminary reports [52,57]. Interestingly, Lam *et al.* found several putative pangolin CoV sequences with 85.5% to 92.4% similarity to 2019-nCoV [52]. Further preliminary studies showing the existence of multiple lineages of pangolin CoVs with genetic similarity to 2019-nCoV further support the hypothesis that pangolins served as a potential intermediate host [52,58]. The currently available data do not fully elucidate if the virus was directly transmitted from bats to humans or indirectly through an intermediate host, nor do they currently rule out convergent evolution as an alternative hypothesis to recombination to explain the discordant phylogenetic trees. Consequentially, more sequence data are needed to confirm the specific source and origin of the 2019-nCoV, which can only be achieved by enhanced collection and monitoring of bat and other wild animal samples.

The topology of a phylogenetic tree with all the currently available spike protein gene sequences of 2019-nCoV shows high similarities between human isolates (Figure 2B), indicating only minimal genetic variation, which is rather unexpected for fast evolving RNA viruses [42]. However, these similarities could be the result of a relatively recent common ancestor, suggesting that the emergence of the virus was a recent event. Furthermore, results are similar to the finding from other preliminary reports that indicate that the virus source of interspecies transmission was highly concentrated or limited, possibly a single event [14,42,43,59]. In addition, the high sequence similarity among the viruses isolated from patients indicates a recent introduction to humans [60]. In all, these results further support the role of Wuhan as the epicenter of the outbreak and there is no evidence for other sources of this 2019-nCoV.

Structure and Function of the Spike Protein of 2019-nCoV, the Major Determinant of Cell Tropism

The spike protein (S) is the major determinant of cell tropism and hence interspecies transmission of CoVs, since it binds the virus to a cellular receptor and subsequently catalyzes virus entry by membrane fusion. The 3D structure of the viral S of 2019-nCoV determined by electron microscopy (Figure 3A, [61]) revealed its similarity to S of other CoVs. This allows deduction of further features from other CoVs. S is a type I trimeric transmembrane protein with an N terminal cleavable signal peptide, one large and heavily *N*-glycosylated ectodomain (60–90 carbohydrates per trimer), a transmembrane region, and a cytoplasmic tail containing a cluster of *S*-acylated cysteine residues. The ectodomain is cleaved by proteases into the between genera highly variable S1 domain, carrying the receptor-binding activities, and the more conserved S2 domain that catalyzes membrane fusion. The S1 domain is further divided into

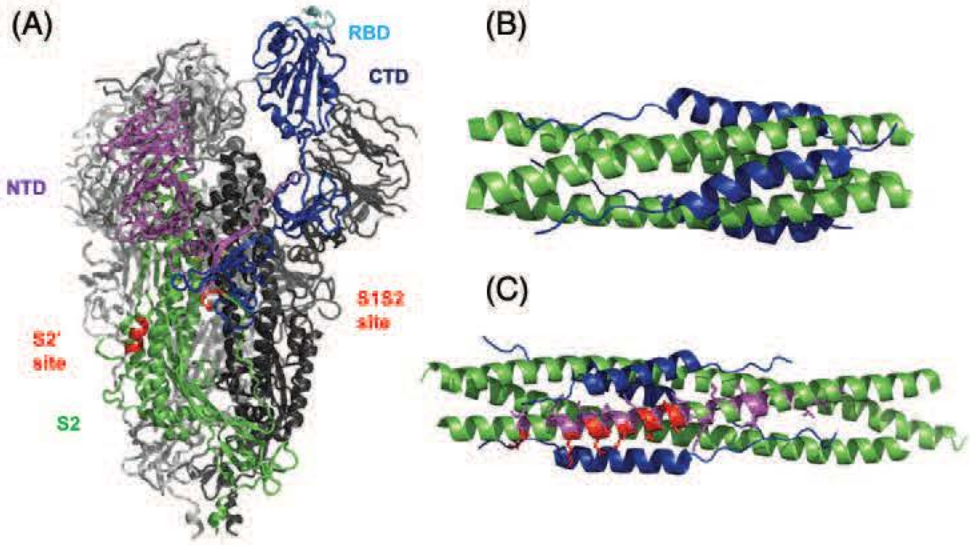

Figure 3. Structure of Spike Protein (S) Before and After Membrane Fusion. (A) Structure of the trimeric ectodomain of S from 2019 nCoV. The S2 subunit in one monomer is shown in green, the N terminal domain (NTD) of S2 in magenta, and the C terminal domain (CTD) of S2 in blue. The CTD is in the 'up conformation', exposing the binding domain for the angiotensin converting enzyme 2 (ACE2) receptor (cyan). The S1/S2 and S2' cleavage sites are indicated in red. The figure was created with Pymol from Protein Data Bank (PDB) file 6VSB. (B) Structure of the heptad repeat (HR) domains of S from severe acute respiratory syndrome coronavirus (SARS CoV). Heptad repeat region 1 (HR1) is labeled green and repeat region 2 (HR2) in blue. Formation of this six helix bundle is supposed to drive membrane fusion. The figure was created with Pymol from PDB file 1ZV8. (C) Structure of the HR1 of S from SARS CoV (green) bound to the pan coronavirus peptide inhibitor EK1 (blue). The amino acids in S essential for binding to EK1 are shown as magenta sticks in one helix. The amino acids in S from 2019 nCoV not conserved in S from SARS CoV are shown as red sticks. Since the nonconserved amino acids are apparently not required for binding to EK1, the fusion inhibitor is likely to prevent cell entry of 2019 nCoV. The figure was created with Pymol from PDB file 5ZVM. Abbreviations: RBD, receptor binding domain.

an N terminal domain (NTD) and a C terminal domain (CTD). The NTD exhibits a structural fold as human galectins, galactose-binding lectins, and hence, in most CoVs, a sugar present at the cell surface serves as an attachment factor. The CTD is responsible for binding to the host receptor angiotensin-converting enzyme 2 (ACE2) in the case of SARS-CoV and 2019-nCoV. The CTD contains two subdomains: a core structure (a five-stranded antiparallel β-sheet) and the actual RBM, which determines the receptor binding specificity. The recently released structure of the RBM ACE2 complex (Figure 4A) revealed that most S residues contacting ACE2 are identical between SARS-CoV and 2019-nCoV. However, some are unique, including an important salt bridge that involves different amino acids in ACE2 to bind S of SARS-CoV and 2019-nCoV. These slight differences might explain the more efficient binding of S from 2019-nCoV to ACE2, but this has not been observed in other preliminary studies [61,62].

The CTD of S has basically the same folding in other CoVs, even if they use different host receptors, such as dipeptidyl peptidase 4 for MERS-CoV. The diversity of receptor usage is an outstanding feature of CoVs and (assuming that they all have derived from a common ancestor) already indicates that they have changed their receptor binding specificity multiple times during evolution [63–65].

After binding to its receptor, S catalyzes fusion of the viral and cellular membrane to allow access of the viral genome to the cytosol. A prerequisite for this activity is the cleavage of S into subunits, a process called priming. The first cleavage site is located at the S1/S2 boundary

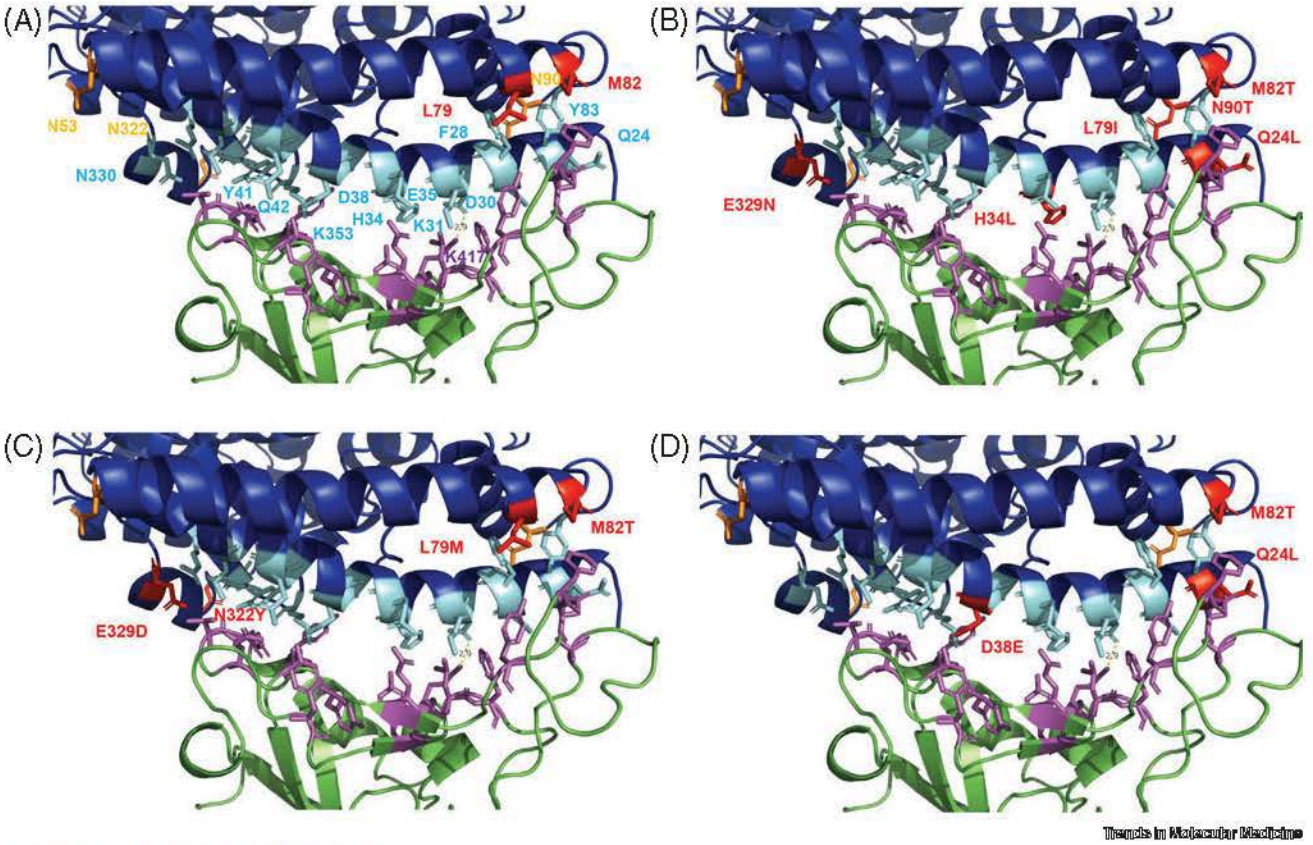

Figure 4. Spike Protein (S) and Its Receptor. (A) Structure of the receptor binding domain of S from 2019 nCoV (green) bound to human angiotensin converting enzyme 2 (ACE2) (blue). Most amino acids involved in binding are highlighted as magenta (S) and cyan (ACE2) sticks. Asparagine (N) that are *N* glycosylation sites (motif N X S/T) in human ACE2 are shown as orange sticks. Amino acids in human ACE2 that are involved in binding, but encode a potential *N* glycosylation site in ACE2 from other species, are shown as red sticks. The dotted line indicates the salt bridge between D30 and K417 (generated with Pymol from Protein Data Bank file6VSB). (B) Amino acid exchanges between human ACE2 and pig ACE2. Amino acid exchanges in ACE2 from pig compared with human ACE2 are highlighted in red. The exchange N90T destroys the *N* glycosylation site in human ACE2. (C) Amino acid exchanges between human ACE2 and cattle ACE2. Amino acid exchanges in ACE2 from cattle compared with human ACE2 are highlighted in red. The exchange N322Y destroys the *N* glycosylation site in human ACE2. ACE2 from sheep exhibits identical amino acid exchanges. (D) Amino acid exchanges between human and cat ACE2. Amino acid exchanges in ACE2 from cat compared with human ACE2 are highlighted in red. All relevant glycosylation sites in human ACE2 are conserved.

and another site (called S2') within S2. CoVs have evolved multiple strategies for proteolytic activation of S, and a large number of host proteases, such as furin, trypsin, trans-membrane protease/serine (TMPRSS), and cathepsins have been identified to process the spike protein. As a rule, furin cleaves S at a polybasic cleavage site (minimal motif R-X-X-R) during its biosynthesis in the trans-Golgi compartments or during virus entry in endosomes. Cleavage by trypsin and TMPRSS family members occurs at monobasic cleavage sites and likely takes place in the extracellular space and at the cell surface. Cathepsins, ubiquitous lysosomal enzymes with a rather broad substrate specificity, cleave S during virus entry [66]. For 2019-nCoV, it was shown that TMPRSS 2 primes S, the cathepsins B and L are only required in the absence of this protease [67]. Interestingly, S of 2019-nCoV has acquired a polybasic motif at the S1/S2 boundary, which is not present in S of the bat CoVs and SARS-CoV [68]. Preliminary data showed that S of 2019-nCoV is cleaved by furin during its biosynthesis [69]. This is reminiscent of low-pathogenic **avian influenza viruses**, which, if introduced into a poultry farm, may acquire a polybasic cleavage motif that causes a deadly outbreak of highly

pathogenic virus. S of MERS-CoV has a similar motif, which is cleaved by furin during biosynthesis of S. The availability and activity of the proteases in a certain cell, tissue, and host species regulates the tropisms of CoVs. However, the fact that S can easily acquire new protease cleavage sites and that various (some of them ubiquitous) proteases can fulfil the same task suggests that CoVs are naturally equipped or can easily adapt to multiply in several cell types.

Cleavage at the internal S2′ site occurs just upstream of the sequence S-F-I-E-D-L-L-F, which is highly conserved between S proteins of CoVs. It likely functions as a fusion peptide that inserts into the cellular membrane once the conformational change that catalyzes membrane fusion has been initiated. What triggers the refolding of S is unclear; the low pH prevailing in the endosome during virus entry is only required to activate cathepsins and binding to the receptor causes only minor conformational changes, but might be required to expose a previously hidden proteolytic cleavage site. The structure of parts of the S2 subunit from SARS-CoV in the postfusion conformation (Figure 3B) revealed a six helix bundle between two heptad repeats (a motif of seven amino acids in which amino acid 1 and 4 are hydrophobic), which is a typical feature of class I fusion proteins, such as hemagglutinin (HA) of influenza virus and Gp160 of HIV. However, the six helix bundle formed by S is longer, indicating its formation released more energy that drives the fusion of two lipid bilayers [70,71]. In summary, an amazingly large number of experimental data have already been worked out for S of 2019-nCoV and these models are still evolving.

Molecular Differences in the ACE2 Receptor between Human and Animal Species

The identification of the contact residues between the receptor-binding domain of S from 2019-nCoV and human ACE2 allows estimation of whether 2019-nCoV could infect other species (Figure 4A) [72]. To do so, we aligned all available ACE2 amino acid sequences with human ACE2. We placed emphasis on the presence of *N*-glycosylation motifs near the binding site, since they might affect attachment of S. Human ACE2 is glycosylated at N53, N90, and N322 (Figure 4A, orange sticks). N53 is conserved in all species. N90 is not a glycosylation site in ACE2 of mouse, pig, *N. procyonoides*, raccoon, civet, ferret, fox, *E. telfairi*, and chicken. N322 is not a glycosylation site in ACE2 of mouse, rat, cattle, sheep, *E. telfairi*, and pangolin. However, ACE2 of some species contain an additional glycosylation motif in this region. Residue L79 is a potential *N*-glycosylation site in chicken and M82 is a potential glycosylation site in *Rhinolophus sinicus*, pangolin, and rat. Notably, glycosylation of residue 82 has been show to prevent binding of S from SARS-CoV to rat ACE2 [73].

Some amino acids in ACE2 affect binding to S of 2019-nCoV are depicted for various species in Table 1. The S binding site of ACE2 from macaque and chimpanzees is identical to human ACE2. ACE2 from other species revealed eleven (chicken), nine and ten (rodents), or only three (cat) amino acid differences compared with human ACE2. Of special interest are ACE2 proteins from farm animals and a pet cat, since they might become another possible reservoir for 2019-nCoV. ACE2 from pig contains six exchanges, but they are mostly located at the periphery of the binding site (Figure 4B). N90T causes the loss of the glycosylation site. E329 forms a salt bridge with R426 in S of SARS-CoV, but S of 2019-nCoV forms a salt bridge with another residue (D30) in ACE2. Thus, the exchange of E329 by N in porcine ACE2 might affect binding to S of SARS-CoV, but not to S from 2019-nCoV. A similar pattern emerges for amino acid differences between human and cattle ACE2 (Figure 4C) and cat ACE2 (Figure 4D). The few exchanges are also located peripheral to the core of the binding region and thus their exchange might not represent a large obstacle for infection of cells from these species with 2019-nCoV.

Table 1. Comparison of Some Important ACE2 Residues among Different Species That Affect Binding to 2019 nCoV Receptor Binding Domain (RBD)

Species	Amino acids (19) in different species ACE2 that affect binding to 2019 nCoV RBD, corresponding positions are based on human ACE2 numbering																			Similarity to human ACE2 (based on 19 amino acids)	GenBank accession number
	24	31	34	35	38	41	42	53	79	82	83	90	322	325	329	330	353	652	710		
Human	Q	K	H	E	D	Y	Q	N	L	M	Y	N	N	Q	E	N	K	R	R	19/19	AAT45083.1
Pig	**L**	K	**L**	E	D	Y	Q	N	**I**	**T**	Y	**T**	N	Q	**N**	N	K	R	R	13/19	XP 020935033.1
Cat	**L**	K	H	E	**E**	Y	Q	N	L	**T**	Y	N	N	Q	E	N	K	R	R	16/19	XP 023104564.1
Macaque	Q	K	H	E	D	Y	Q	N	L	M	Y	N	N	Q	E	N	K	R	R	19/19	XP 011733505.1
Chimpanzee	Q	K	H	E	D	Y	Q	N	L	M	Y	N	N	Q	E	N	K	R	R	19/19	XP 016798468.1
Mouse	**N**	**N**	**Q**	E	D	Y	Q	N	**T**	**S**	**F**	**T**	**H**	Q	**A**	N	**H**	R	R	9/19	ABN80106.1
Rat	**K**	K	**Q**	E	D	Y	Q	N	**I**	**N**	**F**	N	**Q**	**P**	**T**	N	**H**	R	R	10/19	AAW78017.1
Rhinolophus sinicus	**E**	K	**T**	**K**	D	**H**	Q	N	L	**N**	Y	N	N	**E**	**N**	N	K	R	R	12/19	AGZ48803.1
Horse	**L**	K	**S**	E	**E**	**H**	Q	N	L	**T**	Y	N	N	Q	E	N	K	R	R	14/19	XP 001490241.1
Cattle	Q	K	H	E	D	Y	Q	N	**M**	**T**	Y	N	**Y**	Q	**D**	N	K	R	R	15/19	XP 005228485.1
Sheep	Q	K	H	E	D	Y	Q	N	**M**	**T**	Y	N	**Y**	Q	**D**	N	K	R	R	15/19	XP 011961657.1
Nyctereutes procyonoides	**L**	K	**Y**	E	**E**	Y	Q	N	L	**T**	Y	**D**	N	Q	E	N	**R**	R	R	13/19	ABW16956.1
Raccoon	**L**	**N**	**N**	E	**E**	Y	Q	N	**Q**	**T**	Y	**D**	N	Q	E	N	K	R	R	12/19	BAE72462.1
Camel	**L**	**E**	H	E	D	Y	Q	N	**T**	**T**	Y	N	N	Q	**D**	N	K	R	R	14/19	XP 031301717.1
Civet	**L**	**T**	**Y**	E	**E**	Y	Q	N	L	**T**	Y	**D**	N	Q	E	N	K	R	R	13/19	AAX63775.1
Ferret	**L**	K	**Y**	E	**E**	Y	Q	N	**H**	**T**	Y	**D**	N	**E**	**Q**	N	K	R	R	11/19	BAE53380.1
Fox	**L**	K	**Y**	E	**E**	Y	Q	N	L	**T**	Y	**D**	N	Q	E	N	K	R	R	14/19	XP 025842513.1
Echinops telfairi	Q	**T**	**N**	E	**N**	Y	Q	N	L	**K**	**F**	**D**	**P**	Q	**D**	**K**	**L**	R	R	9/19	XP 004710002.1
Chicken	**E**	**E**	**V**	**R**	D	Y	**E**	N	**N**	**R**	**F**	**D**	N	**E**	**T**	N	K	R	R	8/19	XP 416822.2
Pangolin	**E**	K	**S**	E	**E**	Y	Q	N	**I**	**N**	Y	N	**K**	Q	E	N	K	R	R	13/19	XP 017505752.1

Potential Drug Targets in S of 2019-nCoV

No approved antiviral agents are available against the current outbreak, but convalescent sera or monoclonal antibodies inhibit SARS-CoV or MERS-CoV *in vitro* or in animal models. However, sufficient sera and antibodies can hardly be produced during a large outbreak. Moreover, monoclonal antibodies neutralizing SARS-CoV are not (or only poorly) reactive against 2019-nCoV, indicating that the antibody epitopes are highly variable [74]. Inhibitors of the proteases that prime S for fusion also have antiviral activity. However, since S can use various proteases for priming, more than one inhibitor is required.

More promising are drugs directed against the highly conserved S2 subunit, such as peptides that inhibit membrane fusion. The proof of principle is **enfuvirtide**, a 20 amino acid peptide that is identical in sequence to a part of the heptad repeat region 2 (HR2) that forms a six helix bundle with heptad repeat region 1 (HR1). The peptide binds to HR1, which saturates the binding site for HR2, thereby preventing the conformational change that catalyzes membrane fusion. Peptides with a similar mode of action have been developed for the S2 subunit of SARS-CoV and MERS-CoV. They inhibit virus entry, reduce formation of **plaques** *in vitro*, and had beneficial effects in a mouse model. The

most promising peptide is called E1, which binds with high affinity to the HR1 region of S from SARS-CoV [75]. Sequence comparison between HR1 of S from SARS-CoV and 2019-nCoV shows various amino acid exchanges, but none of them is involved in binding to E1 (Figure 3C), indicating that E1 could also be effective against 2019-nCoV.

Another potential drug target might be the cellular enzyme(s) that attach fatty acids to a cluster of cysteines in the cytoplasmic tail of S. The fatty acids are required for S to fuse with the host cell and affect virus assembly, similar to what has been described for other spike proteins, such as HA of influenza virus. Enzymes that attach acyl chains to S have not been identified, but cellular proteins are acylated by one or several of the 23 members of the **ZDHHC family**, which have distinct, only partly overlapping substrate specificities. If only a few of them might acylate S in airway cells of the lung, their blockade might result in suppression of viral replication, while acylation of cellular proteins will not be (or very little) compromised. Although more research is required, targeting acyltransferases might be promising, since the cluster of cysteines is present in S from all CoV genera, regardless of their origin. Acylation might thus be required for a very basic function of S, arguing that even newly emerged CoVs probably will also rely on this modification of S to replicate efficiently [76]. However, since key proteins of the innate immune response are also palmitoylated, acylation inhibitors might be limited if the proteins of the innate immune response are modified by the same enzymes as viral proteins.

Concluding Remarks

Previous studies showed that CoVs genomes display a high degree of plasticity in terms of gene content and recombination. Furthermore, the relatively large CoV genome increases the probabilities for adaptive mutations, with it being relative easy for the spike protein to exploit multiple cellular receptors for virus attachment and entry [52,77–79]. These features are likely the cause of this alarming propensity of CoVs for host-species expansion. Unfortunately, China has seen a number of interspecies transmissions by CoV in recent years [80–82]. Whether this current COVID-19 epidemic 'frizzles out' or expands into a full-blown pandemic remains to be seen. It might also be desirable to monitor farm animals and pet cats for infection with 2019-nCoV, since their ACE2 receptor responsible for 2019-nCoV binding differs in only a few amino acids from human ACE2. Surveillance might prevent the virus establishing itself in another animal species that is in close contact to humans. In addition, in light of the fact that there are multiple species of CoVs circulating in wildlife species and that these animals are constantly interacting with each other, host-species expansion or interspecies transmission of new CoV to humans seems to be inevitable. Major knowledge gaps regarding the emergence of 2019-nCoV remain exists but worldwide scientists are working with unprecedented speed to investigate the virus, rushing to develop targeted therapeutics (see Outstanding Questions). Notwithstanding, a global surveillance network involving veterinarians and animal biologists is urgently needed to monitor, and possibly to predict, potential sources for the emergence of another highly pathogenic CoV. We propose the concept of 'One Health' to facilitate scientific exchange across disciplines, sharing of data, and coordinated efforts in order to prevent future outbreaks.

Outstanding Questions

When and how did COVID 19 emerge? What is or are the natural and intermediate host species for 2019 nCoV? What is the distribution of 2019 nCoV in different mammalian species? Will it infect farm animals or pets?

From surveillance and evolutionary studies on animal viruses, can their zoonotic potential be identified before interspecies transmission occurs?

What are the key interactions between the spike protein (S) of 2019 nCoV and its receptor angiotensin converting enzyme 2 (ACE 2)? Which amino acids in ACE2 determine whether S can bind? Is efficient binding to ACE2 the only determinant that decides whether an animal species can be infected?

Is expression of the trans membrane protease/serine another decisive factor for infection of a cell? Is the newly acquired polybasic cleavage site in S associated with cross species transmission of 2019 nCoV?

What are the similarities and differences of COVID 19 epidemiology in comparison with SARS and MERS? What is the basic reproductive number (R_0), the real incubation period, and the morbidity and mortality rate? Can COVID 19 develop into an endemic or seasonal infectious disease, like the flu?

With the experience of mitigating the outbreaks of SARS and avian influenza, what strategies can be applied in mitigating COVID 19 and future CoV outbreaks? Should veterinarians play more important roles in the prevention and control of emerging zoonoses in the future?

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References

1. Gorbalenya, A.E. *et al.* (2020) Severe acute respiratory syndrome-related coronavirus: the species and its viruses – a statement of the Coronavirus Study Group. *bioRxiv*. Published online February 11, 2020. https://doi.org/10.1101/2020.02.07.937862
2. Resta, S. *et al.* (2018) Isolation and propagation of a human enteric coronavirus. *Science* 4717, 978–981
3. Arabi, Y.M. *et al.* (2017) Middle East Respiratory Syndrome. *N. Engl. J. Med.* 376, 584–594
4. Zhong, N.S. *et al.* (2003) Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet* 362, 1353–1358
5. Drosten, C. *et al.* (2003) Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N. Engl. J. Med.* 348, 1967–1976
6. Guan, Y. *et al.* (2003) Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 302, 276–278
7. Song, H.D. *et al.* (2005) Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. *Proc. Natl. Acad. Sci. U. S. A.* 102, 2430–2435
8. Cohen, J. *et al.* (2019) New SARS-like virus in China triggers alarm. *Science* 6475, 234–235
9. Woo, P.C. *et al.* (2012) Discovery of seven novel mammalian and avian coronaviruses in the genus *Deltacoronavirus* supports bat coronaviruses as the gene source of *Alphacoronavirus* and *Betacoronavirus* and avian coronaviruses as the gene source of *Gammacoronavirus* and *Deltacoronavirus*. *J. Virol.* 86, 3995–4008
10. Chinese SARS Molecular Epidemiology Consortium (2004) Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science* 303, 1666–1669
11. He, B. *et al.* (2014) Identification of diverse alphacoronaviruses and genomic characterization of a novel severe acute respiratory syndrome-like coronavirus from bats in China. *J. Virol.* 88, 7070–7082
12. Li, Q. *et al.* (2020) Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *N. Engl. J. Med.* Published online January 29, 2020. https://doi.org/10.1056/NEJMoa2001316
13. Wang, D. *et al.* (2020) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. Published online February 7, 2020. https://doi.org/10.1001/jama.2020.1585
14. Chan, J.F-W. *et al.* (2020) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395, 514–523
15. Yang, Y. *et al.* (2020) Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. *medRxiv*. Published online February 21, 2020. https://doi.org/10.1101/2020.02.10.20021675
16. Kang, M. *et al.* (2020) Evidence and characteristics of human-to-human transmission of SARS-CoV-2. *medRxiv*. Published online February 17, 2020. https://doi.org/10.1101/2020.02.03.20019141
17. Liu, Y.-C. *et al.* (2020) A locally transmitted case of SARS-CoV-2 infection in Taiwan. *N. Engl. J. Med.* Published online February 12, 2020. https://doi.org/10.1056/NEJMc2001573
18. Chang, D. *et al.* (2020) Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. *JAMA*. Published online February 7, 2020. https://doi.org/10.1001/jama.2020.1623
19. Li, L. *et al.* (2020) An update on the epidemiological characteristics of novel coronavirus pneumonia (COVID-19). *Zhonghua Liu Xing Bing Xue Za Zhi* 41, 139–144
20. Novel Coronavirus Pneumonia Emergency Response Epidemiology Team (2020) The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. *Zhonghua Liu Xing Bing Xue Za Zhi* 41, 145–151
21. Guan, W.-j. *et al.* (2020) Clinical characteristics of 2019 novel coronavirus infection in China. *medRxiv*. Published online February 9, 2020. https://doi.org/10.1101/2020.02.06.20020974
22. Lipsitch, M. *et al.* (2003) Transmission dynamics and control of severe acute respiratory syndrome. *Science* 300, 1966–1970
23. Riley, S. *et al.* (2003) Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions. *Science* 300, 1961–1966
24. Breban, R. *et al.* (2013) Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* 382, 694–699
25. Chowell, G. *et al.* (2004) Model parameters and outbreak control for SARS. *Emerg. Infect. Dis.* 10, 1258–1263
26. Swerdlow, D.L. and Finelli, L. (2020) Preparation for possible sustained transmission of 2019 novel coronavirus: lessons From previous epidemics. *JAMA*. Published online February 11, 2020. https://doi.org/10.1001/jama.2020.1960
27. Park, J.E. *et al.* (2018) MERS transmission and risk factors: a systematic review. *BMC Public Health* 18, 574
28. Paul, L.D. *et al.* (2019) Complexity of the basic reproduction number (R_0). *Emerg. Infect. Dis.* 25, 1–4
29. Chen, N. *et al.* (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513
30. Pongpirul, W.A. *et al.* (2020) Journey of a Thai taxi driver and novel coronavirus. *N. Engl. J. Med.* Published online February 12, 2020. https://doi.org/10.1056/NEJMc2001621
31. Bastola, A. *et al.* (2020) The first 2019 novel coronavirus case in Nepal. *Lancet Infect. Dis.* 20, 279–280
32. Holshue, M.L. *et al.* (2020) First case of 2019 novel coronavirus in the United States. *N Engl. J. Med.* Published online January 31, 2020. https://doi.org/10.1016/S1473-3099(20)30067-0
33. Huang, C., *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497-506
34. Chen, H., *et al.* Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 20, 30360-30363
35. Xu, X. *et al.* (2020) Imaging features of 2019 novel coronavirus pneumonia. *Eur. J. Nucl. Med. Mol. Imaging*. Published online February 14, 2020. https://doi.org/10.1007/s00259-020-04720-2
36. Lin, X. and Gong, Z. (2020) Novel coronavirus pneumonia outbreak in 2019: computed tomographic findings in two cases. *Korean J. Radiol.* 21, 365–368
37. Wei, M. *et al.* (2020) Novel coronavirus infection in hospitalized infants under 1 year of age in China. *JAMA*. Published online February 14, 2020. https://doi.org/10.1001/jama.2020.2131
38. Hoehl, S. *et al.* (2020) Evidence of SARS-CoV-2 infection in returning travelers from Wuhan, China. *N. Engl. J. Med.* Published online February 18, 2020. https://doi.org/10.1056/NEJMc2001899
39. Zhu, H. *et al.* (2020) Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. *Transl. Pediatr.* 9, 51–60
40. Anderson, R.M. *et al.* (2004) Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 359, 1091–1105
41. Chowell, G. *et al.* (2015) Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. *BMC Med.* 13, 210
42. Zhou, P. *et al.* (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. Published online February 3, 2020. https://doi.org/10.1038/s41586-020-2012-7
43. Wu, F. *et al.* (2020) A new coronavirus associated with human respiratory disease in China. *Nature*. Published online February 3, 2020. https://doi.org/10.1038/s41586-020-2008-3
44. Li, G. *et al.* (2018) Organ, genetic diversity, and evolutionary dynamics of novel porcine circovirus 3. *Adv. Sci. (Weinh)* 5, 1800275

45. Huang, Y.-W. *et al.* (2013) Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. *mBio* 4, e00737-00713
46. Tao, Y. *et al.* (2017) Surveillance of bat coronaviruses in Kenya identifies relatives of human coronaviruses NL63 and 229E and their recombination history. *J. Virol.*, e01953-16
47. Yang, L. *et al.* (2019) Broad cross-species infection of cultured cells by bat HKU2-related swine acute diarrhea syndrome coronavirus and identification of its replication in murine dendritic cells in vivo highlight its potential for diverse interspecies transmission. *J. Virol.* 93, e01448-19
48. Karakus, U. *et al.* (2019) MHC class II proteins mediate cross-species entry of bat influenza viruses. *Nature* 7746, 109–112
49. He, W. *et al.* (2019) Genetic analysis and evolutionary changes of porcine circovirus 2. *Mol. Phylogenet. Evol.* 139, 106520
50. Chen, W. *et al.* (2005) SARS-associated coronavirus transmitted from human to pig. *Emerg. Infect. Dis.* 11, 446–448
51. Hu, D. *et al.* (2018) Genomic characterization and infectivity of a novel SARS-like coronavirus in Chinese bats. *Emerg. Microbes Infect.* 7, 154
52. Lam, T.T.-Y. *et al.* (2020) Identification of 2019-nCoV related coronaviruses in Malayan pangolins in southern China. *bioRxiv.* Published online February 18, 2020. https://doi.org/10.1101/2020.02.13.945485
53. Menachery, V.D. *et al.* (2015) A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat. Med.* 21, 1508–1513
54. Paraskevis, D. *et al.* (2020) Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infect. Genet. Evol.* 79, 104212
55. Luk, H.K.H. *et al.* (2019) Molecular epidemiology, evolution and phylogeny of SARS coronavirus. *Infect. Genet. Evol.* 71, 21–30
56. Wong, M.C. *et al.* (2020) Evidence of recombination in coronaviruses implicating pangolin origins of nCoV-2019. *bioRxiv.* Published online February 13, 2020. https://doi.org/10.1101/2020.02.07.939207
57. Xiao, K. *et al.* (2020) Isolation and characterization of 2019-nCoV-like coronavirus from Malayan pangolins. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.17.951335
58. Liu, P. *et al.* (2020) Are pangolins the intermediate host of the 2019 novel coronavirus (2019-nCoV)? *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.18.954628
59. Lu, R. *et al.* (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395, 565–574
60. Hui, K.P. *et al.* (2017) Tropism and innate host responses of influenza A/H5N6 virus: an analysis of ex vivo and in vitro cultures of the human respiratory tract. *Eur. Respir. J.* 49, 1601710
61. Wrapp, D. *et al.* (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* Published online February 19, 2020. https://doi.org/10.1126/science.abb2507
62. Sun, C. *et al.* (2020) SARS-CoV-2 and SARS-CoV spike-RBD structure and receptor binding comparison and potential implications on neutralizing antibody and vaccine development. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.16.951723
63. Li, F. (2016) Structure, function, and evolution of coronavirus spike proteins. *Annu. Rev. Virol.* 3, 237–261
64. Cui, J. *et al.* (2019) Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol.* 17, 181–192
65. Lu, G. *et al.* (2015) Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. *Trends Microbiol.* 23, 468–478
66. Millet, J.K. and Whittaker, G.R. (2015) Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. *Virus Res.* 202, 120–134
67. Hoffmann, M. *et al.* (2020) The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *bioRxiv.* Published online January 31, 2020. https://doi.org/10.1101/2020.01.31.929042
68. Coutard, B. *et al.* (2020) The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antivir. Res.* 176, 104742
69. Walls, A.C. *et al.* (2020) Structure, function and antigenicity of the SARS-CoV-2 spike glycoprotein. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.19.956581
70. Deng, Y. *et al.* (2006) Structures and polymorphic interactions of two heptad-repeat regions of the SARS virus S2 protein. *Structure* 14, 889–899
71. Heald-Sargent, T. and Gallagher, T. (2012) Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. *Viruses* 4, 557–580
72. Lan, J. *et al.* (2020) Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.19.956235
73. Li, F. *et al.* (2005) Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 309, 1864–1868
74. Zumla, A. *et al.* (2016) Coronaviruses - drug discovery and therapeutic options. *Nat. Rev. Drug Discov.* 15, 327–347
75. Xia, S. and Yan, L. (2019) A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci. Adv.* 5, eaav4580
76. Gadalla, M.R. and Veit, M. (2020) Toward the identification of ZDHHC enzymes required for palmitoylation of viral protein as potential drug targets. *Expert Opin. Drug Discov.* 15, 159–177
77. Wang, N. *et al.* (2019) Structural definition of a neutralization-sensitive epitope on the MERS-CoV S1-NTD. *Cell Rep.* 28, 3395–3405
78. Letko, M. *et al.* (2018) Adaptive evolution of MERS-CoV to species variation in DPP4. *Cell Rep.* 24, 1730–1737
79. Zhang, S. *et al.* (2018) Structural definition of a unique neutralization epitope on the receptor-binding domain of MERS-CoV spike glycoprotein. *Cell Rep.* 24, 441–452
80. Su, S. *et al.* (2016) Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends Microbiol.* 24, 490–502
81. Zhou, P. *et al.* (2018) Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* 556, 255–258
82. Hu, B. *et al.* (2017) Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog.* 13, e1006698
83. Katoh, K. *et al.* (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30, 3059–3066
84. Kumar, S. *et al.* (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874
85. Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313

From: Liu, Shan-Lu
To: Saif, Linda; Yount, Jacob
Subject: Columbus Dispatch letter or commentary
Date: Saturday, March 21, 2020 4:03:36 PM
Attachments: Dispatch_commentary .docx
image001.png

Hi Linda and Jacob:

Last few days, I have received numerous requests for interview, including local news media and even fire departments. I had to decline all of them for a variety of reasons. But I thought that it would be helpful for three of us to write a letter or commentary addressing some common questions and concerns people may have regarding the virus (not too much the COIVD-19 disease). With this mind, I just had a draft and would share with you. I would appreciate your comments, edits, etc.

Again, this is just an idea and the draft is rough, kind of outline…

Thanks.

Shan-Lu

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SARS-CoV-2: The Virus that Causes COIVD-19

Shan-Lu Liu, Jacob Yount, and Linda Saif

The Ohio State University

COIVD-19 (coronavirus diseases 2019) is now a global pandemic. The disease originated in Wuhan, the capital city of Hubei Province in China in November 2019. A Huanan seafood wholesale market in the city is thought to be the original source of the virus where wild animals were sold, resulting in the transmission of the virus to humans. As of March 21, 2020, more than XXX,000 confirmed cases of COIVD-19 were reported worldwide, affecting at least XX countries and causing XXX deaths. In the US, there are XXXX confirmed cases, including XX cases in the state of Ohio.

The virus causing COIVD-19 has been named by the International Committee on Taxonomy of Viruses as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The natural reservoir of the virus SARS-CoV-2 is believed to be bats, the only flying animal that harbors many other viruses, including the SARS coronavirus, Ebola virus and Zika virus. Viral phylogenetic analyses show that SARS-CoV-2 shares over 96 % similarity to one of the bat coronaviruses known as RaTG13 found in *Rhinolophus affinis*. However, the intermediate animal species, if there is one, that directly transmit the virus to human is currently clear. Notably, SARS-CoV-2 shares about 90% overall nucleotide sequence identity to another related coronavirus found in the endangered species of small mammals known as pangolins, and both likely use the same receptor

ACE2 to enter the host cell. Recombination between coronaviruses in different animal species may account for the origin of SARS-CoV-2.

Viruses in their natural hosts do not normally cause diseases because of mutual coadaptation. However, when the virus jumps to a new species, including humans, severe infection occurs that results in pathogenesis even deaths. This has been proven to be the case for HIV that causes AIDS pandemic and many viruses. One critical question is whether or not the continued spread of SARS-CoV-2 in humans would result in changes in transmission rates and diseases severity. If the transmission is weakened over time, the outbreak would ultimately end and the virus SARS-CoV-2 be eradicated from humans. However, if effective transmission is sustained, the viral infection will become community-acquired human coronaviruses, such as 229E, OC43, HKU1 and NL63, which are known to cause flu-like common cold. One measurement of the viral transmission rate is the viral reproductive number (R_0); for SARS-CoV-2, it is currently estimated to be 2.7, corresponding to an epidemic doubling time of about 6.4 days. This rate is relatively high compared to that of SARS-CoV, the virus that caused SARS outbreak in 2003 (R_0 less than 2.0). Accurately defining and monitoring the R_0 values should provide informed guidance for the effective control of the SARS-CoV-2 spread.

While SARS-CoV-2 causes severe pulmonary syndromes and even deaths, many infected individuals remain asymptomatic, which constitutes a dangerous source of viral transmission. Hence, social distancing currently taken by the US and other COIVD-19 outbroken countries is critical and the most effective way to contain the viral and

disease spread. In addition to transmission by droplets and close contact, fecal–oral transmission of SARS-CoV has been recently reported; thus, frequent handwashing and clean sanitation may be important. There have also been reports of ocular infection in SARS-CoV-2 infected individuals, so eye protection is needed under certain circumstances.

Animal coronavirus and implications for COIVD-19: Linda please add.

Vaccination is the most effective strategy to prevent occurrence of infectious diseases. Unfortunately, an FDA-approved vaccine for SARS-CoV-2-induced COIVD-19 is currently not available. Encouragingly, a viral mRNA-based vaccine has just entered the first phase of human trial, and if successful, this vaccine, along with many others in the pipeline, will become powerful in the fight of COIVD-19.

The authors of this commentary, SLL, JY and LS, are co-directors of the Viruses and Emerging Pathogens Program, The Infectious Diseases Institute, The Ohio State University.

From: Liu, Shan-Lu
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Cc: Saif, Linda; tcbaric@med.unc.edu
Subject: Visit to The Ohio State University and commentary
Date: Thursday, February 27, 2020 2:20:10 AM
Attachments: No credible evidence supporting claims of the laboratory engineering of SARS CoV 2.pdf
image001.png

Hi Ralph,

See below the link and also the attached PDF file of our newly published commentary.

https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1733440

Kindly let us know your preferred date of the visit to OSU.

Best.

Shan-Lu

From: "Liu, Shan-Lu" <Shan-Lu.Liu@osumc.edu>
Date: Tuesday, February 25, 2020 at 6:34 PM
To: "rbaric@email.unc.edu" <rbaric@email.unc.edu>
Cc: "Saif, Linda" <saif.2@osu.edu>
Subject: Visit to The Ohio State University for a distinguished seminar

Dear Ralph,

It was great to see you at the VirB meeting last week, and I truly enjoyed our discussion, although it was short.

As I mentioned, Linda and I would like to invite you to The Ohio State for a distinguished seminar this year for our Infectious Diseases Institute seminar series. I just looked at our schedule and realized that we will have a workshop focusing on emerging viral pathogenesis and vaccine development on April 15. If you are able to make this time, we will arrange your talk in the morning opening session as a distinguished keynote address. In the afternoon, Dan Barouch from Harvard Medical School will give another keynote lecture.

If the date of April 15 does not work for you, I will discuss with Linda and try to find another time suitable for you. Perhaps you may also suggest some preferred dates from March -June that will work for you.

As promised, I will send you're the link to our Commentary in EMI once it becomes available online – should be online tomorrow or on Thursday.

Best wishes!

Shan-Lu

THE OHIO STATE UNIVERSITY

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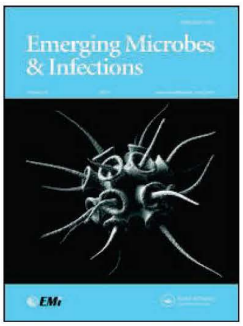

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

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COMMENTARY

OPEN ACCESS Check for updates

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

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ARTICLE HISTORY Received 13 February 2020; Accepted 13 February 2020

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1,100 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

It was proposed that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary

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evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to MA15 chimeric virus with the original human SARS S gene in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1585

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections invol ving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1623

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coro navirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30;395(10223):507 513.

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia out break associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2012 7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24;382(8):727 733.

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evol ution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characteriz ation of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS corona virus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin con verting enzyme 2 is a functional receptor for the

SARS coronavirus. Nature. 2003 Nov 27;426(6965): 450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2008 3
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

From:	Liu, Shan-Lu
To:	Saif, Linda; Lishan Su; Susan Weiss
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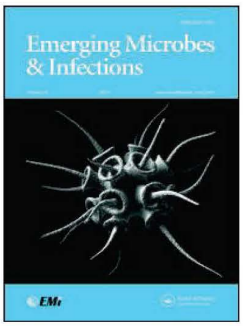

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2020, VOL. 9
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COMMENTARY

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

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ARTICLE HISTORY Received 13 February 2020; Accepted 13 February 2020

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Disclosure statement

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References

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[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections invol ving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1623

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coro navirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30;395(10223):507 513.

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia out break associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2012 7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24;382(8):727 733.

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evol ution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characteriz ation of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS corona virus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin con verting enzyme 2 is a functional receptor for the

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[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2008 3
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

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COMMENTARY

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Q1 Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan Weiss[f] and Lishan Su[g]

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ARTICLE HISTORY Received 13 February 2020; Accepted 13 February 2020

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The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe

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bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

Disclosure statement

No potential conflict of interest was reported by the author(s). Q2

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References Q3

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. Q4

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. Q5

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. Q6

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. Q7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. Q8

[6] Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430–2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508–1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535–538.

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SARS coronavirus. Nature. 2003 Nov 27;426 (6965):450–454.

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[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350–6353.

[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.

[15] Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378–381.

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Perspective Piece

The Origin of COVID-19 and Why It Matters

David M. Morens,[1,2*] Joel G. Breman,[3] Charles H. Calisher,[4] Peter C. Doherty,[5] Beatrice H. Hahn,[6,7] Gerald T. Keusch,[8,9,10] Laura D. Kramer,[11,12] James W. LeDuc,[13] Thomas P. Monath,[3,14] and Jeffery K. Taubenberger[15]

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Abstract. The COVID-19 pandemic is among the deadliest infectious diseases to have emerged in recent history. As with all past pandemics, the specific mechanism of its emergence in humans remains unknown. Nevertheless, a large body of virologic, epidemiologic, veterinary, and ecologic data establishes that the new virus, SARS-CoV-2, evolved directly or indirectly from a β-coronavirus in the sarbecovirus (SARS-like virus) group that naturally infect bats and pangolins in Asia and Southeast Asia. Scientists have warned for decades that such sarbecoviruses are poised to emerge again and again, identified risk factors, and argued for enhanced pandemic prevention and control efforts. Unfortunately, few such preventive actions were taken resulting in the latest coronavirus emergence detected in late 2019 which quickly spread pandemically. The risk of similar coronavirus outbreaks in the future remains high. In addition to controlling the COVID-19 pandemic, we must undertake vigorous scientific, public health, and societal actions, including significantly increased funding for basic and applied research addressing disease emergence, to prevent this tragic history from repeating itself.

In 2007, scientists studying coronaviruses warned: "The presence of a large reservoir of SARS-CoV–like viruses in horseshoe bats... is a time bomb. The possibility of the re-emergence of SARS and other novel viruses... should not be ignored."[1]

Few paid attention following the disappearance of SARS after the initial outbreak in 2002. Now, 18 years later, COVID-19 has emerged as the deadliest respiratory disease pandemic since 1918, when the "Spanish" influenza pandemic killed an estimated 50 million people.[2] We need to understand what happened so that we can prevent it from happening again, and be better prepared to contain similar pandemics at their outsets.

EMERGENCE OF THE COVID-19 PANDEMIC

The agent of COVID-19, SARS-CoV-2, was named after the genetically related SARS-CoV (more recently distinguished by some as SARS-CoV-1), which caused a deadly near-pandemic in 2002–2003.[3] Before 2019, neither SARS-CoV-2 nor its genetic sequences had ever been identified in viruses of humans or animals.

Even so, scientific research conducted over the last two decades provides clues about how and why the COVID-19 pandemic appeared. We must understand these critically important scientific findings, described in the following text, so that we can better address significant existential risks we will continue to face for the foreseeable future.

* Address correspondence to David M. Morens, Room 7A-03, Building 31, 31 Center Drive, Bethesda, Maryland 20892-2520. Email: dm270q@nih.gov

HOW VIRAL DISEASES EMERGE

Viruses are compact nucleic acid packages of either DNA or (in the case of coronaviruses) RNA associated with proteins, and in some cases with lipids. Viruses are not living organisms and can only reproduce inside living cells susceptible to viral entry and with the capacity to replicate viral nucleic acids and translate nucleic acid signals into amino acids to build viral proteins. Viruses are therefore nonliving self-contained genetic programs capable of redirecting a cell's machinery to produce more of themselves.

It follows that when a virus enters a human cell for the first time, it has very recently been transmitted from cells of some other host, that is, from another animal or, for example, an insect vector. Emergence of a pathogen between a vertebrate or an insect has been referred to as host-switching, sometimes described as a spillover event. Most of the human viral and nonviral infectious diseases that have existed for centuries—measles, influenza, cholera, smallpox (eradicated in 1980), falciparum malaria,[4] dengue, HIV, and many others—originated by animal-to-human host-switching.[5] The complex genetic events that underlie host-switching differ greatly from pathogen to pathogen, but general mechanisms have been recognized for many.[6–9]

Host-switching determinants prominently include social, environmental, and biological factors providing the opportunity for host–species interaction; shared host cell receptors; genetic distance between transmitting and receiving hosts; and characteristics and complexity of the viral quasi-species or viral swarm. (RNA viruses in particular are not transmitted to multiple cells as identical virions, but as collections of thousands of different genetically related virions. The ever-changing complexity of the viral swarm varies between infections of genetically distinct but related hosts and in single hosts over time.)

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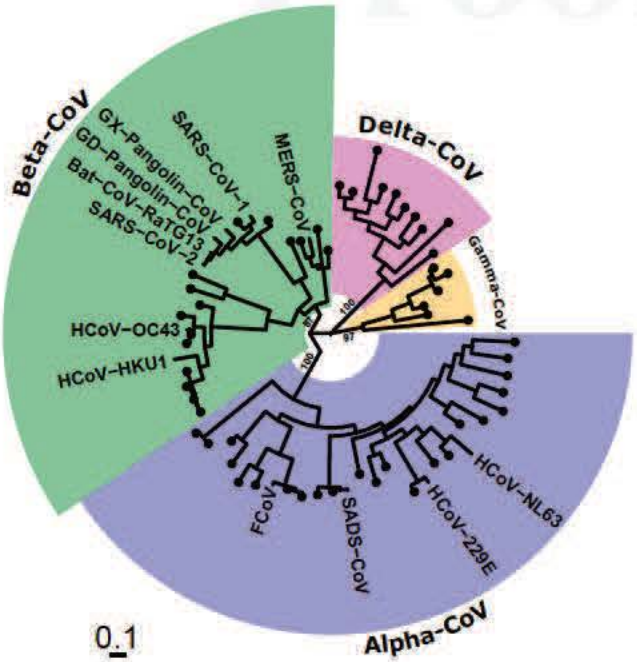

FIGURE 1. Phylogenetic relationships of selected coronaviruses of medical and veterinary importance. Human SARS-CoV and SARS-CoV-2 are closely related to numerous bat and pangolin coronaviruses in a viral genetic grouping called sarbecoviruses, which contains many other viruses very closely related to SARS-CoV and SARS-CoV-2. These viruses belong to the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae* and the four genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. The betacoronaviruses are comprised of two subgenera, *Sarbecovirus* and *Merbecovirus*. The former include SARS-CoV and SARS-CoV-2; the latter includes Middle East respiratory syndrome-related coronavirus (MERS-CoV). Image created by Sebastian M. Gygli, Ph.D., NIAID, NIH, and used with permission.

Studying animal viruses that have previously spilled over into humans provides clues about host-switching determinants. A well-understood example is influenza virus emergence into humans and other mammals.[2] Human pandemic and seasonal influenza viruses arise from enzootic viruses of wild waterfowl and shore birds. From within this natural reservoir, the 1918 pandemic "founder" virus somehow host-switched into humans. We know this from genetic studies comparing avian viruses, the 1918 virus, and its descendants, which have caused three subsequent pandemics, as well as annual seasonal influenza in each of the 102 years since 1918. Similarly, other avian influenza viruses have host-switched into horses, dogs, pigs, seals, and other vertebrates, with as yet unknown pandemic potential.[2,10,11] Although some molecular host-switching events remain unobserved, phylogenetic analyses of influenza viruses allow us to readily characterize evolution and host-switching as it occurs in nature.[2]

CORONAVIRUSES

Coronaviruses are RNA viruses globally distributed in a large but unknown number of animal species. Coronaviruses important for humans are found within phylogenetically distinct taxonomic subgroups, labeled as the α- and β-coronaviruses (Figure 1).[12] Four endemic human coronaviruses, which emerged at some undetermined time in the past, cause (mostly) mild self-limited upper respiratory tract infections (Figure 1).

RECENT CORONAVIRUS EMERGENCES FROM ANIMALS INTO HUMANS

Until recently, relatively little was known about coronaviruses, and research interest in these common cold viruses was minimal. Eighteen years ago, a previously unknown β-coronavirus named SARS-CoV suddenly emerged. Following its initial appearance in China it spread to 29 other countries, causing a near-pandemic and killing 813 of the 8,809 people with confirmed infection before being controlled by aggressive public health measures. It has not been seen since. In 2012, however, another previously unknown β-coronavirus named Middle East respiratory syndrome coronavirus (MERS-CoV), and closely related to SARS-CoV, emerged to cause high case-fatality human infections. Fortunately, this virus does not efficiently transmit between humans, and cases have been largely limited to the Middle East where its intermediary host, the dromedary camel, is present in relatively high numbers. In 2016, yet another novel bat-origin coronavirus, an α-coronavirus, emerged in China to cause a novel epizootic disease in pigs, termed swine acute diarrhea syndrome coronavirus (SADS-CoV). And most recently, at least as early as late November 2019, SARS-CoV-2 was recognized and became the third fatal bat virus–associated human disease

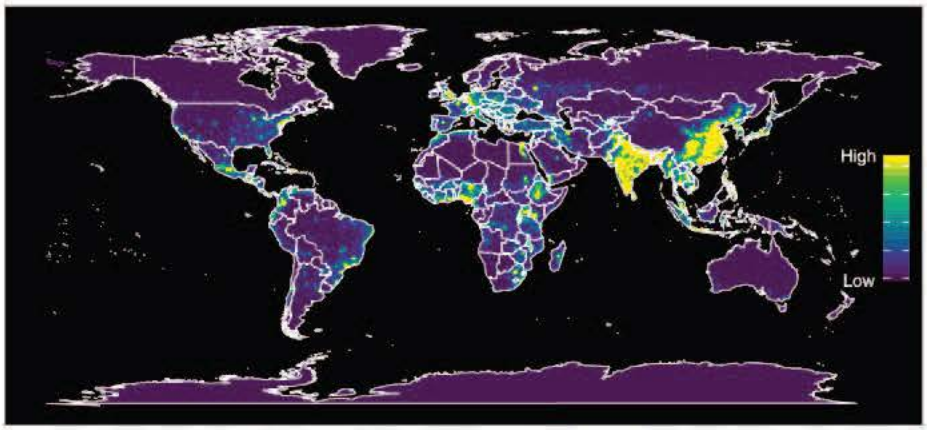

FIGURE 2. Predicted global hotspots for disease emergence, showing estimated risks, adjusted for reporting bias. From a comprehensive global study combining multiple data sources. Reproduced with permission from Allen et al.[14]

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emergence and the fourth bat virus–associated mammalian emergence in 18 years.

CORONAVIRUS EMERGENCE RISKS

An enormous reservoir of coronaviruses infects hundreds of bat species distributed globally. SARS-CoV, MERS-CoV, and SARS-CoV-2 are closely related β-coronaviruses clustering in two adjacent phylogenetic groupings: sarbecovirus (SARS-like viruses) and merbecovirus (MERS-like viruses) (Figure 1). The two SARS viruses, as well as SADS-CoV, are descended from viruses enzootic in rhinolophid (genus, *Rhinolophus*), or horseshoe bats.

Over the past 15 years, scientists have also identified global animal reservoirs of coronaviruses (in Africa, the Americas, the Middle East, Asia and Southeast Asia, and particularly China, the location of three of the four most recent emergences). These efforts have revealed much about coronaviral ecosystems, reservoir hosts, viral movement between hosts, viral evolution, and risk of emergence into humans and other mammals.

Bats of numerous globally distributed genera and species are now known to be the major reservoir of animal coronaviruses. One 20-country study of more than 19,000 animals (predominantly nonhuman primates, bats, and rodents) revealed that bats accounted for more than 98% of coronavirus detections, and that almost 9% of > 12,000 randomly studied bats were infected with one or more coronavirus.[13] Significant interspecies viral transmission between closely and distantly related bats also appears to be important. Bats of some species, including rhinolophids, co-roost with bats of other species, facilitating viral exchanges and enhanced viral evolution associated with genetic recombination. In fact, many such bat coronaviruses have genetic sequences similar to SARS-CoV and SARS-CoV-2.

Investigators have also mapped global hotspots for potential infection emergence, prominently in south/southwest China and contiguous regions and countries (Figure 2),[14] and have identified numerous human–animal interactions that constitute emergence risk factors, for example bat tourism, wet markets, wildlife supply chains for human consumption,[15] land management practices, and environmental perturbations.[16–18] Virologic and risk mapping studies indicate a very high risk of further coronavirus outbreaks.[19–21]

SARS-CoV and SARS-CoV-2 emerged in China, home to bats of more than 100 species, many of which carry α- and/or β-coronaviruses. In one study, more than 780 partial coronavirus genetic sequences were identified from bats of 41 species infected by α- and of 31 species infected by β-coronaviruses.[21] Within the sarbecovirus lineage, encompassing SARS and SARS-like viruses, many identified genetic sequences are very similar to SARS-CoV and SARS-CoV-2.[21–23] One such virus is more than 96% identical to SARS-CoV-2 in its whole genome[23]; another shares more than 97% identity in the 1ab replicase gene, as well as a furin cleavage site insertion.[24] Nature is clearly a cauldron for intense and dangerous coronavirus evolution.

WAS COVID-19 PREDICTED?

A clearer, more worrisome picture of the coronavirus ecosystem has recently come together. A contiguous area encompassing parts of south/southwest China, Laos, Myanmar, and Vietnam constitutes a bat coronavirus "hotspot," featuring intense interspecies viral transmission. In such hotspots, a rich diversity of SARS-like viruses has been found, not only in rhinolophid bats but also in bats of other genera and species to which these viruses had host-switched. The same rhinolophid bats are also implicated in the emergence of SADS-CoV in southern China. Many of these SARS-like viruses bind to human angiotensin-converting enzyme-2 (ACE2) receptors and infect human respiratory epithelial cells in vitro, suggesting their pandemic potential.[19,25]

Ominously, bat-to-human transmission of SARS-like viruses has already been detected,[20] perhaps representing pandemic near-misses. Even the more genetically distant SADS-CoV infects cells of humans and numerous other vertebrates, raising concern about indirect coronavirus emergences. This seems to have occurred with the bat-to-camel-to-human emergence of MERS, and possibly with SARS-CoV emergence into humans, which may have resulted from bat virus infection of masked palm civet cats (*Paguma larvata*), with subsequent human spillover.[12] As a byproduct of the important international surveillance work described above, in 2017, the therapeutic benefit of the antiviral drug remdesivir was suggested; it is now, in 2020, being widely used to treat persons infected with SARS-CoV-2.[26]

Since 2007, when alarming predictions about threatened coronavirus emergences began to appear,[1] understanding of coronavirus ecosystems has become far more complete. Over the past 5 years, Chinese, American, European, and other scientists have begun to renew warnings that humans are intensively interacting with coronavirus-infected bats, that enzootic SARS-related bat coronaviruses have all of the essential components of the SARS virus, that some of these SARS-like viruses can infect laboratory-humanized mice to cause SARS-like disease, that SARS-like viruses have the ability to directly infect and be transmitted between humans, and, therefore, that these viruses are poised for human emergence.[19,21,22] Many scientists have proposed aggressive monitoring of known hotspots to try to predict and prevent viral emergence that might impact human health, including early warning of host-switching events.[19,20,27]

Unfortunately, outside of some members of the scientific community, there has been little interest and no sense of urgency. In 2020, we learned, tragically, what 12 years of unheeded warnings have led to: a bat-derived sarbecovirus—from the very same SARS-like bat virus group that had been warned about by multiple voices for over a decade—emerged and proceeded to cause the COVID-19 pandemic that now sweeps the globe.

SARS-CoV-2 emerged essentially as predicted: a natural event associated with either direct transmission of a bat coronavirus to humans or indirect transmission to humans via an intermediate host such as a Malaysian pangolin (*Manis javanica*) or another, yet-to-be-identified mammal.[28–31]

It should be clarified that theories about a hypothetical man-made origin of SARS-CoV-2 have been thoroughly discredited by multiple coronavirus experts.[21,28,29] SARS-CoV-2 contains neither the genetic fingerprints of any of the reverse genetics systems that have been used to engineer coronaviruses nor does it contain genetic sequences that would have been "forward engineered" from preexisting viruses, including the genetically closest sarbecoviruses. That is, SARS-CoV-2 is unlike any previously identified coronavirus from which it could have been engineered. Moreover, the SARS-CoV-2

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receptor-binding domain, which has affinity for cells of various mammals, binds to human ACE2 receptors via a novel mechanism.

Engineering such a virus would have required 1) published or otherwise available scientific knowledge that did not exist until after COVID-19 recognition; 2) a failure to follow obvious engineering pathways, resulting in an imperfectly constructed virus; and 3) an ability to genetically engineer a new virus without leaving fingerprints of the engineering. Furthermore, the 12 amino acid furin-cleavage site insertion between the SARS-CoV-2 spike protein's S1 and S2 domains, which some have alleged to be a sign of genetic engineering, is found in other bat and human coronaviruses in nature, probably arising via naturally occurring recombination.[24]

It is also highly unlikely that SARS-CoV-2 was released from a laboratory by accident because no laboratory had the virus nor did its genetic sequence exist in any sequence database before its initial GenBank deposition (early January 2020). China's laboratory safety practices, policies, training, and engineering are equivalent to those of the United States and other developed countries,[32] making viral "escape" extremely unlikely, and of course impossible without a viral isolate present. SARS-CoV-2 shares genetic properties with many other sarbecoviruses, lies fully within their genetic cluster, and is thus a virus that emerged naturally.

COVID-19 EMERGENCE MECHANISMS: WHY THEY MATTER

Understanding how COVID-19 emerged is of great importance. We now know that the viruses causing SARS, MERS, and COVID-19 are all members of enormous groups of bat coronaviruses distributed globally, and that many of these viruses are functionally preadapted to human emergence. This preadaptation can be thought of as "accidental" because it must have occurred in nature in the absence of human infection and does not rule out further human adaptation to enable pandemicity. Molecular mechanisms of preadaptation are not fully known, but are undoubtedly related to functional similarities between ACE2 receptors on the cells of numerous mammals (bats, humans, minks, cats, and other domestic and wild animals).[33,34]

The ability of coronaviruses to evolve at a high rate, illustrated by extreme phylogenetic diversity, coupled with the dispersion of new viral variants within an enormous array of wild animal species that can serve as hosts, portends poorly for the future of coronavirus disease emergence. We are already seeing coronavirus mutants with altered affinity for human ACE2. Whether bat coronaviruses evolve independently or by "sampling" various mammalian ACE2 receptors, the result is the same. That bat sarbecoviruses so easily switch between multiple hosts suggests a many-pronged human risk: directly from bats and indirectly from other mammals infected by bat viruses. Because we have only just begun to sample, sequence, and study bat/mammalian coronaviruses, we can be certain that what we now know is but the tip of a very large iceberg.

The findings described earlier reaffirm what has long been obvious: that future coronavirus transmissions into humans are not only possible, but likely. Scientists knew this years ago and raised appropriate alarm. Our prolonged deafness now exacts a tragic price.

The story of COVID-19 emergence sends a powerful message. A quantum leap in bat coronavirus surveillance and research is urgently needed. This work must emphasize virologic and behavioral field studies of humans and animals wherever they interface, and especially in disease hotspots, as well as virologic studies related to human and animal spillover risks and the means of reducing them.[35]

Important research that has languished, been underfunded, or discontinued should be greatly expanded to deal with the urgency of the situation, and more scientists, including scientists working in China and other hotspot countries (Figure 2), should be recruited to these efforts, especially in international research partnerships. Full, open international collaboration involving many countries is essential. In particular, field research on the prevalence and virus-host relationships of coronaviruses, development of platform technologies for diagnostics, vaccines, and animal models for studies of pathogenesis and potential therapeutics is essential to permit, for example, modeling structure/function relationships of specific binding domains from newly identified agents to create critical tools for disease control.

In addition to robust expansion of surveillance and research, there are things that we can do now to lower our risks. We know much about coronavirus hotspots, not only in China but also globally; we can more aggressively surveil these locations to learn more about the local viral ecology and identify initial human spillover events. We also know much about human behaviors that directly and indirectly bring us into contact with bats, including risks from wet markets, bat cave tourism, capturing and eating bats, and perturbing the environment in ways that alter bat habitats and habits. These are behaviors that we can and must change.

We can also strengthen basic public health, including hygiene and sanitation, so that emerging viruses do not have a fertile field in which to amplify replication, and we must build and maintain strong public health infrastructure to respond quickly and efficiently to pathogen emergence. For viruses that have emerged, such as SARS-CoV-2, we need to develop effective antivirals and, ideally, broadly protective vaccines. Education and communication with populations where spillover events occur is also an important component of risk reduction.

We must also realize that the problem is larger than just coronaviruses. In recent years, we have seen emergences and reemergences of numerous other human infectious diseases such as Ebola fever, Lassa fever, hantavirus pulmonary syndrome, human monkeypox, HIV, dengue, chikungunya, Zika, and epizootic avian influenza. We have entered a new pandemic era,[36] one in which epidemic and pandemic emergences are becoming commonplace; some are likely to be highly pathogenic. In 2020, our science is sufficiently robust to have a good chance of controlling pandemic viral emergences within 2–3 years, but dramatically insufficient to prevent and control their emergences in the first place.

We should begin developing broadly protective vaccines and broadly therapeutic antiviral/antimicrobial agents against pathogens within taxonomic groups likely to emerge in the future, including coronaviruses, henipaviruses, and filoviruses, among others. Organizations like the Coalition for Epidemic Preparedness Innovations, among others, should be extended and strengthened, emphasizing, in addition to vaccine development, therapeutics as well as prevention tools. Pandemic prevention should be a global effort on a par with chemical and nuclear weapon prevention.

Unless we reset the equation; invest more in critical and creative laboratory, field, and behavioral research; and start finding

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ways to prevent these emergences, we will soon see additional coronavirus pandemics, as well as global spread of other types of infectious agents not yet imagined, caused by some of the millions of viruses in the natural world, many of which we have not yet had the time and funding to identify and study.[27]

Understanding how COVID-19 emerged is a critical point on a steep learning curve we must quickly master. As we face the mounting deaths and societal upheavals of the COVID-19 pandemic, we must not lose sight of how this pandemic began, how and why we missed the warning signs, and what we can do to prevent it from happening again—and again.

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REFERENCES

1. Cheng VCC, Lau SKP, Woo PCY, Yuen KY, 2007. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clin Microbiol Rev 20:* 660–694.
2. Taubenberger JK, Kash JC, Morens DM, 2019. The 1918 influenza pandemic: 100 years of questions answered and unanswered. *Sci Transl Med 11:* eeaau5485.
3. Ksiazek TG et al., 2003. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med 348:* 1953–1966.
4. Sharp PM, Plenderleith LJ, Hahn BH, 2020. Ape origins of human malaria. *Ann Rev Microbiol 74:* 39–63.
5. Morens DM, Folkers GK, Fauci AS, 2008. Emerging infections: a perpetual challenge. *Lancet Infect Dis 8:* 710–719.
6. Culliton BJ, 1990. Emerging viruses, emerging threat. *Science 247:* 279–280.
7. Kuiken T, Holmes EC, McCauley J, Rimmelzwaan GF, Williams CS, Grenfell BT, 2006. Host species barriers to influenza virus infections. *Science 312:* 394–397.
8. Parrish CR, Holmes EC, Morens DM, Park E-C, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P, 2008. Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiol Mol Biol Rev 72:* 457–470.
9. Geoghegan JL, Holmes EC, 2018. Evolutionary virology at 40. *Genetics 210:* 1151–1162.
10. Morens DM, Taubenberger JK, 2011. Pandemic influenza: certain uncertainties. *Rev Med Virol 21:* 262–284.
11. Sun H et al., 2020. Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection. *Proc Natl Acad Sci U S A*, doi: 10.1073/pnas.1921186117.
12. Corman VM, Muth D, Niemeyer D, Drosten C, 2018. Hosts and sources of endemic human coronaviruses. *Adv Virus Res 100:* 163–188.
13. Anthony SJ et al., 2017. Global patterns in coronavirus diversity. *Virus Evol 3:* vex012.
14. Allen T, Murray KA, Zambtana-Torrelio C, Morse SS, Rondinini C, Marco MD, Breit N, Olival NJ, Daszak P, 2017. Global hotspots and correlates of emerging zoonotic diseases. *Nat Comm 8:* 1124.
15. Huong NQ et al., 2020. Coronavirus testing indicates transmission risk along wildlife supply chains for human consumption in Viet Nam, 2013–2014. bioRxiv, doi: 10.1101/2020.06.05.098590.
16. Li H et al., 2019. Human-animal interactions and bat coronavirus spillover potential among rural residents in Southern China. *Biosaf Health 1:* 84–90.
17. Monagin C et al., 2018. Serologic and behavioral risk survey of workers with wildlife contact in China. *PLoS One 13:* e0194647.
18. Li H-Y et al., 2020. A qualitative study of zoonotic risk factors among rural communities in southern China. *Int Health 12:* 77–85.
19. Hu B et al., 2017. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog 13:* e1006698.
20. Wang N et al., 2018. Serological evidence of bat SARS-related coronavirus infection in humans, China. *Virol Sin 33:* 104–107.
21. Latinne A et al., 2020. Origin and cross-species transmission of bat coronaviruses in China. *Nat Commun* (In press).
22. Menachery VD et al., 2016. SARS-like WIV1-CoV poised for human emergence. *Proc Natl Acad Sci U S A 113:* 3048–3053.
23. Zhou P et al., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature 579:* 270–2734.
24. Zhou H et al., 2020. A novel bat coronavirus reveals natural insertions at the S1/S2 cleavage site of the Spike protein and a possible recombinant origin of HCoV-19. bioRxiv, doi: 10.1101/2020.03.02.974139.
25. Ge XY et al., 2013. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature 503:* 535–538.
26. Sheahan TP et al., 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med 9:* eaal3653.
27. Carroll D, Daszak P, Wolfe ND, Gao GF, Morel CM, Morzaria S, Pablos-Méndez A, Tomori O, Mazet JAK, 2018. The Global Virome Project. *Science 359:* 872–974.
28. Anderson KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF, 2020. The proximal origin of SARS-CoV-2. *Nat Med 26:* 450–452.
29. Zhang Y-Z, Holmes EC, 2020. A genomic perspective on the origin and emergence of SARS-CoV-2. *Cell 181:* 223–226.
30. Lu R et al., 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet 395:* 565–574.
31. Li X, Giorgi EE, Marichann MH, Foley B, Xiao C, Kong X-P, Chen Y, Krober B, Gao F, 2020. Emergence of SARS-CoV-2 through recombination and strong purifying selection. *Sci Adv 6:* eabb9153.
32. Xia H, Huang Y, Ma H, Liu B, Xie W, Song D, Yuan Z, 2019. Biosafety level 4 laboratory user training program, China. *Emerg Infect Dis 25:* e180220.
33. Oreshkova N et al., 2020. SARS-CoV2 infection in farmed mink, Netherlands. *Euro Surveill 25:* pii 2001005.
34. Halfman PJ et al., 2020. Transmission of SARS-CoV-2 in domestic cats. *N Engl J Med*, doi: 10.1056/NEJMc2013400.
35. Letko M, Seifert SN, Olival KJ, Plowright RK, Munster VJ, 2020. Bat-borne virus diversity, spillover, and emergence. *Nat Rev Microbiol 18:* 461–471.
36. Morens DM, Daszak P, Markel H, Taubenberger JK, 2020. Pandemic COVID-19 joins history's pandemic legion. *mBio 11:* e00812-20.

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Subject:	Predicting the Future Trajectory COVID-19
Date:	Thursday, March 26, 2020 3:12:36 AM

Dear Colleagues:

Further to that letter you published in *The Lancet*.
https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30418-9/fulltext

I am writing to you all again. Apart from our attempt to alert the world in *The Lancet* and *The Australian* (early February, below) all our analyses and predictions on the origin and global spread of COVID-19 are now published or *In Press*. We are the only scientific analysts and pundits who have got it right from the Get-go: our explanation is consistent with all the genetic, immunologic, epidemiologic, geophysical, astrophysical and astrobiological data and observations – the principles and key turning point analyses were largely assembled earlier (40 years ago) by Sir Fred Hoyle and Professor N Chandra Wickramasinghe, and now updated here by Professor Chandra Wickramasinghe (in his 81 st year) and his active colleagues for the COVID-19 pandemic (see URLs to recent comprehensive reviews at end of list below) .

Their 1979 book "Diseases from Space" is obligatory reading, a scientific masterpiece written for the general intelligent reader – both Fred and Chandra should have been awarded the Nobel years ago, but human frailty, clay feet, jealously, envy and cowardice made sure that did not happen.

Yours sincerely

Ted Steele

Wickramasinghe, Steele, Gorczynski et al 2020 *Virology Current Research* (In Press)
On the Fragility of Empires and Paradigms
http://viXra.org/abs/2003.0524 **Category**: Physics of Biology
https://www.academia.edu/42310752/Virology_Current_Research_On_The_Fragility_of_Empires_and_Paradigms_Letter_to_the_Editor

Wickramasinghe, Steele, Gorczynski et al 2020 *Virology Current Research* (In Press)
Predicting the Future Trajectory of COVID-19
https://vixra.org/abs/2003.0320 **Category**: Physics of Biology
https://www.academia.edu/42314513/Predicting_the_Future_Trajectory_of_COVID-19

Wickramasinghe, Steele , Gorczynski et al 2020 *Virology Current Research*
Growing Evidence against Global Infection-Driven by Person-to-Person Transfer of COVID-19
http://viXra.org/abs/2003.0042 **Category**: Physics of Biology
https://www.academia.edu/42148293/Growing_Evidence_against_Global_Infection-Driven_by_Person-to-Person_Transfer_of_COVID-19

Wickramasinghe, Steele , Gorczynski et al 2020 *Virology Current Research*
Comments on the Origin and Spread of the 2019 Coronavirus
http://viXra.org/abs/2002.0525 **Category**: Physics of Biology
https://www.academia.edu/42041228/Comments_on_the_Origin_and_Spread_of_the_2019_Coronavirus

Origin of New Emergent Coronavirus and Candida Fungal Disease– Terrestrial or Cosmic?- posted 17.2.20-Chapter 6 for Cosmic Genetic Evolution
Authors: Edward J. Steele, Jiangwen Qu, Reginald M. Gorczynski, Robyn A. Lindley, Gensuke Tokoro, Robert Temple, N. Chandra Wickramasinghe
http://viXra.org/abs/2002.0310
Category: Physics of Biology

Article submitted to *The Australian* 6.2.20, updated 9.2.20 (then rejected by Editor)
The Coronavirus May Have Come From Space

Authors: N. Chandra Wickramasinghe, Edward J Steele
https://vixra.org/abs/2002.0118
http://viXra.org/abs/2002.0118?ref=11085574
Category: Physics of Biology

Draft letter to *The Lancet* at:
viXra:2002.0039 submitted on 2020-02-03 17:33:22 (then rejected by Editor)
http://viXra.org/abs/2002.0039?ref=11076818

Comment on the Origin of the 2019 Novel Coronavirus

Authors: Edward J. Steele, N. Chandra Wickramasinghe, Jiangwen Qu, Robert Temple, Gensuke Tokoro, Reginald M. Gorczynski
Category: Physics of Biology

Steele EJ, Gorczynski RM, Lindley RA, Liu Y, Temple R, Tokoro G, Wickramasinghe DT, Wickramasinghe NC. 2019 "Lamarck and Panspermia - On the Efficient Spread of Living Systems Throughout the Cosmos". Prog. Biophys. Mol. Biol. 2019 149 : 10 -32. https://doi.org/10.1016/j.pbiomolbio.2019.08.010

Steele EJ, Al-Muft S, Augustyn KK, Chandrajith R, Coghlan JP, Coulson SG, Ghosh S, Gillman M. et al 2018 "Cause of Cambrian Explosion: Terrestrial or Cosmic?" Prog. Biophys. Mol. Biol. 136: 3-23, https://doi.org/10.1016/j.pbiomolbio.2018.03.004

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To: daszak@ecohealthalliance.org; rbaric@email.unc.edu; rcolwell@umd.edu; larrabee@ecohealthalliance.org; board@ecohealthalliance.org; imaizel@axoniccap.com; eshedlarz@aol.com; MDBACKER@its.jnj.com; hume.field@ecohealthalliance.org; andre@ecohealthalliance.org; li@ecohealthalliance.org; karesh@ecohealthalliance.org; kessler@ecohealthalliance.org; chmura@ecohealthalliance.org; ejsteele@cyo.edu.au; Charles.Calisher@colostate.edu; bushschoolscowcroft@tamu.edu; b.haagmans@erasmusmc.nl; keusch@bu.edu; lamsk@nipahvirus.org; Juan.Lubroth@fao.org; janewilsonhilton@gmail.com; Lawrence.Madoff@umassmemorial.org; equitech@bigpond.com; ssa16@columbia.edu; sachs@columbia.edu; laknin@sfu.ca; jgallen@hsph.harvard.edu; Andy.Haines@lshtm.ac.uk; Hotez, Peter Jay; phoebe.koundouri@icre8.eu; docmohw@snu.ac.kr; ksrinath.reddy@phfi.org; john.thwaites@climateworksaustralia.org; sdgacademy@unsdsn.org; bottazzi@bcm.edu; yba2101@columbia.edu; COVID19Commission@unsdsn.org; comm@cdrf.org.cn; ok2267@columbia.edu; ime2111@columbia.edu; ebright@waksman.rutgers.edu; sja2127@cumc.columbia.edu; COVID19statement@gmail.com; a.cunningham@ioz.ac.uk; vrr1@columbia.edu; rbcorley@bu.edu; L.Enjuanes@cnb.csic.es; a.e.gorbalenya@lumc.nl; jmhughe@emory.edu; J.Mackenzie@curtin.edu.au; larry.madoff@state.ma.us; jkmazet@ucdavis.edu; peter.palese@mssm.edu; stanley-perlman@uiowa.edu; llmpoon@hku.hk; bernard.roizman@bsd.uchicago.edu; Saif, Linda; dirk.pfeiffer@cityu.edu.hk; JEREMY.PAGE@WSJ.COM; NATASHA.KHAN@WSJ.COM; r.basen@medpagetoday.com; empoweredpatient@cnn.com; munro.peter@abc.net.au; beth.mole@arstechnica.com; news@axios.com; newswatch@bbc.co.uk; edith.bracho.sanchez@gmail.com; cnn@cnn.com; news@coastnewsgroup.com; news@chron.com; letters@dailysignal.com; editor@dnews.com; newsroom@epochtimes.com; editorial@fatherly.com; myron.levin@fairwarning.org; info@eyeonannapolis.net; thaelle@gmail.com; news@fox17online.com; science@theguardian.com; fullmeasurenews@gmail.com; allergy-immunology@healio.com; stories@healthline.com; newsdesk@irishtimes.com; newsroom@idahostatesman.com; KHN-tips@kff.org; dhyde@kuow.org; mike.agogliati@wfsb.com; submit@wired.com; fox2newsdesk@foxtv.com; betsy.mckay@wsj.com; williamplooney@gmail.com; investigate@ap.org; editor@bayareanewsgroup.com; ashermullard@gmail.com; editorial@bigissue.com; tips@buzzfeed.com; stephen.colbert@cbs.com; cfrederick@duluthnews.com; scoops@huffpost.com; kotanews@kotatv.com; news@ksby.com; PRandhawa@KSDK.com; newstips@latimes.com; akrueger@mpr.org; editor@newatlas.com; kanderson@observer-reporter.com; news@politicshome.com; editors@sciam.com; jgoldsteinstreet@seattletimes.com; metro@sfchronicle.com; editors@texasobserver.org; tips@rollingstone.com; info@the-scientist.com; Blake.Montgomery@thedailybeast.com; sciencenetwork@ucsusa.org; newsroom@waaytv.com; newsroom@whyy.org; lrosenbaum@forbes.com; felice.freyer@globe.com; lgustus@sacbee.com; deidre@acbio.org.za; eva@i-sis.org.uk; jimmye@northsidesun.com; kbouffard@detroitnews.com; tips@propublica.org; thackerpd@gmail.com; peter.chianca@boston.com; rowan@rowanjacobsen.com; rasmus.nielsen@politics.ox.ac.uk; editors@medicalnewstoday.com; media@parler.com; chiversthomas@gmail.com; eesTheLancet@lancet.com; richard.horton@lancet.com; editorial@lancet.com; ombudsman@lancet.com; astrid.james@lancet.com; r.cooney@lancet.com; He.wang@lancet.com; child-adolescent@lancet.com; diabetes-endocrinology@lancet.com; robert.brierley@lancet.com; Zoe.Mullan@lancet.com; lan-lan.smith@lancet.com; p.hayward@lancet.com; ideditorial@lancet.com; E.Becker-Barroso@lancet.com; david.collingridge@lancet.com; niall.boyce@lancet.com; audrey.ceschia@lancet.com; emma.grainger@lancet.com; duc.le@lancet.com; jstacey@lancet.com; mkoch@lancet.com; psychiatry@lancet.com; neurology@lancet.com; mxw127@bham.ac.uk; l.hart@latrobe.edu.au; m.wulff@elsevier.com; laura.hart@elsevier.com; helen.brooks@lancet.com; Sabine.Kleinert@lancet.com; naomi.lee@lancet.com; Stuart.Spencer@lancet.com; jocalyn.clark@lancet.com; helen.frankish@lancet.com; Tamara.Lucas@lancet.com; joanna.palmer@lancet.com; lucy.banham@lancet.com; hannah.jones@lancet.com; a.barusic@elsevier.com; j.gibson@lancet.com; m.guenot@lancet.com; Jonathan.Pimm@lancet.com; vcwisdom@lancet.com; r.cooney@elsevier.com; selina.lo@lancet.com; T.Dehnel@lancet.com; olaya.astudillo@lancet.com; helen.penny@lancet.com; emilia.harding@lancet.com; kayleigh.hook@lancet.com; Ashley.cooper@lancet.com; e.dallavecchia@lancet.com; jessica.dwyer@lancet.com; mariam.faruqi@lancet.com; harsimran.flora@lancet.com; kitty.graham@lancet.com; r.hellier@lancet.com; Rhiannon.Howe@lancet.com; anna.johnson@lancet.com; c.leigh@lancet.com; clara.llorentelemm@lancet.com; kate.mcintosh@lancet.com; g.merry@lancet.com; a.sharman@elsevier.com; ashley.steeper@lancet.com; joana.vindeirinho@lancet.com; giulia.vivaldi@lancet.com; sophie.woolven@lancet.com; chris.wortley@lancet.com; j.blott@elsevier.com; bianca.brandon@lancet.com; benjamin.burwood@lancet.com; marcia.costa@lancet.com; danielle.gash@lancet.com; victoria.higgs@lancet.com; eleftheria.kyriacou@lancet.com; 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Subject: Criminal "scientists" caught covering up the cover up of SARS-CoV-2 origin from a Wuhan lab

Date: Thursday, November 19, 2020 3:00:46 PM

EcoHealth Alliance orchestrated key scientists' statement on "natural origin" of SARS-CoV-2

https://www.gmwatch.org/en/news/latest-news/19600

"The emails obtained via public records requests show that EcoHealth Alliance President Peter Daszak drafted the Lancet statement, and that he intended it to "not be identifiable as coming from any one organization or person" but rather to be seen as "simply a letter from leading scientists". Daszak wrote that he wanted "to avoid the appearance of a political statement"."

We know exactly how SARS-CoV-2 originated.

Root cause of COVID-19? Biotechnology's dirty secret: Contamination. Bioinformatics evidence demonstrates that SARS-CoV-2 was created in a laboratory, unlikely to be a bioweapon but most likely a result of sloppy experiments

https://doi.org/10.5281/zenodo.3766462

Coronavirus may have been a 'cell-culture experiment' gone wrong
https://www.skynews.com.au/details/_6158843835001

SARS-CoV-2 is well adapted for humans. What does this mean for re-emergence?

https://www.biorxiv.org/content/10.1101/2020.05.01.073262v1

It grew on human embryonic kidney cells in a Wuhan lab. Is it a surprise that it is well adapted for humans?

Thanks,

Vinu

From: Saif, Linda
To: Peter Daszak
Subject: Re: CGTN World Insight Interview Invite: Conspiracy Theories of COVID-19, Feb. 26, 27
Date: Tuesday, February 25, 2020 6:58:45 PM

Dr Wang said Chinese government has said this!

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Tuesday, February 25, 2020 at 6:53 PM
To: Linda Saif <saif.2@osu.edu>
Subject: RE: CGTN World Insight Interview Invite: Conspiracy Theories of COVID-19, Feb. 26, 27

Not heard of that anywhere, even in China. It's BSL-3 here and likewise prob in China I believe.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. [redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and

wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Saif, Linda [mailto:saif.2@osu.edu]
Sent: Tuesday, February 25, 2020 11:50 AM
To: Peter Daszak
Subject: Re: CGTN World Insight Interview Invite: Conspiracy Theories of COVID-19, Feb. 26, 27

Thanks. Do you know anything about efforts to classify SARS-CoV-2 as BSL4 or is this only in China?

Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Monday, February 24, 2020 at 11:32 PM
To: Linda Saif <saif.2@osu.edu>
Subject: RE: CGTN World Insight Interview Invite: Conspiracy Theories of COVID-19, Feb. 26, 27

I've been on CGTN a few times and they're good. They're state run, of course, but so is every news outlet in mainland China. To me they're a bit like Al Jazeera – an attempt to be an outwardly normal media company, but with a subtle pro-China stance.

If you're willing, it's great publicity.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance

460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Saif, Linda [mailto:saif.2@osu.edu]
Sent: Monday, February 24, 2020 5:31 PM
To: Peter Daszak
Subject: FW: CGTN World Insight Interview Invite: Conspiracy Theories of COVID-19, Feb. 26, 27

Here is latest request for interview. This is state owned media station, so not sure if should follow through with interview.
What are your thoughts?
Thanks
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: 崔若凡 <cui.ruofan@cgtn.com>
Date: Sunday, February 23, 2020 at 9:41 PM
To: Linda Saif <saif.2@osu.edu>
Subject: CGTN World Insight Interview Invite: Conspiracy Theories of COVID-19, Feb. 26, 27

Dear Professor Saif,

Hope this mail finds you well.

This is Ruofan with CGTN World Insight on China's state English broadcaster: China Global Television Network (CGTN). CGTN was formerly the English channel of China Central Television (CCTV). Our show is a current affairs commentary program; we invited experts across the globe to discuss issues related to China and beyond.

I noticed you signed on the statement published on the Lancet to stand to condemn conspiracy theories suggesting that Covid-19 does not have a natural origin, so hope we could have a chance to invite you to join our show and help us analyze more on this pneumonia.

We plan to do an interview focusing on the conspiracy theory on the Covid-19, why the rumor spreads so fast, why people choose to believe in it, why we still know very little on this coronavirus? Can we see the turning point in a short time? As more people out of China are getting infected, how should we control the severe situation?

We could arrange to do the interview on Wednesday and Thursday base on your schedule. Better be on Wednesday or Thursday evening 9:00 pm to 9:30 pm.

We could help to book local studio.

Hope to hear back from you.

Take care!

Ms. Cui Ruofan
Producer, World Insight, CGTN
+86 136 0127 7871

CCTV Headquarters: 32 East 3rd Ring Road Middle, Chaoyang, Beijing, China

From: calisher@cybersafe.net
To: Charles.Calisher@colostate.edu; bushschoolscowcroft@tamu.edu; rcolwell@umd.edu; rbcorley@bu.edu; daszak@ecohealthalliance.org; christian.drosten@charite.de; L.Enjuanes@cnb.csic.es; a.e.gorbalenya@lumc.nl; b.haagmans@erasmusmc.nl; jmhughe@emory.edu; karesh@ecohealthalliance.org; keusch@bu.edu; lamsk@nipahvirus.org; Juan.Lubroth@fao.org; J.Mackenzie@curtin.edu.au; Lawrence.Madoff@umassmemorial.org; jkmazet@ucdavis.edu; peter.palese@mssm.edu; stanley-perlman@uiowa.edu; llmpoon@hku.hk; bernard.roizman@bsd.uchicago.edu; Saif, Linda; kanta.subbarao@influenzacentre.org
Cc: "Jane Hilton"; "Equitech"
Subject: RE: Origin Coronavirus COVID-19
Date: Thursday, February 20, 2020 12:43:27 PM

There's a meteorite circling my house right now. Should I be concerned?

Charlie

From: Ted Steele <e.j.steele@bigpond.com>
Sent: Thursday, February 20, 2020 2:58 AM
To: Charles.Calisher@colostate.edu; bushschoolscowcroft@tamu.edu; rcolwell@umd.edu; rbcorley@bu.edu; daszak@ecohealthalliance.org; christian.drosten@charite.de; L.Enjuanes@cnb.csic.es; a.e.gorbalenya@lumc.nl; b.haagmans@erasmusmc.nl; jmhughe@emory.edu; karesh@ecohealthalliance.org; keusch@bu.edu; lamsk@nipahvirus.org; Juan.Lubroth@fao.org; J.Mackenzie@curtin.edu.au; Lawrence.Madoff@umassmemorial.org; jkmazet@ucdavis.edu; peter.palese@mssm.edu; stanley-perlman@uiowa.edu; llmpoon@hku.hk; bernard.roizman@bsd.uchicago.edu; saif.2@osu.edu; kanta.subbarao@influenzacentre.org
Cc: Jane Hilton <janewilsonhilton@gmail.com>; Equitech <equitech@bigpond.com>
Subject: Origin Coronavirus COVID-19

Dear Colleagues:

We understand why you had to write and sign that letter in this week's *The Lancet*.
https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30418-9/fulltext

The conspiracy theory that COVID-19 is a bioweapon that has been released from Wuhan bioweapons facility c. f. Senator Tom Cotton, is highly implausible.

However we also feel a special responsibility to make contact with biomedical scientists such as yourselves. COVID-19 is the biggest story on the planet right now- knowing how it may have plausibly arisen gives insight into its spread and then decline, and how it should be managed rationally. e.g. those older passengers on the cruise ships (the vulnerable sub-group) should have been advised to not make hand contact with the deck railings outside the sea-side

cabin).

We are experts in the analysis of the origins of sudden emerging diseases just like COVID-19 - and how they also precipitously decline and fade away. Several of us are biomedical immunologists and immunogeneticists. Our explanation handles all the genetic, immunologic, epidemiologic, geophysical and astrophysical (astrobiologic) data surrounding this suddenly emerging COVID-19 mediated disease.

I am sure you will understand our analysis-– we agree it did not come from a Wuhan bio-weapons lab (Why would the Chinese Defence Dept design a low mutation rate, low person-to-person transmitting virus , that only kills older already co-morbid susceptible patients?).

As the key correspondent with you I am a fairly well known senior Australian scientist and immunologist, of 50 years standing. I am widely published in the peer-reviewed scientific literature (Check out EJ Steele on PubMed).

My colleagues ,Professor Chandra Wickramasinghe (University of Buckingham, UK) is ***the*** world expert on sudden disease emergence like this. Together with Professor Reginald Gorczynski MD PhD (clinical immunology scientist and basic researcher,University of Toronto, Canada) we, and our other expert co-authors have analysed all the genetic, immunologic, epidemiological, geophysical and astrophysical data surrounding the origins and spread of this newly emergent Coronavirus. It follows a pattern all too familiar to us (check out our analyses at the URL links)- sudden emergence, then massive induced herd immunity, then sudden decline- this is unfolding right now with COVID-19.

It did not come from animals, it did not come from the Wuhan research facility - all our scientific analysis (in URL links below) indicate it has most plausibly come from a meteorite which burst over central China on the night of October 11 2019. Over the next month the fall-out , much like from an upper atmosphere nuclear test, settled mainly in the central Chinese city of Wuhan and its surrounds. But this fall-out is an infective replicating virus not radioactivity.

The whole central China /Wuhan region and Hubei province has , in our view, been physically contaminated with reasonably high concentrations of COVID-19 virus particles (that replicate in susceptible hosts on landing). As you know it causes a rather mild common cold in humans, and only causes severe pneumonia in older vulnerable , co-morbid, patients. The death rate is low. The mutation rate is low. The actual "cough in your face" human-human transmission is low. It is spread by *environmental contamination* - that is the key to understanding this virus e.g. we believe that at least two cruise ships in the South China Sea/Sea of Japan have been heavily contaminated by this drifting virus fall out dust cloud.

But the panic and hysteria is high- and the ham-fisted and secretive way the Communist Chinese government has behaved has made it even worse. But the Communist Government is acting rationally in trying to disinfect and lock down almost 500 million citizens in Central China (e.g. images of Chinese men in moon suites with disinfectant spray guns spraying down machinery, road ways, etc). Xi and the Communist Party of China knew of the widespread physical contamination, I am certain, by early January- it was a rational decision by Xi to lock down the region. We believe the viral dust cloud hit the *Diamond Princess* cruise ship (and the Dutch *Westerdam* cruise ship), and is these ships are now heavily contaminated. (Cruise ships in the Atlantic and Mediterranean sea are not reporting this ship wide phenomenon). In our view a fragment of the viral dust cloud (or even the same one) made spot in-falls over Japan- all these COVID-19 cases in Japan with NO links to China are factual evidence in favour of our explanation.
"None of Japan's new coronavirus patients had direct China links - Nikkei Asian Review"
https://asia.nikkei.com/Spotlight/Coronavirus/None-of-Japan-s-new-coronavirus-patients-had-direct-China-links

But there is much other evidence consistent with our explanation, and predictions for the future course of the COVID-19 pandemic.

I know many of you will understand the logic our scientific analysis, that is why I

am making contact, as you are all scholars, scientists and analysts who react to hard data. At the URLs click to read the PDF articles of our detailed scientific analyses of this epidemic, now clearly a pandemic at :

Origin of New Emergent Coronavirus and Candida Fungal Disease– Terrestrial or Cosmic?- posted 17.2.20-Chapter 6 for "*Cosmic Genetic Evolution*"
Authors: Edward J. Steele, Jiangwen Qu, N. Chandra Wickramasinghe, Reginald M. Gorczynski, Gensuke Tokoro, Robert Temple, Robyn A. Lindley.
http://viXra.org/abs/2002.0310
Category: Physics of Biology

Article submitted to *The Australian* 6.2.20, updated 9.2.20
The Coronavirus May Have Come From Space
Authors: N. Chandra Wickramasinghe, Edward J Steele
https://vixra.org/abs/2002.0118
http://viXra.org/abs/2002.0118?ref=11085574
Category: Physics of Biology

Letter to *The Lancet* at:
viXra:2002.0039 submitted on 2020-02-03 17:33:22
http://viXra.org/abs/2002.0039?ref=11076818
Comment on the Origin of the 2019 Novel Coronavirus
Authors: Edward J. Steele, N. Chandra Wickramasinghe, Jiangwen Qu, Robert Temple, Gensuke Tokoro, Reginald M. Gorczynski
Category: Physics of Biology

We are happy to be advisors and discuss this further if any of you make contact with us.

Thank you and kind regards. We are genuinely sincere in wanting to communicate the most plausible explanation of the causes of this COVID-19 pandemic

Ted Steele

NB: Some of the letter co-signers did not have an easily recoverable email e.g, Hume Field, Uni QLD; and those with Welcome Trust (Jeremy Farrar, Josie Golding, Mike Turner) . Could those of you who are concerned please forward this email to them.

.....

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Prahran, 3181, Melbourne, VIC Australia
email: e.j.steele@bigpond.com

From:	Madoff, Lawrence
To:	Corley, Ronald B; William B. Karesh; calisher@cybersafe.net; Charles.Calisher@colostate.edu
Cc:	bushschoolscowcroft@tamu.edu; rcolwell@umd.edu; Peter Daszak; christian.drosten@charite.de; L.Enjuanes@cnb.csic.es; a.e.gorbalenya@lumc.nl; b.haagmans@erasmusmc.nl; JMHUGHE@emory.edu; Keusch, Gerald T; lamsk@nipahvirus.org; Juan Lubroth; John MacKenzie; Jonna Mazet; peter.palese@mssm.edu; stanley-perlman@uiowa.edu; llmpoon@hku.hk; bernard.roizman@bsd.uchicago.edu; Saif, Linda; kanta.subbarao@influenzacentre.org; Jane Hilton; Equitech
Subject:	Re: Origin Coronavirus COVID-19
Date:	Thursday, February 20, 2020 4:04:30 PM

I had to check the calendar to see if it was April 1st.
Larry

From: Corley, Ronald B <rbcorley@bu.edu>
Sent: Thursday, February 20, 2020 1:07 PM
To: William B. Karesh; calisher@cybersafe.net; Charles.Calisher@colostate.edu
Cc: bushschoolscowcroft@tamu.edu; rcolwell@umd.edu; Peter Daszak; christian.drosten@charite.de; L.Enjuanes@cnb.csic.es; a.e.gorbalenya@lumc.nl; b.haagmans@erasmusmc.nl; JMHUGHE@emory.edu; Keusch, Gerald T; lamsk@nipahvirus.org; Juan Lubroth; John MacKenzie; Madoff, Lawrence; Jonna Mazet; peter.palese@mssm.edu; stanley-perlman@uiowa.edu; llmpoon@hku.hk; bernard.roizman@bsd.uchicago.edu; saif.2@osu.edu; kanta.subbarao@influenzacentre.org; Jane Hilton; Equitech
Subject: Re: Origin Coronavirus COVID-19

This is rich – thank you!

Ron

From: "William B. Karesh" <karesh@ecohealthalliance.org>
Date: Thursday, February 20, 2020 at 12:58
To: "calisher@cybersafe.net" <calisher@cybersafe.net>, "Charles.Calisher@colostate.edu" <Charles.Calisher@colostate.edu>
Cc: "bushschoolscowcroft@tamu.edu" <bushschoolscowcroft@tamu.edu>, "rcolwell@umd.edu" <rcolwell@umd.edu>, RBC Office <rbcorley@bu.edu>, Peter Daszak <daszak@ecohealthalliance.org>, "christian.drosten@charite.de" <christian.drosten@charite.de>, "L.Enjuanes@cnb.csic.es" <L.Enjuanes@cnb.csic.es>, "a.e.gorbalenya@lumc.nl" <a.e.gorbalenya@lumc.nl>, "b.haagmans@erasmusmc.nl" <b.haagmans@erasmusmc.nl>, "JMHUGHE@emory.edu" <jmhughe@emory.edu>, Gerald Keusch <keusch@bu.edu>, "lamsk@nipahvirus.org" <lamsk@nipahvirus.org>, Juan Lubroth <Juan.Lubroth@fao.org>, John MacKenzie <J.Mackenzie@curtin.edu.au>, "Lawrence.Madoff@umassmemorial.org" <Lawrence.Madoff@umassmemorial.org>, Jonna Mazet <jkmazet@ucdavis.edu>, "peter.palese@mssm.edu" <peter.palese@mssm.edu>, "stanley-perlman@uiowa.edu" <stanley-perlman@uiowa.edu>, "llmpoon@hku.hk" <llmpoon@hku.hk>, "bernard.roizman@bsd.uchicago.edu" <bernard.roizman@bsd.uchicago.edu>, "saif.2@osu.edu" <saif.2@osu.edu>,

"kanta.subbarao@influenzacentre.org" <kanta.subbarao@influenzacentre.org>, Jane Hilton <janewilsonhilton@gmail.com>, Equitech <equitech@bigpond.com>
Subject: Re: Origin Coronavirus COVID-19

Same hypothesis as SARS from the same person!!, and my alternative hypothesis at the time (2003). see attached from an old presentation I used to use.

Billy

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001 USA

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From: Saif, Linda
To: Peter Daszak
Subject: Re: Revised commentary for EMI - final!
Date: Tuesday, February 18, 2020 8:46:50 AM
Attachments: image001.png

Hi Peter,
Once again thanks for tackling this. Glad to see this group has reversed its conclusions once the actual data was analyzed and interpreted on a factual basis!
Hope this too gets to the "Grey House".
Thanks,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Tuesday, February 18, 2020 at 12:27 AM
To: Linda Saif <saif.2@osu.edu>
Subject: RE: Revised commentary for EMI - final!

Definitely – already cited it. It's in review for Nature. Unfortunately this is the exact same group that elevated the potential that this was a lab release all the way to the White House two weeks ago, and helped fuel some of the conspiracy theorists.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Saif, Linda [mailto:saif.2@osu.edu]
Sent: Monday, February 17, 2020 9:28 PM
To: Peter Daszak
Subject: Fwd: Revised commentary for EMI - final!

Hi Peter
Highly relevant posting. Could we still cite it on our statement?
Thanks for funding ideas!
Regards
Linda

Sent from my iPhone

Begin forwarded message:

> **From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Date:** February 17, 2020 at 6:12:34 PM EST
> **To:** "Saif, Linda" <saif.2@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
> **Subject: Re: Revised commentary for EMI - final!**
>
> See a very relevant online posting:
>
> # The Proximal Origin of SARS-CoV-2
>
> http://virological.org/t/the-proximal-origin-of-sars-cov-2/398
>
> Shan-Lu

From: "Saif, Linda" <saif.2@osu.edu>
Date: Sunday, February 16, 2020 at 7:20 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: Revised commentary for EMI - final!

Attached
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 16, 2020 3:14 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: Revised commentary for EMI - final!

See a typo in the title, and the last sentence as we had discussed. Thanks,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 1:55 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: RE: Revised commentary for EMI - final!

Good to me.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 1:45 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>

Subject: Revised commentary for EMI - final!

Please look at this new version, sorry!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
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Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Shan-Lu Liu <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 1:38 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

THE OHIO STATE UNIVERSITY

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Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Saif, Linda
To: Peter Daszak
Subject: FW: SARS reagents
Date: Sunday, April 12, 2020 3:47:29 PM

Hi Peter,
Below is the list of reagents most urgently needed.
Thanks for any help you can give us to procure these as rapidly as possible to undertake our BSL3 SARS CoV-2 research!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Wang, Qiuhong" <wang.655@osu.edu>
Date: Sunday, April 12, 2020 2:06 PM
To: Linda Saif <saif.2@osu.edu>, Anastasia Vlasova <vlasova.1@osu.edu>
Subject: RE: SARS reagents

I suggest that we also request the hyperimmune sera to SARS-CoV-2 nonstructural and structural proteins, respectively, for IHC and IFA assays.

So, the list of reagents are below:

1. SARS-CoV-2 S-pseudovirus;
2. Hyperimmune sera to SARS-CoV-2 nonstructural and structural proteins, respectively;
3. Human antiserum to SARS-CoV-2;
4. Human antiserum to SARS-CoV;
5. Human antiserum to MERS-CoV;
6. Human antiserum to HCoV-OC43;
7. Human antiserum to HCoV-HKU1;
8. Human antiserum to HCoV-229E;
9. Human antiserum to HCoV-NL63;

Thanks,
Qiuhong

From: Saif, Linda <saif.2@osu.edu>
Sent: Sunday, April 12, 2020 12:51 PM
To: Wang, Qiuhong <wang.655@osu.edu>; Vlasova, Anastasia <vlasova.1@osu.edu>
Subject: Re: SARS reagents

From: Linda Saif <saif.2@osu.edu>
Date: Saturday, April 11, 2020 at 1:51 PM
To: "Wang, Qiuhong" <wang.655@osu.edu>, Anastasia Vlasova <vlasova.1@osu.edu>
Subject: FW: SARS reagents

Hi
Maybe opportunity for us.
Please send me the list to forward. Lets also include S pseudotype virus and the Human CoVs and antisera!
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Saturday, April 11, 2020 1:19 PM
To: Linda Saif <saif.2@osu.edu>
Cc: Alison Andre <andre@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>
Subject: RE: nature news request

Hi Linda,

Delayed response, but I'm particularly bad for that at the moment.

I think Ralph will know best how to get access to reagents, but the NIH CoV PI call that I'm on every week with NIH/NIAID is a good place to start.

Can you send me a new email with a bulleted list of the reagents you need right now and I'll forward it to the group, cc'd to you. We'll get a good response I think. Ralph is part of that group, and it's headed up by Erik Stemmy (the program officer for my CoV R01) and includes the CEIRS group (Centers of Excellence for Influenza Research).

Cheers,

Peter

Peter Daszak
President

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New York, NY 10001
USA

Tel.: +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

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From: Saif, Linda <saif.2@osu.edu>
Sent: Friday, February 28, 2020 10:23 PM
To: Peter Daszak <daszak@ecohealthalliance.org>
Subject: Re: nature news request
Importance: High

HI Peter,
Thanks for your reply! I appreciate your perspectives. We have also contacted Ralph about his CoV R01 so we need to see where our research will fit best and is most feasible. We will stay in touch about this and I appreciate your offer to help.
However it is not just for Agriculture to see if pigs are susceptible, but if they are, because they better resemble humans in physiology, metabolism and immunity than rodent models, they could be a better model to test vaccines and antivirals for COVID-19. A major component of my research has been using the pig as a model for human rotavirus vaccines since they are susceptible to disease and infection with human rotaviruses and I have long term NIH support for this research using a pig disease model (also for human noroviruses testing for antivrials!).
Do you know any source for the SARS-CoV-2 reagents I indicated below?

Thanks again for getting back to me so promptly and your willingness to consider our proposal.
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program

OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Friday, February 28, 2020 7:04 PM
To: Linda Saif <saif.2@osu.edu>
Cc: Aleksei Chmura <chmura@ecohealthalliance.org>, Hongying Li <li@ecohealthalliance.org>
Subject: RE: nature news request

Hi Linda…responses below…

Cheers,

Peter

Peter Daszak
President

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460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Saif, Linda [mailto:saif.2@osu.edu]
Sent: Friday, February 28, 2020 6:19 PM
To: Peter Daszak
Subject: Re: nature news request

Hi Peter,
I would be very cautious to imply anything about pigs without any scientific proof. This could cause a major public panic in the face of what likely will be a major outbreak in the US soon and have a

drastic effect on the swine industry and pork in the US and worldwide—like what happened during the concern over influenza spilling over from chickens, when the consumption of poultry plummeted!
[Peter Daszak] Good point and something I normally don't have to think about with our work, but I totally agree. My point to the reporter was that there are other possible pathways than the pangolin, but I think I should probably just say to him that this is a hypothesis with no data other than the ACE2 info. The other people making this point and hypothesis are on the WHO outbreak team, who have commented on the mixed farms that are across China (wildlife farming and pig/poultry farming in the same site).

In all the wildlife markets that I visited in China, I never saw any pigs, but I am sure you would know more about this. Are pigs sold in the wildlife or wet markets? Also my Chinese colleagues have mentioned that the availability of pigs in China is drastically reduced because of ASFV and so many pigs were slaughtered.
[Peter Daszak] except that in many markets they sell mixed livestock and wildlife and I have information that the Huanan Seafood market did sell pork and poultry. In any case, my hypothesis is that pigs may have been infected in a farm and then the virus transferred to the market via infected pigs coming in to slaughter.

Because I do have a great concern about this, Dr Wang and I are trying very hard to find funding and to get the SARS-CoV-2 and inoculate it into pigs in our BSL3 Ag facility for large animals. We also have to fill in tons of forms and it is not clear yet if SARS CoV 2 will be a select agent. The only NIH funding I have seen for COVID-19 is only as supplements to those PIs with an existing NIAID R01 working on CoV which makes it impossible for others outside of this to get funds to work on COVID-19! This is why I asked about your NIH grant. I will also need to develop our own ELISA to detect antibodies in swine specific for SARS-CoV-2, so if you know where I can get cDNA clones for the SARS-CoV-2 spike and N protein, and positive SARS-CoV-2 Ab controls, please let me know as quickly as possible because I can work on this immediately.
[Peter Daszak] I agree – we could put in a supplement to do this, using my grant as the parent. I'm putting in my own supplement, but there's no problem with doing more than one. I'm absolutely fine to do this and we should probably talk with Erik Stemmy (my program officer) if you'd like to go ahead. (cc'ing Aleksei and Hongying so they are aware!)

I noted that CDC in their advisories for the public, mentioned that anyone with pets should not be indirect contact with them if the person gets sick with COVID-19—I agree with this! I think a greater concern is that humans may infect pets, such as cats with related ACE2 receptor (or pigs) and then we will have new animal reservoirs if virus is infectious for pets! This is why I think it is so urgent to set up ELISAs to detect Abs in animals including cats!
[Peter Daszak] And today there is a news item about a positive dog. Unclear yet if it's just picked up virus around the snout from close contact or if it's infected and infectious. The news item is here: https://www.scmp.com/news/hong-kong/health-environment/article/3052874/coronavirus-no-need-panic-hong-kong-veterinarians
(Note that it's a Pomeranian, and particularly cute!)

I would love to hear your perspective on all this and any advise about funding sources and the

reagents (I am checking BEI resources since I deposited all our animal CoV strains and Abs with them after SARS)!
Sorry for such a long reply!
[Peter Daszak] great to get a long reply – I'm so sick of single sentence emails now because everyone's so busy!! Let's plan to call my Program Officer next week to see what's possible and if it's possible I'd be very happy to help out. He's suggesting that supplementary proposals are not too expensive (not the same as an R01). Reading between the lines I expect a budget of $150-200K direct would be what they'd fund. But he might say they can't fund this because it's work aimed at agriculture, so let's talk first, then speak with him…

Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Friday, February 28, 2020 4:59 PM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Robert Kessler <kessler@ecohealthalliance.org>, Linda Saif <saif.2@osu.edu>
Subject: RE: nature news request

Hi Linda,

I'm introducing you to a reporter from Nature who is doing a story on the animal origins of SARS-CoV-2. I mentioned that the pangolin link is likely spurious, i.e. that it's unlikely they were an amplifier of infection at the Wuhan market because they are so rare in the wildlife trade as live animals (mainly dried scales sold for medicine). I also mentioned that one concern is other mammals, e.g. farmed wildlife or pigs could be a potential intermediate or amplifying host because the ACE2 receptors seem able to bind the virus spike protein and because these are a very common animal in and around wildlife and other markets in Wuhan.

Would you be able to comment on this to her? I've cc'd her above and told her you'd be a good independent voice to give an opinion of the possibility that pigs could have played a part.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Friday, February 28, 2020 3:25 AM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

Just to follow up on this – do you know anyone who is seriously investigating this hypothesis? I would be interested in hearing more on this if any further research developments emerge.

Kind regards,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, 25 February 2020 4:26 AM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: RE: nature news request

Hi Smriti,

The pig idea is based on:

- sequence analysis that shows the pig ACE2 receptor can likely bind with SARS-CoV-2, meaning it could likely infect pigs.
- Live pangolins are extremely rare in markets, so are unlikely to have played a significant role in transmission. Pangolin scales (dried, and therefore unlikely to be able to transmit virus) are normally sold.
- We still don't know the history of the pangolins that had the CoV with genetic elements close to SARS-CoV-2, and it's possible they were infected during transit from another intermediate host
- One plausible scenario is that there are farms with the virus circulating in a receptive mammal (e.g. a pig) in rural SW or Central China, and that these animals were taken to the wet markets, slaughtered and butchered, enhancing the transmission of the CoV into people.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Saturday, February 22, 2020 8:25 PM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

I just noticed something in your response and wanted to ask you about it. At the moment, researchers have suggested that pangolins might have been a potential source of the virus spreading to humans. You mentioned pigs. Is there a growing body of research that suggests, or a group of researchers that believe, that it isn't pangolins, but instead pigs?

Thank you,
Smriti

From: Smriti Mallapaty
Sent: Tuesday, 18 February 2020 4:36 PM
To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: Re: nature news request

Thank you again Peter, I just have some follow up questions below from your comments.

Thanks again, and sorry for all the questions!

Kind regards,

Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, February 18, 2020 4:23 PM
To: Smriti Mallapaty
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

No problem – some answers to your questions below…

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Monday, February 17, 2020 10:42 PM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

Thank you for your quick response. Can I also ask another question about the infectiousness of the virus?

How does this study help to explain the infectiousness of the virus?
[Peter Daszak] The identification of potential binding sites in the Receptor Binding Domain of the Spike Protein of the virus suggests that it has enhanced ability to bind to human ACE2 (cell surface receptor protein) relative to the nearest known bat-CoV relative. The binding pattern that this virus gene encodes is different to SARS-CoV suggesting it evolved separately (and there may be other binding patterns in other viruses in bats not yet worked out). The ability to efficiently bind ACE2 may explain some of this viruses' capacity to undergo human-to-human transmission (i.e. infectivity), and other aspects of the illness may also help (respiratory infection that causes a lot of mucus, sneezing, etc. assists in other viral infections).
--Could you please elaborate on this point of other aspects of the illness that help to explain how infectious the virus is?
--Have you seen any other studies pointing to what might make this coronavirus so infectious?
--How would you assess the infectiousness of this virus compared to other viruses? One researcher I spoke to said that the cases on the cruise ship suggest that it is very infectious.

What is the significance of the virus acquiring a polybasic cleavage site?
[Peter Daszak] Unfortunately, we don't have detailed analyses of SARS-CoV-2 and related viruses in

cell culture or animal models, so we're left with a bit of a gap and the authors rightfully say that the significant is not yet known. However, in avian flu, there are low-pathogenicity and high-pathogenicity strains. The high-path strains are extremely lethal to poultry and have caused high mortality in the low numbers of people infected. One of the key differences between them is that the low path strains don't have the polybasic cleavage site and sequential evolution of the cleavage site leads to enhanced proteolytic activity and higher pathogenicity. The low path strains are only able to infect cell types that have lots of trypsin (which is proteolytic) mainly in respiratory cells and GI tract, but the high path AI strains can affect many different organs. The point is that if this has happened with SARS-CoV-2, it might explain why it acquired an ability to be lethal in people and affect them throughout the lungs. There is some evidence to back this up – when a cleavage site is engineered into SARS-CoV it enhances cell-cell fusion (but not viral entry).

And the two options – sustained human-to-human transmission vs involvement of an intermediate host – could either one help to better explain how infectious the virus is?
[Peter Daszak] Both scenarios would give the virus chance to mutate and adapt, particularly if there is a high density of hosts so that any beneficial mutations to the virus can be transmitted readily and out-compete less efficient mutants. Sustained human-to-human transmission would do this but it would be particularly effective if there was a farmed animal intermediate host – e.g. pigs, which are common and in dense populations. The paper then makes important points about the need to 1) identify these potential sources so that we can rule out further spillover and identify the origins of these mutations; and 2) better understanding of the ACE2 receptors across a wide range of animals – this would help understand the capacity of other bat-CoVs to bind and transmit.
I would add a third issue – given that we have already identified 500 or so CoVs in bats in China and we expect many more – we should also have a concerted effort to identify and fully sequence as many bat-CoVs as possible to 1) assess other potential pathways to RBD-ACE2 binding; and 2) be better able to test candidate vaccines and drugs against a wide range of potentially zoonotic CoVs. Currently we have some candidate vaccines against SARS that we know don't work against other bat CoVs we've discovered. As a public health pandemic prevention strategy, we're feeling around blindly in the dark if we don't identify the diversity of potential viral threats out there in wildlife.

Thank you,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, 18 February 2020 1:13 PM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: RE: nature news request
Importance: High

Thanks Smriti,

Yes – I read the paper and here are my thoughts:

First, I'm delighted to see an analysis of SARS-CoV-2 sequence data by this group of leading evolutionary virologists. I think the big take home for me is that their analysis supports what many of us working on bat-origin coronaviruses have said, that there's a high diversity of CoVs in bats in southeast Asia (we've identified over 500 in the last few years), and that these animals have frequent and intimate contact with people, livestock and other wildlife in the region. The paper clearly demonstrates a natural origin for SARS-CoV-2 and strongly refutes the theory that this virus was bioengineered. It also provides a strong argument against hypotheses that this virus was an escape from a lab.

The two most likely hypotheses the authors put forward for the acquisition of polybasic cleavage sites are interesting. Re. the potential for sustained human-to-human transmission prior to the outbreak being noticed – I agree that's possible and it certainly happened with SARS. This may have been happening in a rural site, even as part of the market supply chain – a wildlife hunter, farmer or wildlife trade middleman may have transmitted the virus to people in the Wuhan market as part of trading activities. In support of this, we conducted a small survey in rural Yunnan and Guangxi provinces, S. China a couple of years ago and found 2.93% (6/200) people who live near bat caves in Yunnan to have antibodies to bat coronaviruses (published in *Virologica Sinica*). We don't know which one, or whether this caused any symptoms, but if you look at the human population across the region that *Rhinolophus* spp. bats live in SE Asia, you're looking at a few million people who have likely been exposed in their lifetime, if these numbers hold throughout the region. That's a large interface, and suggests these events are far more common, but that evolution towards a large outbreak is rare – as we'd expect, and as we saw with HIV.

However, I believe the involvement of other animal hosts (so-called 'intermediate' hosts) is even more plausible. Having visited many rural villages, wildlife markets, bat caves, livestock and wildlife farms across South China during the last 15 years, the opportunities for these viruses to spillover across a very active wildlife-livestock-human interface is clear and obvious. There is a booming and lucrative industry breeding wildlife for food, given the scarcity (and often illegal nature) of wild-caught animals. These farms almost invariably stock a diversity of captive-bred wildlife species – civets, porcupines, bamboo rats, coypu, ferret-badgers, raccoon dogs etc., and they're usually mixed in with livestock – pigs, chickens, ducks, geese. And these farms are usually wide open to bats which feed at night above the pens, and some of which roost in the buildings. They are also usually linked to people's houses so that whole families are potentially exposed – and workers who often sleep adjacent to the pens. This is a shocking milieu if you think about it from a viral evolutionary point of view – perfect for a not-quite well-adapted bat CoV to acquire the right mutations to become better at transmission among other mammals, including humans. In support of this hypothesis, Zhou et al. 2020 show that SARS-CoV-2 spike proteins would likely bind to the ACE2 of pigs. We found another bat-CoV (HKU-2, SADS-CoV) causing a die-off of >25,000 pigs in 5 farms in Guangdong province a couple of years ago (published in *Nature*). A scenario I find really likely is that a *Rhinolophus affinis* or related species bat was feeding in a pig farm in rural Hubei or further south and a progenitor virus was transmitted via bat feces to pigs at that farm. These pigs were then butchered and the meat sold, or sold live to one of more markets, which then led to a substantial initial exposure of a number of people, seeding human-to-human transmission in mid- to late-November. The nightmare scenario is that this virus is therefore not only circulating in humans in China, but also, currently unknown to us, in one or a number of pig or wildlife farms in the region. This means that even if the

outbreak is controlled, if we don't get to the animal source, we could see repeated seeding of future epidemics through spillover at these farms. That scenario has been discussed at a number of meetings and calls I've been on, including with WHO at the R&D Blueprint Research Agenda-setting meeting and is something that should be investigated.

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Monday, February 17, 2020 8:06 PM
To: Peter Daszak
Subject: nature news request

Dear Peter,

I am a reporter for nature news, covering the coronavirus.

I assume you have seen this preprint recently posted online: http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

I wanted to know if you had any thoughts on the research and the significance of the findings? I have included a few key points below.

It talks about a cleavage site that is a unique feature of SARS-COV-2. The papers says 'the functional consequence of the polybasic cleavage site in SARS-CoV-2 is unknown' but then goes on to describe

how similar events in other coronavirus have been linked to a virus going from low to high pathogenicity. ***Acquisition of a polybasic cleavage site in HA, by either insertion or recombination, converts low pathogenicity avian influenza viruses into highly pathogenic forms***

The paper also considers whether this and other mutations happened in an intermediary animal before the spillover, or after in humans. If it happened in animals then > ***if SARS-CoV-2 pre-adapted in another animal species then we are at risk of future re-emergence events even if the current epidemic is controlled***. If it happened in humans then > if the adaptive process we describe occurred in humans, then even if we have repeated zoonotic transfers they are unlikely to take-off unless the same series of mutations occurs.

Thank you again,
Smriti

Smriti Mallapaty
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E: smriti.mallapaty@nature.com
W: nature.com/news

From: Saif, Linda
To: Peter Daszak
Subject: Re: nature news request
Date: Saturday, April 11, 2020 1:53:57 PM
Importance: High

Thanks for reply—I know the feeling of being overwhelmed just now! Beside the SARS-CoV-2 and antiserum, we need S pseudotype virus ASAP—will get back to you on other reagents. Does our request have to go thru BEI?
Stay safe,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Saturday, April 11, 2020 1:19 PM
To: Linda Saif <saif.2@osu.edu>
Cc: Alison Andre <andre@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>
Subject: RE: nature news request

Hi Linda,

Delayed response, but I'm particularly bad for that at the moment.

I think Ralph will know best how to get access to reagents, but the NIH CoV PI call that I'm on every week with NIH/NIAID is a good place to start.

Can you send me a new email with a bulleted list of the reagents you need right now and I'll forward it to the group, cc'd to you. We'll get a good response I think. Ralph is part of that group, and it's headed up by Erik Stemmy (the program officer for my CoV R01) and includes the CEIRS group (Centers of Excellence for Influenza Research).

Cheers,

Peter

Peter Daszak
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USA

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Twitter: @PeterDaszak

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

---

From: Saif, Linda <saif.2@osu.edu>
Sent: Friday, February 28, 2020 10:23 PM
To: Peter Daszak <daszak@ecohealthalliance.org>
Subject: Re: nature news request
Importance: High

HI Peter,
Thanks for your reply! I appreciate your perspectives. We have also contacted Ralph about his CoV R01 so we need to see where our research will fit best and is most feasible. We will stay in touch about this and I appreciate your offer to help.
However it is not just for Agriculture to see if pigs are susceptible, but if they are, because they better resemble humans in physiology, metabolism and immunity than rodent models, they could be a better model to test vaccines and antivirals for COVID-19. A major component of my research has been using the pig as a model for human rotavirus vaccines since they are susceptible to disease and infection with human rotaviruses and I have long term NIH support for this research using a pig disease model (also for human noroviruses testing for antivrials!).
Do you know any source for the SARS-CoV-2 reagents I indicated below?

Thanks again for getting back to me so promptly and your willingness to consider our proposal.
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave

Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Friday, February 28, 2020 7:04 PM
To: Linda Saif <saif.2@osu.edu>
Cc: Aleksei Chmura <chmura@ecohealthalliance.org>, Hongying Li <li@ecohealthalliance.org>
Subject: RE: nature news request

Hi Linda…responses below…

Cheers,

Peter

Peter Daszak
President

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Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

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From: Saif, Linda [mailto:saif.2@osu.edu]
Sent: Friday, February 28, 2020 6:19 PM
To: Peter Daszak
Subject: Re: nature news request

Hi Peter,
I would be very cautious to imply anything about pigs without any scientific proof. This could cause a major public panic in the face of what likely will be a major outbreak in the US soon and have a drastic effect on the swine industry and pork in the US and worldwide—like what happened during the concern over influenza spilling over from chickens, when the consumption of poultry

plummeted!
[Peter Daszak] Good point and something I normally don't have to think about with our work, but I totally agree. My point to the reporter was that there are other possible pathways than the pangolin, but I think I should probably just say to him that this is a hypothesis with no data other than the ACE2 info. The other people making this point and hypothesis are on the WHO outbreak team, who have commented on the mixed farms that are across China (wildlife farming and pig/poultry farming in the same site).

In all the wildlife markets that I visited in China, I never saw any pigs, but I am sure you would know more about this. Are pigs sold in the wildlife or wet markets? Also my Chinese colleagues have mentioned that the availability of pigs in China is drastically reduced because of ASFV and so many pigs were slaughtered.
[Peter Daszak] except that in many markets they sell mixed livestock and wildlife and I have information that the Huanan Seafood market did sell pork and poultry. In any case, my hypothesis is that pigs may have been infected in a farm and then the virus transferred to the market via infected pigs coming in to slaughter.

Because I do have a great concern about this, Dr Wang and I are trying very hard to find funding and to get the SARS-CoV-2 and inoculate it into pigs in our BSL3 Ag facility for large animals. We also have to fill in tons of forms and it is not clear yet if SARS CoV 2 will be a select agent. The only NIH funding I have seen for COVID-19 is only as supplements to those PIs with an existing NIAID R01 working on CoV which makes it impossible for others outside of this to get funds to work on COVID-19! This is why I asked about your NIH grant. I will also need to develop our own ELISA to detect antibodies in swine specific for SARS-CoV-2, so if you know where I can get cDNA clones for the SARS-CoV-2 spike and N protein, and positive SARS-CoV-2 Ab controls, please let me know as quickly as possible because I can work on this immediately.
[Peter Daszak] I agree – we could put in a supplement to do this, using my grant as the parent. I'm putting in my own supplement, but there's no problem with doing more than one. I'm absolutely fine to do this and we should probably talk with Erik Stemmy (my program officer) if you'd like to go ahead. (cc'ing Aleksei and Hongying so they are aware!)

I noted that CDC in their advisories for the public, mentioned that anyone with pets should not be indirect contact with them if the person gets sick with COVID-19—I agree with this! I think a greater concern is that humans may infect pets, such as cats with related ACE2 receptor (or pigs) and then we will have new animal reservoirs if virus is infectious for pets! This is why I think it is so urgent to set up ELISAs to detect Abs in animals including cats!
[Peter Daszak] And today there is a news item about a positive dog. Unclear yet if it's just picked up virus around the snout from close contact or if it's infected and infectious. The news item is here: https://www.scmp.com/news/hong-kong/health-environment/article/3052874/coronavirus-no-need-panic-hong-kong-veterinarians
(Note that it's a Pomeranian, and particularly cute!)

I would love to hear your perspective on all this and any advise about funding sources and the reagents (I am checking BEI resources since I deposited all our animal CoV strains and Abs with them after SARS)!

Sorry for such a long reply!
[Peter Daszak] great to get a long reply – I'm so sick of single sentence emails now because everyone's so busy!! Let's plan to call my Program Officer next week to see what's possible and if it's possible I'd be very happy to help out. He's suggesting that supplementary proposals are not too expensive (not the same as an R01). Reading between the lines I expect a budget of $150-200K direct would be what they'd fund. But he might say they can't fund this because it's work aimed at agriculture, so let's talk first, then speak with him...

Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Friday, February 28, 2020 4:59 PM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Robert Kessler <kessler@ecohealthalliance.org>, Linda Saif <saif.2@osu.edu>
Subject: RE: nature news request

Hi Linda,

I'm introducing you to a reporter from Nature who is doing a story on the animal origins of SARS-CoV-2. I mentioned that the pangolin link is likely spurious, i.e. that it's unlikely they were an amplifier of infection at the Wuhan market because they are so rare in the wildlife trade as live animals (mainly dried scales sold for medicine). I also mentioned that one concern is other mammals, e.g. farmed wildlife or pigs could be a potential intermediate or amplifying host because the ACE2 receptors seem able to bind the virus spike protein and because these are a very common animal in and around wildlife and other markets in Wuhan.

Would you be able to comment on this to her? I've cc'd her above and told her you'd be a good independent voice to give an opinion of the possibility that pigs could have played a part.

Cheers,

Peter

Peter Daszak
President

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Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

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From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Friday, February 28, 2020 3:25 AM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

Just to follow up on this – do you know anyone who is seriously investigating this hypothesis? I would be interested in hearing more on this if any further research developments emerge.

Kind regards,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, 25 February 2020 4:26 AM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: RE: nature news request

Hi Smriti,

The pig idea is based on:

- sequence analysis that shows the pig ACE2 receptor can likely bind with SARS-CoV-2, meaning it could likely infect pigs.
- Live pangolins are extremely rare in markets, so are unlikely to have played a significant role in transmission. Pangolin scales (dried, and therefore unlikely to be able to transmit virus) are normally sold.
- We still don't know the history of the pangolins that had the CoV with genetic elements close to SARS-CoV-2, and it's possible they were infected during transit from another intermediate host
- One plausible scenario is that there are farms with the virus circulating in a receptive mammal (e.g. a pig) in rural SW or Central China, and that these animals were taken to the wet markets, slaughtered and butchered, enhancing the transmission of the CoV into people.

Cheers,

Peter

Peter Daszak
President

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Website: www.ecohealthalliance.org
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From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Saturday, February 22, 2020 8:25 PM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

I just noticed something in your response and wanted to ask you about it. At the moment, researchers have suggested that pangolins might have been a potential source of the virus spreading to humans. You mentioned pigs. Is there a growing body of research that suggests, or a group of researchers that believe, that it isn't pangolins, but instead pigs?

Thank you,
Smriti

From: Smriti Mallapaty
Sent: Tuesday, 18 February 2020 4:36 PM
To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: Re: nature news request

Thank you again Peter, I just have some follow up questions below from your comments.

Thanks again, and sorry for all the questions!

Kind regards,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, February 18, 2020 4:23 PM
To: Smriti Mallapaty
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

No problem – some answers to your questions below…

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
th th

460 West 34 Street – 17 Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

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From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Monday, February 17, 2020 10:42 PM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

Thank you for your quick response. Can I also ask another question about the infectiousness of the virus?

How does this study help to explain the infectiousness of the virus?
[Peter Daszak] The identification of potential binding sites in the Receptor Binding Domain of the Spike Protein of the virus suggests that it has enhanced ability to bind to human ACE2 (cell surface receptor protein) relative to the nearest known bat-CoV relative. The binding pattern that this virus gene encodes is different to SARS-CoV suggesting it evolved separately (and there may be other binding patterns in other viruses in bats not yet worked out). The ability to efficiently bind ACE2 may explain some of this viruses' capacity to undergo human-to-human transmission (i.e. infectivity), and other aspects of the illness may also help (respiratory infection that causes a lot of mucus, sneezing, etc. assists in other viral infections).
--Could you please elaborate on this point of other aspects of the illness that help to explain how infectious the virus is?
--Have you seen any other studies pointing to what might make this coronavirus so infectious?
--How would you assess the infectiousness of this virus compared to other viruses? One researcher I spoke to said that the cases on the cruise ship suggest that it is very infectious.

What is the significance of the virus acquiring a polybasic cleavage site?
[Peter Daszak] Unfortunately, we don't have detailed analyses of SARS-CoV-2 and related viruses in cell culture or animal models, so we're left with a bit of a gap and the authors rightfully say that the significant is not yet known. However, in avian flu, there are low-pathogenicity and high-pathogenicity strains. The high-path strains are extremely lethal to poultry and have caused high mortality in the low numbers of people infected. One of the key differences between them is that the low path strains don't have the polybasic cleavage site and sequential evolution of the cleavage site leads to enhanced proteolytic activity and higher pathogenicity. The low path strains are only able to infect cell types that have lots of trypsin (which is proteolytic) mainly in respiratory cells and

GI tract, but the high path AI strains can affect many different organs. The point is that if this has happened with SARS-CoV-2, it might explain why it acquired an ability to be lethal in people and affect them throughout the lungs. There is some evidence to back this up – when a cleavage site is engineered into SARS-CoV it enhances cell-cell fusion (but not viral entry).

And the two options – sustained human-to-human transmission vs involvement of an intermediate host – could either one help to better explain how infectious the virus is?
[Peter Daszak] Both scenarios would give the virus chance to mutate and adapt, particularly if there is a high density of hosts so that any beneficial mutations to the virus can be transmitted readily and out-compete less efficient mutants. Sustained human-to-human transmission would do this but it would be particularly effective if there was a farmed animal intermediate host – e.g. pigs, which are common and in dense populations. The paper then makes important points about the need to 1) identify these potential sources so that we can rule out further spillover and identify the origins of these mutations; and 2) better understanding of the ACE2 receptors across a wide range of animals – this would help understand the capacity of other bat-CoVs to bind and transmit.
I would add a third issue – given that we have already identified 500 or so CoVs in bats in China and we expect many more – we should also have a concerted effort to identify and fully sequence as many bat-CoVs as possible to 1) assess other potential pathways to RBD-ACE2 binding; and 2) be better able to test candidate vaccines and drugs against a wide range of potentially zoonotic CoVs. Currently we have some candidate vaccines against SARS that we know don't work against other bat CoVs we've discovered. As a public health pandemic prevention strategy, we're feeling around blindly in the dark if we don't identify the diversity of potential viral threats out there in wildlife.

Thank you,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, 18 February 2020 1:13 PM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: RE: nature news request
Importance: High

Thanks Smriti,

Yes – I read the paper and here are my thoughts:

First, I'm delighted to see an analysis of SARS-CoV-2 sequence data by this group of leading evolutionary virologists. I think the big take home for me is that their analysis supports what many of us working on bat-origin coronaviruses have said, that there's a high diversity of CoVs in bats in southeast Asia (we've identified over 500 in the last few years), and that these animals have frequent and intimate contact with people, livestock and other wildlife in the region. The paper clearly demonstrates a natural origin for SARS-CoV-2 and strongly refutes the theory that this virus was bioengineered. It also provides a strong argument against hypotheses that this virus was an

escape from a lab.

The two most likely hypotheses the authors put forward for the acquisition of polybasic cleavage sites are interesting. Re. the potential for sustained human-to-human transmission prior to the outbreak being noticed – I agree that's possible and it certainly happened with SARS. This may have been happening in a rural site, even as part of the market supply chain – a wildlife hunter, farmer or wildlife trade middleman may have transmitted the virus to people in the Wuhan market as part of trading activities. In support of this, we conducted a small survey in rural Yunnan and Guangxi provinces, S. China a couple of years ago and found 2.93% (6/200) people who live near bat caves in Yunnan to have antibodies to bat coronaviruses (published in *Virologica Sinica*). We don't know which one, or whether this caused any symptoms, but if you look at the human population across the region that *Rhinolophus* spp. bats live in SE Asia, you're looking at a few million people who have likely been exposed in their lifetime, if these numbers hold throughout the region. That's a large interface, and suggests these events are far more common, but that evolution towards a large outbreak is rare – as we'd expect, and as we saw with HIV.

However, I believe the involvement of other animal hosts (so-called 'intermediate' hosts) is even more plausible. Having visited many rural villages, wildlife markets, bat caves, livestock and wildlife farms across South China during the last 15 years, the opportunities for these viruses to spillover across a very active wildlife-livestock-human interface is clear and obvious. There is a booming and lucrative industry breeding wildlife for food, given the scarcity (and often illegal nature) of wild-caught animals. These farms almost invariably stock a diversity of captive-bred wildlife species – civets, porcupines, bamboo rats, coypu, ferret-badgers, raccoon dogs etc., and they're usually mixed in with livestock – pigs, chickens, ducks, geese. And these farms are usually wide open to bats which feed at night above the pens, and some of which roost in the buildings. They are also usually linked to people's houses so that whole families are potentially exposed – and workers who often sleep adjacent to the pens. This is a shocking milieu if you think about it from a viral evolutionary point of view – perfect for a not-quite well-adapted bat CoV to acquire the right mutations to become better at transmission among other mammals, including humans. In support of this hypothesis, Zhou et al. 2020 show that SARS-CoV-2 spike proteins would likely bind to the ACE2 of pigs. We found another bat-CoV (HKU-2, SADS-CoV) causing a die-off of >25,000 pigs in 5 farms in Guangdong province a couple of years ago (published in *Nature*). A scenario I find really likely is that a *Rhinolophus affinis* or related species bat was feeding in a pig farm in rural Hubei or further south and a progenitor virus was transmitted via bat feces to pigs at that farm. These pigs were then butchered and the meat sold, or sold live to one of more markets, which then led to a substantial initial exposure of a number of people, seeding human-to-human transmission in mid- to late-November. The nightmare scenario is that this virus is therefore not only circulating in humans in China, but also, currently unknown to us, in one or a number of pig or wildlife farms in the region. This means that even if the outbreak is controlled, if we don't get to the animal source, we could see repeated seeding of future epidemics through spillover at these farms. That scenario has been discussed at a number of meetings and calls I've been on, including with WHO at the R&D Blueprint Research Agenda-setting meeting and is something that should be investigated.

Cheers,

Peter

Peter Daszak
President

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From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Monday, February 17, 2020 8:06 PM
To: Peter Daszak
Subject: nature news request

Dear Peter,

I am a reporter for nature news, covering the coronavirus.

I assume you have seen this preprint recently posted online: http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

I wanted to know if you had any thoughts on the research and the significance of the findings? I have included a few key points below.

It talks about a cleavage site that is a unique feature of SARS-COV-2. The papers says 'the functional consequence of the polybasic cleavage site in SARS-CoV-2 is unknown' but then goes on to describe how similar events in other coronavirus have been linked to a virus going from low to high pathogenicity. ***Acquisition of a polybasic cleavage site in HA, by either insertion or recombination, converts low pathogenicity avian influenza viruses into highly pathogenic forms***

The paper also considers whether this and other mutations happened in an intermediary animal before the spillover, or after in humans. If it happened in animals then > ***if SARS-CoV-2 pre-***

adapted in another animal species then we are at risk of future re-emergence events even if the current epidemic is controlled. If it happened in humans then > if the adaptive process we describe occurred in humans, then even if we have repeated zoonotic transfers they are unlikely to take-off unless the same series of mutations occurs.

Thank you again,
Smriti

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W: nature.com/news

From:	Peter Daszak
To:	Peter Daszak
Cc:	Aleksei Chmura; Robert Kessler; Hongying Li
Subject:	Contact for journalists re. today"s statement in the Lancet
Date:	Tuesday, February 18, 2020 9:10:30 AM
Attachments:	20tl1775Corr_2 Corrected proofs.pdf
Importance:	High

Dear all,

We've been in touch with Lancet this morning to get all the final details of our statement of support letter fixed (see corrected proofs attached). We expect this to be published in about 1 hour from now, and have begun reaching out to journalists who might cover the story.

To make sure we have good coverage and a range of voices speaking with reporters, we would very much like to pass on your name and email address as someone for journalists to contact about the issues we're discussing in the letter – i.e. the need to support colleagues working under difficult situations in an outbreak, to reduce rumors and misinformation, and the scientific evidence demonstrating that the conspiracy theories on the virus' origins are unfounded.

If you are willing to talk with reporters, please send your phone numbers (landline and mobile if possible) by return to me and cc'ing Robert Kessler, who will help coordinate press interest. Lancet will also be contacting journalists and we will pass on your contact info to them also.

Ideally, we would have people from Europe, Australia, Asia, and the Americas (all represented on the authorship list) able to talk or email with journalists, so please step forward!

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and

wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Doctopic: Analysis and Interpretation
20TL1775_Corr

Embargo: February 18, 2020—ASAP

Statement in support of the scientists, public health, and medical professionals of china combating COVID-19

We are public health scientists who have closely followed the emergence of 2019 novel coronavirus disease (COVID-19) and are deeply concerned about its impact on global health and wellbeing. We have watched as the scientists, public health, and medical professionals of China, in particular, have worked diligently and effectively to rapidly identify the pathogen behind this outbreak, put in place significant measures to reduce its impact, and share their results transparently with the global health community. This effort has been remarkable.

We sign this statement in solidarity with all scientists and health professionals in China who continue to save lives and protect global health during the challenge of the COVID-19 outbreak. We are all in this together, with our Chinese counterparts in the forefront, against this new viral threat.

The rapid, open, and transparent sharing of data on this outbreak is now being threatened by rumors and misinformation around its origins. We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin. Scientists from multiple countries have published and analysed genomes of the causative agent, severe acute respiratory syndrome corona virus 2 (SARS-CoV-2),[1] and they overwhelmingly conclude that this coronavirus originated in wildlife,[2–10] as have so many other emerging pathogens.[11,12] This is further supported by a letter from the Presidents of the US National Academies of Science, Engineering, and Medicine[13] and by the scientific communities they represent. Conspiracy theories do nothing but create fear, rumors, and prejudice that jeopardise our global collaboration in the fight against this virus. We support the call from the Director General of WHO to promote scientific evidence and unity over misinformation and conjecture.[14] We want you, the science and health professionals of China, to know that we stand with you in your fight against this virus.

We invite others to join us in supporting the scientists, public health, and medical professionals of Wuhan and across China. Stand with our colleagues on the front-line!

We speak in one voice. [A: Any competing interests you'd like to declare? Eg, LM is Editor of ProMED-mail as I have taken this out of the list of affiliations. The information here must match the information provided in your ICMJE form.]

*Charles Calisher, Dennis Carroll, Rita Colwell, Ronald B Corley, *Peter Daszak, Christian Drosten, Luis Enjuanes, Jeremy Farrar, Hume Field, Josie Golding, Alexander Gorbalenya, Bart Haagmans, James M Hughes, William B Karesh, Gerald T Keusch, Sai Kit Lam, Juan Lubroth, John S Mackenzie, Larry Madoff, Jonna Mazet, Peter Palese, Stanley Perlman, Leo Poon, Bernard Roizman, Linda Saif, Kanta Subbarao, Mike Turner*

daszak@ecohealthalliance.org

Colorado State University, Fort Collins, CO, USA (CC); Scowcroft Institute of International Affairs, Texas A&M, College Station, TX, USA (DC); University of Maryland, College Park, MD, USA (RC); NEIDL Institute (RBC), Boston University (GTK), Boston, MA, USA; EcoHealth Alliance, New York, NY [A: zipcode please], USA (PD); Charité – Universitatsmedizin Berlin, Berlin, Germany (CD); National Center of Biotechnology, Madrid, Spain (LE); The Wellcome Trust, London, UK (JF, JG); School of Veterinary Science, The University of Queensland, Brisbane, QLD, Australia (HM); Leiden University Medical Center, Leiden, Netherlands (AG); Erasmus Medical Center, Rotterdam, Netherlands (BH); Emory University, Atlanta, GA, USA (JMH); World Organization for Animal Health (OIE) Working Group on Wildlife, USA (WBK [A: where in the US is the OIE? City, state?]); University of Malaya, Kuala Lumpur, Malaysia (SKL); Food and Agriculture Organization of the United Nations, Rome, Italy (JL); Curtin University, Perth, WA, Australia (JSM); Massachusetts Medical School, Worcester, MA, USA (LM); University of California at Davis, Davis, CA, USA (JM); Department of Microbiology, Icahn School of Medicine, Mt Sinai Hospital, New York, NY, USA (PP); University of Iowa, Roy J and Lucille A Carver College of Medicine, Iowa City, IA, USA (SP); The University of Hong Kong, Hong Kong (LP); University of Chicago, Chigaco, IL, USA (BR); The Ohio State University, Columbus, OH USA (LS); and The University of Melbourne, Melboune, VIC, Australia (KS)

[A: The original ref 1 is now in the margin. Please check the rest of the references.]

1 Gorbalenya AE, Baker SC, Baric RS, et al. Severe acute respiratory syndrome-related coronavirus: the species and its viruses—a statement of the Coronavirus Study Group. *bioRxiv* 2020; published online MM DD [A: please complete]. DOI: 2020.02.07.937862.

2 Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; published online Feb 3. DOI:10.1038/s41586-020-2012-7.

3 Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; published online Jan 30. https://doi.org/10.1016/S0140-6736(20)30251-8.

4 Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *NEJM* 2020; published online Jan 24. DOI:10.1056/NEJMoa2001017.

5 Ren L, Wang Y-M, Wu Z-Q, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J* 2020; published online Feb 11. DOI:10.1097/CM9.0000000000000722.

6 Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infect Genet Evol* 2020; published online Jan 29. DOI:10.1016/j.meegid.2020.104212.

7 Benvenuto D, Giovanetti M, Ciccozzi A, Spoto S, Angeletti S, Ciccozzi M. The 2019-new coronavirus epidemic: evidence for virus evolution. *J Med Virol* 2020; published online Jan 29. DOI:10.1002/jmv.25688.

8 Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *J Virol* 2020; published online Jan 29. DOI:10.1128/JVI.00127-20.

9 CDC. Coronavirus disease 2019 (COVID-19) situation summary. Feb 16, 2020. https://www.cdc.gov/coronavirus/2019-nCoV/summary.html (accessed Feb 8, 2020).

10 Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Feb 16, 2020; http://virological.org/t/the-proximal-origin-of-sars-cov-2/398 (accessed Feb 17, 2020).

11 Bengis R, Leighton F, Fischer J, Artois M, Morner T, Tate C. The role of wildlife in emerging and re-emerging zoonoses. *Rev Sci Tech* 2004; **23**: 497–512.

12 Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. *Emerg Infect Dis* 2005; **11**: 1842–47.

13 NASEM. The National Academies of Science Engineering and Medicine of the USA. NAS, NAE, and NAM Presidents' letter to the White House Office of Science and Technology Policy. Feb 6, 2020. https://www.nationalacademies.org/includes/NASEM%20Response%20to%20OSTP%20re%20Coronavirus_February%206,%202020.pdf (accessed Feb 7, 2020).

14 WHO. Director-General's remarks at the media

Published Online February 18, 2020 https://doi.org/10.1016/PII

For the **SARS-CoV-2 genome analysis** see https://www.gisaid.org/epiflu-applications/next-betacov-app/

Submissions should be made via our electronic submission system at http://ees.elsevier.com/thelancet/

briefing on 2019 novel coronavirus on 8 February 2020. Feb 8, 2020. https://wwwwhoint/dg/speeches/detail/director-general-s-remarks-at-the-media-briefing-on-2019-novel-coronavirus- --8-february-2020 (accessed MM DD, 2020).

From:	Peter Daszak
To:	Peter Daszak
Cc:	Aleksei Chmura; Hongying Li
Subject:	URGENT - need signatures in next few hours: Our statement on COVID-19 will be published this morning US Eastern time in The Lancet
Date:	Tuesday, February 18, 2020 5:51:48 AM
Attachments:	Statement of support COVID-19 China_submission_corrected_clean.docx TL_AuthorSigs 2019.pdf icmje-COI-form to sign.pdf
Importance:	High

Dear All,

I want to let you all know that we received strong support from Richard Horton at *The Lancet*, and our paper will be published today (Tuesday 18th Feb) at 3pm UK time (10am Eastern US time). Thank you also to those of you who sent last minute changes – I've incorporated them where possible (see final version attached). I've also cited a paper that was uploaded yesterday (http://virological.org/t/the-proximal-origin-of-sars-cov-2/398), currently in review in *Nature* (I believe) that clearly refutes the bio-engineered virus hypothesis and strongly supports the conclusion that SARS-CoV-2 is of natural origin.

As we discussed, the authorship will be alphabetical. Unfortunately, it looks like there has to be a single corresponding author, but the editor will put a statement at the top of the authorship list to indicate that we are all speaking in one voice on this. I will see what that looks like when proofs come through in a minute. *The Lancet* have also agreed to publish our Mandarin version of this statement (thanks for the translation Hongying) online, so it reaches a wider audience in Asia and around the world.

I have two urgent requests:

1) Please fill in the attached Conflict of Interest form ASAP
2) Please e-sign the Author signature form ASAP

It will be really important to get this message out to journalists once it's published. Finally, I would ask all of you who can post this to your websites, or on social media, or email to your colleagues, please do so.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

Statement in Support of the Scientists, Public Health and Medical Professionals of China Combating the COVID-19 Outbreak

We, the undersigned, are public health scientists who have closely followed the emergence of COVID-19, and are deeply concerned about its impact on global health and well-being. We have watched as the scientists, public health and medical professionals of China, in particular, have worked diligently and effectively to rapidly identify the pathogen behind this outbreak, put in place significant measures to reduce its impact, and share their results transparently with the global health community. This effort has been remarkable.

We sign this statement in solidarity with all scientists and health professionals in China who continue to save lives and protect global health during the challenge of this novel coronavirus outbreak. We are all in this together, with our Chinese counterparts in the forefront, against this new viral threat.

The rapid, open and transparent sharing of data on this outbreak is now being threatened by rumors and misinformation around its origins. We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin. Scientists from multiple countries have published and analyzed genomes of the causative agent, SARS-CoV-2[1,2], and they overwhelmingly conclude that this coronavirus originated in wildlife[3-12], as have so many other emerging pathogens[13,14]. This is further supported by a letter from the Presidents of the US National Academies of Science, Engineering, and Medicine[15], and by the scientific communities they represent. Conspiracy theories do nothing but create fear, rumors, and prejudice that jeopardize our global collaboration in the fight against this virus. We support the call from the Director-General of the World Health Organization to promote scientific evidence and unity over misinformation and conjecture[16]. We want you, the science and health professionals of China, to know that we stand with you in your fight against this virus.

We invite others to join us in supporting the scientists, public health, and medical professionals of Wuhan and across China. Stand with our colleagues on the front-line!

Signatories

Dr. Charles Calisher, Professor Emeritus, Colorado State University, USA
Dr. Dennis Carroll, Senior Fellow, Scowcroft Institute of International Affairs, Texas A&M University, USA
Dr. Rita Colwell, Distinguished University Professor, University of Maryland College Park, USA
Dr. Ronald B. Corley, Director & Professor, NEIDL Institute, Boston University, USA
Dr. Peter Daszak, President, EcoHealth Alliance, USA
Dr. Christian Drosten, Professor, Charité – Universitatsmedizin Berlin, Germany
Dr. Luis Enjuanes, National Center of Biotechnology, Madrid, Spain
Dr. Jeremy Farrar, Director, The Wellcome Trust, UK
Dr. Hume Field, Honorary Professor, School of Veterinary Science, The University of Queensland, Australia
Dr. Josie Golding, Epidemics Lead, The Wellcome Trust, UK
Dr. Alexander Gorbalenya, Professor, Leiden University Medical Center, The Netherlands

Dr. Bart Haagmans, Researcher, Erasmus Medical Center, The Netherlands
Dr. James M. Hughes, Professor, Emory University, USA
Dr. William B. Karesh, President, World Organization for Animal Health (OIE) Working Group on Wildlife, USA
Dr. Gerald T. Keusch, Professor of Medicine and Global Health, Boston University, USA
Dr. Sai Kit Lam, Professor Emeritus, University of Malaya, Kuala Lumpur, Malaysia
Dr. Juan Lubroth, Former Chief Veterinary Officer, Food and Agriculture Organization of the United Nations, Italy
Dr. John S. Mackenzie, Professor Emeritus, Curtin University, Perth, Australia
Dr. Larry Madoff, Editor & Professor of Medicine, ProMED-mail & University of Massachusetts Medical School, USA
Dr. Jonna Mazet, Professor, University of California at Davis, USA
Dr. Peter Palese, Professor & Head, Dept Microbiology, Icahn School of Medicine, Mt. Sinai Hospital, USA
Dr. Stanley Perlman, Professor, Carver College of Medicine, University of Iowa, USA
Dr. Leo Poon, Professor, The University of Hong Kong, Hong Kong
Dr. Bernard Roizman, Joseph Regenstein Distinguished Service Professor Emeritus of Virology, University of Chicago, USA
Dr. Linda Saif, Distinguished University Professor, The Ohio State University, USA
Dr. Kanta Subbarao, Honorary Professor, The University of Melbourne, Australia
Dr. Mike Turner, Director of Science, The Wellcome Trust, UK

References

1. GISAID. Genomic epidemiology of BetaCoV 2019-2020. 2020. https://www.gisaid.org/epiflu-applications/next-betacov-app/ (accessed February 8th 2020 2020).
2. Gorbalenya AE, Baker SC, Baric RS, et al. Severe Acute Respiratory Syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. *bioRxiv* 2020: 2020.02.07.937862.
3. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020.
4. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* 2020.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 2020.
6. Ren L, Wang Y-M, Wu Z-Q, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J Epub ahead of print* 2020.
7. Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *bioRxiv* 2020.
8. Benvenuto D, Giovanetti M, Ciccozzi A, Spoto S, Angeletti S, Ciccozzi M. The 2019-new coronavirus epidemic: Evidence for virus evolution. *J Med Virol* 2020; **1-5**(n/a).
9. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *Journal of Virology* 2020.

10. Centers for Disease Control and Prevention. 2019 Novel Coronavirus. 2020. https://www.cdc.gov/coronavirus/2019-nCoV/summary.html (accessed Februray 8th 2020.
11. Latinne A, Hu B, Olival KJ, et al. Origin and cross-species transmission of bat coronaviruses in China. *Nature Communications* 2020; **In review**.
12. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The Proximal Origin of SARS-CoV-2. 2020; http://virological.org/t/the-proximal-origin-of-sars-cov-2/398 (accessed February 17th 2020).
13. Bengis R, Leighton F, Fischer J, Artois M, Morner T, Tate C. The role of wildlife in emerging and re-emerging zoonoses. *Revue scientifique et technique-office international des epizooties* 2004; **23**(2): 497-512.
14. Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. *Emerg Infect Dis* 2005; **11**(12): 1842-7.
15. NASEM: The National Academies of Science Engineering and Medicine of the USA. NAS, NAE, and NAM Presidents' letter to the White House Office of Science and Technology Policy (OSTP)2020. https://www.nationalacademies.org/includes/NASEM%20Response%20to%20OSTP%20re%20Coronavirus_February%206,%202020.pdf (accessed February 7th, 2020).
16. The World Health Organization. Director-General's remarks at the media briefing on 2019 novel coronavirus on 8 February 2020. *https://wwwwhoint/dg/speeches/detail/director-general-s-remarks-at-the-media-briefing-on-2019-novel-coronavirus---8-february-2020* 2020.

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Author statements

Please insert the relevant text under the subheadings below. A completed form must be signed by all authors. Please note that we will accept hand-signed and electronic (typewritten) signatures. Please complete multiple forms if necessary, and upload the signed copy with your submission, scan and email to: editorial@lancet.com

Manuscript title:

Corresponding author:

Article type:

For research articles only:
I irrevocably authorise and grant my full consent to the corresponding author of the manuscript to: (1) enter into an exclusive publishing agreement with Elsevier on my behalf, in the relevant form set out at www.elsevier.com/copyright; and (2) unless I am a US government employee, to transfer my copyright or grant an exclusive license of rights to Elsevier as part of that publishing agreement, effective on acceptance of the article for publication. If the article is a work made for hire, I am authorized to confirm this on behalf of my employer. I agree that the copyright status selected by the corresponding author for the article shall apply and that this agreement is subject to the governing law of England and Wales.

Does your manuscript have a reference number? No ☐ Yes ☐ If yes, enter number here:

Does your manuscript have a handling editor? No ☐ Yes ☐ If yes, enter name here:

Authors' contributions
Please insert here the contribution each author made to the manuscript—eg, literature search, figures, study design, data collection, data analysis, data interpretation, writing etc. If all authors contributed equally, please state this. The information provided here must match the contributors' statement in the manuscript.

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Role of the funding source

Please disclose any funding sources and their role, if any, in the writing of the manuscript or the decision to submit it for publication. Examples of involvement include: data collection, analysis, or interpretation; trial design; patient recruitment; or any aspect pertinent to the study. Please also comment whether you have been paid to write this article by a pharmaceutical company or other agency. If you are the corresponding author, please indicate if you had full access to all the data in the study and had final responsibility for the decision to submit for publication. The information provided here must match the role of the funding source statement in the manuscript.

Conflicts of interest

Please complete the ICMJE conflict of interest form, which is available at http://www.thelancet.com/for-authors/forms#icmje-coi. Please ensure that a conflict of interest statement is included at the end of the manuscript, which matches what is declared on the ICMJE conflict of interest form.

Patient consent (if applicable) - completion of this section is mandatory for Clinical Pictures, and Adverse Drug Reactions.

Please sign below to confirm that all necessary consents required by applicable law from any relevant patient, research participant, and/or other individual whose information is included in the article have been obtained in writing. **The signed consent form(s) should be retained by the corresponding author and NOT sent to *The Lancet*.**

I agree with: the plan to submit to *The Lancet;* the contents of the manuscript; to being listed as an author; and to the conflicts of interest statement as summarised. I have had access to all the data in the study (for original research articles) and accept responsibility for its validity.

Title and name:	Highest degree:	Signature:	Date:
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Title and name:	Highest degree:	Signature:	Date:
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Title and name:	Highest degree:	Signature:	Date:
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Corresponding author declaration

I _____, the corresponding author of this manuscript, certify that the contributors' and conflicts of interest statements included in this paper are correct and have been approved by all co-authors.

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

ICMJE Form for Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Intellectual Property.

This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

5. Relationships not covered above.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

Definitions.

Entity: government agency, foundation, commercial sponsor, academic institution, etc.
Grant: A grant from an entity, generally [but not always] paid to your organization
Personal Fees: Monies paid to you for services rendered, generally honoraria, royalties, or fees for consulting , lectures, speakers bureaus, expert testimony, employment, or other affiliations
Non-Financial Support: Examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.
Other: Anything not covered under the previous three boxes
Pending: The patent has been filed but not issued
Issued: The patent has been issued by the agency
Licensed: The patent has been licensed to an entity, whether earning royalties or not
Royalties: Funds are coming in to you or your institution due to your patent

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name)

2. Surname (Last Name)

3. Date
18-February-2020

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name
Peter Daszak

5. Manuscript Title
Statement in Support of the Scientists, Public Health and Medical Professionals of China Combating the COVID-19 Outbreak

6. Manuscript Identifying Number (if you know it)
THELANCET-D-20-01775

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☐ Yes ☐ No

Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were **present during the 36 months prior to publication**.

Are there any relevant conflicts of interest? ☐ Yes ☐ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☐ No

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 5. Relationships not covered above

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☐ Yes, the following relationships/conditions/circumstances are present (explain below):

☐ No other relationships/conditions/circumstances that present a potential conflict of interest

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Section 6. Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

Evaluation and Feedback

Please visit http://www.icmje.org/cgi-bin/feedback to provide feedback on your experience with completing this form.

From: Saif, Linda
To: Peter Daszak
Cc: Aleksei Chmura; Hongying Li
Subject: Re: COVID-19 statement of support for Scientists and Public Health Professionals in China, and a condemnation of conspiracy theories on the origin of the virus
Date: Sunday, February 16, 2020 11:11:24 PM
Attachments: Liu et al_EMI Commentary_Revision_Final-sls.docx
Importance: High

Hi Peter,
Thanks again for taking the lead on this—I saw the segment about Cotton which makes this statement all the more timely.
Attached is a commentary from me, Susan Weiss and 2 of my US Chinese American colleagues that we just submitted to EMI.
Hopefully NAS will put together a task force to address COVID-19 and these issues, especially since NAS was active in sending NAS members (I went) and others to Wuhan and Harbin to tour the new BSL4 facilities and foster relations with our Chinese CAS counterparts.
Regards,
Linda

Regards,
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Sunday, February 16, 2020 10:48 PM
To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Aleksei Chmura <chmura@ecohealthalliance.org>, Hongying Li <li@ecohealthalliance.org>
Subject: COVID-19 statement of support for Scientists and Public Health Professionals in China, and a condemnation of conspiracy theories on the origin of the virus

Dear All,

Firstly, I want to thank each of you for your kindness, academic integrity, and openness in signing this statement of support. I've attached the current version of it for your records, and you can see the list of the eminent public health scientists from 8 countries who have co-signed with you. These include former heads of government agencies, current heads of world class research groups and major organizations, members of the US National Academies of Medicine and Science, an Academician from Malaysia, an Officer of the Order of Australia, and others who have achieved great success in public health and infectious disease research.

Secondly, I want to mention that the situation as regards conspiracy theories has worsened over the last few days, having been given credence through reporting yesterday in a UK tabloid (*Express*), regurgitation on prime time TV yesterday in the USA by US senator Tom Cotton, and discussions at a very high level within government in the USA and China. At the same time, some colleagues in China have received violent threats to their families and themselves.

For these reason, and as a way to get our statement across directly to the senior leaders in the governments of China and around the world, Jeremy Farrar (Director of the Wellcome Trust, and co-signatory) suggested that I submit this letter to the editor of *The Lancet* for possible publication. I have done so just now, out of a sense of urgency, and await his response (I have also asked if he is willing to sign).

I realize that all of you agreed to having this letter published and distributed, and I believe this would be an extremely appropriate platform to do so. Please let me know if you feel otherwise by responding to this email. Note that, for equity and impact, I have assigned authorship of the statement alphabetically, and will ask that no one person act as corresponding author.

I will, of course, let you know of the response as soon as I hear back.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

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[2] Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a

close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable

to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that

could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More

studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by scientific community and governments.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

From:	Peter Daszak
To:	Peter Daszak
Cc:	Aleksei Chmura; Hongying Li
Subject:	COVID-19 statement of support for Scientists and Public Health Professionals in China, and a condemnation of conspiracy theories on the origin of the virus
Date:	Sunday, February 16, 2020 10:51:24 PM
Attachments:	Statement of support COVID-19 China 021620.docx
Importance:	High

Dear All,

Firstly, I want to thank each of you for your kindness, academic integrity, and openness in signing this statement of support. I've attached the current version of it for your records, and you can see the list of the eminent public health scientists from 8 countries who have co-signed with you. These include former heads of government agencies, current heads of world class research groups and major organizations, members of the US National Academies of Medicine and Science, an Academician from Malaysia, an Officer of the Order of Australia, and others who have achieved great success in public health and infectious disease research.

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I will, of course, let you know of the response as soon as I hear back.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

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Statement in Support of the Scientists, Public Health and Medical Professionals of China Combating the Novel Coronavirus (COVID-19) Outbreak

We, the undersigned, are public health scientists who have closely followed the emergence of COVID-19, and are deeply concerned about its impact on global health and well-being. We have watched as the scientists, public health and medical professionals of China, in particular, have worked diligently and effectively to rapidly identify the pathogen behind this outbreak, put in place significant measures to reduce its impact, and share their results transparently with the global health community. This effort has been remarkable.

We sign this statement in solidarity with all scientists and health professionals in China who continue to save lives and protect global health during the challenge of this novel coronavirus outbreak. We are all in this together, with our Chinese counterparts in the forefront, against this new viral threat.

The rapid, open and transparent sharing of data on this outbreak is now being threatened by rumors and misinformation around its origins. We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin. Scientists from multiple countries have published and analyzed SARS-CoV-2 genomes[1], and they overwhelmingly conclude that this virus originated in wildlife[2-10], as have so many other emerging diseases[11,12]. This is further supported by a letter from the Presidents of the US National Academies of Science, Engineering, and Medicine[13], and by the scientific communities they represent. Conspiracy theories do nothing but create fear, rumors, and prejudice that jeopardize our global collaboration in the fight against this virus. We support the call from the Director-General of the World Health Organization to promote scientific evidence and unity over misinformation and conjecture[14]. We want you, the science and health professionals of China, to know that we stand with you in your fight against this virus.

We invite others to join us in supporting the scientists, public health, and medical professionals of Wuhan and across China. Stand with our colleagues on the front-line!

Signatories

Dr. Charles Calisher, Professor Emeritus, Colorado State University, USA
Dr. Dennis Carroll, Senior Fellow, Scowcroft Institute, Texas A&M, USA
Dr. Rita Colwell, Distinguished University Professor, University of Maryland College Park, USA
Dr. Ronald B. Corley, Director & Professor, NEIDL Institute, Boston University, USA
Dr. Peter Daszak, President, EcoHealth Alliance, USA
Dr. Christian Drosten, Professor, Charité – Universitatsmedizin Berlin, Germany
Dr. Jeremy Farrar, Director, The Wellcome Trust, UK
Dr. Hume Field, Honorary Professor, School of Veterinary Science, The University of Queensland, Australia
Dr. Josie Golding, Programme Officer for Epidemic Preparedness and Response, The Wellcome Trust, UK
Dr. Alexander Gorbalenya, Professor, Leiden University Medical Center, The Netherlands
Dr. Bart Haagmans, Researcher, Erasmus Medical Center, The Netherlands
Dr. James M. Hughes, Professor, Emory University, USA

Dr. William B. Karesh, President, World Organization for Animal Health (OIE) Working Group on Wildlife, USA
Dr. Gerald T. Keusch, Professor of Medicine and Global Health, Boston University, USA
Dr. Sai Kit Lam, Professor Emeritus, University of Malaya, Kuala Lumpur, Malaysia
Dr. Juan Lubroth, Former Chief Veterinary Officer, Food and Agriculture Organization of the United Nations, Italy
Dr. John S. Mackenzie, Professor Emeritus, Curtin University, Perth, Australia
Dr. Larry Madoff, Editor & Professor of Medicine, ProMED-mail & University of Massachusetts Medical School, USA
Dr. Jonna Mazet, Professor, University of California at Davis, USA
Dr. Peter Palese, Professor & Head, Dept Microbiology, Icahn School of Medicine, Mt. Sinai Hospital, USA
Dr. Stanley Perlman, Professor, University of Iowa, Carver College of Medicine, USA
Dr. Leo Poon, Professor, The University of Hong Kong, Hong Kong
Dr. Bernard Roizman, Joseph Regenstein Distinguished Service Professor Emeritus of Virology, University of Chicago, USA
Dr. Linda Saif, Distinguished University Professor, The Ohio State University, USA
Dr. Kanta Subbarao, Honorary Professor, The University of Melbourne, Australia

References

1. GISAID. Genomic epidemiology of BetaCoV 2019-2020. 2020. https://www.gisaid.org/epiflu-applications/next-betacov-app/ (accessed February 8th 2020 2020).
2. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020.
3. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* 2020.
4. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 2020.
5. Ren L, Wang Y-M, Wu Z-Q, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J Epub ahead of print* 2020.
6. Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *bioRxiv* 2020.
7. Benvenuto D, Giovanetti M, Ciccozzi A, Spoto S, Angeletti S, Ciccozzi M. The 2019-new coronavirus epidemic: Evidence for virus evolution. *J Med Virol* 2020; **1-5**(n/a).
8. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *Journal of Virology* 2020.
9. Centers for Disease Control and Prevention. 2019 Novel Coronavirus. 2020. https://www.cdc.gov/coronavirus/2019-nCoV/summary.html (accessed Februray 8th 2020.
10. Latinne A, Hu B, Olival KJ, et al. Origin and cross-species transmission of bat coronaviruses in China. *Nature Communications* 2020; **In review**.
11. Bengis R, Leighton F, Fischer J, Artois M, Morner T, Tate C. The role of wildlife in emerging and re-emerging zoonoses. *Revue scientifique et technique-office international des epizooties* 2004; **23**(2): 497-512.

12. Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. *Emerg Infect Dis* 2005; **11**(12): 1842-7.
13. NASEM: The National Academies of Science Engineering and Medicine of the USA. NAS, NAE, and NAM Presidents' letter to the White House Office of Science and Technology Policy (OSTP)2020. https://www.nationalacademies.org/includes/NASEM%20Response%20to%20OSTP%20re%20Coronavirus_February%206,%202020.pdf (accessed February 7th, 2020).
14. The World Health Organization. Director-General's remarks at the media briefing on 2019 novel coronavirus on 8 February 2020. *https://wwwwhoint/dg/speeches/detail/director-general-s-remarks-at-the-media-briefing-on-2019-novel-coronavirus---8-february-2020* 2020.

From: Peter Daszak
To: Saif, Linda
Subject: Confidential - re. NASEM Standing Committee
Date: Wednesday, April 1, 2020 2:31:03 AM
Importance: High

Linda, apologies for not responding sooner, but as you can see from the timestamp on this email, I'm overworked right now as we all are.

I too am a bit surprised at the makeup of the Standing Committee. Obviously, I'm pleased to be on, not least because it began in response to a question about the potential bioengineered origin of SARS-CoV-2 which was a major shock to me as a close collaborator with the alleged conspirators. My joining was in itself was a struggle, as I had to explain my relationship with the Wuhan Institute of Virology to the group, given the conspiracy theories that started this whole request from OSTP to the NASEM.

I was very surprised to not see Ralph and yourself on there. I think Ralph is even more in the crosshairs of the conspiracy theorists, so that may be one reason, even though it's inappropriate, the politics are such that we have an administration with people who might tend to believe these theories! Additionally, I saw that you weren't on, and neither are many well-known and well-experienced EID outbreak people, like Jim Hughes, Jerry Keusch etc. Meanwhile we have few if any real epidemiologists, too many sequence phylogeny people, and some who just don't have the gravitas I'd have liked to have seen.

One explanation may be that although this is an NASEM committee, it was pushed heavily by Victor Dzau, so maybe it's weighted a bit to the NAM. By the way – that's my membership – I'm in NAM, not NAS, although I am being nominated for NAS this year by Rita Colwell.

All that said, I did mention your absence to Julie Pavlin (obviously without mentioning your email), who's staff director of the Board on Global Health at NAM and heavily involved in this committee. She agreed it is surprising that you aren't on and said she'd mention it to Andrew Pope, who's sort of managing the committee on NASEM staff.

The committee will also be setting up a series of Working Groups, including one on One Health, and another on Epidemiology etc. That might be an opportunity for your involvement, albeit that you really should be running one of these…

I'm glad you're organizing a statement from NAS. Please let me know if there's anything more I can do or something more direct. I'll definitely push as much as possible to get you involved – your expertise is sorely needed. I'll do this confidentially at this point, and openly if you say I should!

Cheers,

Peter

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USA

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Twitter: @PeterDaszak

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

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From: Saif, Linda <saif.2@osu.edu>
Sent: Tuesday, March 24, 2020 10:37 AM
To: Peter Daszak <daszak@ecohealthalliance.org>
Subject: COVID-19

Hi Peter,
I am very disappointed that as one of only a few members of NAS who has extensive experience working on CoVs (more than 40 years), that I have not been asked to serve on the Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats to address the issues pertaining to COVID-19. I know that you are a member and as one of the few members of NAS with CoV expertise I too would like to volunteer to offer my extensive expertise on CoVs. I am trying to keep up with the COVID-19 literature on this and I could also offer long term perspectives based on my broad experience on CoVs across many species.

As you are aware here are many ominous political issues now that will impact our ability to mitigate or suppress COVID-19 in the US. I plan to write a letter to Dr McNutt and the other NAS officers to ask them now to prepare a letter or whitepaper, signed by NAS members if helpful, to send to the President and Congressional elected officials and governors to provide the factual information about the epidemiologic estimates for the projected numbers of cases in the US, hospitalizations and deaths with and without the various mitigation or suppression strategies, based on the data from China, Italy and the Imperial College report. I think the congress and governors need to see these figures in front of them for their decision making. We were created to advise the nation and are supposed to be the scientific advisors to the US: ie "we provide independent, objective advice to inform policy with evidence, spark progress and innovation, and confront challenging issues for the benefit of society." As one of the

biggest disease crises in US history, the NAS should not abdicate a leadership role in dealing with the COVID-19 outbreak.

Please let me know your thoughts on this—I am drafting my letter this week!

Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From:	Peter Daszak
To:	Ralph Baric (rbaric@email.unc.edu); Saif, Linda
Cc:	Robert Kessler
Subject:	confidential (ish) our paper will be out on preprint server this week with >700 novel sequences (RdRp) and analysis of bat-CoVs China
Date:	Wednesday, April 1, 2020 2:20:34 AM
Attachments:	China bat CoVs_R2_PD.docx Talking points from Latinne et al. Origin and cross transmission of bat CoVs in China.docx
Importance:	High

Ralph and Linda,

We've got a paper just accepted in Nature Communications that analyzes a tone of RdRp sequences of bat-CoVs from China. They're making us upload it onto a preprint server, which we'll do Wednesday night. I've attached the uncorrected version here, and some talking points that I drafted.

I don't know if we'll get reporters asking about it, but we are in the middle of a pandemic caused by a relative of one of these, so it's possible. If so, I'd like to suggest you as alternate voices to speak to them. I hope that's ok.

Hope you and your families are well and staying safe. I already have a relative in the UK who has now (last night) died of COVID-19 – my father-in-law. Tragic and shocking that this is a bat-origin CoV, but gives my more drive to do this work. Likewise, thanks for both of your work on these viruses for many years – we're right now seeing how valuable that is.

Cheers,

Peter

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

Origin and cross-species transmission of bat coronaviruses in China

Alice Latinne[1¶], Ben Hu[2¶], Kevin J. Olival[1], Guangjian Zhu[1], Libiao Zhang[3], Hongying Li[1], Aleksei A. Chmura[1], Hume E. Field[1,4], Carlos Zambrana-Torrelio[1], Jonathan H. Epstein[1], Bei Li[2], Wei Zhang[2], Lin-Fa Wang[5], Zhengli Shi[2*], Peter Daszak[1*]

[1]EcoHealth Alliance, New York, USA;

[2]Key laboratory of special pathogens and biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China;

[3]Guangdong Institute of Applied Biological Resources, Guangdong Academy of Sciences, Guangzhou, China;

[4]School of Veterinary Science, The University of Queensland, Brisbane, Australia.

[5]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore.

[¶]Authors contributed equally to this paper

[*]Correspondence should be addressed to: daszak@ecohealthalliance.org; zlshi@wh.iov.cn

Abstract

Bats harbor a large diversity of coronaviruses (CoVs) and have been identified as the likely natural reservoirs and evolutionary origin of several zoonotic coronaviruses, including Severe Acute Respiratory Syndrome (SARS)-CoV that emerged in China in 2002 and SARS-CoV-2 ~~2019-nCoV~~ that emerged in Hubei, China and is currently causing a global pandemic. However, the evolution and diversification of CoVs in their bat hosts remain poorly understood. Here, we use an extensive dataset (including 732 novel CoV sequences) and Bayesian statistical framework to study the macroevolution of ~~bat-CoV~~bat-CoVs, their

cross-species transmission dynamics, and dispersal in China. Our findings reveal that alpha-CoVs have switched hosts more frequently, and among more distantly related taxa, than beta-CoVs during their evolution. Phylogenetic distance among hosts was found to represent a higher constraint on host switches for beta- than for alpha-CoVs. We also show that Rhinolophidae and the genus *Rhinolophus* were involved in more inter-family and inter-genus significant host switching events than any other family or genus. We use our analyses to identify the host taxa and geographic regions that together define hotspots of CoV evolutionary diversity in China. This provides a strategy for better targeting of bat-borne CoV discovery and proactive zoonotic disease surveillance. Finally, we provide the most comprehensive analysis to date, including all known bat-CoVs, to show that the emerging SARS-CoV-2 ~~2019-nCoV~~ has a likely origin in *Rhinolophus* spp. bats.

Introduction

Coronaviruses (CoVs) are RNA viruses causing respiratory and enteric diseases with varying pathogenicity in humans and animals. All CoVs known to infect humans are zoonotic, or of animal origin, with many thought to originate in bat hosts[1,2]. Due to their large genome size (the largest non-segmented RNA viral genome), frequent recombination and high genomic plasticity, CoVs are prone to cross-species transmission and are able to rapidly adapt to new hosts[1,3]. This phenomenon is thought to have led to the emergence of a number of CoVs affecting livestock and human health[4-9]. Three of these causing significant outbreaks originated in China during the last two decades. Severe Acute Respiratory Syndrome (SARS)-CoV emerged first in humans in Guangdong province, southern China, in 2002 and spread globally, causing fatal respiratory infections in close to 800 people[10-12]. Subsequent investigations identified horseshoe bats (genus *Rhinolophus*) as the natural reservoirs of SARS-CoV[13-16]. In 2016, Swine Acute Diarrhea Syndrome (SADS)-CoV caused the death of over 25,000 pigs in farms within Guangdong province[17]. This virus appears to have originated within *Rhinolophus* spp. bats, and belongs to the HKU2-CoV clade previously detected in bats in the region[17-19]. ~~Very recently~~In 2019, a novel coronavirus (SARS-CoV-2~~2019-nCoV~~) ~~was identified as the cause of~~caused an outbreak of respiratory illness (COVID-19) first detected in Wuhan, Hubei province, China, which has since become a pandemic. This emerging human virus is closely related to SARS-CoV, and also appears to have originated in horseshoe bats -- with its full genome 96% similar to a virus we discovered in *Rhinolophus affinis*[20].

A growing body of research has identified bats as the evolutionary sources of SARS- and Middle East Respiratory Syndrome (MERS)-CoVs [13,14,21-23], and as ~~evolutionary~~ the source of progenitors for the human CoVs, NL63 and 229E[24,25]. The emergence of SARS-CoV-2 ~~2019-nCoV~~ further underscores the importance of bat-origin CoVs ~~for~~ to global health, and understanding their origin and cross-species transmission ~~of~~ is a high priority for pandemic preparedness[20,26]. Bats harbor the largest diversity of

CoVs among mammals and two CoV genera, alpha- and beta-CoVs (α- and β-CoVs), have been widely detected in bats from most regions of the world[27,28]. ~~Bat-CoV~~Bat-CoV diversity seems to be correlated with host taxonomic diversity globally, the highest CoV diversity being found in areas ~~where~~ with the highest bat species richness ~~is the highest~~[29]. Host switching of viruses over evolutionary time is an important mechanism driving the evolution of bat coronaviruses in nature and appears to vary geographically[29,30]. However, detailed analyses of host-switching have been hampered by incomplete or opportunistic sampling, typically with relatively low numbers of viral sequences from any given region[31].

China has a rich bat fauna, with more than 100 described bat species and several endemic species representing both the Palearctic and Indo-Malay regions[32]. Its situation at the crossroads of two zoogeographic regions heightens China's potential to harbor a unique and distinctive CoV diversity. Since the emergence of SARS-CoV in 2002, China has been the focus of an intense viral surveillance and a large number of diverse ~~bat-CoV~~bat-CoVs has been discovered in the region[33-41]. However, the macroevolution of CoVs in their bat hosts in China and their cross-species transmission dynamics remain poorly understood.

In this study, we analyze an extensive field-collected dataset of ~~bat-CoV~~bat-CoV sequences from across China. We use a phylogeographic Bayesian statistical framework to reconstruct virus transmission history between different bat host species and virus spatial spread over evolutionary time. Our objectives were to compare the macroevolutionary patterns of α- and β-CoVs and identify the hosts and geographical regions that act as centers of evolutionary diversification for ~~bat-CoV~~bat-CoVs in China. These analyses aim to improve our understanding of how CoVs evolve, diversify, circulate among, and transmit between bat families and genera to help identify bat hosts and regions where the risk of CoV spillover is the highest.

Results

Taxonomic and geographic sampling

We generated 732 partial sequences (440 nt) of the RNA-dependent RNA polymerase (RdRp) gene from bat rectal swabs collected in China and added 508 ~~bat-CoV~~bat-CoV sequences from China available in GenBank to our datasets (list of GenBank accession numbers available in Supplementary Material). For each CoV genus, two datasets were created: one including all sequences with known host (host dataset) and one including all sequences with known sampling location at the province level (geographic dataset). To create a geographically discrete partitioning scheme that was more ecologically relevant than administrative borders for our phylogeographic reconstructions, we defined six zoogeographic regions within China by clustering provinces with similar mammalian diversity using hierarchical clustering[42] (see Methods): South western region (SW), Northern region (NO), Central northern region (CN), Central region (CE), Southern region (SO) and Hainan island (HI) (Fig. 1 and Fig. S1).

Our host datasets included 718 α-CoV sequences (XX new sequences, including XX new SADSr-CoV sequences) from 41 bat species (14 genera, five families) and 544 β-CoV sequences (XX new sequences, including XX new SARSr-CoV sequences) from 31 bat species (15 genera, four families) (Table S1). Our geographic datasets included 694 α-CoV sequences from six zoogeographic regions (22 provinces) and 519 β-CoV sequences from five zoogeographic regions (21 provinces) (Fig 1). As some regions or hosts were overrepresented in our datasets, we also created and ran our analyses using a more uniform subset of our sequence data that included ~30 randomly-selected sequences per host family or region to mitigate sampling and surveillance intensity bias.

Ancestral hosts and cross-species transmission

We used a Bayesian discrete phylogeographic approach implemented in BEAST[43] to reconstruct the ancestral host of each node in the phylogenetic tree using bat host family as a discrete character state. The phylogenetic reconstructions for α-CoVs in China suggest an evolutionary origin within rhinolophid

and vespertilionid bats (Fig 2A). The first α-CoV lineage to diverge historically corresponds to the subgenus *Rhinacovirus* (L1), originating within rhinolophid bats, and includes sequences related to HKU2-CoV and SADS-CoV (Fig S2). Then several lineages, labelled L2 to L7, emerged from vespertilionid bats (Fig 2A). The subgenus *Decacovirus* (L2) includes sequences mostly associated with the Rhinolophidae and Hipposideridae and related to HKU10-CoV (Fig S3), while the subgenera *Myotacovirus* (L3) and *Pedacovirus* (L5) as well as an unidentified lineage (L4) include CoVs mainly from vespertilionid bats and related to HKU6-, HKU10-, and 512-CoVs (Fig S4-S5). Finally, a well-supported node comprises the subgenera *Nyctacovirus* (L6) from vespertilionid bats and *Minunacovirus* (L7) from miniopterid bats, and includes HKU7-, HKU8-, 1A-, and 1B-CoVs (Fig S6). These seven α-CoV lineages are mostly associated with a single host family but each also included several sequences identified from other bat families (Fig 2A, S2-S6 and Table S1), suggesting frequent cross-species transmission events have occurred among bats. Ancestral host reconstructions based on the random data subset, to normalize sampling effort, gave very similar results with rhinolophids and vespertilionids being the most likely ancestral hosts of most α-CoV lineages too (Fig S7A). However, the topology of the tree based on the random subset was slightly different as the lineage L5 was paraphyletic.

Chinese β-CoVs likely originated from vespertilionid and rhinolophid bats (Fig 2B). The MCC tree was clearly structured into four main lineages: *Merbecovirus* (Lineage C), including MERS-related (MERSr-) CoVs, HKU4- and HKU5-CoVs and strictly restricted to vespertilionid bats (Fig S8); *Nobecovirus* (lineage D), originating from pteropodid bats and corresponding to HKU9-CoV (Fig S9); *Hibecovirus* (lineage E) comprising sequences isolated in hipposiderid bats (Fig S10) and *Sarbecovirus* (Lineage B) including sequences related to HKU3- and SARS-related (SARSr-) CoVs originating in rhinolophid bats (Fig S11). We show that SARS-CoV-2 ~~2019-nCoV~~ forms a divergent clade within *Sarbecovirus* and is most closely related to viruses sampled from *Rhinolophus affinis* (Fig 3). Similar tree topology and ancestral host inference were obtained with the random subset (Fig S7B).

We used a Bayesian Stochastic Search Variable Selection (BSSVS) procedure[44] to identify viral host switches (transmission over evolutionary time) between bat families and genera that occurred along the branches of the MCC annotated tree and calculated Bayesian Factor (BF) to estimate the significance of these switches (Fig 4). We identified nine highly supported (BF > 10) inter-family host switches for α-CoVs and three for β-CoVs (Fig 4A and 4B). These results are robust over a range of sample sizes, with seven of these nine switches for α-CoVs and the exact same three host switches for β-CoVs having strong BF support (BF > 10) when analyzing our random subset (Tables S2 and S3). To quantify the magnitude of these host switches, we estimated the number of host switching events (Markov jumps)[45,46] along the significant inter-family switches (Fig 4C and 4D) and estimated the rate of inter-family host switching events per unit of time for each CoV genus. The rate of inter-family host switching events was ~~more than 10~~five times higher in the evolutionary history of α- (~~90/0.703=12.8~~0.010) than β-CoVs (~~11/0.926=1.2~~0.002) in China. For α-CoVs, host switching events from the Rhinolophidae and the Miniopteridae were greater than from other bat families while rhinolophids were the highest donor family for β-CoVs. The Rhinolophidae and the Vespertilionidae for α-CoVs and the Hipposideridae for β-CoVs received the highest numbers of switching events (Fig 4C and 4D). When using the random dataset, similar results were obtained for β-CoVs while rhinolophids were only the highest donor family for α-CoVs (Tables S4 and S5).

At the genus level, we identified 20 highly supported inter-genus host switches for α-CoVs, 17 of them were also highly significant using the random subset (Fig 5A and Table S6). *Rhinolophus* and *Myotis* were the donor genera in four of these switches while *Miniopterus* and *Rhinolophus* were each the recipients of four of these switches (Fig 5A). Sixteen highly supported inter-genus switches were identified for β-CoVs (Fig 5B). Similar results were obtained for the random β-CoV subset (Table S7). *Cynopterus* was the most common donor and *Myotis* the most common recipient of these switches (Fig 5B). Most of the significant cross-genus CoV switches for α-CoVs, 15 of 20 (75%), were between genera in different bat

families, while this proportion was only 6 of 16 (37.5%) for β-CoVs. The estimated rate of inter-genus host switching events (Markov jumps) was ~~more than two times higher~~similar for α- (~~123/0.703=175~~0.014) and~~than~~ β-CoVs (~~70/0.926=76~~0.014). For α-CoVs, *Rhinolophus* and *Miniopterus* were the greatest donor genera and *Rhinolophus* was the greatest receiver (Table S8). For β-CoVs, *Rousettus* was the greatest donor and *Eonycteris* the greatest receiver genus (Table S9).

CoV spatiotemporal dispersal in China

We used our Bayesian discrete phylogeographic model with zoogeographic regions as character states to reconstruct the spatiotemporal dynamics of CoV dispersal in China. Eleven and seven highly significant (BF > 10) dispersal routes within China were identified for α- and β-CoVs, respectively (Fig 6). Seven and five of these dispersal routes, respectively, remained significant when using our random subsets (Tables S10 and S11). The *Rhinacovirus* lineage (L1) that includes HKU2 and SADS-CoV likely originated in the SO region while all other α-CoV lineages historically arose in SW China and spread to other regions before several dispersal events from SO and NO in all directions (Fig 6A and Fig S12). A roughly similar pattern of α-CoV dispersal was obtained using the random subset (Tables S10 and S12).

The oldest inferred dispersal movements for β-CoVs occurred among the SO and SW regions (Fig 6B). The SO region was the likely origin of *Merbecovirus* (Lineage C, including HKU4 and HKU5) and *Sarbecovirus* subgenera (Lineage B, including HKU 3 and SARSr-CoVs) while the *Nobecovirus* (lineage D, including HKU9) and *Hibecovirus* (lineage E) subgenera originated in SW China (Fig S12). Then several dispersal movements likely originated from SO and CE (Fig 6B). More recent southward dispersal from NO was observed. Similar spatiotemporal dispersal patterns were observed using the random subset of β-CoVs (Tables S11 and S13).

The estimated rate of migration events per unit of time along these significant dispersal routes was ~~five~~ more than two times higher for α- (~~227/0.703=322.9~~0.026) than β-CoVs (~~57/0.926=61.6~~0.011) and SO

was the region involved in the greatest total number of migration events for both α- and β-CoVs. SO had the highest number of outbound and inbound migration events for α-CoVs (Fig 6C and Table S12). For β-CoVs, the highest number of outbound migration events was estimated to be from NO and SO while SO and SW had the highest numbers of inbound migration events (Fig 6D and Table S13).

Phylogenetic diversity

In order to identify the hotspots of CoV phylogenetic diversity in China and evaluate phylogenetic clustering of CoVs, we calculated the Mean Phylogenetic Distance (MPD) and the Mean Nearest Taxon Distance (MNTD) statistics[47] and their standardized effect size (SES).

We found significant and negative SES MPD values, indicating significant phylogenetic clustering, within all bat families and genera for both α- and β-CoVs, except within the *Aselliscus* and *Tylonycteris* for α-CoVs (Fig 7A and 7B). Negative and mostly significant SES MNTD values, reflecting phylogenetic structure closer to the tips, were also observed within most bat families and genera for α- and β-CoVs but we found non-significant positive SES MNTD value for vespertilionid bats and *Pipistrellus* for β-CoVs (Fig 7A and 7B). In general, we observed lower phylogenetic diversity for β- than α-CoVs within all bat families and most genera when looking at SES MPD, but the difference in the level of diversity between α- and β-CoVs is less important when looking at SES MNTD (Fig 7). These results suggest stronger basal clustering (reflected by larger SES MPD values) for β-CoVs than α-CoVs, indicating stronger host structuring effect and phylogenetic conservatism for β-CoVs. Very similar results were obtained with the random subsets for both α- and β-CoVs (Tables S14-S21).

We found negative and mostly significant values of MPD and MNTD (Fig 7C and Tables S22-S25) indicating significant phylogenetic clustering of CoV lineages in bat communities within the same zoogeographic region. However, SES MPD values for α-CoVs in SW were positive (significant for the random subset) indicating a greater evolutionary diversity of CoVs in that region than others (Fig 7 and

Tables S22-S25). We used a linear regression analysis to assess the relationship between CoV phylogenetic diversity and bat species richness in China and determine if bat richness is a significant predictor of ~~bat-CoV~~bat-CoV diversity and evolution. α-CoV phylogenetic diversity (MPD) was not significantly correlated to total bat species richness or sampled bat species richness in zoogeographic regions or provinces (Table S26). Non-significant correlations between bat species richness and β-CoV phylogenetic diversity were also observed at the zoogeographic region level (Table S27). However, a significant correlation was observed between sampled bat species richness and β-CoV phylogenetic diversity at the province level (Table S27). Similar results were obtained when using the random subsets (Table S26 and S27). These findings suggest that bat host diversity is not the main driver of CoV diversity in China and that other ecological or biogeographic factors may influence this diversity. We observed higher CoV diversity than expected in several southern or central provinces (Hainan, Guangxi, Hunan) given their underlying total or sampled bat diversity (Fig S13 and S14).

We also assessed patterns of CoV phylogenetic turnover/differentiation among Chinese zoogeographic regions and bat host families by measuring the inter-region and inter-host values of MPD (equivalent to a measure of phylogenetic β diversity) and their SES. We found positive inter-family SES MPD values, except between Pteropodidae and Hipposideridae for α-CoVs and between Rhinolophidae and Hipposideridae for β-CoVs (Fig 8A and 8B and Tables S28 and S29), suggesting higher phylogenetic differentiation of CoVs among most bat families than among random communities. Our phylo-ordination based on inter-family MPD values indicated that α-CoVs from vespertilionids and miniopterids, and from hipposiderids and pteropodids; as well as β-CoVs from rhinolophids and hipposiderids are phylogenetically closely related (Fig 8A and 8B). We also observed strong phylogenetic turnover between α-CoV strains from rhinolophids and all other bat families, and between β-CoV strains from vespertilionids and all other bat families. Phylo-ordination among bat genera based on inter-genus MPD confirmed these results and indicated that CoV strains from genera belonging to the same bat

family were mostly more closely related to each other than to genera from other families (Fig 8C and 8D and Tables S30 and S31).

We observed high and positive inter-region SES MPD values between SW/HI and all other regions, suggesting that these two regions host higher endemic diversity (Fig 9 and Tables S32 and S31). Negative inter-region SES MPD values suggested that the phylogenetic turnover among other regions was less important than expected among random communities. Our phylo-ordination among zoogeographic regions also reflected the high phylogenetic turnover and deep evolutionary distinctiveness of both α- and β-CoVs from SW and HI regions (Fig 9 and Tables S32 and S33). Similar results were obtained using the random subset (Tables S32 and S33).

Mantel tests

Mantel tests revealed a positive and significant correlation between CoV genetic differentiation (F_{ST}) and geographic distance matrices, both with and without provinces including fewer than four viral sequences, for α- (r = 0.25, p = 0.0097; r = 0.32, p = 0.0196; respectively) and β-CoVs (r = 0.22, p = 0.0095; r = 0.23, p = 0.0336; respectively). We also detected a positive and highly significant correlation between CoV genetic differentiation (F_{ST}) and their host phylogenetic distance matrices, both with and without genera including fewer than four viral sequences, for β-CoVs (r = 0.41, p = 0; r = 0.39, p = 0.0012; respectively) but not for α-CoVs (r = -0.13, p = 0.8413; r = 0.02, p = 0.5019; respectively).

Discussion

Our dataset and analyses represent the most comprehensive investigation of bat-origin CoVs in China to date. Our phylogenetic analysis shows a high diversity of CoVs from bats sampled in China, with most bat genera included in this study (10/16) infected by both α- and β-CoVs. In the most comprehensive phylogenetic analysis published to date, that includes all known ~~bat-CoV~~bat-CoVbat-CoVs from China, we find that the emerging SARS-CoV-2 ~~2019-nCoV~~ is likely derived from a clade of viruses originating in

horseshoe bats (*Rhinolophus* spp.) from Yunnan province. This analysis also demonstrates that a significant amount of cross-species transmission has occurred among bat hosts over evolutionary time. Our Bayesian phylogeographic inference and Bayesian analysis of host switching showed varying levels of viral connectivity among bat hosts and allowed us to identify significant host transitions that appear to have occurred during ~~bat CoV~~bat-CoV evolution in China.

We found that bats in the family Rhinolophidae (horseshoe bats) played a key role in the evolution and cross-species transmission history of α-CoVs. The family Rhinolophidae and the genus *Rhinolophus* were involved in more inter-family and inter-genus highly significant host switching of α-CoVs than any other family or genus. They were the greatest receivers of α-CoV host switching events and second greatest donors after Miniopteridae/*Miniopterus*. The Rhinolophidae, together with the Hipposideridae, also played an important role in the evolution of β-CoVs, being at the origin of most inter-family host switching events. Chinese horseshoe bats are characterized by a distinct and evolutionary divergent α-CoV diversity, while their β-CoV diversity is similar to that found in the Hipposideridae. The Rhinolophidae comprises a single genus, *Rhinolophus*, and is the most speciose bat family after the Vespertilionidae in China[48], with 20 known species, just under a third of global *Rhinolophus* diversity, mostly in Southern China[32]. This family likely originated in Asia[49,50], but some studies suggest an African origin[51,52]. Rhinolophid fossils from the middle Eocene (38 - 47.8 Mya) have been found in China, suggesting a westward dispersal of the group from eastern Asia to Europe[53]. The ancient likely origin of the Rhinolophidae in Asia and China in particular may explain the central role they played in the evolution and diversification of ~~bat CoV~~bat-CoVs in this region, including SARS-CoV-2~~2019-nCoV~~, SARSr-CoVs and SADSr-CoVs, which are important human and livestock pathogens. Horseshoe bats are known to share roosts with genera from all other bat families in this study[54], which may also favor CoV cross-species transmission from and to rhinolophids[31]. A global meta-analysis showing higher rates of viral sharing among co-roosting cave bats supports this finding[55].

Vespertilionid and miniopterid bats (largely within the *Myotis* and *Miniopterus* genera) also appear to have been involved in several significant host switches during α-CoV evolution. However, no significant transition from vespertilionid bats was identified for β-CoVs and these bats exhibit a divergent β-CoV diversity compared to other bat families. Vespertilionid and miniopterid bats are characterized by strong basal phylogenetic clustering but high recent CoV diversification rates, indicating a more rapid evolutionary radiation of CoVs in these bat hosts. At the genus level, similar findings were observed for the genera *Myotis*, *Pipistrellus* and *Miniopterus*.

A significant correlation between geographic distance and genetic differentiation of both α- and β-CoVs has been detected, even if only a relatively small proportion of the variance is explained by geographic distance. We also revealed a significant effect of host phylogeny on β-CoV evolution while it had a minimal effect on α-CoV diversity. Contrary to the α-CoV phylogeny, the basal phylogenetic structure of β-CoVs mirrored the phylogeny of their bat hosts, with a clear distinction between the Yangochiroptera, encompassing the Vespertilionidae and Miniopteridae, and the Yinpterochiroptera, which includes the megabat family Pteropodidae and the microbat families Rhinolophidae and Hipposideridae, as evidenced in recent bat phylogenies[49,56]. These findings suggest a profound co-macroevolutionary process between β-CoVs and their bat hosts, even if host switches also occurred throughout their evolution as our study showed. The phylogenetic structure of α-CoVs, with numerous and closely related lineages identified in the Vespertilionidae and Miniopteridae, contrasts with the β-CoV macroevolutionary pattern and suggests α-CoVs have undergone an adaptive radiation in these two Yangochiroptera families. Our BSSVS procedure and Markov jump estimates revealed higher connectivity, both qualitatively and quantitatively, among bat families and genera in the α-CoV cross-species transmission history. Larger numbers of highly significant host transitions and higher rates of switching events along these pathways were inferred for α- than β-CoVs, especially at the host family level. These findings suggest that α-CoVs are able to switch hosts more frequently and between more

distantly related taxa, and that phylogenetic distance among hosts represents a higher constraint on host switches for β- than α-CoVs. This is supported by more frequent dispersal events in the evolution of α- than β-CoVs in China.

Variation in the extent of host jumps between α and β-CoVs within the same hosts in the same environment may be due to virus-specific factors such as differences in receptor usage between α- and β-CoVs[57-59]. Coronaviruses use a large diversity of receptors, and their entry into host cells is mediated by the spike protein with an ectodomain consisting of a receptor-binding subunit S1 and a membrane-fusion subunit S2[60]. However, despite differences in the core structure of their S1 receptor binding domains (RBD), several α- and β-CoV species are able to recognize and bind to the same host receptors[61]. Other factors such as mutation rate, recombination potential, or replication rate might also be involved in differences in host switching potential between α- and β-CoVs. A better understanding of receptor usage and other biological characteristics of these ~~bat-CoV~~bat-CoVs may help predict their cross-species transmission and zoonotic potential.

We also found that some bat genera were infected by a single CoV genus: *Miniopterus* (Miniopteridae) and *Murina* (Vespertilionidae) carried only α-CoVs, while *Cynopterus*, *Eonycteris*, *Megaerops* (Pteropodidae) and *Pipistrellus* (Vespertilionidae) hosted only β-CoVs. This was found despite using the same conserved pan-CoV PCR assays for all specimens screened and it can't be explained by differences in sampling effort for these genera (Table S1): for example, >250 α-CoV sequences but no β-CoV were discovered in *Miniopterus* bats in China during our recent fieldwork. These migratory bats, which seem to have played a key role in the evolution of α-CoVs, share roosts with several other bat genera hosting β-CoVs in China[54], suggesting high likelihood of being exposed to β-CoVs. Biological or ecological properties of miniopterid bats may explain this observation and clearly warrant further investigation.

Our Bayesian ancestral reconstructions revealed the importance of South western and Southern China as centers of diversification for both α- and β-CoVs. These two regions are hotspots of CoV phylogenetic diversity, harboring evolutionarily old and phylogenetically diverse lineages of α- and β-CoVs. South western China acted as a refugium during Quaternary glaciation for numerous plant and animal species including several bat species, such as *Rhinolophus affinis*[62], *Rhinolophus sinicus*[63], *Myotis davidii*[64], and *Cynopterus sphinx*[65]. The stable and long-term persistence of bats and other mammals throughout the Quaternary may explain the deep macroevolutionary diversity of ~~bat-CoV~~bat-CoVs in these regions[66]. Several highly significant and ancient CoV dispersal routes from these two regions have been identified in this study. Other viruses, such as the Avian Influenza A viruses H5N6, H7N9 and H5N1, also likely originated in South western and Southern Chinese regions[67,68].

Our findings suggest that bat host diversity is not the main driver of CoV diversity in China and that other ecological or biogeographic factors may influence this diversity. Overall, there were no significant correlations between CoV phylogenetic diversity and bat species diversity (total or sampled) for each province or biogeographic region, apart from a weak correlation between β-CoV phylogenetic diversity and the number of bat species sampled at the province level. Yet, we observed higher than expected phylogenetic diversity in several southern provinces (Hainan, Guangxi, Hunan). These results and main conclusions are consistent and robust even when we account for geographic biases in sampling effort by analyzing random subsets of the data.

Despite being the most exhaustive study of ~~bat-CoV~~bat-CoVs in China, this study had several limitations that must be taken into consideration when interpreting our results. First, only partial RdRp sequences were generated in this study and used in our phylogenetic analysis as the non-invasive samples (rectal swabs/feces) collected in this study prevented us from generating longer sequences in many cases. The RdRp gene is a suitable marker for this kind of study as it reflects vertical ancestry and is less prone to recombination than other regions of the CoV genome such as the spike protein gene[16,69]. While using

long sequences is always preferable, our phylogenetic trees are well supported and their topology consistent with trees obtained using longer sequences or whole genomes[27,70]. Second, most sequences in this study were obtained by consensus PCR using primers targeting highly conserved regions. Even if this broadly reactive PCR assay designed to detect widely variant CoVs has proven its ability to detect a large diversity of CoVs in a wide diversity of bats and mammals[29,71-74], we may not rule out that some ~~bat CoV~~bat-CoV variants remained undetected. Using deep sequencing techniques would allow to detect this unknown and highly divergent diversity.

In this study, we identified the host taxa and geographic regions that together define hotspots of CoV phylogenetic diversity and centers of diversification in China. These findings may provide a strategy for targeted discovery of bat-borne CoVs of zoonotic or livestock infection potential, and for early detection of ~~bat CoV~~bat-CoV outbreaks in livestock and people, as proposed elsewhere[75]. Our results suggest that future sampling and viral discovery should target two hotspots of CoV diversification in Southern and South western China in particular. These regions are characterized by a subtropical to tropical climate; dense, growing and rapidly urbanizing populations of people; a high degree of poultry and livestock production; and high rates of consumption of wildlife, including bats – all factors which may promote cross-species transmission and disease emergence[75-77]. Additionally, faster rates of evolution in the tropics have been described for other RNA viruses which could favor cross-species transmission of RNA viruses in these regions[78]. Both SARS-CoV and SADS-CoV emerged in this region, and several bat SARSr-CoVs with high zoonotic potential have recently been reported from there, although the dynamics of their circulation in wild bat populations remain poorly understood[16,58]. Importantly, the closest known relative of SARS-CoV-2~~2019-nCoV~~, a SARS-related virus, was found in a *Rhinolophus* sp. bat in this region. The significant public health and food security implications of these outbreaks reinforces the need for enhanced, targeted sampling and discovery of novel CoVs. Our finding that *Rhinolophus* spp. are most likely to be involved in host-switching events makes them a key target for future longitudinal

surveillance programs, but surveillance targeted the genera *Hipposideros* and *Aselliscus* may also be fruitful as they share numerous β-CoVs with *Rhinolophus* bats.

In the aftermath of the SARS-CoV and MERS-CoV outbreaks, β-CoVs have been the main focus of ~~bat CoV~~bat-CoV studies in China, Africa, and Europe[17,29,33,58,79]. However, we have shown that α-CoVs have a higher propensity to switch host within their natural bat reservoirs, and therefore also have a high cross-species transmission potential and risk of spillover. This is exemplified by the recent emergence of SADS-CoV in pigs in Guangdong province[17]. Two human α-CoVs, NL63 and 229E, also likely originated in bats[24,25], reminding us that past spillover events from bat species can readily be established in the human population. Future work discovering and characterizing the biological properties of bat α-CoVs may therefore be of potential value for public and livestock health. Our study, and recent analysis of viral discovery rates[80], suggest that a substantially wider sampling and discovery net will be required to capture the complete diversity of coronaviruses in their natural hosts and assess their potential for cross-species transmission. The bat genera *Rhinolophus*, *Hipposideros*, *Myotis* and *Miniopterus*, all involved in numerous naturally-occurring host switches throughout α-CoV evolution, should be a particular target for α-CoV discovery in China, with *in vitro* and experimental characterization to better understand their potential to infect people or livestock and cause disease.

Material and Methods

Bat sampling

Bat oral and rectal swabs and fecal pellets were collected from 2010 to 2015 in numerous Chinese provinces (Anhui, Beijing, Guangdong, Guangxi, Guizhou, Hainan, Henan, Hubei, Hunan, Jiangxi, Macau, Shanxi, Sichuan, Yunnan, and Zhejiang). Fecal pellets were collected from tarps placed below bat colonies. Bats were captured using mist nets at their roost site or feeding areas. Each captured bat was stored into a cotton bag, all sampling was non-lethal and bats were released at the site of capture

immediately after sample collection. A wing punch was also collected for barcoding purpose. Bat-handling methods were approved by Tufts University IACUC committee (proposal #G2017-32) and Wuhan Institute of Virology Chinese Academy of Sciences IACUC committee (proposal WIVA05201705). Samples were stored in viral transport medium at -80°C directly after collection.

RNA extraction and PCR screening

RNA was extracted from 200 µl swab rectal samples or fecal pellets with the High Pure Viral RNA Kit (Roche) following the manufacturer's instructions. RNA was eluted in 50 µl elution buffer and stored at -80°C. A one-step hemi-nested RT-PCR (Invitrogen) was used to detect coronavirus RNA using a set of primers targeting a 440-nt fragment of the RdRp gene and optimized for ~~bat-CoV~~bat-CoV detection (CoV-FWD3: GGTTGGGAYTAYCCHAARTGTGA; CoV-RVS3: CCATCATCASWYRAATCATCATA; CoV-FWD4/Bat: GAYTAYCCHAARTGTGAYAGAGC)[81]. For the first round PCR, the amplification was performed as follows: 50°C for 30 min, 94°C for 2 min, followed by 40 cycles consisting of 94°C for 20 sec, 50°C for 30 sec, 68°C for 30 sec, and a final extension step at 68°C for 5 min. For the second round PCR, the amplification was performed as follows: 94°C for 2 min followed by 40 cycles consisting of 94°C for 20 sec, 59°C for 30 sec, 72°C for 30 sec, and a final extension step at 72°C for 7 min. PCR products were gel purified and sequenced with an ABI Prism 3730 DNA analyzer (Applied Biosystems, USA). PCR products with low concentration or bad sequencing quality were cloned into pGEM-T Easy Vector (Promega) for sequencing. Positive results detected in bat genera that were not known to harbor a specific CoV lineage previously were repeated a second time (PCR + sequencing) as a confirmation. Species identifications from the field were also confirmed and re-confirmed by cytochrome (cytb) DNA barcoding using DNA extracted from the feces or swabs[82]. Only viral detection and barcoding results confirmed at least twice were included in this study.

Sequence data

We also added ~~bat-CoV~~bat-CoV RdRp sequences from China available in GenBank to our dataset. All sequences for which sampling year and host or sampling location information was available either in GenBank metadata or in the original publication were included (as of March 15, 2018). Our final datasets include 732 sequences generated for this study and 508 sequences from GenBank (list of GenBank accession numbers available in Supplementary Material, Tables S34 and S35). Nucleotide sequences were aligned using MUSCLE and trimmed to 360 base pair length to reduce the proportion of missing data in the alignments. All phylogenetic analyses were performed on both the complete data and random subset, and for α- and β-CoVs separately.

Defining zoogeographic regions in China for phylogeographic analyses

Hierachical clustering was used to define zoogeographic regions within China by clustering provinces with similar mammalian diversity[42]. Hierarchical cluster analysis classifies several objects into small groups based on similarities between them. To do this, we created a presence/absence matrix of all extant terrestrial mammals present in China using data from the IUCN spatial database[83] and generated a cluster dendrogram using the function *hclust* with average method of the R package stats. Hong Kong and Macau were included within the neighboring Guangdong province. We then visually identified geographically contiguous clusters of provinces for which CoV sequences are available (Fig 1 and Fig S1).

We identified six zoogeographic regions within China based on the similarity of the mammal community in these provinces: South western region (SW; Yunnan province), Northern region (NO; Xizang, Gansu, Jilin, Anhui, Henan, Shandong, Shaanxi, Hebei and Shanxi provinces and Beijing municipality), Central northern region (CN; Sichuan and Hubei provinces), Central region (CE; Guangxi, Guizhou, Hunan, Jiangxi and Zhejiang provinces), Southern region (SO; Guangdong and Fujian provinces, Hong Kong, Macau and Taiwan), and Hainan island (HI). Hunan and Jiangxi, clustering with the SO provinces in our dendrogram, were included within the central region to create a geographically contiguous Central cluster (Fig S1).

These six zoogeographic regions are very similar to the biogeographic regions traditionally recognized in China[84]. The three β-CoV sequences from HI were included in the SO region to avoid creating a cluster with a very small number of sequences.

Model selection and phylogenetic analysis

Bayesian phylogenetic analysis were performed in BEAST 1.8.4[43]. Sampling years were used as tip dates. Preliminary analysis were run to select the best fitting combination of substitution models (HKY/GTR), codon partition scheme, molecular clock (strict/lognormal uncorrelated relaxed clock) and coalescent models (constant population size/exponential growth/GMRF Bayesian Skyride). Model combinations were compared and the best fitting model was selected using a modified Akaike information criterion (AICM) implemented in Tracer 1.6[85]. We also used TEMPEST[86] to assess the temporal structure within our α- and β-CoV datasets. TEMPEST showed that both datasets did not contain sufficient temporal information to accurately estimate substitution rates or time to the most recent common ancestor (TMRCA). Therefore we used a fixed substitution rate of 1.0 for all our BEAST analysis.

All subsequent BEAST analysis were performed under the best fitting model including a HKY substitution model with two codons partitions ((1+2), 3), a strict molecular clock and a constant population size coalescent model. Each analysis was run for 2.5 x 10^8 generations, with sampling every 2 x 10^4 steps. All BEAST computations were performed on the CIPRES Science Getaway Portal[87]. Convergence of the chain was assessed in Tracer so that the effective sample size (ESS) of all parameters was > 200 after removing at least 10% of the chain as burn-in.

Ancestral state reconstruction and transition rates

A Bayesian discrete phylogeographic approach implemented in BEAST 1.8.4 was used to reconstruct the ancestral state of each node in the phylogenetic tree for three discrete traits: host family, host genus and zoogeographic region. An asymmetric trait substitution model was applied. These analyses were

performed for each trait on the complete dataset and random subsets. Maximum clade credibility (MCC) tree annotated with discrete traits were generated in TreeAnnotator and visualized using the software SpreaD3[88].

For each analysis, a Bayesian stochastic search variable selection (BSSVS) was applied to estimate the significance of pairwise switches between trait states using Bayesian Factor (BF) as a measure of statistical significance[44]. BF were computed in SpreaD3. BF support was interpreted according to Jeffreys 1961[89] (BF > 3: substantial support, BF > 10: strong support, BF > 30: very strong support, BF > 100: decisive support) and only strongly supported transitions were presented in most figures, following a strategy used in other studies[90,91]. We also estimated the count of state switching events (Markov jumps)[45,46] along the branches of the phylogenetic tree globally (for the three discrete traits) and for each strongly supported (BF > 10) transition between character states (for bat families and ecoregions only). Convergence of the MCMC runs was confirmed using Tracer. The rate of state switching events per unit of time was estimated for each CoV genus by dividing the total estimated number of state switching events by the total ~~height~~ branch length of the MCC tree.

To assess the phylogenetic relationships among SARS-CoV-2 and other CoVs from the *Sarbecovirus* subgenus, we also reconstructed a MCC tree in BEAST 1.8.4 and median-joining network in Network[92] including all *Sarbecovirus* sequences and two sequences of SARS-CoV-2 isolated in humans (GenBank accession numbers: MN908947 and MN975262) and one sequence of SARS-CoV (GenBank accession number: NC_004718).

Phylogenetic diversity

The Mean Phylogenetic Distance (MPD) and the Mean Nearest Taxon Distance (MNTD) statistics[47] and their standardized effect size (SES) were calculated for each zoogeographic region, bat family and genus using the R package ~~picante[92]~~picante[93]. MPD measures the mean phylogenetic distance among all pairs

of CoVs within a host or a region. It reflects phylogenetic structuring across the whole phylogenetic tree and assesses the overall divergence of CoV lineages in a community. MNTD is the mean distance between each CoV and its nearest phylogenetic neighbor in a host or region, and therefore it reflects the phylogenetic structuring closer to the tips and shows how locally clustered taxa are. SES MPD and SES MNTD values correspond to the difference between the phylogenetic distances in the observed communities versus null communities. Low and negative SES values denote phylogenetic clustering, high and positive values indicate phylogenetic over-dispersion while values close to 0 show random dispersion. The SES values were calculated by building null communities by randomly reshuffling tip labels 1000 times along the entire phylogeny. Phylogenetic diversity computations were performed on both the complete dataset and random subset for each trait. A linear regression analysis was performed in R to assess the correlation between CoV phylogenetic diversity (MPD) and bat species richness in China. Total species richness per province or region was estimated using data from the IUCN spatial database while sampled species richness corresponds to the number of bat species sampled and tested for CoV per province or region in our datasets.

The inter-region and inter-host values of MPD (equivalent to phylogenetic β diversity), corresponding to the mean phylogenetic distance among all pairs of CoVs from two distinct hosts or regions, and their SES were estimated using the function *comdist* of the R package ~~phylocomr[93]~~phylocomr[94]. The matrices of inter-region and inter-host MPD were used to cluster zoogeographic regions and bat hosts in a dendrogram according to their evolutionary similarity (phylo-ordination) using the function *hclust* with complete linkage method of the R package stats (R core team). These computations were performed on both the complete dataset and random subset.

Mantel tests and isolation by distance

Mantel tests performed in ARLEQUIN 3.~~5~~[94]-5[95] were used to compare the matrix of viral genetic differentiation (F_{ST}) to matrices of host phylogenetic distance and geographic distance in order to evaluate the role of geographic isolation and host phylogeny in shaping CoV population structure. The correlation between these matrices was assessed using 10,000 permutations. To gain more resolution into the process of evolutionary diversification, these analyses were also performed at the host genus and province levels. To calculate phylogenetic distances among bat genera, we reconstructed a phylogenetic tree including a single sequence for all bat species included in our dataset. Pairwise patristic distances among tips were computed using the function *distTips* in the R package ~~adephylo~~[95]adephylo[96]. We then averaged all distances across genera to create a matrix of pairwise distances among bat genera. Pairwise Euclidian distances were measured between province centroids and log transformed. Mantel tests were performed with and without genera and provinces including less than four viral sequences to assess the impact of low sample size on our results.

References

1. Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular Evolution of Human Coronavirus Genomes. *Trends in Microbiology* **25**, 35-48 (2017).
2. Tao, Y. *et al.* Surveillance of Bat Coronaviruses in Kenya Identifies Relatives of Human Coronaviruses NL63 and 229E and Their Recombination History. *Journal of Virology* **91**(2017).
3. Graham, R.L. & Baric, R.S. Recombination, Reservoirs, and the Modular Spike: Mechanisms of Coronavirus Cross-Species Transmission. **84**, 3134-3146 (2010).
4. Vijgen, L. *et al.* Evolutionary history of the closely related group 2 coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus OC43. *Journal of virology* **80**, 7270-7274 (2006).

5. Zhang, X. *et al.* Quasispecies of bovine enteric and respiratory coronaviruses based on complete genome sequences and genetic changes after tissue culture adaptation. *Virology* **363**, 1-10 (2007).

6. Parrish, C.R. *et al.* Cross-Species Virus Transmission and the Emergence of New Epidemic Diseases. *Microbiology and Molecular Biology Reviews* **72**, 457-470 (2008).

7. Li, D.L. *et al.* Molecular evolution of porcine epidemic diarrhea virus and porcine deltacoronavirus strains in Central China. *Research in Veterinary Science* **120**, 63-69 (2018).

8. Cui, J., Li, F. & Shi, Z.-L. Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology* **17**, 181-192 (2019).~~Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular Evolution of Human Coronavirus Genomes. *Trends Microbiol* **25**, 35-48 (2017).~~

9. Lau, S.K.P. & Chan, J.F.W. Coronaviruses: emerging and re-emerging pathogens in humans and animals. *Virology Journal* **12**, 209 (2015).

10. Drosten, C. *et al.* Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* **348**, 1967-76 (2003).

11. Heymann, D.L. The international response to the outbreak of SARS in 2003. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 1127-1129 (2004).

12. World Health Organization. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. Vol. 2019 (World Health Organization, 2004).

13. Ge, X.-Y. *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).

14. Li, W. *et al.* Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**, 676-9 (2005).

15. Lau, S.K.P. *et al.* Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 14040-14045 (2005).

16. Hu, B. *et al.* Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathogens* **13**, e1006698 (2017).

17. Zhou, P. *et al.* Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* **556**, 255-258 (2018).

18. Gong, L. *et al.* A New Bat-HKU2-like Coronavirus in Swine, China, 2017. *Emerging infectious diseases* **23**, 1607-1609 (2017).

19. Pan, Y. *et al.* Discovery of a novel swine enteric alphacoronavirus (SeACoV) in southern China. *Veterinary Microbiology* **211**, 15-21 (2017).

20. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, ~~in press~~**579**, 270-273 (2020).

21. Corman, V.M. *et al.* Rooting the Phylogenetic Tree of Middle East Respiratory Syndrome Coronavirus by Characterization of a Conspecific Virus from an African Bat. *Journal of Virology* **88**, 11297-11303 (2014).

22. Anthony, S.J. *et al.* Further Evidence for Bats as the Evolutionary Source of Middle East Respiratory Syndrome Coronavirus. *mBio* **8**, e00373-17 (2017).

23. Lau, S.K.P. *et al.* Receptor Usage of a Novel Bat Lineage C Betacoronavirus Reveals Evolution of Middle East Respiratory Syndrome-Related Coronavirus Spike Proteins for Human Dipeptidyl Peptidase 4 Binding. *The Journal of Infectious Diseases*, jiy018-jiy018 (2018).

24. Corman, V.M. *et al.* Evidence for an Ancestral Association of Human Coronavirus 229E with Bats. *Journal of Virology* **89**, 11858-11870 (2015).

25. Huynh, J. *et al.* Evidence Supporting a Zoonotic Origin of Human Coronavirus Strain NL63. *Journal of Virology* **86**, 12816-12825 (2012).

26. Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., *et al.* Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*~~,~~ **395**, 565-574 ~~in press~~ (2020).

27. Wong, A.C.P., Li, X., Lau, S.K.P. & Woo, P.C.Y. Global Epidemiology of Bat Coronaviruses. *Viruses* **11**, 174 (2019).

28. Drexler, J.F., Corman, V.M. & Drosten, C. Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Research* **101**, 45-56 (2014).

29. Anthony, S.J. *et al.* Global patterns in coronavirus diversity. *Virus Evolution* **3**, vex012-vex012 (2017).

30. Leopardi, S. *et al.* Interplay between co-divergence and cross-species transmission in the evolutionary history of bat coronaviruses. *Infection, Genetics and Evolution* **58**, 279-289 (2018).

31. Cui, J. *et al.* Evolutionary relationships between bat coronaviruses and their hosts. *Emerging Infectious Diseases* **13**, 1526-1532 (2007).

32. Smith, A.T. & Xie, Y. *A Guide to the Mammals of China*, (Princeton University Press, Princeton, USA, 2008).

33. Lin, X.-D. *et al.* Extensive diversity of coronaviruses in bats from China. *Virology* **507**, 1-10 (2017).

34. Ge, X.-Y. *et al.* Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* **31**, 31-40 (2016).

35. Woo, P.C.Y. *et al.* Molecular diversity of coronaviruses in bats. *Virology* **351**, 180-187 (2006).

36. Wu, Z. *et al.* Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. *The Isme Journal* **10**, 609-620 (2016).

37. Tang, X.C. *et al.* Prevalence and Genetic Diversity of Coronaviruses in Bats from China. *Journal of Virology* **80**, 7481-7490 (2006).

38. Woo, P.C.Y. *et al.* Comparative Analysis of Twelve Genomes of Three Novel Group 2c and Group 2d Coronaviruses Reveals Unique Group and Subgroup Features. *Journal of Virology* **81**, 1574-1585 (2007).

39. Ge, X. *et al.* Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *J Virol* **86**, 4620-4630 (2012).

40. Xu, L. *et al.* Detection and characterization of diverse alpha- and betacoronaviruses from bats in China. *Virologica Sinica* **31**, 69-77 (2016).

41. Luo, Y. *et al.* Longitudinal Surveillance of Betacoronaviruses in Fruit Bats in Yunnan Province, China During 2009–2016. **33**, 87-95 (2018).

42. Legendre, P. & Legendre, L.F. *Numerical ecology*, (Elsevier, 2012).

43. Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969-1973 (2012).

44. Lemey, P., Rambaut, A., Drummond, A.J. & Suchard, M.A. Bayesian Phylogeography Finds Its Roots. *PLoS Computational Biology* **5**, e1000520 (2009).

45. Minin, V.N. & Suchard, M.A. Counting labeled transitions in continuous-time Markov models of evolution. *Journal of Mathematical Biology* **56**, 391-412 (2008).

46. O'Brien, J.D., Minin, V.N. & Suchard, M.A. Learning to Count: Robust Estimates for Labeled Distances between Molecular Sequences. *Molecular Biology and Evolution* **26**, 801-814 (2009).

47. Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M.J. Phylogenies and Community Ecology. **33**, 475-505 (2002).

48. Simmons, N.B. Order Chiroptera. in *Mammal Species of the World: A Taxonomic and Geographic Reference* (eds. Wilson, D.E. & Reeder, D.M.) 312-529 (Johns Hopkins University Press, 2005).

49. Teeling, E.C. *et al.* A Molecular Phylogeny for Bats Illuminates Biogeography and the Fossil Record. **307**, 580-584 (2005).

50. Stoffberg, S., Jacobs, D.S., Mackie, I.J. & Matthee, C.A. Molecular phylogenetics and historical biogeography of *Rhinolophus* bats. *Molecular Phylogenetics and Evolution* **54**, 1-9 (2010).

51. Foley, N.M. *et al.* How and Why Overcome the Impediments to Resolution: Lessons from rhinolophid and hipposiderid Bats. *Molecular Biology and Evolution* **32**, 313-333 (2014).

52. Eick, G.N., Jacobs, D.S. & Matthee, C.A. A Nuclear DNA Phylogenetic Perspective on the Evolution of Echolocation and Historical Biogeography of Extant Bats (Chiroptera). *Molecular Biology and Evolution* **22**, 1869-1886 (2005).

53. Ravel, A., Marivaux, L., Qi, T., Wang, Y.-Q. & Beard, K.C. New chiropterans from the middle Eocene of Shanghuang (Jiangsu Province, Coastal China): new insight into the dawn horseshoe bats (Rhinolophidae) in Asia. **43**, 1-23 (2014).

54. Luo, J. *et al.* Bat conservation in China: should protection of subterranean habitats be a priority? *Oryx* **47**, 526-531 (2013).

55. Willoughby, A.R., Phelps, K.L., Consortium, P. & Olival, K.J. A Comparative Analysis of Viral Richness and Viral Sharing in Cave-Roosting Bats. *Diversity* **9**, 35 (2017).

56. Tsagkogeorga, G., Parker, J., Stupka, E., Cotton, James A. & Rossiter, S.J. Phylogenomic Analyses Elucidate the Evolutionary Relationships of Bats. *Current Biology* **23**, 2262-2267 (2013).

57. Yang, Y. *et al.* Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proceedings of the National Academy of Sciences* **111**, 12516-12521 (2014).

58. Menachery, V.D. *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nature Medicine* ~~**advance online publication**~~**21**, 1508-1513 (2015).

59. Li, W. *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).

60. Li, F. Receptor Recognition Mechanisms of Coronaviruses: a Decade of Structural Studies. **89**, 1954-1964 (2015).

61. Li, F. Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annual Review of Virology* **3**, 237-261 (2016).

62. Mao, X.G., Zhu, G.J., Zhang, S. & Rossiter, S.J. Pleistocene climatic cycling drives intra-specific diversification in the intermediate horseshoe bat (*Rhinolophus affinis*) in Southern China. *Molecular Ecology* **19**, 2754-2769 (2010).

63. Mao, X. *et al.* Multiple cases of asymmetric introgression among horseshoe bats detected by phylogenetic conflicts across loci. *Biological Journal of the Linnean Society* **110**, 346-361 (2013).

64. You, Y. *et al.* Pleistocene glacial cycle effects on the phylogeography of the Chinese endemic bat species, *Myotis davidii*. *BMC Evolutionary Biology* **10**, 208 (2010).

65. Chen, J.P. *et al.* Contrasting Genetic Structure in Two Co-Distributed Species of Old World Fruit Bat. *PLoS ONE* **5** (2010).

66. Krasnov, B.R., Pilosof, S., Shenbrot, G.I. & Khokhlova, I.S. Spatial variation in the phylogenetic structure of flea assemblages across geographic ranges of small mammalian hosts in the Palearctic. *International Journal for Parasitology* **43**, 763-770 (2013).

67. Bi, Y. *et al.* Novel avian influenza A (H5N6) viruses isolated in migratory waterfowl before the first human case reported in China, 2014. *Scientific Reports* **6**, 29888 (2016).

68. Bui, C.M., Adam, D.C., Njoto, E., Scotch, M. & MacIntyre, C.R. Characterising routes of H5N1 and H7N9 spread in China using Bayesian phylogeographical analysis. *Emerging Microbes & Infections* **7**, 184 (2018).

69. Gouilh, M.A., Puechmaille, S.J., Gonzalez, J.-P., Teeling, E., Kittayapong, P. & Manuguerra, J.-C. SARS-Coronavirus ancestor's foot-prints in South-East Asian bat colonies and the refuge theory. *Infection Genetics and Evolution* **11**, 1690-1702 (2011).

70. Hu, B., Ge, X., Wang, L.-F. & Shi, Z. Bat origin of human coronaviruses. *Virology Journal* **12**, 1-10 (2015).

71. Anthony, S.J., Ojeda-Flores, R., Rico-Chávez, O., Navarrete-Macias, I., Zambrana-Torrelio, C.M., Rostal, M.K., Epstein, J.H., Tipps, T., Liang, E., Sanchez-Leon, M., *et al.* Coronaviruses in bats from Mexico. *Journal of General Virology* **94**, 1028-1038 (2013).

72. Corman, V.M., Kallies, R., Philipps, H., Göpner, G., Müller, M.A., Eckerle, I., Brünink, S., Drosten, C. & Drexler, J.F. Characterization of a novel betacoronavirus related to MERS-CoV in European hedgehogs. *Journal of Virology* **88**, 717-724 (2014).

73. Munster, V.J., Adney, D.R., van Doremalen, N., Brown, V.R., Miazgowicz, K.L., Milne-Price, S., Bushmaker, T., Rosenke, R., Scott, D., Hawkinson, A., *et al.* Replication and shedding of MERS-CoV in Jamaican fruit bats (*Artibeus jamaicensis*). *Scientific Reports* **6**, 21878 (2016).

74. Joyjinda, Y., Rodpan, A., Chartpituck, P., Suthum, K., Yaemsakul, S., Cheun-Arom, T., Bunprakob, S., Olival, K.J., Stokes, M.M., Hemachudha, T., *et al.* First Complete Genome Sequence of Human Coronavirus HKU1 from a Nonill Bat Guano Miner in Thailand. *Microbiology Resource Announcements* **8**, e01457-01418 (2019).

75. Carroll, D., Daszak, P., Wolfe, N.D., Gao, G.F., Morel, C.M., Morzaria, S., Pablos-Méndez, A., Tomori, O. & Mazet, J.A.K. The Global Virome Project. *Science* **359**, 872-874 (2018).

76. Fountain-Jones, N.M. *et al.* Towards an eco-phylogenetic framework for infectious disease ecology. **93**, 950-970 (2018).

77. Allen, T. *et al.* Global hotspots and correlates of emerging zoonotic diseases. *Nature Communications* **8**, 1124 (2017).

78. Streicker, D.G., Lemey, P., Velasco-Villa, A. & Rupprecht, C.E. Rates of Viral Evolution Are Linked to Host Geography in Bat Rabies. *PLoS Pathog* **8**, e1002720 (2012).

79. Hu, B. *et al.* Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLOS Pathogens* **13**(2017).

80. Carroll, D. *et al.* The global virome project. *Science* **359**, 872-874 (2018).

81. Watanabe, S. *et al.* Bat Coronaviruses and Experimental Infection of Bats, the Philippines. *Emerging Infectious Diseases* **16**, 1217-1223 (2010).

82. Irwin, D.M., Kocher, T.D. & Wilson, A.C. Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution* **32**, 128-144 (1991).

83. IUCN. The IUCN Red List of Threatened Species. Version 2015.2, http://www.iucnredlist.org. (2018).

84. Xie, Y., MacKinnon, J., Li, D.J.B. & Conservation. Study on biogeographical divisions of China. **13**, 1391-1417 (2004).

85. Baele, G., Li, W.L.S., Drummond, A.J., Suchard, M.A. & Lemey, P. Accurate Model Selection of Relaxed Molecular Clocks in Bayesian Phylogenetics. *Molecular Biology and Evolution* **30**, 239-243 (2013).

86. Rambaut, A., Lam, T.T., Max Carvalho, L. & Pybus, O.G. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evolution* **2**, vew007 (2016).

87. Miller, M.A., Pfeiffer, W. & Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA*, 1-8 (2010).

88. Bielejec, F. *et al.* SpreaD3: Interactive Visualization of Spatiotemporal History and Trait Evolutionary Processes. *Molecular Biology and Evolution* **33**, 2167-2169 (2016).

89. Jeffreys, H. Theory of probability. Oxford: Clarendon. (1961).

90. Faria, N.R., Suchard, M.A., Rambaut, A., Streicker, D.G. & Lemey, P. Simultaneously reconstructing viral cross-species transmission history and identifying the underlying constraints. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**, 20120196 (2013).

91. Kamath, P.L., Foster, J.T., Drees, K.P., Luikart, G., Quance, C., Anderson, N.J., Clarke, P.R., Cole, E.K., Drew, M.L., Edwards, W.H., *et al.* Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. *Nature Communications* **7**, 11448 (2016).

92. Bandelt, H.J., Forster, P., & Rohl, A. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37-48 (1999).

~~92~~93. Kembel, S.W. *et al.* Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463-1464 (2010).

~~93~~94. Ooms, J., Chamberlain, S., Webb, C.O., Ackerly, D.D. & Kembel, S.W. phylocomr: Interface to 'Phylocom'. *R package version 0.1.2* (2018).

~~94~~95. Excoffier, L. & Lischer, H.E.L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567 (2010).

~~95~~96. Jombart, T. & Dray, S. adephylo: exploratory analyses for the phylogenetic comparative method. R package version 1.1-11. (2008).

Data availability

GenBank accession numbers of sequences generated in this study and previously published sequences included in our analysis are available in the Supplementary Material (Tables S34 and S35).

Acknowledgements

This study was funded by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (Award Number R01AI110964) and the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT project (cooperative agreement number GHN-A-OO-09-00010-00), the strategic priority research program of the Chinese Academy of Sciences (XDB290101001), and National Natural Science Foundation of China (31770175, 31830096). Coronavirus research in L-FW's group is funded by grants from Singapore National Research Foundation (NRF2012NRF-CRP001-056 and NRF2016NRF-NSFC002-013).

Author contributions

K.J.O., H.E.F, J.H.E., L-F.W., Z.S. and P.D. created the study design, initiated field work and set up sample collection and testing protocols. B.H., G.Z., L.Z., H.L., A.A.C and Z.L. provided samples or data. B.H., B.L., and W.Z. performed laboratory work. A.L. carried out the analyses and drafted the manuscript with K.J.O, C.Z.-T. and P.D. All authors reviewed and edited the manuscript

Competing interests: The authors declare no competing interests.

Figure legends

Fig. 1 Pie chart (A) showing the number of sequences of each CoV genus (α-CoVs and β-CoVs) available for each zoogeographic region and map of China provinces (B) showing the number of RdRp sequences available for each province, in bold grey for α-CoVs and black for β-CoVs. Province colors correspond to the zoogeographic region to which they belong: NO, Northern region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region; HI, Hainan island. The three β-CoV sequences from HI were included in the SO region. Provinces colored in grey are those where CoV sequences are not available.

Fig. 2 α-CoV (A) and β-CoV (B) maximum clade credibility annotated trees using complete datasets of RdRp sequences and bat host family as discrete character state. Pie charts located at the root and close to the deepest nodes show the state posterior probabilities for each bat family. Branch colors correspond to the inferred ancestral family with the highest probability. Branch lengths are scaled according to relative time units (clock rate = 1.0). Well-supported nodes (posterior probability > 0.95) are indicated with a black dot. The ICTV approved CoV subgenera were highlighted: *Rhinacovirus* (L1), *Decacovirus* (L2), *Myotacovirus* (L3), *Pedacovirus* (L5), *Nyctacovirus* (L6), *Minunacovirus* (L7) and an unidentified lineage (L4) for α-CoVs; and *Merbecovirus* (Lineage C), *Nobecovirus* (lineage D), *Hibecovirus* (lineage E) and *Sarbecovirus* (Lineage B) for β-CoVs.

Fig. 3 Maximum clade credibility tree (A) including 201 RdRp sequences from the *Sarbecovirus* lineage isolated in bats and two sequences of ~~2019-nCoV~~SARS-CoV-2 isolated in humans (GenBank accession numbers: MN908947 and MN975262). Well-supported nodes (posterior probability > 0.95) are indicated with a black dot. Tip colors correspond to the bat host genus, SARS-CoV-2 ~~2019-nCoV~~ sequences are highlighted in yellow. Median-joining network (B) including 201 RdRp sequences from the *Sarbecovirus* lineage isolated in bats and two sequences of SARS-CoV-2 isolated in humans (GenBank accession numbers: MN908947 and MN975262) and one sequence of SARS-CoV (GenBank accession number: NC_004718). Colored circles correspond to distinct CoV sequences, circle size is proportional to the number of identical sequences in the data set. Small black circles represent median vectors (ancestral or unsampled intermediate sequences). Branch length is proportional to the number of mutational steps between haplotypes.

Fig. 4 Strongly supported host switches between bat families for α- (A) and β-CoVs (B). Arrows indicate the direction of the switch; arrow thickness is proportional to the switch significance level, only host switches supported by strong Bayes factor (BF) > 10 are shown. Histograms of total number of host

776 switching events (state changes counts using Markov jumps) from/to each bat family along the
777 significant inter-family switches for α- (C) and β-CoVs (D).

778 **Fig. 5** Strongly supported host switches between bat genera for α- (A) and β-CoVs (B) and their
779 significance level (Bayes factor, BF). Only host switches supported by strong BF values > 10 are shown.
780 Line thickness is proportional to the switch significance level. Red lines correspond to host switches
781 among bat genera belonging to different families, black lines correspond to host switches among bat
782 genera from the same family. Arrows indicate the direction of the switch. Genus names are colored
783 according to the family they belong to using the same colors as in Figures 2 and 3.

784 **Fig. 6** Strongly supported dispersal routes (BF > 10) over recent evolutionary history among China
785 zoogeographic regions for α- (A) and β-CoVs (B). Arrows indicate the direction of the dispersal route;
786 arrow thickness is proportional to the dispersal route significance level. Darker arrow colors indicate
787 older dispersal events. Histograms of total number of dispersal events (Markov jumps) from/to each
788 region along the significant dispersal routes for α- (C) and β-CoVs (D). NO, Northern region; CN, Central
789 northern region; SW, South western region; CE, Central region; SO, Southern region; HI, Hainan island.

790 **Fig. 7** Metrics of CoV phylogenetic diversity within each bat family (A), genus (B) and zoogeographic
791 regions (C): standardized effect size of Mean Phylogenetic Distance (SES MPD), on the left panels; and
792 standardized effect size of Mean Nearest Taxon Distance (SES MNTD), on the right panels. Values
793 departing significantly from the null model (p-value < 0.05) are indicated with an asterisk. NO, Northern
794 region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region;
795 HI, Hainan island.

796 **Fig. 8** Phylogenetic β-diversity (standardized effect size of Mean Phylogenetic Distance, SES MPD) and
797 phylogenetic ordination among bat host families (A, B) and genera (C, D) for α- and β-CoVs. Boxplots for
798 each host family and genus show the mean (cross), median (dark line within the box), interquartile range

(box), 95% confidence interval (whisker bars), and outliers (dots), calculated from all pairwise comparisons between bat families and genera.

Fig. 9 Phylogenetic β-diversity (standardized effect size of Mean Phylogenetic Distance, SES MPD) and phylogenetic ordination among zoogeographic regions for α- (A) and β-CoVs (B). Boxplots for each region show the mean (cross), median (dark line within the box), interquartile range (box), 95% confidence interval (whisker bars), and outliers (dots), calculated from all pairwise comparisons between regions. NO, Northern region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region; HI, Hainan island.

808 **Figure 1**

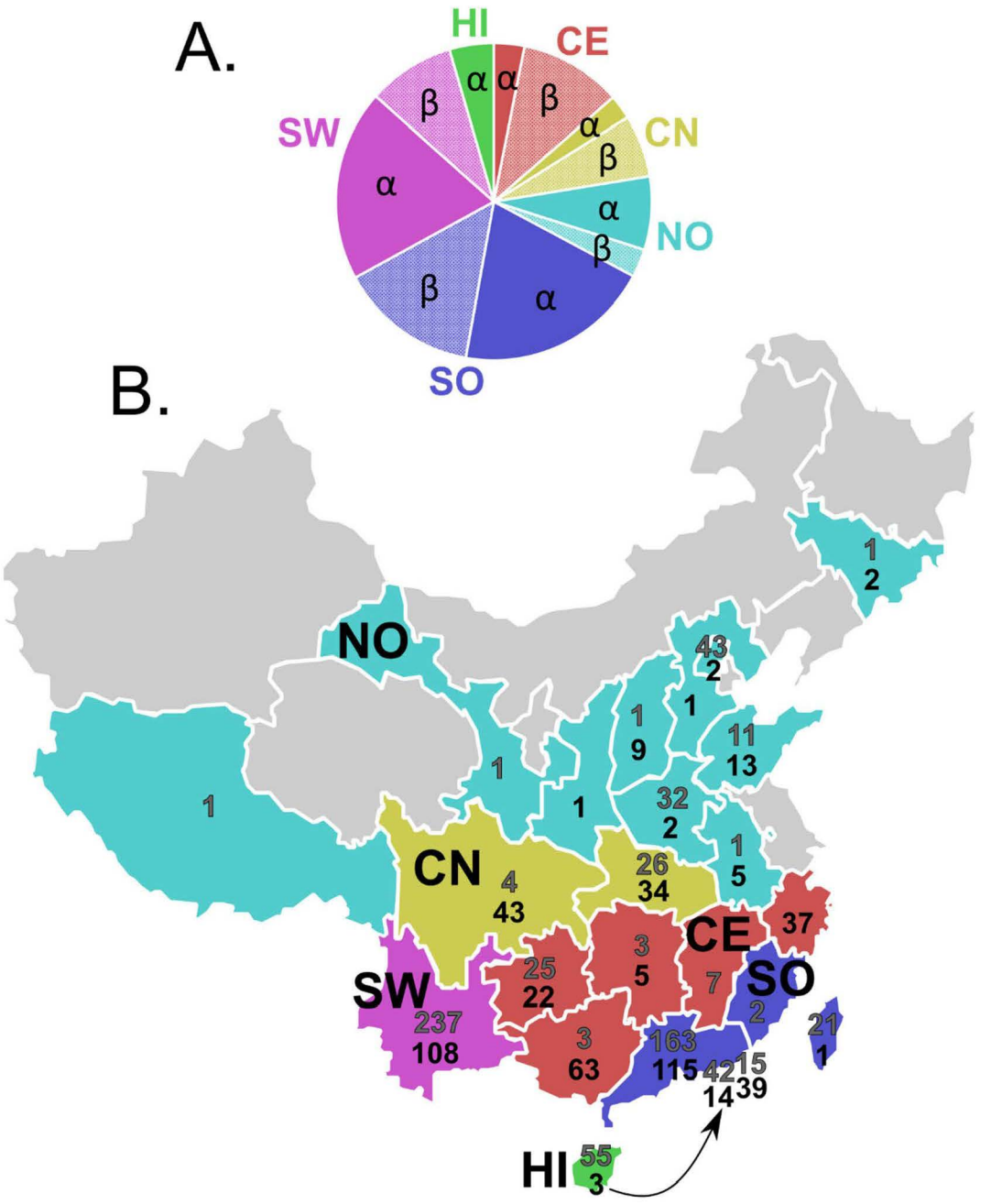

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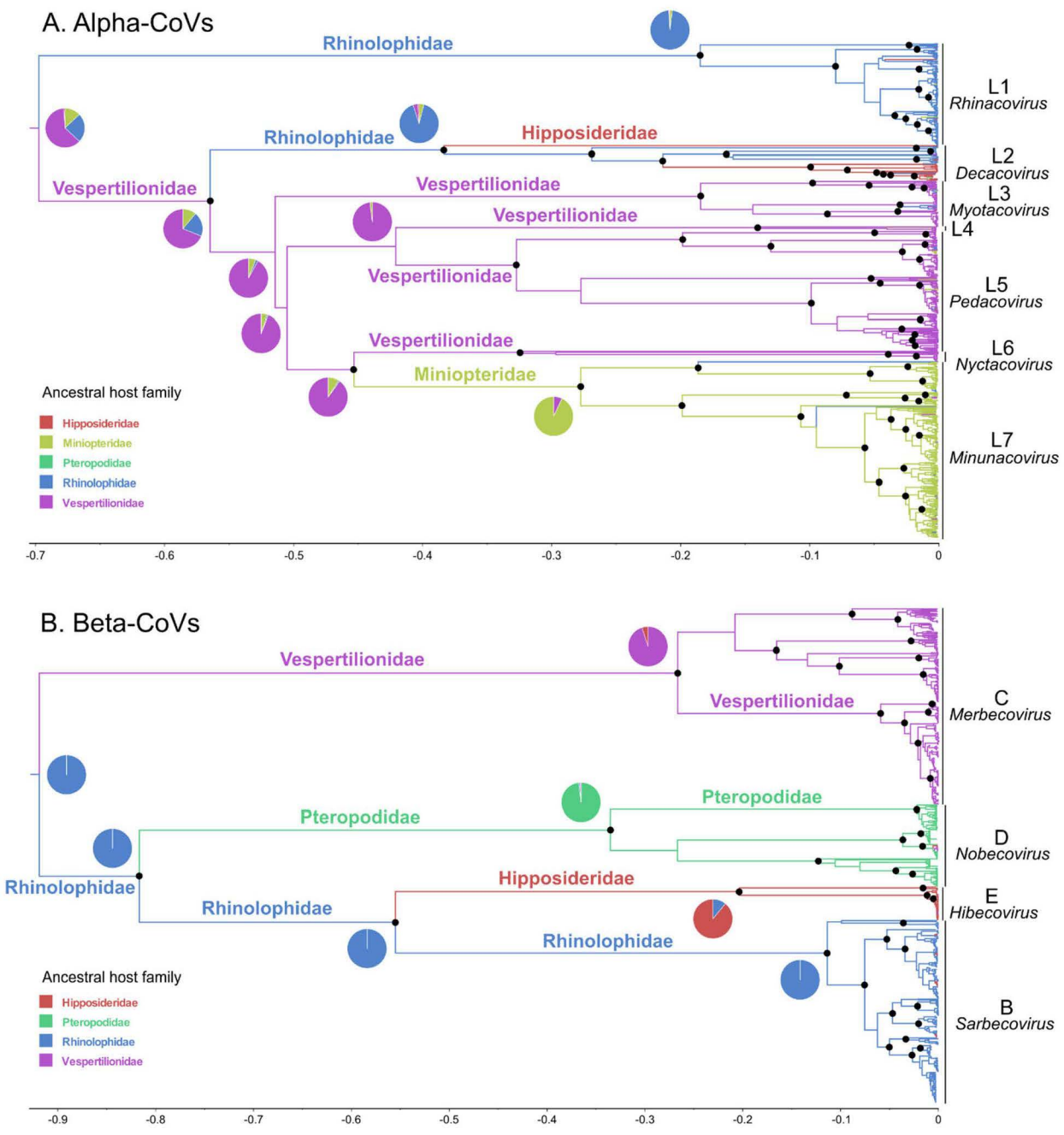
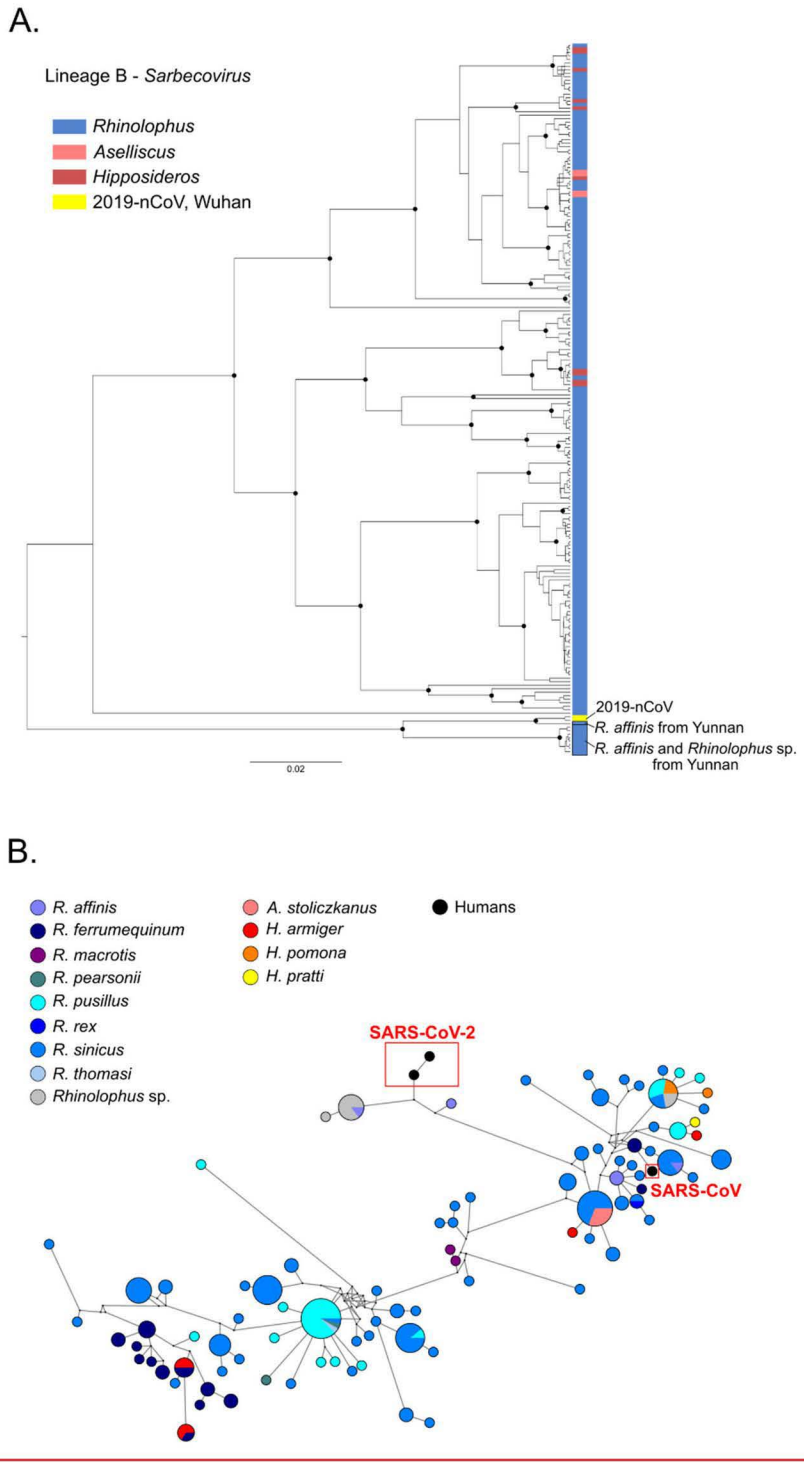
Figure 2

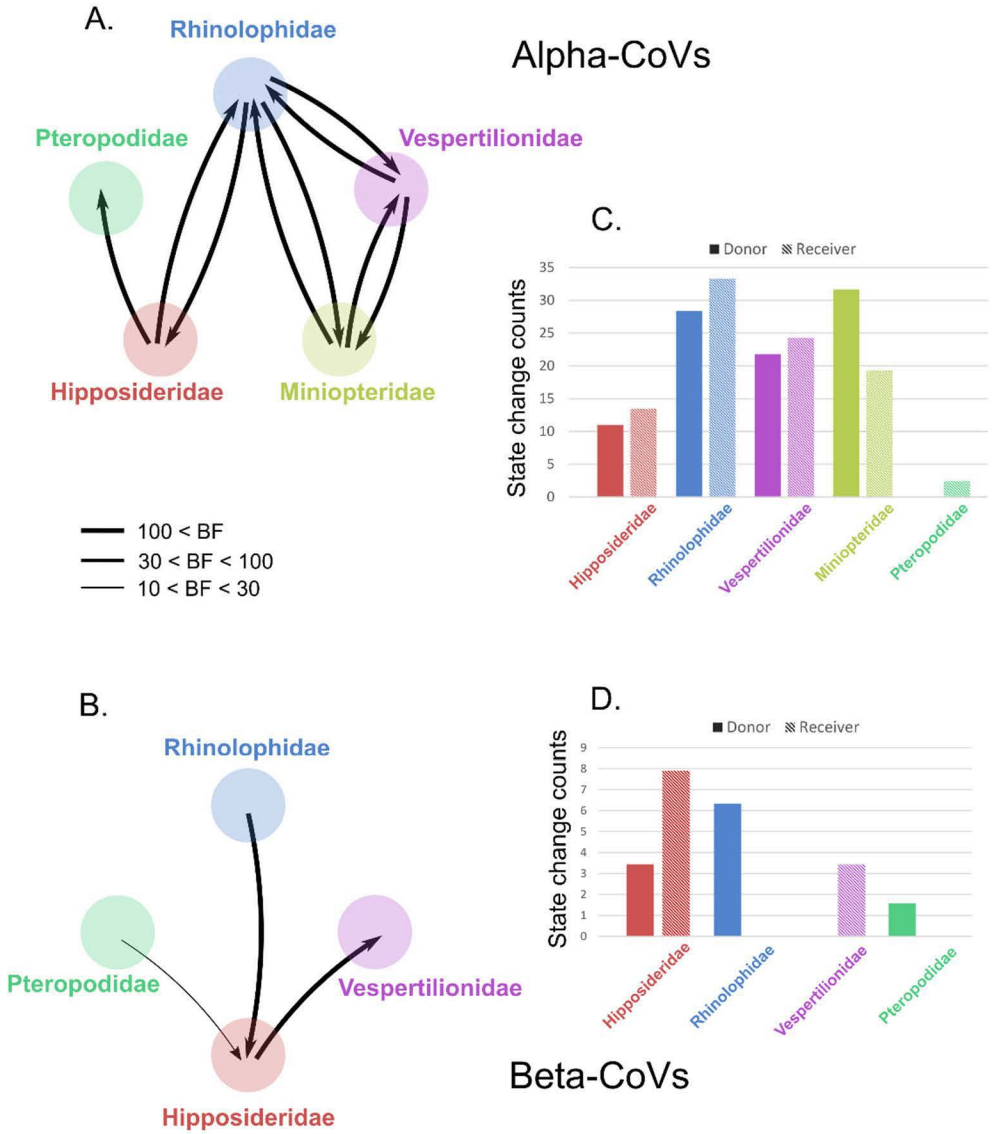

A. Alpha-CoVs
Rhinolophidae
Rhinolophidae
Hipposideridae
Vespertilionidae
Vespertilionidae
Vespertilionidae
Vespertilionidae
Vespertilionidae
Miniopteridae
L1
Rhinacovirus
L2
Decacovirus
L3
Myotacovirus
L4
L5
Pedacovirus
L6
Nyctacovirus
L7
Minunacovirus
Ancestral host family
Hipposideridae
Miniopteridae
Pteropodidae
Rhinolophidae
Vespertilionidae
-0.7
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0
B. Beta-CoVs
Vespertilionidae
Vespertilionidae
Pteropodidae
Pteropodidae
Rhinolophidae
Hipposideridae
Rhinolophidae
Rhinolophidae
C
Merbecovirus
D
Nobecovirus
E
Hibecovirus
B
Sarbecovirus
Ancestral host family
Hipposideridae
Pteropodidae
Rhinolophidae
Vespertilionidae
-0.9
-0.8
-0.7
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0

816 **Figure 3**

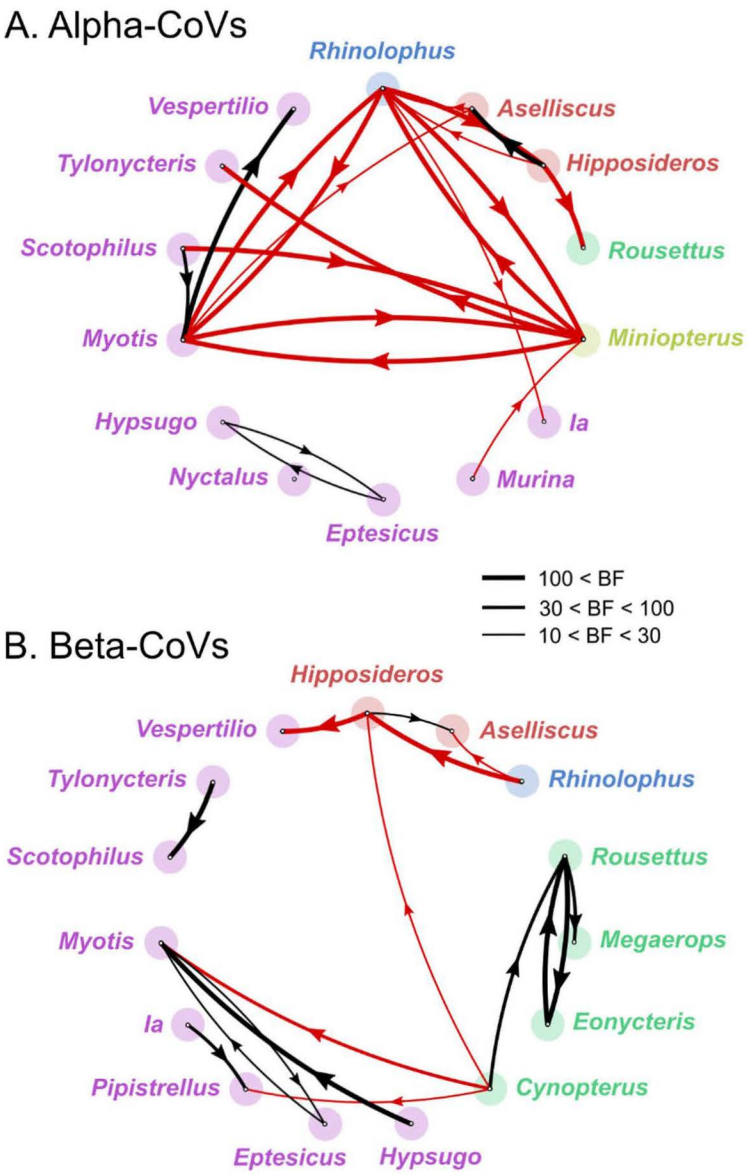


817

Figure 4


A.
Rhinolophidae
Alpha-CoVs
Pteropodidae
Vespertilionidae
Hipposideridae
Miniopteridae
100 < BF
30 < BF < 100
10 < BF < 30
C.
Donor
Receiver
State change counts
35
30
25
20
15
10
5
0
Hipposideridae
Rhinolophidae
Vespertilionidae
Miniopteridae
Pteropodidae
B.
Rhinolophidae
Pteropodidae
Vespertilionidae
Hipposideridae
D.
Donor
Receiver
State change counts
9
8
7
6
5
4
3
2
1
0
Hipposideridae
Rhinolophidae
Vespertilionidae
Pteropodidae
Beta-CoVs

821 **Figure 5**



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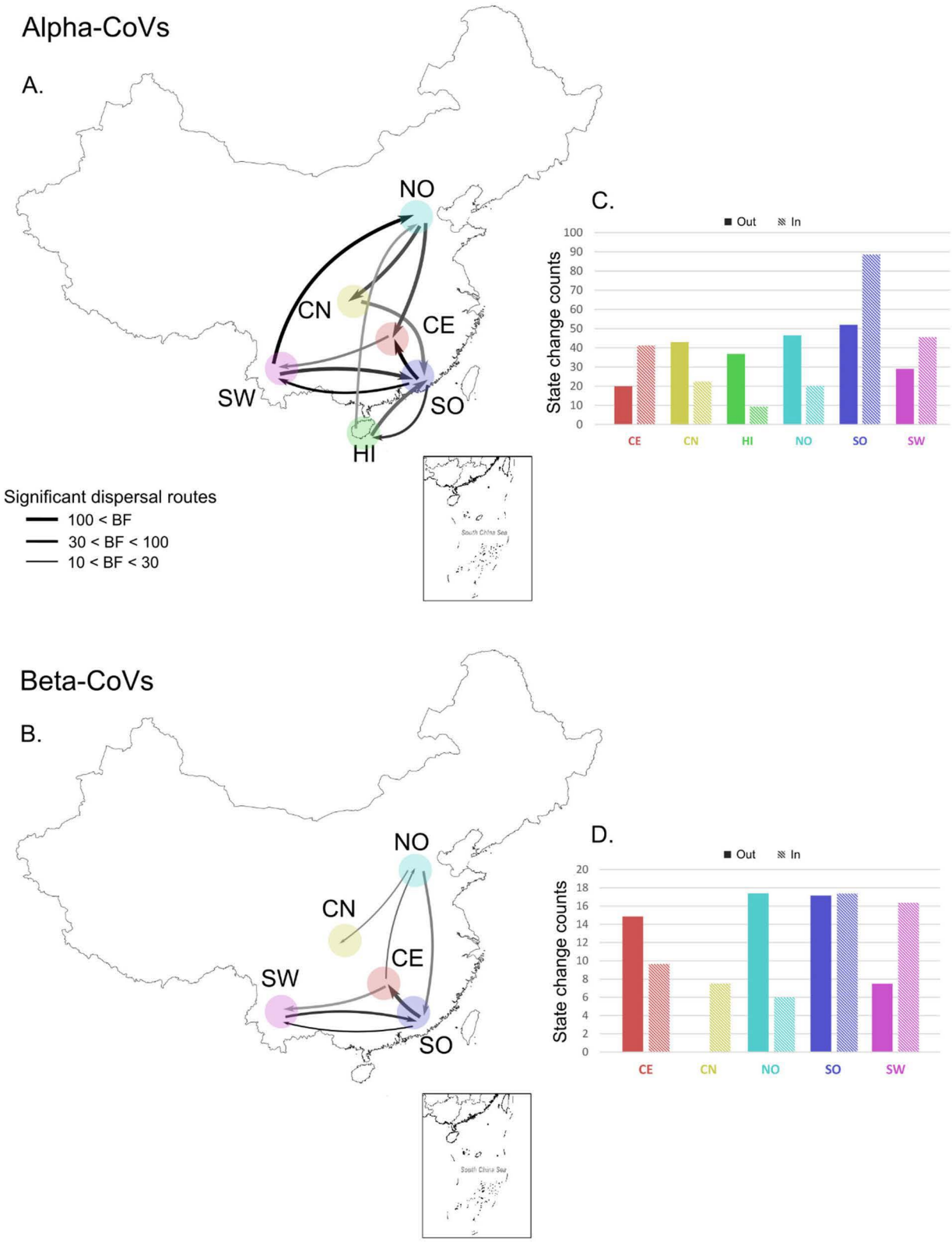
824 **Figure 6**



825

826 **Figure 7**

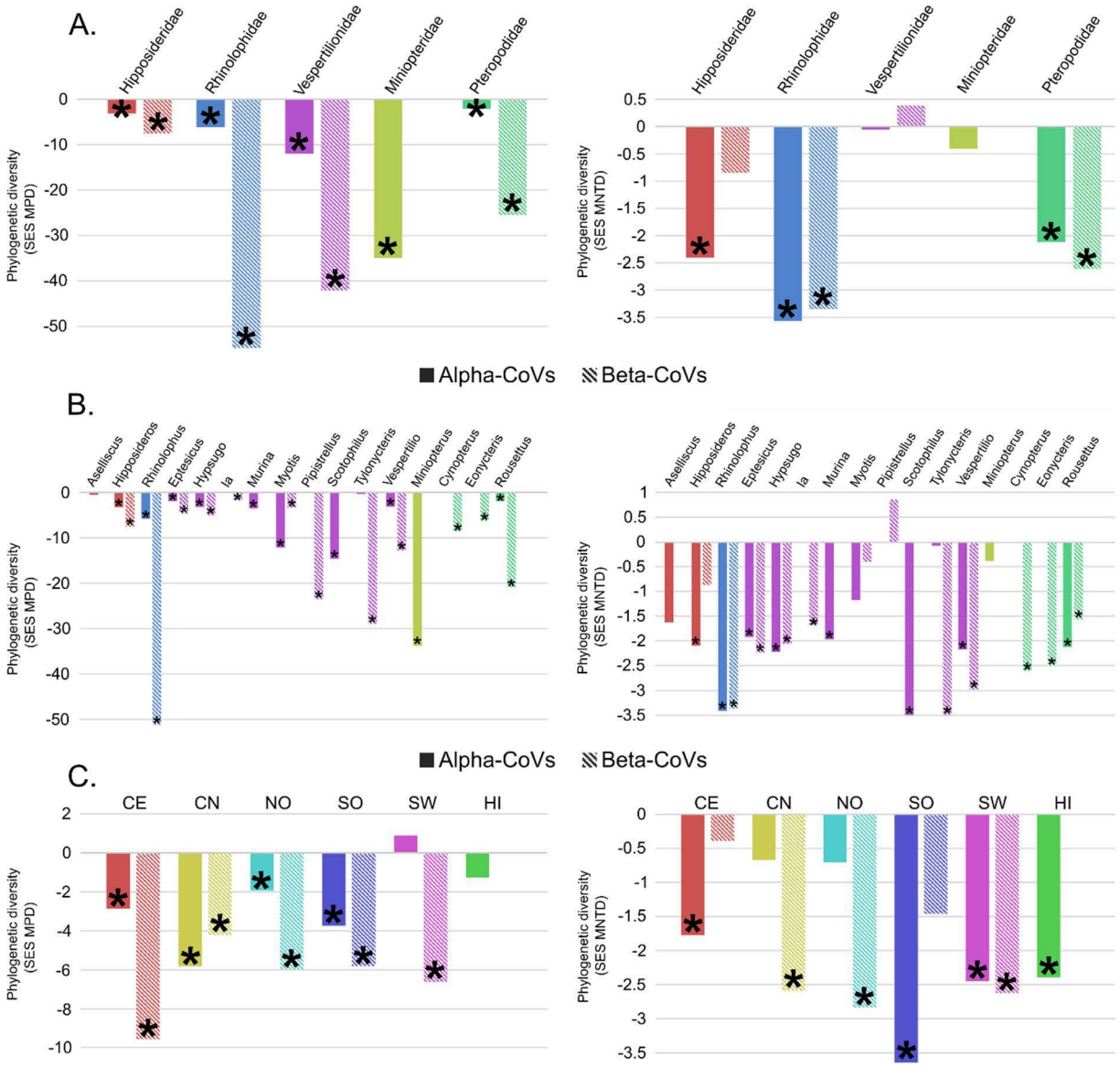


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Figure 8

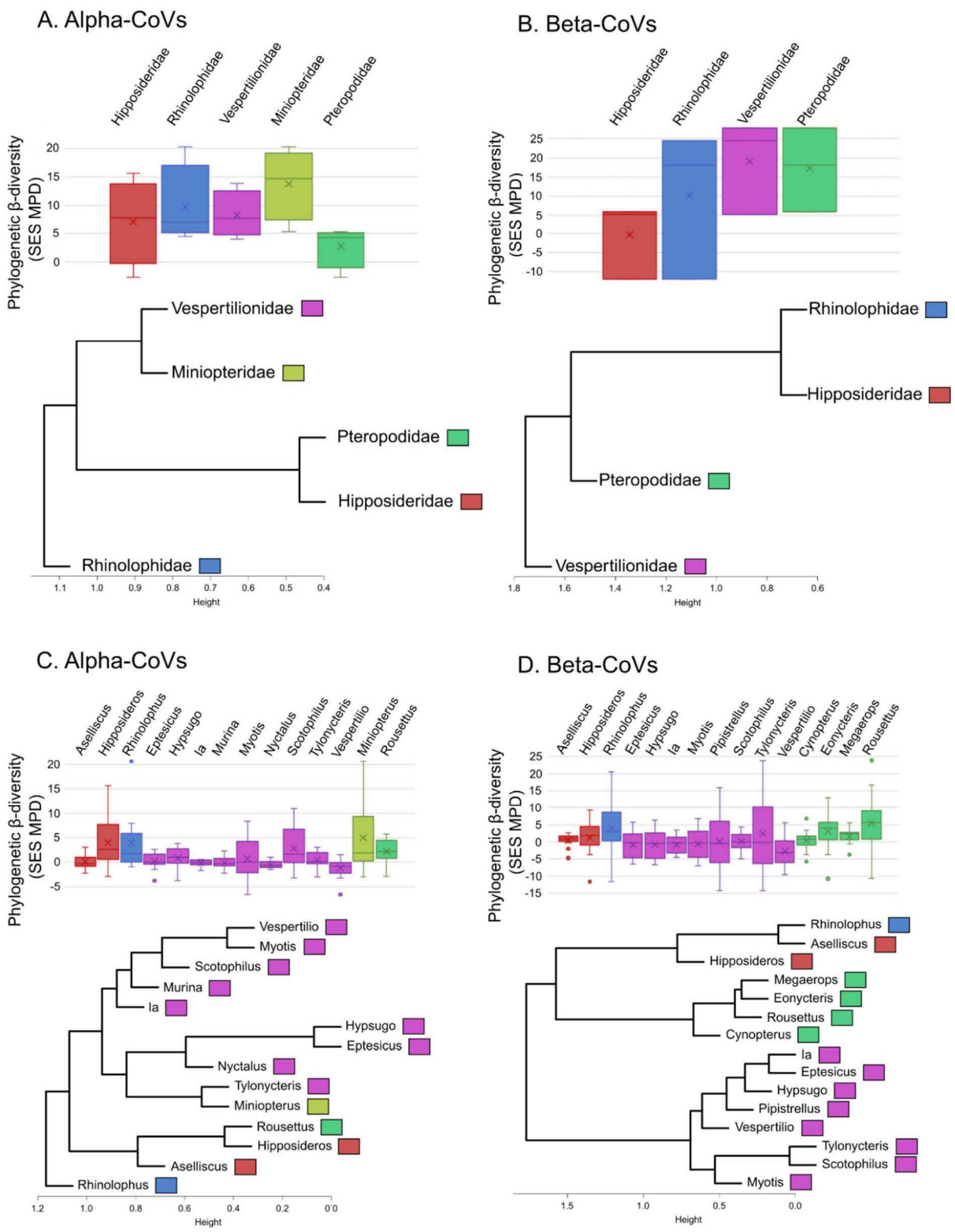
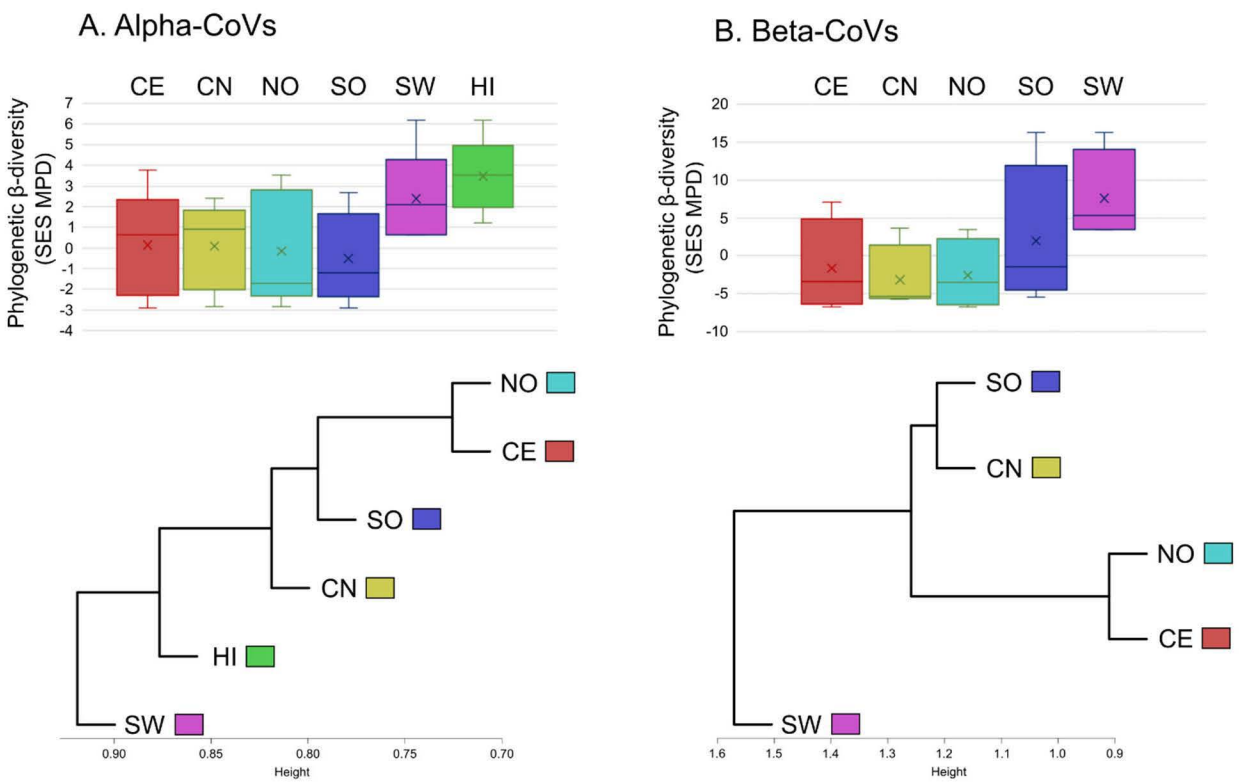

A. Alpha-CoVs
Hipposideridae
Rhinolophidae
Vespertilionidae
Miniopteridae
Pteropodidae
Phylogenetic β-diversity (SES MPD)
Vespertilionidae
Miniopteridae
Pteropodidae
Hipposideridae
Rhinolophidae
Height
B. Beta-CoVs
Hipposideridae
Rhinolophidae
Vespertilionidae
Pteropodidae
Phylogenetic β-diversity (SES MPD)
Rhinolophidae
Hipposideridae
Pteropodidae
Vespertilionidae
Height
C. Alpha-CoVs
Aselliscus
Hipposideros
Rhinolophus
Eptesicus
Hypsugo
Ia
Murina
Myotis
Nyctalus
Scotophilus
Tylonycteris
Vespertilio
Miniopterus
Rousettus
Phylogenetic β-diversity (SES MPD)
Vespertilio
Myotis
Scotophilus
Murina
Ia
Hypsugo
Eptesicus
Nyctalus
Tylonycteris
Miniopterus
Rousettus
Hipposideros
Aselliscus
Rhinolophus
Height
D. Beta-CoVs
Aselliscus
Hipposideros
Rhinolophus
Eptesicus
Hypsugo
Ia
Myotis
Pipistrellus
Scotophilus
Tylonycteris
Vespertilio
Cynopterus
Eonycteris
Megaerops
Rousettus
Phylogenetic β-diversity (SES MPD)
Rhinolophus
Aselliscus
Hipposideros
Megaerops
Eonycteris
Rousettus
Cynopterus
Ia
Eptesicus
Hypsugo
Pipistrellus
Vespertilio
Tylonycteris
Scotophilus
Myotis
Height

Figure 9


A. Alpha-CoVs
CE CN NO SO SW HI
Phylogenetic β-diversity (SES MPD)
7 6 5 4 3 2 1 0 -1 -2 -3 -4
NO
CE
SO
CN
HI
SW
0.90 0.85 0.80 0.75 0.70
Height
B. Beta-CoVs
CE CN NO SO SW
Phylogenetic β-diversity (SES MPD)
20 15 10 5 0 -5 -10
SO
CN
NO
CE
SW
1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9
Height

Talking points from Latinne et al. Origin and cross transmission of bat CoVs in China

Study funded by NIH/NIAID & USAID/PREDICT

1. Most comprehensive analysis of bat coronavirus evolutionary origins ever conducted
    a. Includes 732 novel sequences not previously reported from bats in China
    b. XX alpha-CoVs (includes SADS-CoV); XX beta-CoVs (includes SARS-CoV & SARS-CoV-2)
    c. XX SARSr-CoVs, XX SADSr-CoVs
    d. Includes closest known relatives of SARS-CoV and SARS-CoV-2. Strongly supports their origin in bats

2. Helps understand why China is a hotspot
    a. Not just because of high bat diversity
    b. Ecological or biogeographic factors – sharing roosts with other species, ancient origin of horseshoe bats,
    c. Higher CoV diversity than expected in some S. China provinces (Hainan, Guangxi, Hunan)

3. Significant cross-species transmission of CoVs among bats over evolutionary time
    a. Rhinolophidae and *Rhinolophus* (Horseshoe) bats involved in more inter-family and inter-genus highly significant host switching of α-CoVs than any other family or genus
    b. Rhinolophidae (Horseshoe) & Hipposideridae at the origin of most inter-family host switching events for β-CoVs
    c. Overall, β-CoVs (incl. SARS group) also had strong evidence of co-evolution with their bat hosts. Their ability to diversify as bats evolve, and switch hosts, may have helped produce higher diversity of strains.
    d. α-CoVs (incl. SADS-CoV) are able to switch hosts more frequently and between more distantly related bats.
    e. Differences between these viral groups may be explained by subtle differences in host cell receptor binding, mutation rate, recombination potential, or replication rate.

4. Southern China is a hotspot for evolutionary diversification of bat-coronaviruses
    a. South western and Southern China are centers of diversification for both α- and β-CoVs
    b. They harbor evolutionarily old and phylogenetically diverse lineages of α- and β-CoVs
    c. SW China was Quaternary glacial refugium for bat species incl. *Rhinolophus* spp. & may have allowed survival of older viral strains leading to increased diversity.
    d. Similar theories for avian flu origins.

Relevance for pandemic risk:

1. There is an extraordinary diversity of coronaviruses in bats in southern China, some of which have already emerged in people and livestock, others that are poised to, still others about which we know very little. This represents a significant potential pandemic risk, and threat to food security through livestock disease. Our study alone identified >700 novel sequences and we expect there are many more to be discovered.

2. Evolution and human ecology collide to produce high risk of CoV emergence in S. China: The hotspots of CoV diversification in S & SW China also are regions with a subtropical to tropical climate; dense, growing and rapidly urbanizing populations of people; a high degree of poultry and livestock production; and high rates of consumption of wildlife, including bats – all factors which may promote cross-species transmission and disease emergence.

3. Targeting bats in these regions for surveillance will help identify novel coronaviruses that may emerge in future, helping generate vaccines and control programs to stop them emerging.

4. We should target coronaviruses broadly, not just those similar to SARS-CoV or SARS-CoV-2: We show that α-CoVs have a higher propensity to switch host within their natural bat reservoirs, and therefore high cross-species transmission potential and risk of spillover. These include SADS-CoV in pigs in Guangdong (also can infect human cells) & two human CoVs that likely originated in bats historically: NL63 and 229E. There may be more in the future, and targeted surveillance should be urgently conducted to identify whole diversity of this group.

5. This study provides rationale for programs of viral discovery (like the Global Virome Project) and capacity building/intervention programs to prevent pandemics (like PREDICT) in regions like S. and SW China.

Note limitations of study:

- Short sequences used (RdRp), may not reflect evolutionary patterns of whole viral genomes. However, consistent with evolutionary patterns seen using whole genomes.
- PCR technique builds on known viruses (consensus sequences), and may have missed some unknown viruses.

From:	Su Yadana
To:	ECKERLE Isabella; danielle.anderson_duke-nus; Hume Field; das Neves, Carlos Goncalo; spwa_hotmail; Keusch, Gerald T; Perlman, Stanley; malik; amuas001@umn.edu; Prof Lam Sai Kit; Saif, Linda
Cc:	Peter Daszak; Robert Kessler
Subject:	Draft press release: The Lancet COVID-19 Commission Taskforce
Date:	Monday, November 16, 2020 1:40:09 AM
Attachments:	Lancet Commission Taskforce on origins announcement for distribution.docx Taskforce on origins, early control and one health solutions to future pandemic threats.docx

Dear all,
Attached is the draft press release. Please see the following message from Peter:

Please see attached a draft press release about the Lancet COVID-19 Commission's Taskforce on the "Origins and early spread of COVID-19, and One Health solutions to future pandemics". You may have seen that there's a lot of interest in the press on the Taskforce's goals, and how we'll go about the work (e.g. this report in Washington Post from a couple of days ago: https://www.washingtonpost.com/opinions/global-opinions/the-coronaviruss-origins-are-still-a-mystery-we-need-a-full-investigation/2020/11/13/cbf4390e-2450-11eb-8672-c281c7a2c96e_story.html)

The aim of this release is just to get some basic information out about 1) the make-up of the taskforce; 2) the general goals of what we'll do. As reporters ask for interviews, we'll be able to go into a bit more detail, but for now we were hoping to make this short and sweet.

Please read through this release, make any comments or edits that you would like and send back to me and Su. We've also suggested a place in this release for a quote from you so that you can generate some press for your own institution, if you would like to. Please send back your version with those quotes so that we can track how it goes.

The goal is to release this to the press this week, probably Thursday if possible (US Eastern time, morning). We would like any release from your own institution to coordinate exactly at that time – please keep in touch with Robert Kessler ('cc in this email), who's EHA's Director of Communications for that.

Also, one point that's really important as you speak to reporters. Please make sure that you stick to 1) the goals of the taskforce as laid out in the attached doc and in our meetings, and 2) your own expertise and why you have been invited to be part of this group. It's really critical that we don't give opinions about any of the results of the work before we've done it, especially with so many conspiracy theories about the origins. We will be looking at all of them, with an objective and scientific view to see what evidence is available and what gaps exist. For now, that's all we can really say because we've not yet got into the work.

Cheers,

Peter

Best,
--

Su Yadana, MPH
Research Scientist

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation.

EcoHealth Alliance

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FOR IMMEDIATE RELEASE

MEMBERS OF *THE LANCET* COVID COMMISSION TASKFORCE ON THE ORIGINS OF SARS-COV-2 NAMED

NEW YORK – November TK, 2020 – As cases surge globally and disruption continues, many questions remain about COVID-19 and SARS-CoV-2, the virus behind this pandemic. Chief among them: Where did it come from, how did it escape our control, and how can we stop future pandemics like COVID-19? These questions will be the focus of an international taskforce led by Dr. Peter Daszak, president of EcoHealth Alliance, a nonprofit working at the intersection of animal, environmental, and human health on a global scale. The taskforce is part of *The Lancet* COVID-19 Commission, chaired by Jeffrey D. Sachs of Columbia University.

"We intend to conduct a thorough and rigorous investigation into the origins and early spread of SARS-CoV-2," Dr. Daszak, a disease ecologist who's spent years studying coronavirus transmission in China and Southeast Asia. "Our group will use the findings to formulate One Health solutions for managing future zoonotic disease risk."

FOR TASKFORCE MEMBERS AND INSTITUTIONS TO INSERT QUOTES: As a member of the taskforce, and a leader in XXXXX (e.g. Virology, One Health, lab biosecurity etc.), Dr. XXXXXX of XXXXX adds: " INSERT QUOTE HERE……" (e.g. comment on how important this work is, how your expertise will be used, what specific aspect you are most intrigued by etc.).

The Lancet taskforce has 12 members who come from a diverse set of scientific disciplines and backgrounds, with expertise in One Health, outbreak investigation, virology, lab biosecurity and disease ecology. They are:

Dr. Peter Daszak, Chair
Peter Daszak, PhD, is the President of EcoHealth Alliance. A member of the U.S. National Academy of Medicine, he chairs the National Academies of Science, Engineering, and Medicine's Forum on Microbial Threats.

Dr. John Amuasi, MD PhD
John Amuasi, MD PhD, is Director of the African Research Network for Neglected Tropical Diseases. He lectures at the School of Public Health, Kwame Nkrumah University of Science and Technology and is Group Leader of the Global Health and Infectious Diseases Research Group at the Kumasi Centre for Collaborative Research in Tropical Medicine in Ghana.

Dr. Danielle Anderson
Danielle Anderson, PhD, is the Scientific Director of the BSL-3 laboratory at the Duke-NUS Medical School in Singapore. Dr. Anderson conducts research on negative-stranded RNA viruses such as measles, mumps, and Nipah virus and was the first to isolate SARS-CoV-2 in Singapore.

Dr. Isabella Eckerle
Isabella Eckerle, MD, is a leading virologist, Head of the Centre for Emerging Viral Diseases at the Université de Genève, and has led research on MERS-CoV, SARS-CoV and SARS-CoV-2.

Dr. Hume E. Field
Hume E. Field, DVM PhD, is an Honorary Professor at the University of Queensland. Dr. Field led the original World Health Organization veterinary investigation into the origins of SARS-CoV at wet markets in China's Guangdong Province.

Dr. Gerald Keusch
Gerald Keusch, MD, is Associate Director of the BSL-4 National Emerging Infectious Diseases Laboratories laboratory at Boston University. He is the former Director of the NIH Fogarty International Center and a member of the U.S. National Academy of Medicine.

Dr. Dato' Sai Kit (Ken) Lam
Dato' Sai Kit (Ken) Lam, PhD, is Professor Emeritus at the University of Malaya, a member of the Malaysian Academy of Sciences and discovered Nipah virus following its initial outbreak in peninsular Malaysia, for which he won the prestigious Merdaka Award.

Dr. Carlos das Neves, DVM PhD
Carlos das Neves, DVM PhD, is the Director for Research and Internationalization at the Norwegian Veterinary Institute, President of the International Wildlife Disease Association, and the former Hon. Consul of the Portuguese Republic in Norway.

Dr. Malik Peiris
Malik Peiris, PhD FRS holds the Tam Wah-Ching Professorship, Division of Public Health Laboratory Sciences at the University of Hong Kong. Dr. Peiris was the first person to isolate SARS-CoV and is a global leader in coronavirus and influenza virus research.

Dr. Stanley Perlman, MD PhD
Stanley Perlman, MD PhD, is a Professor of Microbiology and Immunology as well as the Mark Stinski Chair of Virology at the University of Iowa, Carver College of Medicine. Dr. Perlman conducts research on several respiratory human coronaviruses including SARS-CoV, MERS-CoV, SARS-CoV-2, human coronavirus-OC43, and human coronavirus-NL63.

Dr. Linda J. Saif
Linda J. Saif, PhD, is a Professor at the Dept of Veterinary Preventative Medicine at Ohio State University. Dr. Saif is a member of the U.S. National Academy of Sciences, and has worked on coronaviruses since before the SARS outbreak.

Dr. Supaporn Wacharapluesadee
Supaporn Wacharapluesadee, PhD, is in the Faculty of Medicine at Chulalongkorn University in Bangkok and Deputy Director of the Thai Red Cross Emerging Infectious Diseases-Health Science Centre. Dr. Wachaeapluesadee's team was the first to positively identify a human COVID-19 infection outside of China.

In its investigation, the taskforce will recreate a complete timeline of the outbreak of COVID-19, starting from the discovery of RaTG13–the closest known viral relative of SARS-CoV-2–in 2013 and up to the WHO's declaration of COVID-19 as a Public Health Emergency of International Concern on January 30, 2020. They will analyze the available evidence for each of the hypotheses put forward on the origins of COVID-19, and compare its early spread and outbreak control to previous outbreaks to identify strategies that might assist future pandemic prevention.

"There is a great deal of interest in understanding how COVID-19 emerged and spread, but there is a deeper reason for this taskforce's work," *The Lancet* COVID-19 Commission lead Jeff Sachs said. "If we can understand why this pandemic began, we can design solutions to prevent the next one."

More information on *The Lancet* COVID-19 Commission and this taskforce can be found here.

Commented [RK1]: Link Tk

About EcoHealth Alliance

Building on over 45 years of groundbreaking science, EcoHealth Alliance is a global nonprofit organization dedicated to protecting wildlife, environmental, and public health from the emergence of disease. Approximately 60 percent of emerging infectious diseases like Ebola, HIV, Zika, SARS, MERS, West Nile virus, and, now, SARS-CoV-2 have all originated in animals before spilling over to human populations. Using environmental and health data covering the past 60 years, EcoHealth Alliance scientists created the first-ever global disease hotspots map that identified at-risk regions to determine where research and field work are needed to help predict and prevent the next pandemic crisis. That work is the foundation of EcoHealth Alliance's rigorous, science-based approach working in nearly 30 countries worldwide. EcoHealth Alliance's strength is founded on innovations in research, training, global partnerships, capacity building, and policy initiatives.

For more information, please visit www.ecohealthalliance.org.

About XXXXX

PLEASE INSERT A SHORT PARAGRAPH ON YOUR OWN ORGANIZATION HERE

The Lancet COVID-19 Commission

Taskforce on the Origins, Early Control of the Pandemic, and One Health Solutions to Future Pandemic Threats

Significance

Better understanding of the *origin* of SARS-CoV-2 may:

- Identify potential continued risk of re-emergence or emergence of future CoVs or other agents.
- Provide a strategy to heighten biosecurity, design behavior change programs, and introduce legislation/policies to reduce risk of future emergence in China, SE Asia and beyond
- Inform and potentially undermine a politically-divisive strategy to 'blame' countries for the outbreak.

Assessing *early control* of the pandemic may:

- Identify specific points at which future epidemics can be contained more effectively before amplification and international spread
- Identify specific strategies, agencies, policies to improve future control of pandemics as close as possible to initial spillover event.

Identifying *One Health* approaches to controlling future pandemics will:

- Examine the underlying drivers of COVID-19 in the context of other emerging diseases and pandemics
- Identify potential synergistic effects and return-on-investment of taking a multisectoral approach to outbreak investigation and pandemic prevention that includes Animal Health, Human Health, Environmental Health aspects
- Identify key strategies, organizations and mechanisms to fund and deliver a coordinated One Health approach to *preventing* future pandemics

Logistics:

Taskforce lead is Peter Daszak, EcoHealth Alliance (daszak@ecohealthalliance.org). Project coordinator for the Taskforce at EcoHealth is Su Yadana BS MPH (yadana@ecohealthalliance.org) who is based in New York, originally from Myanmar, and has worked in Singapore (DukeNUS) and has an MPH from Columbia University School of Public Health. Point of Contact for our taskforce on the COVID-19 Commission is Dr. Özge Karadag Caman (ok2267@columbia.edu) at the Center for Sustainable Development, currently back in Turkey. Dr. Caman is also part of the Secretariat for the Lancet Commission on COVID-19 and is involved in One Health.

We will meet by zoom in October then again in November to discuss strategy and initial draft plan. We will draft a 10-page report by Dec 1st 2020 to sum up our initial approach, findings. We will conduct background research, zoom meetings throughout Winter, Spring, Summer 2021 to analyze available data, interview key leaders involved in outbreak investigation, conduct background research, draft report. Report Due: Sept. 2021.

Strategy

1. Assemble an international group of trusted experts on emerging disease to review scientific evidence on key theories of COVID-19 origins & control. Expertise on:
    a. Virology, sequence analysis
    b. Ecology of viral emergence

c. Outbreak investigation, epidemiology
d. Social science of risk behavior in developing countries
e. Wildlife ecology/One Health
f. Wildlife trade
g. Biosecurity lab safety

2. On the **origin** question: Use 'Preponderance of Evidence' approach to analyze data on all leading theories for origin. What do we know? What don't we know?
    a. Work backwards from the Huanan Market, as well as forwards from the rural Yunnan sites of nearest known relatives in wildlife
    b. Approach key members of the outbreak investigation teams, virological labs analyzing early cases in China to seek further support or lack thereof for each theory
    c. Build a detailed timeline of the outbreak, stretching from discovery of nearest relatives (2012) through to declaration of COVID-19 as a PHEIC by WHO (Jan 30th 2020)
    d. Weigh the evidence for and against each theory on COVID origins. Identify critical gaps in data and recommend strategies that can be adopted to address them.

3. On the **early control** issue: Document outbreak investigation and control efforts from China, WHO and other countries within the timeline up to Jan 30th 2020.
    a. Compare these with other recent emerging diseases (e.g. Nipah virus, H1N1, West Africa Ebola, H7N9)
    b. Identify critical points in the investigation and control efforts that alternative strategies could have been adopted for,
    c. Identify gaps in our understanding of early control
    d. Recommend strategies for future efforts for control

4. **One Health and Preventing Future Pandemics**: Identify when a One Health approach would have benefits to preventing future pandemics, how this would be funded, and what organizations would be involved
    a. Review common features among COVID-19 and other pandemics that have origins in wildlife, livestock and are driven to emerge by underlying environmental changes
    b. Identify potential synergistic effects and return-on-investment of taking a multisectoral approach to outbreak investigation and pandemic prevention that includes Animal Health, Human Health, Environmental Health aspects
    c. Identify key strategies, organizations and mechanisms to fund and deliver a coordinated One Health approach to *preventing* future pandemics at the intergovernmental and national levels

What we know:

- SARS-CoV and SARS–CoV-2 are both Clade 2b β-coronaviruses. Closest relatives (RaTG13, RmYN02) are from bats.
- There are 528 β-CoV sequences in Genbank which includes 100+ SARSr-CoV sequences. Only a handful have not been reported from bats (sequenced from pangolins)
- Majority are from China, but this reflects collecting bias. Others reported from across SE Asia
- Phylogenetic analysis points to S. China (Yunnan province) or Myanmar/Laos/Vietnam as evolutionary hotspot for this clade.

- What that tells us is that it's extremely likely that SARS-CoV-2 evolved from within this cluster of bat CoVs, probably from an insectivorous bat, probably from Yunnan, S. China, near the border of Myanmar, Laos, & Vietnam.
- Some of these viruses can infect human cells directly, although SARS-CoV (and maybe SARS-CoV-2) infected mammalian 'intermediate' hosts.
- Role of pangolins may be incidental: animals were seized in China after prob. many weeks in transit. Wildlife trade is known to heighten CoV prevalence, pangolins at start of wildlife trade are CoV-free.

Main theories that have been proposed for the origin of COVID-19:

1) Yunnan bat-> hunter-> Wuhan. The virus evolved in S. China from a bat SARSr-CoV lineage and infected a person directly – e.g. a bat hunter – and this person got sick and transmitted it to their social network, which is people in the wildlife trade, so the virus moved through the trade network to Wuhan. Would need to assess all potential pathways of human exposure by bats in the region.
2) Yunnan bat-> traded/farmed wildlife intermediate host-> Wuhan. SARS-CoV-2 was in a bat that was captured by a hunter, or flew into a farm where people have wildlife in cages and infected animals the hunter/farmer was ready to sell into the wildlife trade. The animals carried the virus to the Wuhan market as they were trucked into Wuhan. The animals could be civets, porcupines, raccoon dogs or another one of the animals commonly raised for food or fur in China
3) Hubei bat-> via hunter, intermediate host or direct to Wuhan market. The virus is from a bat endemic to Hubei (the province where Wuhan is), and either of the above two pathways began there. Need to take into account timing of spillover vs. first cluster of cases and assess whether and when bats hibernate in that region.
4) Origin in another region in China or neighboring countries. This happened in another part of China, e.g. Guangdong, or even in countries over the border from Yunnan where the same bats and prob. similar viruses circulate.
5) Origin in another more distant country. Assess hypotheses on US or European origin. Analyze data on proposed first findings of evidence of COVID outside China (e.g. patient in France, sewage in Spain etc.).
6) Role of pangolins as intermediate hosts. The virus moved from bats into pangolins in the wildlife trade and then into people. Assess sequence data from all close relative CoVs, assess volume of live or frozen pangolins traded, analyze ability of pangolin scales to transmit virus
7) It was bioengineered in the Wuhan BSL-4 lab. This has been discounted by everyone who works in the field because there is no evidence from the genetic sequence that the virus has been genetically manipulated, and there almost certainly would be, had that happened.
8) It is derived from a bat virus that was accidentally released from WIV, Wuhan CDC or Wuhan University lab. This theory suggests it was cultured in the lab and accidentally infected a lab worker, or was discarded with animals used in experiments, or infected people sampling bats in caves. Would need to assess what samples were present in the labs, what the routine protocols were, the number of people with access to samples or bat caves for sampling, evidence of safety violations or lack of biosecurity.

Invited members:

1. Peter Daszak Ph.D., Chair. President of EcoHealth Alliance, New York. Member of US National Academy of Medicine, Chair NASEM Forum on Microbial Threats. *Viral Discovery, Epidemiology, Ecology* **USA/UK. Male**
2. John Amuasi MD Ph.D., Director of Africa Ctr for Neglected Tropical Diseases & Sr. Lecturer, Kwame Nkrumah University of Science and Technology (KNUST), Accra, Ghana. *MD, One Health, Global Health Policy* **Ghana, Male**
3. Danielle Anderson Ph.D., Director BSL-3 lab, Duke-NUS, Singapore. First non-Chinese citizen to work in the Wuhan Institute of Virology BSL-4 lab. *Lab Biosafety, virology.* **Australia, Female**
4. Isabella Eckerle MD, Head of Centre for Emerging Diseases, Univ. Geneva. *MD, Virologist.* **Switzerland/German, Female**
5. Hume E. Field DVM Ph.D., School of Veterinary Science, Univ Queensland – Led the original WHO veterinary investigation into the origin of SARS-CoV in wet markets in Guangdong. *Veterinarian, One Health* **Australia, Male**
6. Manish Kakkar MD, Public Health Foundation of India – long term experience in zoonoses research and policy, involvement in WHO SEARO. *MD, Zoonoses research, Public Health Policy* **India, Male**
7. Gerald Keusch MD, Boston University, Head of BSL-4 lab (NEIDL), Former Director of NIH Fogarty Intl. Center, Member National Academy of Medicine. *Lab Biosafety.* **USA, Male**
8. Dato' Sai Kit (Ken) Lam Ph.D., Professor Emeritus Univ Malaya. Discovered Nipah virus, Member Malaysian Academy of Science. *Medical emerging disease virologist.* **Malaysia, Male**
9. Carlos das Neves DVM Ph.D., Director for Research & Internationalization, Norwegian Veterinary Institute, President of International Wildlife Disease Association, Former Hon. Consul of Portuguese Republic in Norway. *One Health*. **Portuguese, Norwegian, Male**
10. Malik Peiris Ph.D. FRS Legion d'honneur, Hong Kong University. Key researcher with deep knowledge of coronaviruses, influenza viruses and Chinese research. *Medical virology.* **Sri Lankan/Hong Kong China, Male**. Alternate: Leo Poon, HKU.
11. Stanley Perlman MD Ph.D., Univ Iowa, Coll Medicine, Rapid Falls – long-time CoV expert, no links to Chinese labs. *Long term Coronavirus virologist.* **USA, Male**
12. Linda Saif Ph.D. – Ohio State Univ, Columbus – Has worked on coronaviruses pre-SARS and was one of the team that inspected the Wuhan lab a few yrs ago from the NAS. Member of US National Academy of Sciences. *Long term Coronavirus research animal models.* **USA, Female**
13. Supaporn Wacharapluesadee Ph.D., WHO Collaborating Ctr, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn University, Bangkok – good virologist, knows the set up in China well. *Virologist.* **Thailand, Female**

Agenda for First meeting

1. Specific non-field research we should do to fill out some of our knowledge gaps:
    - Origins:

        - Background rate of spillover of bat-CoVs, possibility of origin in neighboring countries
    - Early control:
        - Comparison to other outbreak responses over the past 30 yrs (Nipah –COVID-19 timeline)
    - One Health Solutions:
        - Assessment

2. Background consultation we'd like to engage in:
    - Origins
    - Early Control
    - One Health Solutions

3. Membership of sub-teams?
    - Origins
        - Danielle Anderson
        - **Jerry Keusch**
        - Malik Peiris
        - Stanley Perlman
    - Early Control
        - Elizabeth Eckerle
        - **Hume Field**
        - Sai Kit Lam
        - Supaporn Wacharapluesadee
    - One Health Solutions
        - John Amuasi
        - Manish Kakkar
        - **Carlos das Neves**
        - Linda Saif

4. External Communication

5. Timelines

From:	Su Yadana
To:	Stanley; Saif, Linda; spwa_hotmail; amuas001@umn.edu; malik; ECKERLE Isabella; danielle.anderson_duke-nus; das Neves, Carlos Goncalo; Keusch, Gerald T; Hume Field; Prof Lam Sai Kit; Ozge Karadag Caman
Cc:	Alison Andre; Peter Daszak; Aleksei Chmura
Subject:	Meeting notes from Task Force 1st meeting and press release
Date:	Tuesday, November 10, 2020 10:23:56 AM
Attachments:	Meeting Notes 10.29.20 (1st Task Force Meeting- The Lancet Commission).docx

Dear all,
I have attached the meeting minutes from our first meeting including the work plan the group discussed.

The WHO has released Terms of Reference and a report of their initial mission to China to look into the Animal Origins of COVID-19 and it might be of interest to read: https://www.who.int/publications/m/item/who-convened-global-study-of-the-origins-of-sars-cov-2.

We'll also be sending around a draft press release ASAP.

Best,
Su

--

Su Yadana, MPH
Research Scientist

EcoHealth Alliance
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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation.

Meeting Notes
Oct 29th, 2020

***The Lancet* COVID-19 Commission:** Taskforce on the Origins, Early Control of the Pandemic, and One Health Solutions to Future Pandemic Threats

Attendees:
Danielle Anderson,
Isabella Eckerle
Hume Field
Jerry Keusch
Sai Kit Lam
Carlos das Neves
Stanley Perlman
Linda Saif
Su Yadana (Research Scientist)
Supaporn Wacharapluesadee

Apologies:
John Amuasi
Manish Kakkar
Malik Peiris

1. Introductions:

Peter Daszak thanked everyone for volunteering for the Task Force. Everyone introduced themselves, shared their background and expertise. Peter briefly introduced three people who couldn't join the meeting.

Peter highlighted gender-balanced and nationality-balanced nature of this group and highlighted the members' work on broad topics of relevance to the Taskforce's proposed work: biosafety, virology, epidemiology, outbreak investigation, experience in China/SE Asia.

2. Brief Summary of The Lancet COVID-19 Commission and Task Forces (Ozge Karadag Caman)

Dr. Ozge Karadag Caman, who is a member of the Secretariat of the Lancet COVID-19 Commission and a focal point for communications between the Commission and the Task Force, gave a short presentation on The Lancet commission, shared the commission's key aims and topics and the work of other Task Forces. Dr. Caman also highlighted the COVID-19 data portal of the Lancet Commission which is updated daily. The Lancet COVID-19 Commission had their first statement in Sep 14, 2020 and will have a second statement in early 2021 and the third statement on Sep, 2021. All the Task Forces will have their own statements in addition to the Commission's statements.

3. Overview & Goals (Peter Daszak)

Peter Daszak mentioned that this Task Force's first deliverable is a 10-12 page report by Dec 1st, 2020 which will lay out the Task Force's mission and goals, who the members are, what we are planning to do and how we will do it. Peter highlighted that the conversations and the documents shared among members are confidential.

4. Open Discussion on key issues on potential background research

On the origins: Peter referred to the document shared with the members during informal invitations. He pointed out some work of the Task force, particularly the pandemic's origin overlaps with WHO's mission. A WHO team of 2 people has already travelled to China, spoke with Chinese officials and published Terms of Reference for deeper analysis of the virus' "animal origins" (https://www.who.int/publications/m/item/who-convened-global-study-of-the-origins-of-sars-cov-2). There is currently a 2 week quarantine for all foreign visitors to China, and obtaining permission to conduct work on the origin of COVID-19 is sensitive. For this reason, and the fact that the WHO will launch a detailed mission that includes fieldwork in China, the Lancet Commission Task Force is unlikely to need to visit China. Instead, we will aim to interview key contacts who have information from their work on the ground on SARS-CoV-2. We also need to make sure that this Task Force's work has to be different but complementary to WHO-mission on investigating the origin of SARS-CoV-2.

Hume Field brought up challenges with this work, especially because of the politicizing of the topic. There is a blaming for the virus outbreak, which is a great concern. Going forward, we need to work together like we did with previous SARS outbreak.

Peter Daszak commented that an approach we can use to provide useful analysis of the origins could be to examine where the "Preponderance of Evidence" for each hypothesis' validity lies. We can analyze data on what we know, what we don't know and point to gaps in our knowledge that could be filled by future resarch. Our review should be both objective and science based – looking for scientific data that provides evidence for each of the hypotheses on origins..

On the early spread: Jerry Keush mentioned we should put our focus on the preparedness side. What needs to be done in a practical way to enhance future preparedness.

Peter Daszak agreed and added that we could review what happened with covid-19 in the context of what happened in previous outbreak investigations. Was the approach usual, unusual? Were there major gaps compared with, say, the investigations into Nipah, hendra virus, or the emergence of SARS

Isabella Eckerele pointed out we can use MERS example which has been around but didn't cause a pandemic. From syndromic surveillance in Bangladesh, we see that there are MERS cases every year. These things are going on all the time. Scientists know it but the public does not.

Linda Saif said that it is good to give a historical perspective for coronaviruses and put together a timeline on SARS, and MERS discovery. That gives you perspectives on human coronaviruses that have been circling seasonally.

Carlos de Neves agreed and emphasized that laying out a timeline, with evidence and details for each part of the origin and early spread is important to give public data so that 'fake news' and conspiracy theories are put into context.

Jerry Keusch raised the question of who our audience is going to be. If the public is included in the audience, they would need basic background on how the viruses emerge and circulate.

Dani Anderson shared her personal experience of interviews re. her lab work in the Wuhan Institute of Virology from government bodies. Surprisingly, there was a lack of basic understanding of the typical work that happens in a lab, e.g. whether people work with bats in a lab, and dispose of carcasses by selling them at markets. This suggests a key role for our Task Force in helping explain to the public how a typical BSL-3 or -4 lab functions. Likewise the public also has little knowledge that scientific work is based on collaboration and on extensive collaborative work internationally. It highlights that the public will need very basic background knowledge if the work of the Task Force is targeted for the public.

Ozge Caman clarified that the audience is mainly global leaders, policy makers, academics, NGOs and alliances. Main aim is to increase awareness for policy makers and global leaders who don't understand technical aspects for future preparedness for these outbreaks. Task force can still publish their work for the general audience.

Peter Daszak mentioned that it is hard to convince conspiracy theorists since they have a strong belief that there is a secret behind things. But what is possible is to educate people and why this outbreak has occurred. We should do press conferences, talk to reporters and media outlets.

Jerry Keusch shared his own experience with BSL-3 opening in a residential area in Boston that there was a huge push-back. How they got the residents to agree is by talking to the kids and explaining the science and then the kids convinced their parents. So it is crucial that the work of this Task Force be available and accessible for that audience.

Peter Daszak highlighted the need to add a history timeline on what policy measures were taken for the previous SARS outbreak. Hongying Li from EHA wrote a paper on this topic and has a lot of information about it. Maybe she can join one of the meetings in the future and share what she knows.

Stanley Perlman pointed out the timeline from mid-December, 2019 or even November 2019 will be important for early outbreak timeline where hospitals were talking to each other, but it wasn't accepted as an outbreak of a new virus until it got to the higher level. China CDC didn't find out until Dec 30. What animals did they look at? What tissues did they take?

Ozge Caman asked if the Task Force planned to interview infectious disease senior professors to understand their experience with this virus compared to experience with other viruses. Peter Daszak said that speaking to outside infectious disease senior physicians, in addition to existing expertise among this Task Force will be useful, in terms of when we found out human to human transmission.

External communication plan and expectations

Peter Daszak proposed to draft a press release about the Task Force, its members, what we intend to do, what we don't intend to do. We will target a release date of the week of **November 16th 2020**. A

draft release will be shared with all members for edits/comments, and can then be adapted to fit their home institutions. We will plan to release this jointly at the same time.

Ken Lam pointed out that the publicity of the Task Force will also bring outside expertise among the scientific community in getting new information.

Work plan

(i) Peter Daszak to prepare and send the press release statement to members next two weeks (by the week of Nov 8th, 2020)

(ii) Members to email Peter Daszak and cc Su Yadana if they do not want to be involved publicly

(iii) Peter will email members individuals for different tasks that need to be finished before the **Task Force's 2nd meeting - Nov 23rd, 8-10 am EST** (after Day light saving ends, so non-US time-zones will be an hour later than today's meeting time)

(iv) Su Yadana & Peter Daszak will begin work on:

- a detailed timeline of COVID-19 origins, early spread
- list of hypotheses with references
- fleshed out workplan for each section: Origins, early spread, One Health solutions to future pandemics

From: Smriti Mallapaty
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler; Saif, Linda
Subject: RE: nature news request
Date: Sunday, March 1, 2020 6:33:06 PM

Thank you Peter.

Dear Linda,

I'd be interested in speaking more with you on this subject – the latest WHO/China CDC report suggests that more research is needed to identify any intermediate amplification host of the virus, and I wonder if you have any insights on this, or know any researchers studying it.

I am based in Sydney, so your afternoons would be a good time to speak.

Thank you,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Saturday, 29 February 2020 8:59 AM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>; Saif, Linda <saif.2@osu.edu>
Subject: RE: nature news request

Hi Linda,

I'm introducing you to a reporter from Nature who is doing a story on the animal origins of SARS-CoV-2. I mentioned that the pangolin link is likely spurious, i.e. that it's unlikely they were an amplifier of infection at the Wuhan market because they are so rare in the wildlife trade as live animals (mainly dried scales sold for medicine). I also mentioned that one concern is other mammals, e.g. farmed wildlife or pigs could be a potential intermediate or amplifying host because the ACE2 receptors seem able to bind the virus spike protein and because these are a very common animal in and around wildlife and other markets in Wuhan.

Would you be able to comment on this to her? I've cc'd her above and told her you'd be a good independent voice to give an opinion of the possibility that pigs could have played a part.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 [redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Friday, February 28, 2020 3:25 AM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

Just to follow up on this – do you know anyone who is seriously investigating this hypothesis? I would be interested in hearing more on this if any further research developments emerge.

Kind regards,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, 25 February 2020 4:26 AM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: RE: nature news request

Hi Smriti,

The pig idea is based on:

- sequence analysis that shows the pig ACE2 receptor can likely bind with SARS-CoV-2, meaning it could likely infect pigs.
- Live pangolins are extremely rare in markets, so are unlikely to have played a significant role in transmission. Pangolin scales (dried, and therefore unlikely to be able to transmit virus)

are normally sold.

- We still don't know the history of the pangolins that had the CoV with genetic elements close to SARS-CoV-2, and it's possible they were infected during transit from another intermediate host
- One plausible scenario is that there are farms with the virus circulating in a receptive mammal (e.g. a pig) in rural SW or Central China, and that these animals were taken to the wet markets, slaughtered and butchered, enhancing the transmission of the CoV into people.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 [redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Saturday, February 22, 2020 8:25 PM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

I just noticed something in your response and wanted to ask you about it. At the moment, researchers have suggested that pangolins might have been a potential source of the virus spreading to humans. You mentioned pigs. Is there a growing body of research that suggests, or a group of researchers that believe, that it isn't pangolins, but instead pigs?

Thank you,
Smriti

From: Smriti Mallapaty
Sent: Tuesday, 18 February 2020 4:36 PM
To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: Re: nature news request

Thank you again Peter, I just have some follow up questions below from your comments.

Thanks again, and sorry for all the questions!

Kind regards,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, February 18, 2020 4:23 PM
To: Smriti Mallapaty
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

No problem – some answers to your questions below…

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 [redacted]

Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Monday, February 17, 2020 10:42 PM
To: Peter Daszak
Cc: Alison Andre; Aleksei Chmura; Robert Kessler
Subject: RE: nature news request

Dear Peter,

Thank you for your quick response. Can I also ask another question about the infectiousness of the virus?

How does this study help to explain the infectiousness of the virus?
[Peter Daszak] The identification of potential binding sites in the Receptor Binding Domain of the Spike Protein of the virus suggests that it has enhanced ability to bind to human ACE2 (cell surface receptor protein) relative to the nearest known bat-CoV relative. The binding pattern that this virus gene encodes is different to SARS-CoV suggesting it evolved separately (and there may be other binding patterns in other viruses in bats not yet worked out). The ability to efficiently bind ACE2 may explain some of this viruses' capacity to undergo human-to-human transmission (i.e. infectivity), and other aspects of the illness may also help (respiratory infection that causes a lot of mucus, sneezing, etc. assists in other viral infections).
--Could you please elaborate on this point of other aspects of the illness that help to explain how infectious the virus is?
--Have you seen any other studies pointing to what might make this coronavirus so infectious?
--How would you assess the infectiousness of this virus compared to other viruses? One researcher I spoke to said that the cases on the cruise ship suggest that it is very infectious.

What is the significance of the virus acquiring a polybasic cleavage site?
[Peter Daszak] Unfortunately, we don't have detailed analyses of SARS-CoV-2 and related viruses in cell culture or animal models, so we're left with a bit of a gap and the authors rightfully say that the significant is not yet known. However, in avian flu, there are low-pathogenicity and high-pathogenicity strains. The high-path strains are extremely lethal to poultry and have caused high mortality in the low numbers of people infected. One of the key differences between them is that the low path strains don't have the polybasic cleavage site and sequential evolution of the cleavage site leads to enhanced proteolytic activity and higher pathogenicity. The low path strains are only able to infect cell types that have lots of trypsin (which is proteolytic) mainly in respiratory cells and GI tract, but the high path AI strains can affect many different organs. The point is that if this has happened with SARS-CoV-2, it might explain why it acquired an ability to be lethal in people and affect them throughout the lungs. There is some evidence to back this up – when a cleavage site is engineered into SARS-CoV it enhances cell-cell fusion (but not viral entry).

And the two options – sustained human-to-human transmission vs involvement of an intermediate host – could either one help to better explain how infectious the virus is?
[Peter Daszak] Both scenarios would give the virus chance to mutate and adapt, particularly if there is a high density of hosts so that any beneficial mutations to the virus can be transmitted readily and out-compete less efficient mutants. Sustained human-to-human transmission would do this but it would be particularly effective if there was a farmed animal intermediate host – e.g. pigs, which are common and in dense populations. The paper then makes important points about the need to 1) identify these potential sources so that we can rule out further spillover and identify the origins of these mutations; and 2) better understanding of the ACE2 receptors across a wide range of animals – this would help understand the capacity of other bat-CoVs to bind and transmit.
I would add a third issue – given that we have already identified 500 or so CoVs in bats in China and we expect many more – we should also have a concerted effort to identify and fully sequence as many bat-CoVs as possible to 1) assess other potential pathways to RBD-ACE2 binding; and 2) be better able to test candidate vaccines and drugs against a wide range of potentially zoonotic CoVs. Currently we have some candidate vaccines against SARS that we know don't work against other bat CoVs we've discovered. As a public health pandemic prevention strategy, we're feeling around blindly in the dark if we don't identify the diversity of potential viral threats out there in wildlife.

Thank you,
Smriti

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Tuesday, 18 February 2020 1:13 PM
To: Smriti Mallapaty <smriti.mallapaty@nature.com>
Cc: Alison Andre <andre@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Robert Kessler <kessler@ecohealthalliance.org>
Subject: RE: nature news request
Importance: High

Thanks Smriti,

Yes – I read the paper and here are my thoughts:

First, I'm delighted to see an analysis of SARS-CoV-2 sequence data by this group of leading evolutionary virologists. I think the big take home for me is that their analysis supports what many of us working on bat-origin coronaviruses have said, that there's a high diversity of CoVs in bats in southeast Asia (we've identified over 500 in the last few years), and that these animals have frequent and intimate contact with people, livestock and other wildlife in the region. The paper clearly demonstrates a natural origin for SARS-CoV-2 and strongly refutes the theory that this virus was bioengineered. It also provides a strong argument against hypotheses that this virus was an escape from a lab.

The two most likely hypotheses the authors put forward for the acquisition of polybasic cleavage sites are interesting. Re. the potential for sustained human-to-human transmission prior to the

outbreak being noticed – I agree that's possible and it certainly happened with SARS. This may have been happening in a rural site, even as part of the market supply chain – a wildlife hunter, farmer or wildlife trade middleman may have transmitted the virus to people in the Wuhan market as part of trading activities. In support of this, we conducted a small survey in rural Yunnan and Guangxi provinces, S. China a couple of years ago and found 2.93% (6/200) people who live near bat caves in Yunnan to have antibodies to bat coronaviruses (published in *Virologica Sinica*). We don't know which one, or whether this caused any symptoms, but if you look at the human population across the region that *Rhinolophus* spp. bats live in SE Asia, you're looking at a few million people who have likely been exposed in their lifetime, if these numbers hold throughout the region. That's a large interface, and suggests these events are far more common, but that evolution towards a large outbreak is rare – as we'd expect, and as we saw with HIV.

However, I believe the involvement of other animal hosts (so-called 'intermediate' hosts) is even more plausible. Having visited many rural villages, wildlife markets, bat caves, livestock and wildlife farms across South China during the last 15 years, the opportunities for these viruses to spillover across a very active wildlife-livestock-human interface is clear and obvious. There is a booming and lucrative industry breeding wildlife for food, given the scarcity (and often illegal nature) of wild-caught animals. These farms almost invariably stock a diversity of captive-bred wildlife species – civets, porcupines, bamboo rats, coypu, ferret-badgers, raccoon dogs etc., and they're usually mixed in with livestock – pigs, chickens, ducks, geese. And these farms are usually wide open to bats which feed at night above the pens, and some of which roost in the buildings. They are also usually linked to people's houses so that whole families are potentially exposed – and workers who often sleep adjacent to the pens. This is a shocking milieu if you think about it from a viral evolutionary point of view – perfect for a not-quite well-adapted bat CoV to acquire the right mutations to become better at transmission among other mammals, including humans. In support of this hypothesis, Zhou et al. 2020 show that SARS-CoV-2 spike proteins would likely bind to the ACE2 of pigs. We found another bat-CoV (HKU-2, SADS-CoV) causing a die-off of >25,000 pigs in 5 farms in Guangdong province a couple of years ago (published in *Nature*). A scenario I find really likely is that a *Rhinolophus affinis* or related species bat was feeding in a pig farm in rural Hubei or further south and a progenitor virus was transmitted via bat feces to pigs at that farm. These pigs were then butchered and the meat sold, or sold live to one of more markets, which then led to a substantial initial exposure of a number of people, seeding human-to-human transmission in mid- to late-November. The nightmare scenario is that this virus is therefore not only circulating in humans in China, but also, currently unknown to us, in one or a number of pig or wildlife farms in the region. This means that even if the outbreak is controlled, if we don't get to the animal source, we could see repeated seeding of future epidemics through spillover at these farms. That scenario has been discussed at a number of meetings and calls I've been on, including with WHO at the R&D Blueprint Research Agenda-setting meeting and is something that should be investigated.

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Smriti Mallapaty [mailto:smriti.mallapaty@nature.com]
Sent: Monday, February 17, 2020 8:06 PM
To: Peter Daszak
Subject: nature news request

Dear Peter,

I am a reporter for nature news, covering the coronavirus.

I assume you have seen this preprint recently posted online: http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

I wanted to know if you had any thoughts on the research and the significance of the findings? I have included a few key points below.

It talks about a cleavage site that is a unique feature of SARS-COV-2. The papers says 'the functional consequence of the polybasic cleavage site in SARS-CoV-2 is unknown' but then goes on to describe how similar events in other coronavirus have been linked to a virus going from low to high pathogenicity. ***Acquisition of a polybasic cleavage site in HA, by either insertion or recombination, converts low pathogenicity avian influenza viruses into highly pathogenic forms***

The paper also considers whether this and other mutations happened in an intermediary animal before the spillover, or after in humans. If it happened in animals then > ***if SARS-CoV-2 pre-adapted in another animal species then we are at risk of future re-emergence events even if the current epidemic is controlled***. If it happened in humans then > if the adaptive process we describe occurred in humans, then even if we have repeated zoonotic transfers they are unlikely to take-off unless the same series of mutations occurs.

Thank you again,
Smriti

Smriti Mallapaty
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From:	Saif, Linda
To:	Peter Daszak
Subject:	Re: Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"
Date:	Thursday, October 29, 2020 12:55:57 PM
Attachments:	Origin SARS-CoV-2 AJTM 2020 tpmd200849.pdf

Hi Peter,
Good meeting today and I look forward to working with you and the team on this important task.
I want to strongly recommend one more person whose experience and expertise would greatly contribute to this Taskforce and also its impact. This is Dr James Le Duc (jwleduc@UTMB.EDU). He is the director of the BSL4 Galveston National Laboratory, professor, Microbiology and Immunology and the John Sealy Distinguished Chair in Tropical and Emerging Virology, University of Texas Medical Branch, Galveston Texas. Dr. Le Duc joined UTMB in late 2006 from the Centers for Disease Control and Prevention in Atlanta, where he was the influenza coordinator and director of the Division of Viral and Rickettsial Diseases. With more than four decades of experience working in the fields of biodefense and public health, his work has taken him around the world, from West Africa, where he began his professional career as a field biologist working for the Smithsonian Institution, to Brazil and Panama during a 23-year career as a U.S. Army officer in the medical research and development command.

You too have interacted with him during our NAS talks with Chinese scientists—he always provides an astute summary at the end. He went with the NAS group on all our biosecurity visits to China to tour the BSL4 labs in Wuhan and Harbin, so he too has extensive first hand knowledge about BSL4 labs and the Chinese BSL4 labs and their operation. In his interactions with our Chinese counterparts, he was always very calm, well-spoken and perceptive about the issues involved and how to frame the key questions. He is also very familiar with media interviews and does a great job with interviews, as well as having experience in working in a government agency, the CDC. He contributed to the attached highly relevant paper that was very perceptive and may help to frame our report as well.
I strongly suggest inviting him to be a member of our taskforce. He would be a major contributor based on his extensive background on international emerging diseases and provide additional background and perspectives that are not present within current members of our working team.
Thanks
Linda

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Wednesday, October 21, 2020 at 12:27 AM
To: linda saif <saif.2@osu.edu>
Cc: Su Yadana <yadana@ecohealthalliance.org>
Subject: RE: Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"

Great to know that you'll be able to take part in this Linda! I'm cc'ing Su Yadana, who works at EHA

and will be the point of contact for the taskforce. She'll organize a formal invitation from the COVID-19 Commission Chair, Jeff Sachs.

I'll send another email re. SADS, cc'ing Hongying…

Cheers,

Peter

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

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From: Saif, Linda <saif.2@osu.edu>
Sent: Thursday, October 15, 2020 10:42 PM
To: Peter Daszak <daszak@ecohealthalliance.org>
Subject: Re: Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"
Importance: High

Hi Peter,
Thanks for your invitation. The topic is very timely and it will be an honor to serve on the taskforce. I hope that we can talk about both zoonoses and reverse zoonoses regarding COVID-19 or other emerging diseases ! I also hope that this does not once again become totally politicized (origin of SARS-CoV-2) as we have all experienced before! Did you hear any report from the WHO task force to find the animal reservoir in China?

It will be a pleasure to work with you on these important topics!

I have gotten a lot of questions since Ralph's PNAS paper on SADS in human cells about its impact on swine and pork production and possible spillover to humans.
Can you please clarify several points for me for which I am unsure.

1. Is there information on whether this virus is still circulating in swine in S China—any further outbreaks or serology studies in swine?

   Or were most of the swine herds culled because of ASFV so that SADS could have been eliminated?

2. Was there ever any serology or evidence for SADS infections in humans in the region of the original SADS outbreaks?

Thanks,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave Wooster, Ohio 44691

CENTER TO STOP COVID

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Monday, October 12, 2020 11:50 PM
To: Linda Saif <saif.2@osu.edu>
Cc: Su Yadana <yadana@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>
Subject: Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"

Dear Linda,

I hope all's well with you and that you're staying healthy and busy during these difficult times.

I've been asked to join the Lancet COVID-19 Commission and run a 12 month Taskforce to conduct a review of the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats". The Lancet COVID-19 Commission has been created to help speed up global, equitable, and lasting solutions to the pandemic. Two key aims of this Commission are (i) to speed up the awareness and adoption worldwide of successful strategies to suppress transmission and (ii) to ensure that any new COVID-19 vaccines and other key technologies are equitably accessible across the world. There is information on the membership and details of the goals on its main website: https://covid19commission.org/, and also in an article recently published in *The Lancet*:

https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)31494-X.pdf

The goal of the taskforces are to focus on specific dimensions of the pandemic, review all available information and provide a 'state-of-the-art' assessment of an issue and point to future directions, including intergovernmental policy initiatives, research gaps, regional and national policies and other issues that might benefit global health and equity.

I would very much like you to serve on the taskforce that I'll be running. I've attached a brief summary of the goals and workplan, and names of others who've been invited. Please consider this an informal request at the moment – if you indicate your willingness, I'll send a formal invitation co-signed by Commission Chair, Jeff Sachs.

Please note that if you are willing to serve, your involvement will be voluntary and the time commitment will be until September, 2021 when the final report is due. There may be a possibility of on-the-ground work if travel allows and the cost will be covered by the Commission, but given the timeline and the continued disruption of travel by COVID-19, I believe this is unlikely. The taskforce is expected to meet via monthly Zoom calls. Our first meeting will be in October and the first draft report is aimed for Dec, 2020. Please also note that I'm cc'ing Su Yadana, who will be the point person for running the workings of the taskforce here at EHA, as well as my assistant Alison Andre. Please cc both of them on all correspondence.

I'm confident that the taskforce will do significant and meaningful work towards understanding and providing lasting solutions to the pandemic and that your expertise and involvement will strengthen its efforts.

I really hope that you will accept my invitation to join and will then be able to set up dates for our first call.

Cheers,

Peter

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

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Perspective Piece

The Origin of COVID-19 and Why It Matters

David M. Morens,[1,2*] Joel G. Breman,[3] Charles H. Calisher,[4] Peter C. Doherty,[5] Beatrice H. Hahn,[6,7] Gerald T. Keusch,[8,9,10] Laura D. Kramer,[11,12] James W. LeDuc,[13] Thomas P. Monath,[3,14] and Jeffery K. Taubenberger[15]

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Abstract. The COVID-19 pandemic is among the deadliest infectious diseases to have emerged in recent history. As with all past pandemics, the specific mechanism of its emergence in humans remains unknown. Nevertheless, a large body of virologic, epidemiologic, veterinary, and ecologic data establishes that the new virus, SARS-CoV-2, evolved directly or indirectly from a β-coronavirus in the sarbecovirus (SARS-like virus) group that naturally infect bats and pangolins in Asia and Southeast Asia. Scientists have warned for decades that such sarbecoviruses are poised to emerge again and again, identified risk factors, and argued for enhanced pandemic prevention and control efforts. Unfortunately, few such preventive actions were taken resulting in the latest coronavirus emergence detected in late 2019 which quickly spread pandemically. The risk of similar coronavirus outbreaks in the future remains high. In addition to controlling the COVID-19 pandemic, we must undertake vigorous scientific, public health, and societal actions, including significantly increased funding for basic and applied research addressing disease emergence, to prevent this tragic history from repeating itself.

In 2007, scientists studying coronaviruses warned: "The presence of a large reservoir of SARS-CoV–like viruses in horseshoe bats... is a time bomb. The possibility of the re-emergence of SARS and other novel viruses... should not be ignored."[1]

Few paid attention following the disappearance of SARS after the initial outbreak in 2002. Now, 18 years later, COVID-19 has emerged as the deadliest respiratory disease pandemic since 1918, when the "Spanish" influenza pandemic killed an estimated 50 million people.[2] We need to understand what happened so that we can prevent it from happening again, and be better prepared to contain similar pandemics at their outsets.

EMERGENCE OF THE COVID-19 PANDEMIC

The agent of COVID-19, SARS-CoV-2, was named after the genetically related SARS-CoV (more recently distinguished by some as SARS-CoV-1), which caused a deadly near-pandemic in 2002–2003.[3] Before 2019, neither SARS-CoV-2 nor its genetic sequences had ever been identified in viruses of humans or animals.

Even so, scientific research conducted over the last two decades provides clues about how and why the COVID-19 pandemic appeared. We must understand these critically important scientific findings, described in the following text, so that we can better address significant existential risks we will continue to face for the foreseeable future.

* Address correspondence to David M. Morens, Room 7A-03, Building 31, 31 Center Drive, Bethesda, Maryland 20892-2520. Email: dm270q@nih.gov

HOW VIRAL DISEASES EMERGE

Viruses are compact nucleic acid packages of either DNA or (in the case of coronaviruses) RNA associated with proteins, and in some cases with lipids. Viruses are not living organisms and can only reproduce inside living cells susceptible to viral entry and with the capacity to replicate viral nucleic acids and translate nucleic acid signals into amino acids to build viral proteins. Viruses are therefore nonliving self-contained genetic programs capable of redirecting a cell's machinery to produce more of themselves.

It follows that when a virus enters a human cell for the first time, it has very recently been transmitted from cells of some other host, that is, from another animal or, for example, an insect vector. Emergence of a pathogen between a vertebrate or an insect has been referred to as host-switching, sometimes described as a spillover event. Most of the human viral and nonviral infectious diseases that have existed for centuries—measles, influenza, cholera, smallpox (eradicated in 1980), falciparum malaria,[4] dengue, HIV, and many others—originated by animal-to-human host-switching.[5] The complex genetic events that underlie host-switching differ greatly from pathogen to pathogen, but general mechanisms have been recognized for many.[6–9]

Host-switching determinants prominently include social, environmental, and biological factors providing the opportunity for host–species interaction; shared host cell receptors; genetic distance between transmitting and receiving hosts; and characteristics and complexity of the viral quasi-species or viral swarm. (RNA viruses in particular are not transmitted to multiple cells as identical virions, but as collections of thousands of different genetically related virions. The ever-changing complexity of the viral swarm varies among species, genetically distinct but related individuals of the same species, and in single hosts over time.)

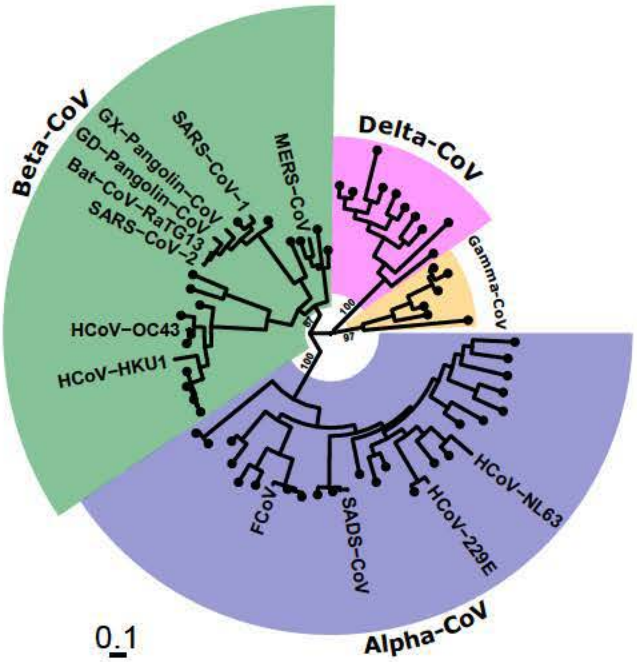

FIGURE 1. Phylogenetic relationships of selected coronaviruses of medical and veterinary importance. Human SARS-CoV and SARS-CoV-2 are closely related to numerous bat and pangolin coronaviruses in a viral genetic grouping called sarbecoviruses, which contains many other viruses very closely related to SARS-CoV and SARS-CoV-2. These viruses belong to the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae* and the four genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. The betacoronaviruses are comprised of two subgenera, *Sarbecovirus* and *Merbecovirus*. The former include SARS-CoV and SARS-CoV-2; the latter includes Middle East respiratory syndrome-related coronavirus (MERS-CoV). Image created by Sebastian M. Gygli, Ph.D., NIAID, NIH, and used with permission.

Studying animal viruses that have previously spilled over into humans provides clues about host-switching determinants. A well-understood example is influenza virus emergence into humans and other mammals.[2] Human pandemic and seasonal influenza viruses arise from enzootic viruses of wild waterfowl and shore birds. From within this natural reservoir, the 1918 pandemic "founder" virus somehow host-switched into humans. We know this from genetic studies comparing avian viruses, the 1918 virus, and its descendants, which have caused three subsequent pandemics, as well as annual seasonal influenza in each of the 102 years since 1918. Similarly, other avian influenza viruses have host-switched into horses, dogs, pigs, seals, and other vertebrates, with as yet unknown pandemic potential.[2,10,11] Although some molecular host-switching events remain unobserved, phylogenetic analyses of influenza viruses allow us to readily characterize evolution and host-switching as it occurs in nature.[2]

CORONAVIRUSES

Coronaviruses are RNA viruses globally distributed in a large but unknown number of animal species. Coronaviruses important for humans are found within phylogenetically distinct taxonomic subgroups, labeled as the α- and β-coronaviruses (Figure 1).[12] Four endemic human coronaviruses, which emerged at some undetermined time in the past, cause (mostly) mild self-limited upper respiratory tract infections (Figure 1).

RECENT CORONAVIRUS EMERGENCES FROM ANIMALS INTO HUMANS

Until recently, relatively little was known about coronaviruses, and research interest in these common cold viruses was minimal. Eighteen years ago, a previously unknown β-coronavirus named SARS-CoV suddenly emerged. Following its initial appearance in China it spread to 29 other countries, causing a near-pandemic and killing 813 of the 8,809 people with confirmed infection before being controlled by aggressive public health measures. It has not been seen since. In 2012, however, another previously unknown β-coronavirus named Middle East respiratory syndrome coronavirus (MERS-CoV), and closely related to SARS-CoV, emerged to cause high case-fatality human infections. Fortunately, this virus does not efficiently transmit between humans, and cases have been largely limited to the Middle East where its intermediary host, the dromedary camel, is present in relatively high numbers. In 2016, yet another novel bat-origin coronavirus, an α-coronavirus, emerged in China to cause a novel epizootic disease in pigs, termed swine acute diarrhea syndrome coronavirus (SADS-CoV). And most recently, at least as early as late November 2019, SARS-CoV-2 was recognized and became the third fatal bat virus–associated human disease

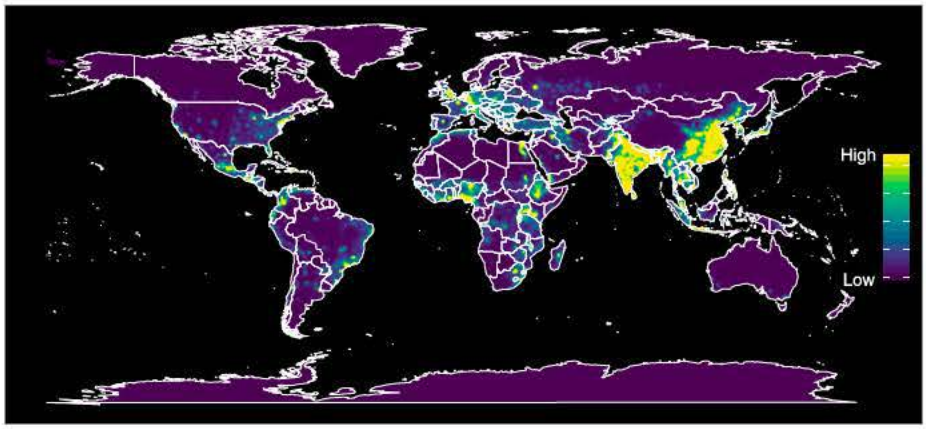

FIGURE 2. Predicted global hotspots for disease emergence, showing estimated risks, adjusted for reporting bias. From a comprehensive global study combining multiple data sources. Reproduced with permission from Allen et al.[14]

emergence and the fourth bat virus–associated mammalian emergence in 18 years.

CORONAVIRUS EMERGENCE RISKS

An enormous reservoir of coronaviruses infects hundreds of bat species distributed globally. SARS-CoV, MERS-CoV, and SARS-CoV-2 are closely related β-coronaviruses clustering in two adjacent phylogenetic groupings: sarbecovirus (SARS-like viruses) and merbecovirus (MERS-like viruses) (Figure 1). The two SARS viruses, as well as SADS-CoV, are descended from viruses enzootic in rhinolophid (genus, *Rhinolophus*), or horseshoe bats.

Over the past 15 years, scientists have also identified global animal reservoirs of coronaviruses (in Africa, the Americas, the Middle East, Asia and Southeast Asia, and particularly China, the location of three of the four most recent emergences). These efforts have revealed much about coronaviral ecosystems, reservoir hosts, viral movement between hosts, viral evolution, and risk of emergence into humans and other mammals.

Bats of numerous globally distributed genera and species are now known to be the major reservoir of animal coronaviruses. One 20-country study of more than 19,000 animals (predominantly nonhuman primates, bats, and rodents) revealed that bats accounted for more than 98% of coronavirus detections, and that almost 9% of > 12,000 randomly studied bats were infected with one or more coronavirus.[13] Significant interspecies viral transmission between closely and distantly related bats also appears to be important. Bats of some species, including rhinolophids, co-roost with bats of other species, facilitating viral exchanges and enhanced viral evolution associated with genetic recombination. In fact, many such bat coronaviruses have genetic sequences similar to SARS-CoV and SARS-CoV-2.

Investigators have also mapped global hotspots for potential infection emergence, prominently in south/southwest China and contiguous regions and countries (Figure 2),[14] and have identified numerous human–animal interactions that constitute emergence risk factors, for example bat tourism, wet markets, wildlife supply chains for human consumption,[15] land management practices, and environmental perturbations.[16–18] Virologic and risk mapping studies indicate a very high risk of further coronavirus outbreaks.[19–21]

SARS-CoV and SARS-CoV-2 emerged in China, home to bats of more than 100 species, many of which carry α- and/or β-coronaviruses. In one study, more than 780 partial coronavirus genetic sequences were identified from bats of 41 species infected by α- and of 31 species infected by β-coronaviruses.[21] Within the sarbecovirus lineage, encompassing SARS and SARS-like viruses, many identified genetic sequences are very similar to SARS-CoV and SARS-CoV-2.[21–23] One such virus is more than 96% identical to SARS-CoV-2 in its whole genome[23]; another shares more than 97% identity in the 1ab replicase gene, as well as a furin cleavage site insertion.[24] Nature is clearly a cauldron for intense and dangerous coronavirus evolution.

WAS COVID-19 PREDICTED?

A clearer, more worrisome picture of the coronavirus ecosystem has recently come together. A contiguous area encompassing parts of south/southwest China, Laos, Myanmar, and Vietnam constitutes a bat coronavirus "hotspot," featuring intense interspecies viral transmission. In such hotspots, a rich diversity of SARS-like viruses has been found, not only in rhinolophid bats but also in bats of other genera and species to which these viruses had host-switched. The same rhinolophid bats are also implicated in the emergence of SADS-CoV in southern China. Many of these SARS-like viruses bind to human angiotensin-converting enzyme-2 (ACE2) receptors and infect human respiratory epithelial cells in vitro, suggesting their pandemic potential.[19,25]

Ominously, bat-to-human transmission of SARS-like viruses has already been detected,[20] perhaps representing pandemic near-misses. Even the more genetically distant SADS-CoV infects cells of humans and numerous other vertebrates, raising concern about indirect coronavirus emergences. This seems to have occurred with the bat-to-camel-to-human emergence of MERS, and possibly with SARS-CoV emergence into humans, which may have resulted from bat virus infection of masked palm civet cats (*Paguma larvata*), with subsequent human spillover.[12] As a byproduct of the important international surveillance work described above, in 2017, the therapeutic benefit of the antiviral drug remdesivir was suggested; it is now, in 2020, being widely used to treat persons infected with SARS-CoV-2.[26]

Since 2007, when alarming predictions about threatened coronavirus emergences began to appear,[1] understanding of coronavirus ecosystems has become far more complete. Over the past 5 years, Chinese, American, European, and other scientists have begun to renew warnings that humans are intensively interacting with coronavirus-infected bats, that enzootic SARS-related bat coronaviruses have all of the essential components of the SARS virus, that some of these SARS-like viruses can infect laboratory-humanized mice to cause SARS-like disease, that SARS-like viruses have the ability to directly infect and be transmitted between humans, and, therefore, that these viruses are poised for human emergence.[19,21,22] Many scientists have proposed aggressive monitoring of known hotspots to try to predict and prevent viral emergence that might impact human health, including early warning of host-switching events.[19,20,27]

Unfortunately, outside of some members of the scientific community, there has been little interest and no sense of urgency. In 2020, we learned, tragically, what 12 years of unheeded warnings have led to: a bat-derived sarbecovirus—from the very same SARS-like bat virus group that had been warned about by multiple voices for over a decade—emerged and proceeded to cause the COVID-19 pandemic that now sweeps the globe.

SARS-CoV-2 emerged essentially as predicted: a natural event associated with either direct transmission of a bat coronavirus to humans or indirect transmission to humans via an intermediate host such as a Malaysian pangolin (*Manis javanica*) or another, yet-to-be-identified mammal.[28–31]

It should be clarified that theories about a hypothetical man-made origin of SARS-CoV-2 have been thoroughly discredited by multiple coronavirus experts.[21,28,29] SARS-CoV-2 contains neither the genetic fingerprints of any of the reverse genetics systems that have been used to engineer coronaviruses nor does it contain genetic sequences that would have been "forward engineered" from preexisting viruses, including the genetically closest sarbecoviruses. That is, SARS-CoV-2 is unlike any previously identified coronavirus from which it could have been engineered. Moreover, the SARS-CoV-2

receptor-binding domain, which has affinity for cells of various mammals, binds to human ACE2 receptors via a novel mechanism.

Engineering such a virus would have required 1) published or otherwise available scientific knowledge that did not exist until after COVID-19 recognition; 2) a failure to follow obvious engineering pathways, resulting in an imperfectly constructed virus; and 3) an ability to genetically engineer a new virus without leaving fingerprints of the engineering. Furthermore, the 12 amino acid furin-cleavage site insertion between the SARS-CoV-2 spike protein's S1 and S2 domains, which some have alleged to be a sign of genetic engineering, is found in other bat and human coronaviruses in nature, probably arising via naturally occurring recombination.[24]

It is also highly unlikely that SARS-CoV-2 was released from a laboratory by accident because no laboratory had the virus nor did its genetic sequence exist in any sequence database before its initial GenBank deposition (early January 2020). China's laboratory safety practices, policies, training, and engineering are equivalent to those of the United States and other developed countries,[32] making viral "escape" extremely unlikely, and of course impossible without a viral isolate present. SARS-CoV-2 shares genetic properties with many other sarbecoviruses, lies fully within their genetic cluster, and is thus a virus that emerged naturally.

COVID-19 EMERGENCE MECHANISMS: WHY THEY MATTER

Understanding how COVID-19 emerged is of great importance. We now know that the viruses causing SARS, MERS, and COVID-19 are all members of enormous groups of bat coronaviruses distributed globally, and that many of these viruses are functionally preadapted to human emergence. This preadaptation can be thought of as "accidental" because it must have occurred in nature in the absence of human infection and does not rule out further human adaptation to enable pandemicity. Molecular mechanisms of preadaptation are not fully known, but are undoubtedly related to functional similarities between ACE2 receptors on the cells of numerous mammals (bats, humans, minks, cats, and other domestic and wild animals).[33,34]

The ability of coronaviruses to evolve at a high rate, illustrated by extreme phylogenetic diversity, coupled with the dispersion of new viral variants within an enormous array of wild animal species that can serve as hosts, portends poorly for the future of coronavirus disease emergence. We are already seeing coronavirus mutants with altered affinity for human ACE2. Whether bat coronaviruses evolve independently or by "sampling" various mammalian ACE2 receptors, the result is the same. That bat sarbecoviruses so easily switch between multiple hosts suggests a many-pronged human risk: directly from bats and indirectly from other mammals infected by bat viruses. Because we have only just begun to sample, sequence, and study bat/mammalian coronaviruses, we can be certain that what we now know is but the tip of a very large iceberg.

The findings described earlier reaffirm what has long been obvious: that future coronavirus transmissions into humans are not only possible, but likely. Scientists knew this years ago and raised appropriate alarm. Our prolonged deafness now exacts a tragic price.

The story of COVID-19 emergence sends a powerful message. A quantum leap in bat coronavirus surveillance and research is urgently needed. This work must emphasize virologic and behavioral field studies of humans and animals wherever they interface, and especially in disease hotspots, as well as virologic studies related to human and animal spillover risks and the means of reducing them.[35]

Important research that has languished, been underfunded, or discontinued should be greatly expanded to deal with the urgency of the situation, and more scientists, including scientists working in China and other hotspot countries (Figure 2), should be recruited to these efforts, especially in international research partnerships. Full, open international collaboration involving many countries is essential. In particular, field research on the prevalence and virus-host relationships of coronaviruses, development of platform technologies for diagnostics, vaccines, and animal models for studies of pathogenesis and potential therapeutics is essential to permit, for example, modeling structure/function relationships of specific binding domains from newly identified agents to create critical tools for disease control.

In addition to robust expansion of surveillance and research, there are things that we can do now to lower our risks. We know much about coronavirus hotspots, not only in China but also globally; we can more aggressively surveil these locations to learn more about the local viral ecology and identify initial human spillover events. We also know much about human behaviors that directly and indirectly bring us into contact with bats, including risks from wet markets, bat cave tourism, capturing and eating bats, and perturbing the environment in ways that alter bat habitats and habits. These are behaviors that we can and must change.

We can also strengthen basic public health, including hygiene and sanitation, so that emerging viruses do not have a fertile field in which to amplify replication, and we must build and maintain strong public health infrastructure to respond quickly and efficiently to pathogen emergence. For viruses that have emerged, such as SARS-CoV-2, we need to develop effective antivirals and, ideally, broadly protective vaccines. Education and communication with populations where spillover events occur is also an important component of risk reduction.

We must also realize that the problem is larger than just coronaviruses. In recent years, we have seen emergences and reemergences of numerous other human infectious diseases such as Ebola fever, Lassa fever, hantavirus pulmonary syndrome, human monkeypox, HIV, dengue, chikungunya, Zika, and epizootic avian influenza. We have entered a new pandemic era,[36] one in which epidemic and pandemic emergences are becoming commonplace; some are likely to be highly pathogenic. In 2020, our science is sufficiently robust to have a good chance of controlling pandemic viral emergences within 2–3 years, but dramatically insufficient to prevent and control their emergences in the first place.

We should begin developing broadly protective vaccines and broadly therapeutic antiviral/antimicrobial agents against pathogens within taxonomic groups likely to emerge in the future, including coronaviruses, henipaviruses, and filoviruses, among others. Organizations like the Coalition for Epidemic Preparedness Innovations, among others, should be extended and strengthened, emphasizing, in addition to vaccine development, therapeutics as well as prevention tools. Pandemic prevention should be a global effort on a par with chemical and nuclear weapon prevention.

Unless we reset the equation; invest more in critical and creative laboratory, field, and behavioral research; and start finding

ways to prevent these emergences, we will soon see additional coronavirus pandemics, as well as global spread of other types of infectious agents not yet imagined, caused by some of the millions of viruses in the natural world, many of which we have not yet had the time and funding to identify and study.[27]

Understanding how COVID-19 emerged is a critical point on a steep learning curve we must quickly master. As we face the mounting deaths and societal upheavals of the COVID-19 pandemic, we must not lose sight of how this pandemic began, how and why we missed the warning signs, and what we can do to prevent it from happening again—and again.

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Disclosure: The views in this article are those of the authors and not of their institutions, or the NIAID, NIH, DHHS.

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REFERENCES

1. Cheng VCC, Lau SKP, Woo PCY, Yuen KY, 2007. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clin Microbiol Rev 20:* 660–694.
2. Taubenberger JK, Kash JC, Morens DM, 2019. The 1918 influenza pandemic: 100 years of questions answered and unanswered. *Sci Transl Med 11:* eeaau5485.
3. Ksiazek TG et al., 2003. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med 348:* 1953–1966.
4. Sharp PM, Plenderleith LJ, Hahn BH, 2020. Ape origins of human malaria. *Ann Rev Microbiol 74:* 39–63.
5. Morens DM, Folkers GK, Fauci AS, 2008. Emerging infections: a perpetual challenge. *Lancet Infect Dis 8:* 710–719.
6. Culliton BJ, 1990. Emerging viruses, emerging threat. *Science 247:* 279–280.
7. Kuiken T, Holmes EC, McCauley J, Rimmelzwaan GF, Williams CS, Grenfell BT, 2006. Host species barriers to influenza virus infections. *Science 312:* 394–397.
8. Parrish CR, Holmes EC, Morens DM, Park E-C, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P, 2008. Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiol Mol Biol Rev 72:* 457–470.
9. Geoghegan JL, Holmes EC, 2018. Evolutionary virology at 40. *Genetics 210:* 1151–1162.
10. Morens DM, Taubenberger JK, 2011. Pandemic influenza: certain uncertainties. *Rev Med Virol 21:* 262–284.
11. Sun H et al., 2020. Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection. *Proc Natl Acad Sci U S A*, doi: 10.1073/pnas.1921186117.
12. Corman VM, Muth D, Niemeyer D, Drosten C, 2018. Hosts and sources of endemic human coronaviruses. *Adv Virus Res 100:* 163–188.
13. Anthony SJ et al., 2017. Global patterns in coronavirus diversity. *Virus Evol 3:* vex012.
14. Allen T, Murray KA, Zambtana-Torrelio C, Morse SS, Rondinini C, Marco MD, Breit N, Olival NJ, Daszak P, 2017. Global hotspots and correlates of emerging zoonotic diseases. *Nat Comm 8:* 1124.
15. Huong NQ et al., 2020. Coronavirus testing indicates transmission risk along wildlife supply chains for human consumption in Viet Nam, 2013–2014. bioRxiv, doi: 10.1101/2020.06.05.098590.
16. Li H et al., 2019. Human-animal interactions and bat coronavirus spillover potential among rural residents in Southern China. *Biosaf Health 1:* 84–90.
17. Monagin C et al., 2018. Serologic and behavioral risk survey of workers with wildlife contact in China. *PLoS One 13:* e0194647.
18. Li H-Y et al., 2020. A qualitative study of zoonotic risk factors among rural communities in southern China. *Int Health 12:* 77–85.
19. Hu B et al., 2017. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog 13:* e1006698.
20. Wang N et al., 2018. Serological evidence of bat SARS-related coronavirus infection in humans, China. *Virol Sin 33:* 104–107.
21. Latinne A et al., 2020. Origin and cross-species transmission of bat coronaviruses in China. *Nat Commun* (In press).
22. Menachery VD et al., 2016. SARS-like WIV1-CoV poised for human emergence. *Proc Natl Acad Sci U S A 113:* 3048–3053.
23. Zhou P et al., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature 579:* 270–2734.
24. Zhou H et al., 2020. A novel bat coronavirus reveals natural insertions at the S1/S2 cleavage site of the Spike protein and a possible recombinant origin of HCoV-19. bioRxiv, doi: 10.1101/2020.03.02.974139.
25. Ge XY et al., 2013. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature 503:* 535–538.
26. Sheahan TP et al., 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med 9:* eaal3653.
27. Carroll D, Daszak P, Wolfe ND, Gao GF, Morel CM, Morzaria S, Pablos-Méndez A, Tomori O, Mazet JAK, 2018. The Global Virome Project. *Science 359:* 872–974.
28. Anderson KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF, 2020. The proximal origin of SARS-CoV-2. *Nat Med 26:* 450–452.
29. Zhang Y-Z, Holmes EC, 2020. A genomic perspective on the origin and emergence of SARS-CoV-2. *Cell 181:* 223–226.
30. Lu R et al., 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet 395:* 565–574.
31. Li X, Giorgi EE, Marichann MH, Foley B, Xiao C, Kong X-P, Chen Y, Krober B, Gao F, 2020. Emergence of SARS-CoV-2 through recombination and strong purifying selection. *Sci Adv 6:* eabb9153.
32. Xia H, Huang Y, Ma H, Liu B, Xie W, Song D, Yuan Z, 2019. Biosafety level 4 laboratory user training program, China. *Emerg Infect Dis 25:* e180220.
33. Oreshkova N et al., 2020. SARS-CoV2 infection in farmed mink, Netherlands. *Euro Surveill 25:* pii 2001005.
34. Halfman PJ et al., 2020. Transmission of SARS-CoV-2 in domestic cats. *N Engl J Med*, doi: 10.1056/NEJMc2013400.
35. Letko M, Seifert SN, Olival KJ, Plowright RK, Munster VJ, 2020. Bat-borne virus diversity, spillover, and emergence. *Nat Rev Microbiol 18:* 461–471.
36. Morens DM, Daszak P, Markel H, Taubenberger JK, 2020. Pandemic COVID-19 joins history's pandemic legion. *mBio 11:* e00812-20.

From: Su Yadana
To: Saif, Linda
Cc: Alison Andre; Peter Daszak
Subject: Re: Fw: Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"
Date: Tuesday, October 20, 2020 4:53:55 PM
Attachments: 9A460A58-FDD9-490E-B5AC-7CD0FD5C7C51[43].png

Dear Dr. Saif,
I will be helping coordinate this Task Force for Dr. Daszak. Would you be available to join the first Task Force meeting in the morning of Oct 29th (Thursday) from 8-10am (EST)? For the second meeting in November, we are tentatively scheduling it for November 23rd either from 8:30 to 10AM or 9 to 10:30AM (EST) but I will send out another email with the set time in November.
Looking forward to your response.

Best,
Su

From: Saif, Linda <saif.2@osu.edu>
Sent: Thursday, October 15, 2020 10:41 PM
To: Peter Daszak
Subject: Re: Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"

Hi Peter,
Thanks for your invitation. The topic is very timely and it will be an honor to serve on the taskforce. I hope that we can talk about both zoonoses and reverse zoonoses regarding COVID-19 or other emerging diseases ! I also hope that this does not once again become totally politicized (origin of SARS-CoV-2) as we have all experienced before! Did you hear any report from the WHO task force to find the animal reservoir in China?
It will be a pleasure to work with you on these important topics!

I have gotten a lot of questions since Ralph's PNAS paper on SADS in human cells about its impact on swine and pork production and possible spillover to humans.
Can you please clarify several points for me for which I am unsure.

1. Is there information on whether this virus is still circulating in swine in S China—any further outbreaks or serology studies in swine?

   Or were most of the swine herds culled because of ASFV so that SADS could have been eliminated?
2. Was there ever any serology or evidence for SADS infections in humans in the region of

the original SADS outbreaks?

Thanks,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave Wooster, Ohio 44691

CENTER TO STOP COVID

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Monday, October 12, 2020 11:50 PM
To: Linda Saif <saif.2@osu.edu>
Cc: Su Yadana <yadana@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>
Subject: Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"

Dear Linda,

I hope all's well with you and that you're staying healthy and busy during these difficult times.

I've been asked to join the Lancet COVID-19 Commission and run a 12 month Taskforce to conduct a review of the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats". The Lancet COVID-19 Commission has been created to help speed up global, equitable, and lasting solutions to the pandemic. Two key aims of this Commission are (i) to speed up the awareness and adoption worldwide of successful strategies to suppress transmission and (ii) to ensure that any new COVID-19 vaccines and other key technologies are equitably accessible across the world. There is information on the membership and details of the goals on its main website: https://covid19commission.org/, and also in an article recently published in *The Lancet*: https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)31494-X.pdf

The goal of the taskforces are to focus on specific dimensions of the pandemic, review all available information and provide a 'state-of-the-art' assessment of an issue and point to future directions, including intergovernmental policy initiatives, research gaps, regional and national policies and other issues that might benefit global health and equity.

I would very much like you to serve on the taskforce that I'll be running. I've attached a brief summary of the goals and workplan, and names of others who've been invited. Please consider this an informal request at the moment – if you indicate your willingness, I'll send a formal invitation co-signed by Commission Chair, Jeff Sachs.

Please note that if you are willing to serve, your involvement will be voluntary and the time commitment will be until September, 2021 when the final report is due. There may be a possibility of on-the-ground work if travel allows and the cost will be covered by the Commission, but given the timeline and the continued disruption of travel by COVID-19, I believe this is unlikely. The taskforce is expected to meet via monthly Zoom calls. Our first meeting will be in October and the first draft report is aimed for Dec, 2020. Please also note that I'm cc'ing Su Yadana, who will be the point person for running the workings of the taskforce here at EHA, as well as my assistant Alison Andre. Please cc both of them on all correspondence.

I'm confident that the taskforce will do significant and meaningful work towards understanding and providing lasting solutions to the pandemic and that your expertise and involvement will strengthen its efforts.

I really hope that you will accept my invitation to join and will then be able to set up dates for our first call.

Cheers,

Peter

Peter Daszak

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

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From:	Rusek, Benjamin
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Subject:	RE: Some bullets following our US-China dialogue discussion on Friday
Date:	Monday, October 19, 2020 11:57:58 PM
Attachments:	3-month follow-up-JP Weng.pdf

Greetings,

Thanks again for participating in the China bio dialogue sessions last week. And thank you Peter and others who sent me feedback and thoughts on the future of the dialogue. Additional thoughts and comments are welcome.

Re next steps: The general plan is to try and hold another two night session in 2-3 months, when we have more information to share on vaccines, durability of immunity and the evaluation and uses of different types of tests. More discussion on the origin or "natural history" of the virus focused on preventing future outbreaks (since George Gao seems to be open to it) might be possible as well.

PS I have attached the ppt on learning from Covid patients from the dialogue.

Kind regards,

Ben

Benjamin Rusek
The U.S. National Academy of Sciences
1-[redacted]

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: Monday, October 19, 2020 12:21 AM
To: Rusek, Benjamin <BRusek@nas.edu>; 'relman@stanford.edu' <relman@stanford.edu>; rbaric_email.unc <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>; 'Nancy Connell' <NancyConnell@jhu.edu>; 'Dave Franz (davidrfranz@gmail.com)' <davidrfranz@gmail.com>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; antoinette_baric.med <antoinette_baric@med.unc.edu>; Alison Andre <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; Cervenka, Nicole <NCervenka@nas.edu>; Sharples, Fran <FSharples@nas.edu>; Block, Bruce <BBlock@nas.edu>
Subject: Some bullets following our US-China dialogue discussion on Friday

Importance: High

Thanks for a good discussion on Friday Ben,

I fully support a continued dialog and noted, as did some of those on the call, that George Gao and others were more open in their discussion of investigations into animal reservoirs of SARS-CoV-2 – i.e. discussion about the origin. We discussed ways we could frame a future topic that would allow us to talk about some important issues around the 'natural history' of SARS-CoV-2, that might also be comfortable for our Chinese colleagues. Here are a couple of bullets along the lines you asked me for:

1. Summary of recent findings re. the ability of SARS-CoV-2 to infect other species of animals in the lab, and in the wild, around the world (e.g. mink farm infections Europe and US, experimental infections of ferrets & raccoon dogs, risk assessments of SARS-CoV-2 infecting bats in other countries)

2. From the natural history of the virus, what do we know about the diversity of alpha and beta CoVs in wildlife reservoirs, and in potential intermediate hosts in various countries in Asia.

3. What information can we identify from the receptor binding domain of SARS-related CoVs that might help us predict future potential for emergence of CoVs from other countries

I think a good strategy would be to have the US side give the opening slide deck so that we sort of set the parameters and open up some of the discussion that I'm sure would lead to interesting information. I'd be happy to help on the first 2 points, and I'm sure Ralph could talk to the 3rd point. Linda and Stanley have a great deal of knowledge and could provide supporting comments…

Cheers,

Peter

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President

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EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

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Sent: Thursday, October 15, 2020 1:18 PM
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Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; antoinette_baric.med <antoinette_baric@med.unc.edu>; Alison Andre <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; Cervenka, Nicole <NCervenka@nas.edu>; Sharples, Fran <FSharples@nas.edu>; Block, Bruce <BBlock@nas.edu>
Subject: RE: Virtual U.S. China dialogue meeting October 13 and 14 - agenda with zoom links

Greetings,

Thank you for participating in the China bio dialogue sessions on Tuesday and Wednesday this week. We have scheduled a short hotwash session so the American participants can discuss the virtual dialogue discussions (from this week and earlier this year) and your get your ideas on future topics and other issues.

The session will take place tomorrow from 5:30-6:30 PM, Zoom link is below. Sorry for the short notice, if you can't make it tomorrow feel free to weigh in by email.

Topic: China Bio Post Dialogue Meeting Discussion
Time: Oct 16, 2020 5:30 PM ET / 4:30 PM CT / 2:30 PM PT
Meeting Link: https://nasem.zoom.us/j/92476126782?pwd=a0VUaDI1dEVORjlKOC9xaXRuTGpRdz09
Password: 604638

PS I have asked CAS for the ppts from last night, will send those out as soon as I get them.

Kind regards,

Ben

Benjamin Rusek

The U.S. National Academy of Sciences
1-[redacted]

From: Rusek, Benjamin
Sent: Wednesday, October 14, 2020 7:32 PM
To: 'relman@stanford.edu' <relman@stanford.edu>; 'rbaric@email.unc.edu' <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'daszak@ecohealthalliance.org' <daszak@ecohealthalliance.org>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>; 'Nancy Connell' <NancyConnell@jhu.edu>; 'Dave Franz (davidrfranz@gmail.com)' <davidrfranz@gmail.com>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; 'antoinette_baric@med.unc.edu' <antoinette_baric@med.unc.edu>; 'andre@ecohealthalliance.org' <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; Cervenka, Nicole <NCervenka@nas.edu>; Sharples, Fran <FSharples@nas.edu>; Block, Bruce <BBlock@nas.edu>
Subject: RE: Virtual U.S. China dialogue meeting October 13 and 14 - agenda with zoom links

Greetings,

Thank you for joining the dialogue session last night. I have attached the slides from the three presentations.

FYI CAS has invited two additional CCDC experts to the session tonight.

Dr. Huaqing Wang, PI, Immunization program, Chinese Center for Disease Control and Prevention
Dr. Zundong Yin, Director of National Immunization Program, Chinese Center for Disease Control and Prevention

Looking forward seeing and hearing from you all in a few hours.
Session 2: Wednesday October 14, **9-11 PM ET / 6-8 PM PT in U.S.**
Meeting Link: https://nasem.zoom.us/j/98420889232?pwd=NFllKzF1eWgxT0xDZHQzQWxMbnJPdz09
Password: 375761

Kind regards,

Ben

Benjamin Rusek
The U.S. National Academy of Sciences

1-[redacted]

From: Rusek, Benjamin
Sent: Monday, October 12, 2020 12:36 PM
To: 'relman@stanford.edu' <relman@stanford.edu>; 'rbaric@email.unc.edu' <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'daszak@ecohealthalliance.org' <daszak@ecohealthalliance.org>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>; 'Nancy Connell' <NancyConnell@jhu.edu>; 'Dave Franz (davidrfranz@gmail.com)' <davidrfranz@gmail.com>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; 'antoinette_baric@med.unc.edu' <antoinette_baric@med.unc.edu>; 'andre@ecohealthalliance.org' <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; Cervenka, Nicole <NCervenka@nas.edu>; Sharples, Fran <FSharples@nas.edu>; Block, Bruce <BBlock@nas.edu>
Subject: RE: Virtual U.S. China dialogue meeting October 13 and 14 - agenda with zoom links
Importance: High

Greetings,

I have attached what should be the final agenda for the U.S. China dialogue meeting sessions on Tuesday, October 13 and Wednesday October 14. It includes the Chinese participant list at the end. Links to join the Zooms are also below.

Looking forward to seeing/talking to you all on Tuesday and Wednesday evening.

Kind regards,

Ben

Benjamin Rusek
The U.S. National Academy of Sciences
1-[redacted]

***

Session 1: Tuesday, October 13, **9-11 PM ET / 6-8 PM PT in U.S.**
Meeting Link: https://nasem.zoom.us/j/92754903815?pwd=QUV2R3BPdDdibDdZZ24vcGd4VmJoUT09
Password: 833624

Session 2: Wednesday October 14, **9-11 PM ET / 6-8 PM PT in U.S.**

Meeting Link: https://nasem.zoom.us/j/98420889232?pwd=NFllKzF1eWgxT0xDZHQzQWxMbnJPdz09
Password: 375761

From: Rusek, Benjamin
Sent: Friday, October 9, 2020 5:43 PM
To: 'relman@stanford.edu' <relman@stanford.edu>; 'rbaric@email.unc.edu' <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'daszak@ecohealthalliance.org' <daszak@ecohealthalliance.org>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>; 'Nancy Connell' <NancyConnell@jhu.edu>; 'Dave Franz (davidrfranz@gmail.com)' <davidrfranz@gmail.com>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; 'antoinette_baric@med.unc.edu' <antoinette_baric@med.unc.edu>; 'andre@ecohealthalliance.org' <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; Cervenka, Nicole <NCervenka@nas.edu>; Sharples, Fran <FSharples@nas.edu>
Subject: RE: Virtual U.S. China dialogue meeting October 13 and 14 - agenda with zoom links
Importance: High

Greetings,

We are looking forward to the two virtual bio dialogue sessions set to take place on Tuesday, October 13 (9-11 PM ET / 6-8 PM PT) and Wednesday, October 14 (9-11 PM ET / 6-8 PM PT) next week. Like in previous sessions we expect that the Chinese expert participants will lead the discussion on the majority of the topics and that the format will be more of a discussion among the participants instead of a series of formal presentations. I have attached an agenda that includes the Zoom connection links for both days along with the U.S. participant list. If I get the Chinese participant list before the session I will send that out to you all.

Feel free to respond to this email with any thoughts, points of emphasis or issues that you would like to discuss among the U.S. group before the sessions. Also let me know if you have any questions or concerns.

Kind regards,

Ben

Benjamin Rusek
The U.S. National Academy of Sciences
1-[redacted]

From: Rusek, Benjamin
Sent: Monday, September 21, 2020 9:01 PM
To: 'relman@stanford.edu' <relman@stanford.edu>; 'rbaric@email.unc.edu' <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'daszak@ecohealthalliance.org' <daszak@ecohealthalliance.org>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>; 'Nancy Connell' <NancyConnell@jhu.edu>; Dave Franz (davidrfranz@gmail.com) <davidrfranz@gmail.com>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; 'antoinette_baric@med.unc.edu' <antoinette_baric@med.unc.edu>; 'andre@ecohealthalliance.org' <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>
Subject: Virtual U.S. China dialogue meeting October 13 and 14
Importance: High

Greetings,

I hope you all had a good summer and are staying safe and healthy. The Chinese Academy of Sciences has agreed to hold another (4th) virtual bio dialogue meeting with NASEM on **1) vaccine development and delivery** and **2) immunity, testing and diagnostics.** The agreed topics for the session are below. **We have reserved Tuesday, October 13 (9-11 PM ET / 6-8 PM PT) and Wednesday, October 14 (9-11 PM ET / 6-8 PM PT**) to discuss the topics on the agenda.

We hope you are able to participate. Please let me know if you are available and if so save the dates and times on your calendar. We will get back to you with more information and a detailed agenda for the sessions soon.

Kind regards,

Ben

Benjamin Rusek
The U.S. National Academy of Sciences
1-[redacted]

***

Vaccine development and delivery

Human

1) **Current status of CoVID-19 vaccine development in China and the U.S.**
2) **Chinse vaccination of military personnel and other Chinese populations**
3) **Vaccination of pediatric populations**

4) **Active surveillance strategies for monitoring adverse events observed after vaccination (as well as immunogenicity)**
5) **Adapting current vaccine platforms to novel mass vax strategies and other mass vaccination strategic issues**
6) **Progress on a universal influenza vaccines**
7) **Vaccine for enterovirus D68**

Animal

1) **Status of corona virus vaccination for animals - kinds of vaccine, efficacy, complications, etc**
2) **ASF in China and ASF vaccine progress**
3) **New swine coronavirus**
4) **Vaccination strategy for H5N1 avian influenza and domestic poultry**

Immunity, testing and diagnostics

1) **Correlates of immunity including the possibility of background immunity from circulating "common cold" coronaviruses**
2) **Chinese diagnostic testing strategies for testing large populations quickly**
3) **Antibody and antibody testing topics, importance of T-cell responses**
4) **Long-term sequela following COVID-19 infection—lung function, neurologic issues, others**

From: Rusek, Benjamin
Sent: Thursday, June 4, 2020 1:25 PM
To: 'relman@stanford.edu' <relman@stanford.edu>; 'rbaric@email.unc.edu' <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'daszak@ecohealthalliance.org' <daszak@ecohealthalliance.org>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; 'antoinette_baric@med.unc.edu' <antoinette_baric@med.unc.edu>; 'andre@ecohealthalliance.org' <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; 'davidrfranz@gmail.com' <davidrfranz@gmail.com>; 'Nancy Connell' <NancyConnell@jhu.edu>
Subject: RE: 3rd Virtual U.S. China dialogue meeting on COVID-19 Tuesday, June 9, 9-11PM ET
Importance: High

Greetings,

I have attached the American version of the agenda for the 3rd U.S. China virtual dialogue meeting on immunity and related topics set to take place on **Tuesday night, June 9 from 9:00-11:00 PM ET**

(9-11 AM the next morning, Beijing time).

As you can see we have incorporated George Gao's questions into the agenda, we hope that **Harvey Fineberg** can provide information on and lead the discussion of 1) serologic investigation in the U.S. 2) strategy in the U.S. for the second half of this year 3) vaccine availability in the U.S. and that **Ralph Baric** can do the same re progress in the development of vaccine in the U.S. (especially mRNA vaccine).

We have also listed delegation member names after the other Immunity questions. Like previously, folks are listed simply so someone is responsible for getting an answer from the Chinese to each question during the discussion. I will send a version to CAS without those names listed but will let them know who we have asked to answer George's questions.

Please let us know if you have any thoughts or comments on the agenda or this plan. I will send you the Zoom link later tonight or tomorrow morning.

Kind regards,

Ben

Benjamin Rusek
The U.S. National Academy of Sciences
1-[redacted]

From: Rusek, Benjamin
Sent: Monday, June 1, 2020 10:03 AM
To: 'relman@stanford.edu' <relman@stanford.edu>; 'rbaric@email.unc.edu' <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'daszak@ecohealthalliance.org' <daszak@ecohealthalliance.org>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; 'antoinette_baric@med.unc.edu' <antoinette_baric@med.unc.edu>; 'andre@ecohealthalliance.org' <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; 'davidrfranz@gmail.com' <davidrfranz@gmail.com>
Subject: RE: 3rd Virtual U.S. China dialogue meeting on COVID-19 Tuesday, June 9, 9-11PM ET
Importance: High

Greetings,

Good news, just heard back from CAS. They agreed to hold the 3rd dialogue meeting on the

proposed immunity topics on **Tuesday, June 9 from 9:00-11:00 PM ET** (9-11 AM the next morning, Beijing time). Please hold that time on your calendar.

CAS let us know that George Gao wants to add the following questions to the discussion:

- Could any US participant introduce the progress in the development of vaccine in the US, especially mRNA vaccine?
- How is the overall situation of serologic investigation in the US?
- What is the COVID-19 prevention and control strategy in the US for the second half of this year? When do you expect COVID-19 vaccine to be available in the US ?

We will send out a new agenda and Zoom link for the meeting later in the week.

Kind regards,

Ben

Benjamin Rusek
The U.S. National Academy of Sciences
1-[redacted]

From: Rusek, Benjamin
Sent: Friday, May 22, 2020 3:55 PM
To: 'relman@stanford.edu' <relman@stanford.edu>; 'rbaric@email.unc.edu' <rbaric@email.unc.edu>; 'saif.2@osu.edu' <saif.2@osu.edu>; 'stanley-perlman@uiowa.edu' <stanley-perlman@uiowa.edu>; 'daszak@ecohealthalliance.org' <daszak@ecohealthalliance.org>; 'harvey.fineberg@moore.org' <harvey.fineberg@moore.org>; 'dgriffi6@jhmi.edu' <dgriffi6@jhmi.edu>; 'peggy@hbfam.net' <peggy@hbfam.net>; 'jwleduc@UTMB.EDU' <jwleduc@UTMB.EDU>; 'peshi@UTMB.EDU' <peshi@UTMB.EDU>; Dzau, Victor J. <VDzau@nas.edu>
Cc: 'fsharples_3@hotmail.com' <fsharples_3@hotmail.com>; Lowenthal, Micah <mlowenth@nas.edu>; 'antoinette_baric@med.unc.edu' <antoinette_baric@med.unc.edu>; 'andre@ecohealthalliance.org' <andre@ecohealthalliance.org>; 'jennifer.ryan@moore.org' <jennifer.ryan@moore.org>; Bowman, Katherine <KBowman@nas.edu>; Kanarek, Morgan <MKanarek@nas.edu>; 'Raymond JEANLOZ' <jeanloz@berkeley.edu>; Hare, Hope <HHare@nas.edu>; 'davidrfranz@gmail.com' <davidrfranz@gmail.com>
Subject: RE: 3rd Virtual U.S. China dialogue meeting on COVID-19
Importance: High

Greetings,

Good news: Last night CAS leadership agreed to hold the 3rd virtual dialogue (Zoom meeting) on the list of Immunity topics we proposed. However they would like to push the dates for the 3rd meeting back to the first or second week of June (so no Zoom meeting on Tuesday night next week). Some of our Chinese counterparts are involved in China's National People's Congress taking place next week.

We are targeting **one night on June 1-4 or on June 8-11** (at 9-11 PM ET for the Americans, the following morning for the Chinese group.)

Please send your availability to participate on those nights (or maybe simply send the nights you can't participate) to Hope Hare [HHare@nas.edu] so we can propose a date or dates to CAS that works best for the American group.

Thanks again for your availability and willingness to participate in this initiative and I hope you have a good long weekend.

Kind regards,

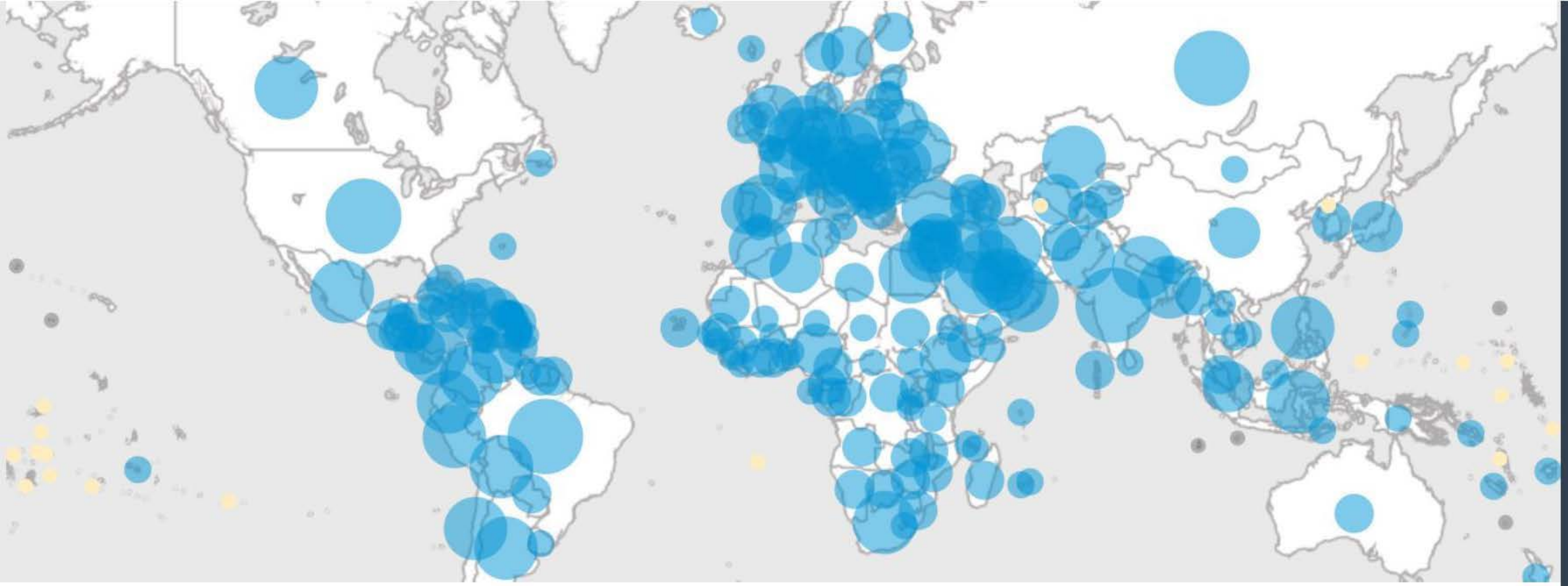
Ben

Benjamin Rusek
The U.S. National Academy of Sciences
1-[redacted]

Learning from following up of COVID-19 patients

Jianping Weng October 15, 2020

中国科学技术大学
University of Science and Technology of China



COVID-19 global pandemic: a historical challenge

- Globally, as of 5:06pm CEST, 13 October 2020
- **37,704,153** confirmed cases
- Causing **1,079,029** deaths

1. https://covid19.who.int/ (accessed October 14, 2020)

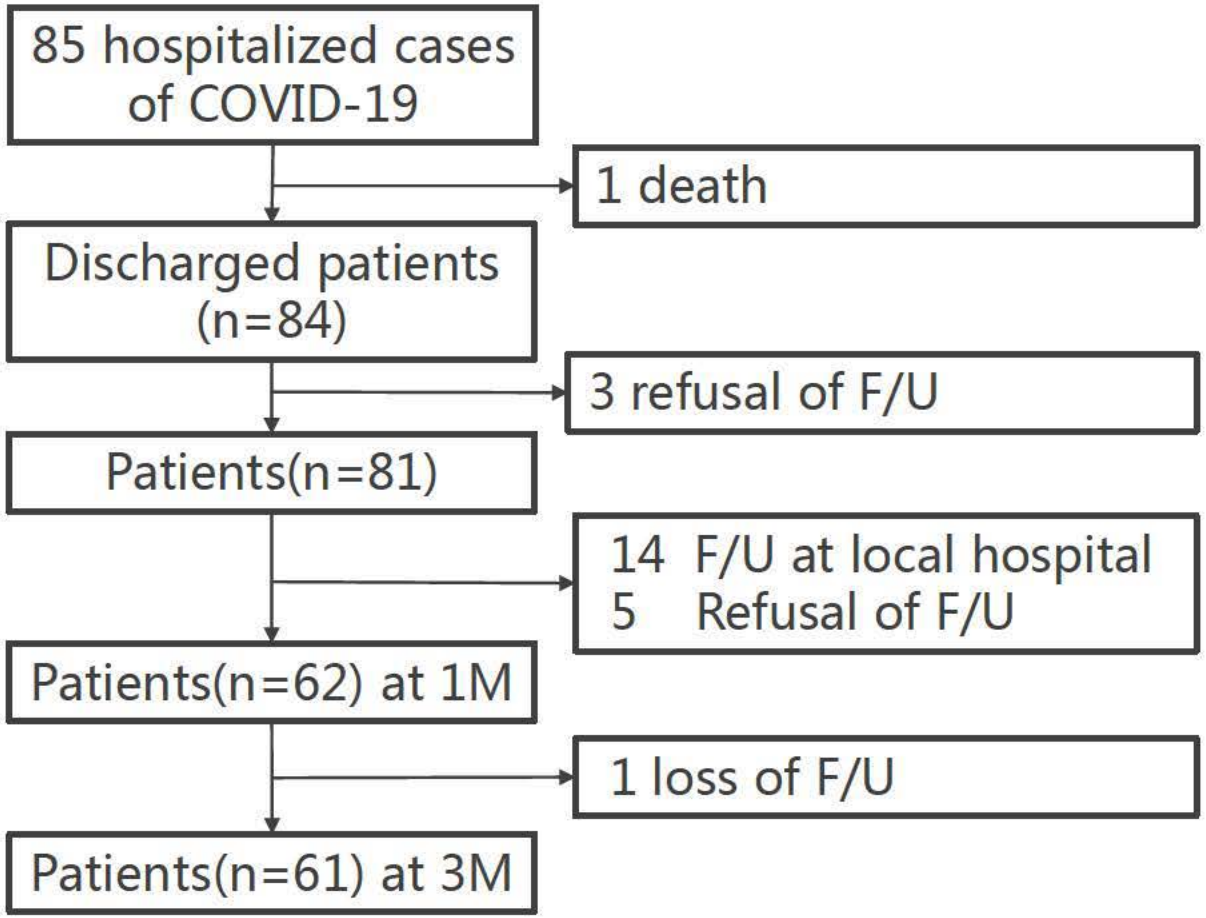
Epidemiology of COVID-19 among Infant and Children in China

- Pediatric cases accounted for approx. 1% of all cases (728/80,000, estimated via China CDC case series)
- Communicability of infection amongst children has been tracked and, as expected, infected children shed virus although, as noted above, they are frequently asymptomatic or only mildly symptomatic.
- Negative breast milk, Amniotic fluid, cord blood, and neonatal throat swab samples by RT-PCR from mothers with COVID-19 reported.
- Cases series of babies breastfed by mothers with overt COVID-19 – not infected

1. Dong Y, et al. Pediatrics. 2020 Jun;145(6):e20200702. 2. Chen H, et al. Lancet. 2020;395(10226):809–815.
3. Liu W, et al. J Hum Lact. In press. 4. Zhu H, et al. Transl Pediatr. 2020;9(1):51–60.
5. Bi Q, et al. Lancet Infect Dis. 2020;20(8):911–919.

Clinical follow-up of COIVD-19 patients after discharge

- A single-center, prospective observational follow-up study to characterize the outcomes in patients with COVID-19 at 1, 3 and 6 months after discharge
- Currently, analysis has finished with the 1- and 3-month data



Manuscript under review

At 3-month COVID-19 patients were not fully recovered

- Baseline characteristics: 58% male; median age 45 years, IQR(34-55) ; 11% had smoking history; 37% had chronic disorders;
- At 3-month (n=61)
  - 1 re-activated virus RT-PCR on D100
  - 38% symptoms persisted: dyspnea(18%), coughing(15%), fatigue(8%)
  - 54% CT scans abnormalities: GGOs (15%), fibrosis (5%)
  - pulmonary ventilating function & physical activity (6MWD) gradually recovering

Potentially more prompt recovery at 3-month compared to SARS

- Compared with SARS, COVID-19 appears to be associated with a prompter resolution on chest CT during the recovery phase.
- Our findings indicate potentially more prompt recovery of COVID-19 patients at 3M in 6MWD compared to those with SARS.
- Preferable to combine FEV1 with DLCO in identifying pulmonary function impairment with higher sensitivity
- No significant difference among the discharged survivors with different severity pneumonia regarding other pulmonary function measures

1. Ng CK, et al.. Thorax. 2004;59(10):889-891.
2. Hui DS, et al. Chest. 2005;128(4):2247-2261.
3. Mo X, et al. Eur Respir J. 2020:2001217.

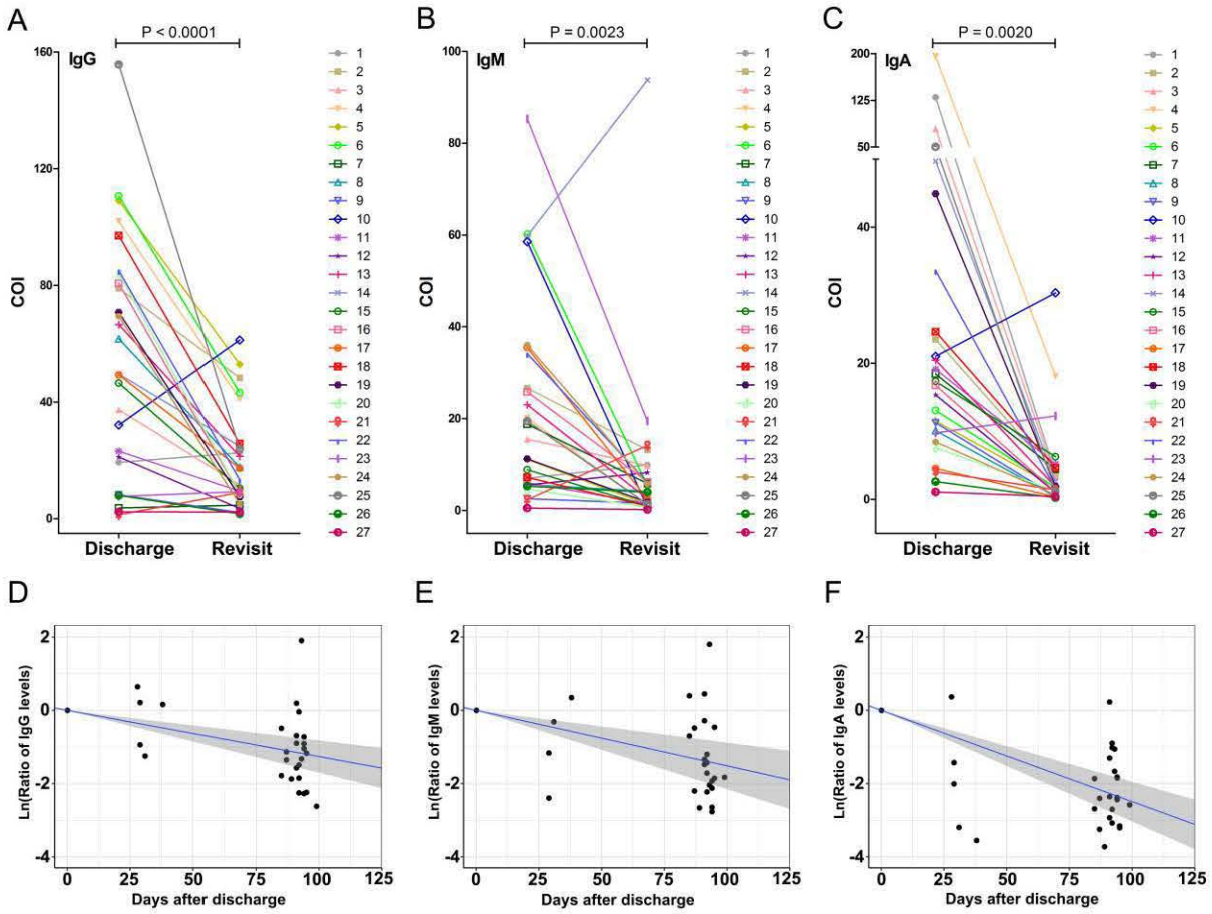
Serological study of COVID-19 patients after recovery

- Previous studies suggest that there is a significant reduction of neutralizing antibodies in the serum of COVID-19 patients in their early convalescent stage.
- Patients recovered from COVID-19 might not have protection against re-infection

1. Robbiani DF, et al. Nature. 2020 Aug;584(7821):437-442. doi: 10.1038/s41586-020-2456-9.
2. Long Q-X, et al. Nat Med. 2020 Aug;26(8):1200-1204. doi: 10.1038/s41591-020-0965-6.

Decline of SARS-CoV-2 specific antibodies in convalescent patients

- Serological study based on 27 patients followed-up after discharge
  - 100% IgG (COI 1.67-61.26) remains positive, 81.5% (COI 0.15-93.73) for IgM and 77.78% (COI 0.25-30.36) for IgA
  - Substantial decline of antibodies level at 3 months



1. Ma et al. Sci China Life Sci, 2020, doi: 10.1007/s11427-020-1805-0

Decline of SARS-CoV-2 specific antibodies in convalescent patients

- IgG antibody would become undetectable after discharge for 273 days
- IgM and IgA would be 150 and 108 days
- Our result suggests humoral immunity diminish in short period, losing the protection for the virus
- Together with previous studies, triggering strong cellular immune response and immune memory is the key for SARS-CoV-2 vaccine development.

1. Ma et al. Sci China Life Sci, 2020, doi: 10.1007/s11427-020-1805-0

Next-generation sequencing revealed influenza and *Chlamydia* infection in recurrent pneumonia in a recovered COVID-19 patient

A 53-year-old man was admitted to hospital with SARS-CoV-2 on January 25, 2020

↓

Discharged on February 9, 2020

↓ 82 days

Recurrence of suspected COVID-19 GGO-like pneumonia On May 1, 2020

↓

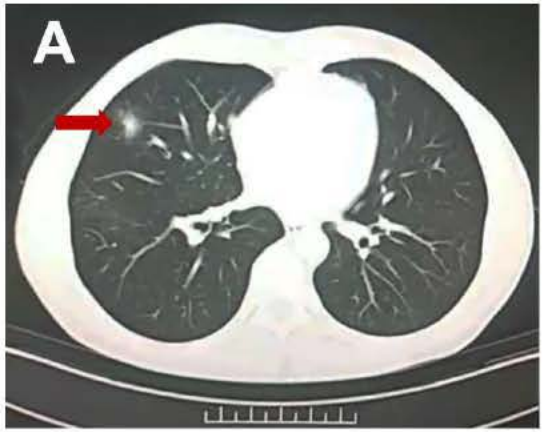
NGS showed co-infection with influenza and Chlamydia

↓

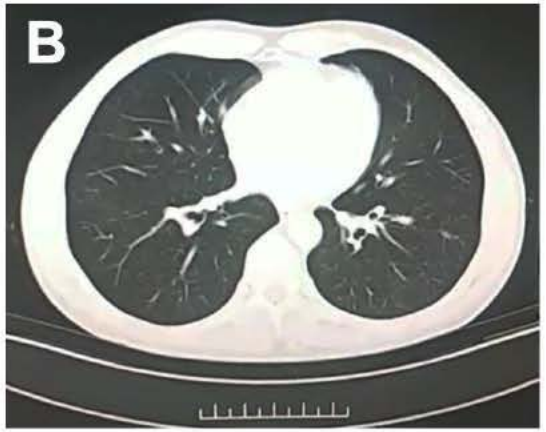
Discharged on May 28, 2020

Table 1 The main pathogens of alveolar lavage fluid sequenced by next generation sequencing.

No.	%	Reads	Genus	No.	%	Reads	Genus
1	72.1	28366988	unclassified	15	0.233	91578	Listeria
2	6.948	2733676	Chlamydia	16	0.205	80778	Idiomarina
3	4.764	1874455	cannot be assigned to a genus	17	0.197	77399	Klebsiella
4	2.387	939292	Enterococcus	18	0.195	76888	Salmonella
5	1.956	769431	Lingulodinium	19	0.18	70859	Epulopiscium
6	1.381	543303	Bacillus	20	0.162	63717	Curvibacter
7	1.278	502795	Acinetobacter	21	0.142	55903	Clostridioides
8	1.152	453243	Plasmodium	22	0.111	43483	Sarcocystis
9	0.55	216421	Pseudomonas	23	0.109	42722	Kangiella
10	0.491	193240	Clostridium	24	0.107	41987	Neisseria
11	0.384	151225	Streptococcus	25	0.096	37916	Enterobacter
12	0.362	142335	Escherichia	26	0.095	37467	Burkholderia
13	0.34	133773	Mycobacterium	27	0.095	37280	Viruses
14	0.322	126861	Staphylococcus				



CT on May 1, 2020



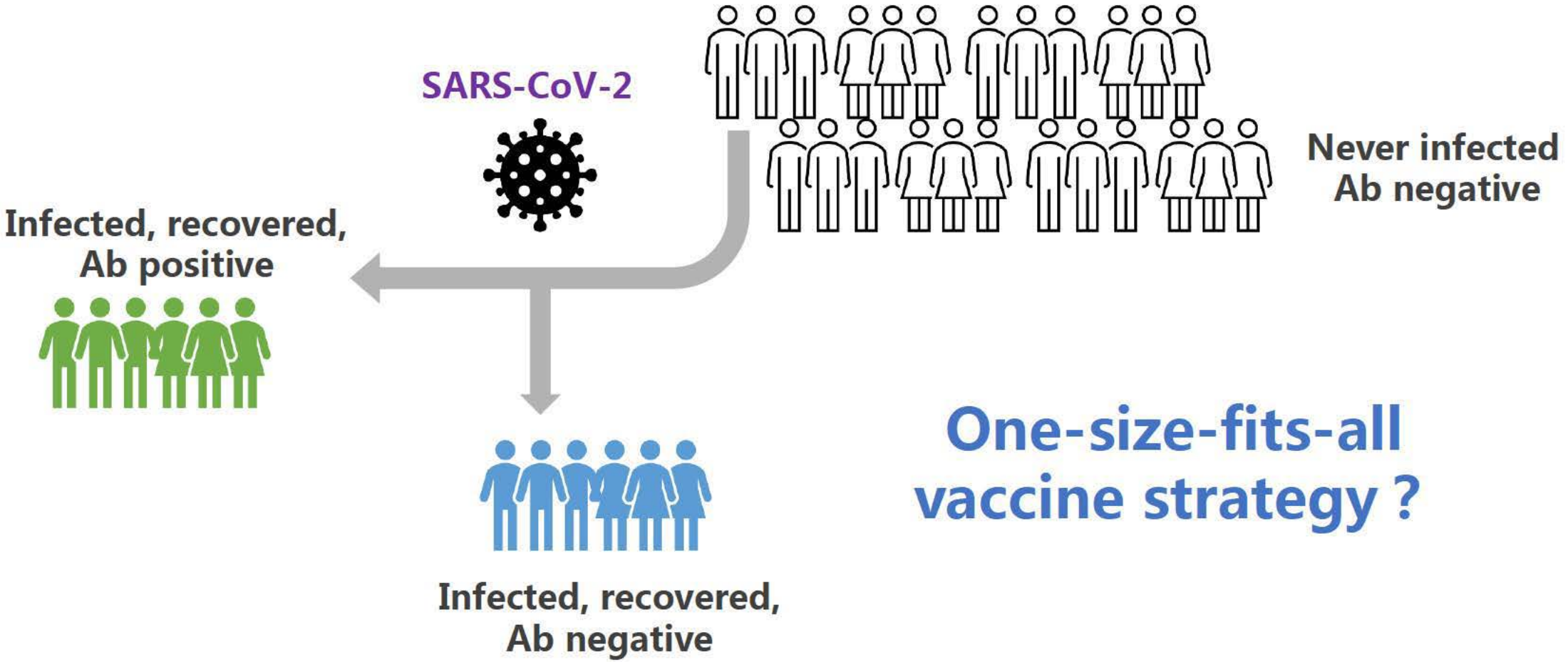
CT on May 28, 2020

Table 2 The information of influenza viruses sequenced by next generation sequencing.

No.	Reads	Virus	Subtype	Description
1	40	Influenza B virus	Influenza B virus	B/Connecticut/Flu110/2013
2	11	Influenza A virus	H1N1 subtype	A/Brazil/RS-3335/2009
3	4	Influenza A virus		
4	2	Influenza A virus	H3N2 subtype	A/Brazil/RS-3335/2009
5	1	Influenza A virus	H1N2 subtype	
6	1	Influenza A virus	H10N6 subtype	A/American green-winged teal/Alaska/11508/2006

*Wen W#, Zhang G#, Luo S#, Bai Z, Tao H, Guo M, Hu S, Liu W, Zhang K, Wang L, Zhu S. 2020. **Precision Clinical Medicine**, doi:10.1093/pcmedi/pbaa033.

Concerns in developing vaccines…



One-size-fits-all vaccine strategy ?

Thank you for listening!

Jianping Weng

中国科学技术大学
University of Science and Technology of China

From:	Peter Daszak
To:	Saif, Linda
Cc:	Su Yadana; Alison Andre
Subject:	Invitation to join a Lancet COVID-19 Commission Taskforce on the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats"
Date:	Monday, October 12, 2020 11:50:59 PM
Attachments:	Taskforce on origins, early control and one health solutions to future pandemic threats.pdf
Importance:	High

Dear Linda,

I hope all's well with you and that you're staying healthy and busy during these difficult times.

I've been asked to join the Lancet COVID-19 Commission and run a 12 month Taskforce to conduct a review of the "Origins, early control of COVID-19 and One Health solutions to future pandemic threats". The Lancet COVID-19 Commission has been created to help speed up global, equitable, and lasting solutions to the pandemic. Two key aims of this Commission are (i) to speed up the awareness and adoption worldwide of successful strategies to suppress transmission and (ii) to ensure that any new COVID-19 vaccines and other key technologies are equitably accessible across the world. There is information on the membership and details of the goals on its main website: https://covid19commission.org/, and also in an article recently published in *The Lancet*: https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)31494-X.pdf

The goal of the taskforces are to focus on specific dimensions of the pandemic, review all available information and provide a 'state-of-the-art' assessment of an issue and point to future directions, including intergovernmental policy initiatives, research gaps, regional and national policies and other issues that might benefit global health and equity.

I would very much like you to serve on the taskforce that I'll be running. I've attached a brief summary of the goals and workplan, and names of others who've been invited. Please consider this an informal request at the moment – if you indicate your willingness, I'll send a formal invitation co-signed by Commission Chair, Jeff Sachs.

Please note that if you are willing to serve, your involvement will be voluntary and the time commitment will be until September, 2021 when the final report is due. There may be a possibility of on-the-ground work if travel allows and the cost will be covered by the Commission, but given the timeline and the continued disruption of travel by COVID-19, I believe this is unlikely. The taskforce is expected to meet via monthly Zoom calls. Our first meeting will be in October and the first draft report is aimed for Dec, 2020. Please also note that I'm cc'ing Su Yadana, who will be the point person for running the workings of the taskforce here at EHA, as well as my assistant Alison Andre. Please cc both of them on all correspondence.

I'm confident that the taskforce will do significant and meaningful work towards understanding and providing lasting solutions to the pandemic and that your expertise and involvement will strengthen its efforts.

I really hope that you will accept my invitation to join and will then be able to set up dates for our

first call.

Cheers,

Peter

Peter Daszak
President

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USA

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Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

The Lancet COVID-19 Commission

Taskforce on the Origins, Early Control of the Pandemic, and One Health Solultions to Future Pandemic Threats

Significance

Better understanding of the *origin* of SARS-CoV-2 may:

- Identify potential continued risk of re-emergence or emergence of future CoVs or other agents.
- Provide a strategy to heighten biosecurity, design behavior change programs, and introduce legislation/policies to reduce risk of future emergence in China, SE Asia and beyond
- Inform and potentially undermine a politically-divisive strategy to 'blame' countries for the outbreak.

Assessing *early control* of the pandemic may:

- Identify specific points at which future epidemics can be contained more effectively before amplification and international spread
- Identify specific strategies, agencies, policies to improve future control of pandemics as close as possible to initial spillover event.

Identifying *One Health* approaches to controlling future pandemics will:

- Examine the underlying drivers of COVID-19 in the context of other emerging diseases and pandemics
- Identify potential synergistic effects and return-on-investment of taking a multisectoral approach to outbreak investigation and pandemic prevention that includes Animal Health, Human Health, Environmental Health aspects
- Identify key strategies, organizations and mechanisms to fund and deliver a coordinated One Health approach to *preventing* future pandemics

Logistics:

Taskforce lead is Peter Daszak, EcoHealth Alliance (daszak@ecohealthalliance.org). Project coordinator for the Taskforce at EcoHealth is Su Yadana BS MPH (yadana@ecohealthalliance.org) who is based in New York, originally from Myanmar, and has worked in Singapore (DukeNUS) and has an MPH from Columbia University School of Public Health. Point of Contact for our taskforce on the COVID-19 Commission is Dr. Özge Karadag Caman (ok2267@columbia.edu) at the Center for Sustainable Development, currently back in Turkey. Dr. Caman is also part of the Secretariat for the Lancet Commission on COVID-19 and is involved in One Health.

We will meet by zoom in October then again in November to discuss strategy and initial draft plan. We will draft a 10-page report by Dec 1st 2020 to sum up our initial approach, findings. We will conduct background research, zoom meetings throughout Winter, Spring, Summer 2021 to analyze available data, interview key leaders involved in outbreak investigation, conduct background research, draft report. Report Due: Sept. 2021.

Strategy

1. Assemble an international group of trusted experts on emerging disease to review scientific evidence on key theories of COVID-19 origins & control. Expertise on:
    a. Virology, sequence analysis
    b. Ecology of viral emergence

c. Outbreak investigation, epidemiology
d. Social science of risk behavior in developing countries
e. Wildlife ecology/One Health
f. Wildlife trade
g. Biosecurity lab safety

2. On the **origin** question: Use 'Preponderance of Evidence' approach to analyze data on all leading theories for origin. What do we know? What don't we know?
    a. Work backwards from the Huanan Market, as well as forwards from the rural Yunnan sites of nearest known relatives in wildlife
    b. Approach key members of the outbreak investigation teams, virological labs analyzing early cases in China to seek further support or lack thereof for each theory
    c. Build a detailed timeline of the outbreak, stretching from discovery of nearest relatives (2012) through to declaration of COVID-19 as a PHEIC by WHO (Jan 30th 2020)
    d. Weigh the evidence for and against each theory on COVID origins. Identify critical gaps in data and recommend strategies that can be adopted to address them.

3. On the **early control** issue: Document outbreak investigation and control efforts from China, WHO and other countries within the timeline up to Jan 30th 2020.
    a. Compare these with other recent emerging diseases (e.g. Nipah virus, H1N1, West Africa Ebola, H7N9)
    b. Identify critical points in the investigation and control efforts that alternative strategies could have been adopted for,
    c. Identify gaps in our understanding of early control
    d. Recommend strategies for future efforts for control

4. **One Health and Preventing Future Pandemics**: Identify when a One Health approach would have benefits to preventing future pandemics, how this would be funded, and what organizations would be involved
    a. Review common features among COVID-19 and other pandemics that have origins in wildlife, livestock and are driven to emerge by underlying environmental changes
    b. Identify potential synergistic effects and return-on-investment of taking a multisectoral approach to outbreak investigation and pandemic prevention that includes Animal Health, Human Health, Environmental Health aspects
    c. Identify key strategies, organizations and mechanisms to fund and deliver a coordinated One Health approach to *preventing* future pandemics at the intergovernmental and national levels

What we know:

- SARS-CoV and SARS–CoV-2 are both Clade 2b β-coronaviruses. Closest relatives (RaTG13, RmYN02) are from bats.
- There are 528 β-CoV sequences in Genbank which includes 100+ SARSr-CoV sequences. Only a handful have not been reported from bats (sequenced from pangolins)
- Majority are from China, but this reflects collecting bias. Others reported from across SE Asia
- Phylogenetic analysis points to S. China (Yunnan province) or Myanmar/Laos/Vietnam as evolutionary hotspot for this clade.

- What that tells us is that it's extremely likely that SARS-CoV-2 evolved from within this cluster of bat CoVs, probably from an insectivorous bat, probably from Yunnan, S. China, near the border of Myanmar, Laos, & Vietnam.
- Some of these viruses can infect human cells directly, although SARS-CoV (and maybe SARS-CoV-2) infected mammalian 'intermediate' hosts.
- Role of pangolins may be incidental: animals were seized in China after prob. many weeks in transit. Wildlife trade is known to heighten CoV prevalence, pangolins at start of wildlife trade are CoV-free.

Main theories that have been proposed for the origin of COVID-19:

1) Yunnan bat-> hunter-> Wuhan. The virus evolved in S. China from a bat SARSr-CoV lineage and infected a person directly – e.g. a bat hunter – and this person got sick and transmitted it to their social network, which is people in the wildlife trade, so the virus moved through the trade network to Wuhan. Would need to assess all potential pathways of human exposure by bats in the region.
2) Yunnan bat-> traded/farmed wildlife intermediate host-> Wuhan. SARS-CoV-2 was in a bat that was captured by a hunter, or flew into a farm where people have wildlife in cages and infected animals the hunter/farmer was ready to sell into the wildlife trade. The animals carried the virus to the Wuhan market as they were trucked into Wuhan. The animals could be civets, porcupines, raccoon dogs or another one of the animals commonly raised for food or fur in China
3) Hubei bat-> via hunter, intermediate host or direct to Wuhan market. The virus is from a bat endemic to Hubei (the province where Wuhan is), and either of the above two pathways began there. Need to take into account timing of spillover vs. first cluster of cases and assess whether and when bats hibernate in that region.
4) Origin in another region in China or neighboring countries. This happened in another part of China, e.g. Guangdong, or even in countries over the border from Yunnan where the same bats and prob. similar viruses circulate.
5) Origin in another more distant country. Assess hypotheses on US or European origin. Analyze data on proposed first findings of evidence of COVID outside China (e.g. patient in France, sewage in Spain etc.).
6) Role of pangolins as intermediate hosts. The virus moved from bats into pangolins in the wildlife trade and then into people. Assess sequence data from all close relative CoVs, assess volume of live or frozen pangolins traded, analyze ability of pangolin scales to transmit virus
7) It was bioengineered in the Wuhan BSL-4 lab. This has been discounted by everyone who works in the field because there is no evidence from the genetic sequence that the virus has been genetically manipulated, and there almost certainly would be, had that happened.
8) It is derived from a bat virus that was accidentally released from WIV, Wuhan CDC or Wuhan University lab. This theory suggests it was cultured in the lab and accidentally infected a lab worker, or was discarded with animals used in experiments, or infected people sampling bats in caves. Would need to assess what samples were present in the labs, what the routine protocols were, the number of people with access to samples or bat caves for sampling, evidence of safety violations or lack of biosecurity.

Invited members:

1. Peter Daszak Ph.D., Chair. President of EcoHealth Alliance, New York. Member of US National Academy of Medicine, Chair NASEM Forum on Microbial Threats. *Viral Discovery, Epidemiology, Ecology* **USA/UK. Male**
2. Hume E. Field DVM Ph.D., School of Veterinary Science, Univ Queensland – Led the original WHO veterinary investigation into the origin of SARS-CoV in wet markets in Guangdong. *Veterinarian, One Health* **Australia, Male**
3. Manish Kakkar MD, Public Health Foundation of India – long term experience in zoonoses research and policy, involvement in WHO SEARO. *MD, Zoonoses research, Public Health Policy* **India, Male**
4. John Amuasi MD Ph.D., Director of Africa Ctr for Neglected Tropical Diseases & Sr. Lecturer, Kwame Nkrumah University of Science and Technology (KNUST), Accra, Ghana. *MD, One Health, Global Health Policy* **Ghana, Male**
5. Danielle Anderson Ph.D., Director BSL-3 lab, Duke-NUS, Singapore. First non-Chinese citizen to work in the Wuhan Institute of Virology BSL-4 lab. *Lab Biosafety, virology.* **Australia, Female**
6. Stanley Perlman MD Ph.D., Univ Iowa, Coll Medicine, Rapid Falls – long-time CoV expert, no links to Chinese labs. *Long term Coronavirus virologist.* **USA, Male**
7. Linda Saif Ph.D. – Ohio State Univ, Columbus – Has worked on coronaviruses pre-SARS and was one of the team that inspected the Wuhan lab a few yrs ago from the NAS. Member of US National Academy of Sciences. *Long term Coronavirus research animal models.* **USA, Female**
8. Supaporn Wacharapluesadee Ph.D., WHO Collaborating Ctr, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn University, Bangkok – good virologist, knows the set up in China well. *Virologist.* **Thailand, Female**
9. Dato' Sai Kit (Ken) Lam Ph.D., Professor Emeritus Univ Malaya. Discovered Nipah virus, Member Malaysian Academy of Science. *Medical emerging disease virologist.* **Malaysia, Male**
10. Malik Peiris Ph.D. FRS Legion d'honneur, Hong Kong University. Key researcher with deep knowledge of coronaviruses, influenza viruses and Chinese research. *Medical virology.* **Sri Lankan/Hong Kong China, Male**. Alternate: Leo Poon, HKU.
11. Isabella Eckerle MD, Head of Centre for Emerging Diseases, Univ. Geneva. *Epidemiologist*. **Switzerland/German, Female**
12. Gerald Keusch MD, Boston University, Head of BSL-4 lab (NEIDL), Former Director of NIH Fogarty Intl. Center, Member National Academy of Medicine. *Lab Biosafety.* **USA, Male**
13. Carlos das Neves VMD, Director for Research & Internationalization, Norwegian Veterinary Institute, President of International Wildlife Disease Association, Advisor to Norwegian Minister of Agriculture, Hon. Consul of Portuguese Republic in Norway. *One Health*. **Portuguese, Norwegian, Male**

From:	William B. Karesh
To:	calisher@cybersafe.net; Charles.Calisher@colostate.edu
Cc:	bushschoolscowcroft@tamu.edu; rcolwell@umd.edu; Corley, Ronald B; Peter Daszak; christian.drosten@charite.de; L.Enjuanes@cnb.csic.es; a.e.gorbalenya@lumc.nl; b.haagmans@erasmusmc.nl; JMHUGHE@emory.edu; Gerald Keusch; lamsk@nipahvirus.org; Juan Lubroth; John MacKenzie; Lawrence.Madoff@umassmemorial.org; Jonna Mazet; peter.palese@mssm.edu; stanley-perlman@uiowa.edu; llmpoon@hku.hk; bernard.roizman@bsd.uchicago.edu; Saif, Linda; kanta.subbarao@influenzacentre.org; Jane Hilton; Equitech
Subject:	Re: Origin Coronavirus COVID-19
Date:	Thursday, February 20, 2020 12:59:37 PM
Attachments:	space.ppt ATT00001.htm

Same hypothesis as SARS from the same person!!, and my alternative hypothesis at the time (2003). see attached from an old presentation I used to use.

Billy

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001 USA

Is SARS from outer space?

By Lauren Compton
CNN
Friday, May 23, 2003

LONDON, England (CNN) –

The SARS virus might have originated in outer space, according to a scientist in Britain.
In a letter to **The Lancet** medical journal, professor Chandra Wickramasinghe of Cardiff University suggests the virus was introduced to Earth on a comet or meteorite.


M. Gilbert / WCS

From:	David Morens
To:	Peter Daszak
Cc:	Saif, Linda; Gerald Keusch; nbhadeli@bu.edu; dcarroll008@gmail.com; Dr. Ralph Baric (rsbaric@gmail.com); Toni C Baric (tcbaric@gmail.com); Robert Kessler
Subject:	Re: paper coming out next week on bioRxiv reporting 780+ partial sequences of bat-CoVs in China
Date:	Friday, May 29, 2020 4:25:23 PM
Attachments:	Talking points Latinne et al. Bat Coronaviruses, China.docx China bat CoVs_R2_v5 changes accepted.docx

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Peter, thanks i just got this and havent had time to read, just scanned the abstract, but it looks important

Yes, definitely, you can refer reporters to me, but if you have the chance please convey the following. All on the record discussions with the press need to be cleared by hhs and the white house, and that includes tony. They often wont let even tony talk to the press and knowing what i have said and written in the past, they might not let me speak on this particular issue. Knowing this, even our own nih media office might try to steer the press away

If the want to speak to anyone in particular such as me they should insist and that might or might not work

If it doesnt work i can still speak to them on background. Dont worry, i am not afraid to speak out

On a different but related matter, i am still waiting for you to send me info we discussed. In the meantime i have been going over a number of you papers i have including the one by Hu. I am only an epidemiologist and struggle to understand the viral genetics so this may be an off the mark Q. That Hu paper seems to show, if i understand it correctly, rather remarkable variability in the rbd Sequences of the bat viruses studied. What does this mean? Is it possible these viruses are "sampling" various receptors of different bat species, ie, doing alot of inter-species host switching, or even to non bat species? This gets at the Q of how similar are Ace2 receptors of different species and are they sufficiently similar To allow frequent host-swItching of these sars-like bat viruses and over time the shaping Of "generalized" rbds in individual viruses or in quasispecies. If this were the case it might mean that these viruses could be pre-adapted to humans even if they hadn't ever seen human cells. What does this all mean. TY. David

Sent from my iPhone
David M Morens
OD, NIAID, NIH

> On May 29, 2020, at 15:29, Peter Daszak <daszak@ecohealthalliance.org> wrote:
>
> Hello all,
>
> Just emailing to give you advance info on a paper that we're uploading onto bioRxiv this weekend. I've attached the draft here and some talking points I've written out for journalists. We've spoken with one NY Times reporter, and depending on what

happens next week, it might get some pick up in the news. Is it OK for me to suggest your names to journalists as people who are knowledgeable on COVID-19, CoVs, pandemics, high impact viral diseases etc.? I think this paper helps a little bit to show that there's nothing unusual about COVID-19 being a bat virus that got into people naturally. There's also the fact that the other close virus (RmYN02) is in the same clade as SARS-2 and RaTG13, and that virus has an insertion in the Spike protein, disproving one of the conspiracy theories, but that won't stop them.

I feel like I should add a disclaimer to emails along the lines of "if you do speak to journalists about this work you may end up being targeted by nutjobs and potentially have one or two of your grants terminated by El Guru-in-Chief!"

BTW – please use Ralph's gmail address so I don't get FoIA'd

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
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Twitter: @PeterDaszak

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

Talking points from Latinne et al. Origin and cross transmission of bat CoVs in China

In press at *Nature Communications*. Already peer-reviewed, this version is almost final. Uploaded to bioRxiv June 1st

Natural origin of COVID-19. The paper provides further evidence that SARS-CoV-2 emerged naturally from bats, casting even more doubt on the lab-origin conspiracy theory:

- The high diversity of bat-CoVs in the wild (781 novel genetic sequences) with 2 known to infect people, others known to infect human cells, and one that has caused an outbreak in pigs suggests ample opportunity for spillover of bat-CoVs into people on a regular basis.
- We find particularly high diversity of the group that contains SARS-CoV & SARS-CoV-2 (106 novel genetic sequences from *Sarbecovirus* genus – SARS-related CoVs).
- Pangolin viruses are close to SARS-CoV-2, but two bat viruses are more closely related, supporting its origin in bats (RaTG13 & RmYN02 – both from *Rhinolophus* horseshoe bats in S. China).
- SARS-CoV-2 has a furin-cleavage insertion between the S1 and S2 genes of the spike protein (the part that binds to human cells) which some have suggested is bioengineered. RmYN02 from bats has a similar insertion and is in the same clade as RaTG13 and the pangolin CoVs, proving that these insertions occur naturally within the same clade of viruses, even though RaTG13 doesn't have this.

Geographic origin of COVID-19. SARS-CoV-2 (cause of COVID-19) likely emerged from a clade of viruses in bats in SW China (Yunnan province) or countries bordering Yunnan (Myanmar, Lao PDR, Vietnam)

- This is the most thorough analysis of viruses related to SARS and COVID-19 ever published. We analyzed 781 novel genetic sequences that we discovered, published here for the first time, along with 509 previously known bat-CoVs. This means our conclusions on origin are the most accurate.
- However, we only sampled bats within China and the bats carrying closest relatives of SARS-CoV-2 (*Rhinolophus affinis* & *R. malayanus*) also occur in neighboring countries, across SE Asia and into S. Asia. It is likely that the same or similar viruses occur in these bats in other countries.
- Many of sampling sites for SARSr-CoV positives were close to the border of Myanmar and Lao PDR.
- SW China was Quaternary glacial refugium for bat species incl. Rhinolophus spp. & may have allowed survival of older viral strains leading to increased diversity.

Importance for pandemic risk.

- This work was funded by the NIH grant that was recently terminated 'for convenience' following political interference from the White House - as reported in 60 Minutes and other outlets, and recently criticized by 77 Nobel Laureates and 31 Scientific Societies. All of the genetic sequences reported here are fragments, and our future plans were to sequence whole genomes and particularly the Receptor Binding Domains to see if any of these viruses are likely able to infect humans. That work will not happen without the funding from NIH.
- There is an high diversity of bat coronaviruses in southern China, some of which have already emerged in people and livestock, others that are poised to, still others about which we know very little. This represents a significant potential pandemic risk, and threat to food security through livestock disease. Even though we have found a few hundred new CoVs, we expect there to be many more across SE Asia and globally (perhaps as many as 10-15,000 bat-CoVs yet-to-be-discovered).
- Bats in SE Asia should be targeted for focused surveillance/viral discovery to help identify novel coronaviruses that may emerge in future. Sequences can be used to test vaccines, drugs. Control programs to stop them emerging can be targeted to where the risk is highest.

Other key findings:

Evolution and human ecology collide to produce high risk of CoV emergence in S. China: Hotspots of CoV diversification in S & SW China have subtropical to tropical climate; dense, growing and rapidly urbanizing populations of people; a high degree of poultry and livestock production; high rates of consumption of wildlife, including bats – all factors that promote viral spillover & disease emergence.

Coronaviruses are a broad pandemic threat, not just those similar to SARS-CoV or SARS-CoV-2: We show that α-CoVs have a higher propensity to switch host within their natural bat reservoirs, and therefore high cross-species transmission potential and risk of spillover. These include SADS-CoV in pigs in Guangdong (also can infect human cells) & two human CoVs that likely originated in bats historically: NL63 and 229E. Targeted surveillance should be conducted to identify whole diversity of this group.

This study provides rationale for programs of viral discovery (like the Global Virome Project) and capacity building/intervention programs to prevent pandemics (like PREDICT) in Southeast Asia.

Summary of paper's findings

1. Most comprehensive analysis of bat coronavirus evolutionary origins ever conducted
    a. 781 novel sequences from bats in China; 509 previously published
    b. 106 novel sequences of SARS-related viruses – the clade (genus *Sarbecovirus*) that contains the cause of SARS and of COVID-19.
2. Helps understand why China is a hotspot
    a. Not just because of high bat diversity: Ecological or biogeographic factors – sharing roosts with other species, ancient origin of horseshoe bats.
    b. Higher CoV diversity than expected in some S. China provinces (Hainan, Guangxi, Hunan)
3. Significant cross-species transmission of CoVs among bats over evolutionary time
    a. Rhinolophidae and *Rhinolophus* (Horseshoe) bats involved in more inter-family and inter-genus highly significant host switching of α-CoVs than any other family or genus
    b. Rhinolophidae (Horseshoe) & Hipposideridae at the origin of most inter-family host switching events for β-CoVs
    c. β-CoVs (incl. SARS group) had strong evidence of co-evolution with their bat hosts. Their ability to diversify, and switch hosts, may have helped produce higher diversity of strains.
    d. α-CoVs (incl. SADS-CoV) are able to switch hosts more frequently and between more distantly related bats.
    e. Differences between these viral groups may be explained by subtle differences in host cell receptor binding, mutation rate, recombination potential, or replication rate.
4. S. China/neighboring countries represent hotspot for evolution/diversification of bat-CoVs.
    a. South western and Southern China are centers of diversification for both α- and β-CoVs
    b. They harbor evolutionarily old and phylogenetically diverse lineages of α- and β-CoVs
    c. SW China was Quaternary glacial refugium for bat species incl. *Rhinolophus* spp. & may have allowed survival of older viral strains leading to increased diversity.
    d. Similar theories for avian flu origins.

Note limitations of study:

- Short sequences used (RdRp), may not reflect evolutionary patterns of whole viral genomes. However, consistent with evolutionary patterns seen using whole genomes.
- PCR technique builds on known viruses (consensus sequences), and may have missed some unknown viruses.

Origin and cross-species transmission of bat coronaviruses in China

Alice Latinne[1§¶], Ben Hu[2¶], Kevin J. Olival[1], Guangjian Zhu[1], Libiao Zhang[3], Hongying Li[1], Aleksei A. Chmura[1], Hume E. Field[1,4], Carlos Zambrana-Torrelio[1], Jonathan H. Epstein[1], Bei Li[2], Wei Zhang[2], Lin-Fa Wang[5], Zheng-Li Shi[2*], Peter Daszak[1*]

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[2]Key laboratory of special pathogens and biosafety, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, China;

[3]Guangdong Institute of Applied Biological Resources, Guangdong Academy of Sciences, Guangzhou, China;

[4]School of Veterinary Science, The University of Queensland, Brisbane, Australia.

[5]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore.

[§]Current Address: Wildlife Conservation Society, Viet Nam Country Program, Ha Noi, Viet Nam; Wildlife Conservation Society, Health Program, Bronx, New York, USA;

[¶]Authors contributed equally to this paper

[*]Correspondence should be addressed to: daszak@ecohealthalliance.org; zlshi@wh.iov.cn

Abstract

Bats are presumed reservoirs of diverse coronaviruses (CoVs) including progenitors of Severe Acute Respiratory Syndrome (SARS)-CoV and SARS-CoV-2, the causative agent of COVID-19. However, the evolution and diversification of these coronaviruses remains poorly understood. We used a Bayesian statistical framework and sequence data from all known bat-CoVs (including 781 novel CoV sequences)

23 to study their macroevolution, cross-species transmission, and dispersal in China. We find that host-
24 switching was more frequent and across more distantly related host taxa in alpha- than beta-CoVs, and
25 more highly constrained by phylogenetic distance for beta-CoVs. We show that inter-family and -genus
26 switching is most common in Rhinolophidae and the genus *Rhinolophus*. Our analyses identify the host
27 taxa and geographic regions that define hotspots of CoV evolutionary diversity in China that could help
28 target bat-CoV discovery for proactive zoonotic disease surveillance. Finally, we present a phylogenetic
29 analysis suggesting a likely origin for SARS-CoV-2 in *Rhinolophus* spp. bats.

30

Introduction

Coronaviruses (CoVs) are RNA viruses causing respiratory and enteric diseases with varying pathogenicity in humans and animals. All CoVs known to infect humans are zoonotic, or of animal origin, with many thought to originate in bat hosts[1,2]. Due to their large genome size (the largest non-segmented RNA viral genome), frequent recombination and high genomic plasticity, CoVs are prone to cross-species transmission and are able to rapidly adapt to new hosts[1,3]. This phenomenon is thought to have led to the emergence of a number of CoVs affecting livestock and human health[4-9]. Three of these causing significant outbreaks originated in China during the last two decades. Severe Acute Respiratory Syndrome (SARS)-CoV emerged first in humans in Guangdong province, southern China, in 2002 and spread globally, causing fatal respiratory infections in close to 800 people[10-12]. Subsequent investigations identified horseshoe bats (genus *Rhinolophus*) as the natural reservoirs of SARS-related CoVs and the likely origin of SARS-CoV[13-16]. In 2016, Swine Acute Diarrhea Syndrome (SADS)-CoV caused the death of over 25,000 pigs in farms within Guangdong province[17]. This virus appears to have originated within *Rhinolophus* spp. bats, and belongs to the HKU2-CoV clade previously detected in bats in the region[17-19]. In 2019, a novel coronavirus (SARS-CoV-2) caused an outbreak of respiratory illness (COVID-19) first detected in Wuhan, Hubei province, China, which has since become a pandemic. This emerging human virus is closely related to SARS-CoV, and also appears to have originated in horseshoe bats[20] (Zhou et al 2020)- with its full genome 96% similar to a viral sequence reported from *Rhinolophus affinis*[20]. Closely related sequences were also identified in Malayan pangolins (Lam et al, 2020; Xiao et al, 2020).

A growing body of research has identified bats as the evolutionary sources of SARS- and Middle East Respiratory Syndrome (MERS)-CoVs [13,14,21-23], and as the source of progenitors for the human CoVs, NL63 and 229E[24,25]. The emergence of SARS-CoV-2 further underscores the importance of bat-origin CoVs to global health, and understanding their origin and cross-species transmission is a high priority for

pandemic preparedness[20,26]. Bats harbor the largest diversity of CoVs among mammals and two CoV genera, alpha- and beta-CoVs (α- and β-CoVs), have been widely detected in bats from most regions of the world[27,28]. Bat-CoV diversity seems to be correlated with host taxonomic diversity globally, the highest CoV diversity being found in areas with the highest bat species richness[29]. Host switching of viruses over evolutionary time is an important mechanism driving the evolution of bat coronaviruses in nature and appears to vary geographically[29,30]. However, detailed analyses of host-switching have been hampered by incomplete or opportunistic sampling, typically with relatively low numbers of viral sequences from any given region[31].

China has a rich bat fauna, with more than 100 described bat species and several endemic species representing both the Palearctic and Indo-Malay regions[32]. Its situation at the crossroads of two zoogeographic regions heightens China's potential to harbor a unique and distinctive CoV diversity. Since the emergence of SARS-CoV in 2002, China has been the focus of an intense viral surveillance and a large number of diverse bat-CoVs has been discovered in the region[33-41]. However, the macroevolution of CoVs in their bat hosts in China and their cross-species transmission dynamics remain poorly understood.

In this study, we analyze an extensive field-collected dataset of bat-CoV sequences from across China. We use a phylogeographic Bayesian statistical framework to reconstruct virus transmission history between different bat host species and virus spatial spread over evolutionary time. Our objectives were to compare the macroevolutionary patterns of α- and β-CoVs and identify the hosts and geographical regions that act as centers of evolutionary diversification for bat-CoVs in China. These analyses aim to improve our understanding of how CoVs evolve, diversify, circulate among, and transmit between bat families and genera to help identify bat hosts and regions where the risk of CoV spillover is the highest.

Results

Taxonomic and geographic sampling

We generated 781 partial sequences (440 nt) of the *RNA-dependent RNA polymerase* (*RdRp*) gene from bat rectal swabs collected in China and added 509 bat-CoV and 8 pangolin CoV sequences from China available in GenBank or GISAID to our datasets (list of GenBank and GISAID accession numbers available in Supplementary Note 1). For each CoV genus, two datasets were created: one including all sequences with known host (host dataset) and one including all sequences with known sampling location at the province level (geographic dataset). To create a geographically discrete partitioning scheme that was more ecologically relevant than administrative borders for our phylogeographic reconstructions, we defined six zoogeographic regions within China by clustering provinces with similar mammalian diversity using hierarchical clustering[42] (see Methods): South western region (SW), Northern region (NO), Central northern region (CN), Central region (CE), Southern region (SO) and Hainan island (HI) (Fig. 1 and Supplementary Fig. 1).

Our host datasets included 718 α-CoV sequences (464 new sequences, including 134 new SADSr-CoV sequences (*Rhinacovirus*)) from 41 bat species (14 genera, five families) and 544 β-CoV sequences (317 new sequences, including 106 new SARSr-CoV sequences (*Sarbecovirus*)) from 31 bat species (15 genera, four families) (Supplementary Table 1). Our geographic datasets included 694 α-CoV sequences from six zoogeographic regions (22 provinces) and 519 β-CoV sequences from five zoogeographic regions (21 provinces) (Fig. 1). As some regions or hosts were overrepresented in our datasets, we also created and ran our analyses using a more uniform subset of our sequence data that included ~30 randomly-selected sequences per host family or region to mitigate sampling and surveillance intensity bias.

Ancestral hosts and cross-species transmission

We used a Bayesian discrete phylogeographic approach implemented in BEAST[43] to reconstruct the ancestral host of each node in the phylogenetic tree using bat host family as a discrete character state.

100 The phylogenetic reconstructions for α-CoVs in China suggest an evolutionary origin within rhinolophid
101 and vespertilionid bats (Fig. 2A). The first α-CoV lineage to diverge historically corresponds to the
102 subgenus *Rhinacovirus* (L1), originating within rhinolophid bats, and includes sequences related to
103 HKU2-CoV and SADS-CoV (Supplementary Fig. 2). Then several lineages, labelled L2 to L7, emerged from
104 vespertilionid bats (Fig. 2A). The subgenus *Decacovirus* (L2) includes sequences mostly associated with
105 the Rhinolophidae and Hipposideridae and related to HKU10-CoV (Supplementary Fig. 3), while the
106 subgenera *Myotacovirus* (L3) and *Pedacovirus* (L5) as well as an unidentified lineage (L4) include CoVs
107 mainly from vespertilionid bats and related to HKU6-, HKU10-, and 512-CoVs (Supplementary Fig. 4-5).
108 Finally, a well-supported node comprises the subgenera *Nyctacovirus* (L6) from vespertilionid bats and
109 *Minunacovirus* (L7) from miniopterid bats, and includes HKU7-, HKU8-, 1A-, and 1B-CoVs
110 (Supplementary Fig. 6). These seven α-CoV lineages are mostly associated with a single host family but
111 each also included several sequences identified from other bat families (Fig. 2A, Supplementary Fig. 2-6
112 and Supplementary Table 1), suggesting frequent cross-species transmission events have occurred
113 among bats. Ancestral host reconstructions based on the random data subset, to normalize sampling
114 effort, gave very similar results with rhinolophids and vespertilionids being the most likely ancestral
115 hosts of most α-CoV lineages too (Supplementary Fig. 7A). However, the topology of the tree based on
116 the random subset was slightly different as the lineage L5 was paraphyletic.

117 Chinese β-CoVs likely originated from vespertilionid and rhinolophid bats (Fig. 2B). The MCC tree was
118 clearly structured into four main lineages: *Merbecovirus* (Lineage C), including MERS-related (MERSr-)
119 CoVs, HKU4- and HKU5-CoVs and strictly restricted to vespertilionid bats (Supplementary Fig. 8);
120 *Nobecovirus* (lineage D), originating from pteropodid bats and corresponding to HKU9-CoV
121 (Supplementary Fig. 9); *Hibecovirus* (lineage E) comprising sequences isolated in hipposiderid bats
122 (Supplementary Fig. 10) and *Sarbecovirus* (Lineage B) including sequences related to HKU3- and SARS-
123 related (SARSr-) CoVs originating in rhinolophid bats (Supplementary Fig. 11). We show that SARS-CoV-2

forms a divergent clade within *Sarbecovirus* and is most closely related to viruses sampled from *Rhinolophus malayanus* and *R. affinis* and from Malayan pangolins (*Manis javanica*) (Fig. 3). Similar tree topology and ancestral host inference were obtained with the random subset (Supplementary Fig. 7B).

We used a Bayesian Stochastic Search Variable Selection (BSSVS) procedure[44] to identify viral host switches (transmission over evolutionary time) between bat families and genera that occurred along the branches of the MCC annotated tree and calculated Bayesian Factor (BF) to estimate the significance of these switches (Fig. 4). We identified nine highly supported (BF > 10) inter-family host switches for α-CoVs and three for β-CoVs (Fig. 4A and 4B). These results are robust over a range of sample sizes, with seven of these nine switches for α-CoVs and the exact same three host switches for β-CoVs having strong BF support (BF > 10) when analyzing our random subset (Supplementary Tables 2 and 3). To quantify the magnitude of these host switches, we estimated the number of host switching events (Markov jumps)[45,46] along the significant inter-family switches (Fig. 4C and 4D) and estimated the rate of inter-family host switching events per unit of time for each CoV genus. The rate of inter-family host switching events was five times higher in the evolutionary history of α- (0.010 host switches/unit time) than β-CoVs (0.002 host switches/unit time) in China. For α-CoVs, host switching events from the Rhinolophidae and the Miniopteridae were greater than from other bat families while rhinolophids were the highest donor family for β-CoVs. The Rhinolophidae and the Vespertilionidae for α-CoVs and the Hipposideridae for β-CoVs received the highest numbers of switching events (Fig. 4C and 4D). When using the random dataset, similar results were obtained for β-CoVs while rhinolophids were only the highest donor family for α-CoVs (Supplementary Tables 4 and 5).

At the genus level, we identified 20 highly supported inter-genus host switches for α-CoVs, 17 of them were also highly significant using the random subset (Fig. 5A and Supplementary Table 6). Sixteen highly supported inter-genus switches were identified for β-CoVs (Fig. 5B). Similar results were obtained for the random β-CoV subset (Supplementary Table 7). Most of the significant cross-genus CoV switches for

α-CoVs, 15 of 20 (75%), were between genera in different bat families, while this proportion was only 6 of 16 (37.5%) for β-CoVs. The estimated rate of inter-genus host switching events (Markov jumps) was similar for α- (0.014 host switches/unit time) and β-CoVs (0.014 host switches/unit time). For α-CoVs, *Rhinolophus* and *Miniopterus* were the greatest donor genera and *Rhinolophus* was the greatest receiver (Supplementary Table 8). For β-CoVs, *Rousettus* was the greatest donor and *Eonycteris* the greatest receiver genus (Supplementary Table 9).

CoV spatiotemporal dispersal in China

We used our Bayesian discrete phylogeographic model with zoogeographic regions as character states to reconstruct the spatiotemporal dynamics of CoV dispersal in China. Eleven and seven highly significant (BF > 10) dispersal routes within China were identified for α- and β-CoVs, respectively (Fig. 6). Seven and five of these dispersal routes, respectively, remained significant when using our random subsets (Supplementary Tables 10 and 11). The *Rhinacovirus* lineage (L1) that includes HKU2 and SADS-CoV likely originated in the SO region while all other α-CoV lineages historically arose in SW China and spread to other regions before several dispersal events from SO and NO in all directions (Fig. 6A and Supplementary Fig. 12). A roughly similar pattern of α-CoV dispersal was obtained using the random subset (Supplementary Tables 10 and 12).

The oldest inferred dispersal movements for β-CoVs occurred among the SO and SW regions (Fig. 6B). The SO region was the likely origin of *Merbecovirus* (Lineage C, including HKU4 and HKU5) and *Sarbecovirus* subgenera (Lineage B, including HKU 3 and SARSr-CoVs) while the *Nobecovirus* (lineage D, including HKU9) and *Hibecovirus* (lineage E) subgenera originated in SW China (Supplementary Fig. 12). Then several dispersal movements likely originated from SO and CE (Fig. 6B). More recent southward dispersal from NO was observed. Similar spatiotemporal dispersal patterns were observed using the random subset of β-CoVs (Supplementary Tables 11 and 13).

The estimated rate of migration events per unit of time along these significant dispersal routes was more than two times higher for α- (0.026 host switches/unit time) than β-CoVs (0.011 host switches/unit time) and SO was the region involved in the greatest total number of migration events for both α- and β-CoVs. SO had the highest number of outbound and inbound migration events for α-CoVs (Fig. 6C and Supplementary Table 12). For β-CoVs, the highest number of outbound migration events was estimated to be from NO and SO while SO and SW had the highest numbers of inbound migration events (Fig. 6D and Supplementary Table 13).

Phylogenetic diversity

In order to identify the hotspots of CoV phylogenetic diversity in China and evaluate phylogenetic clustering of CoVs, we calculated the Mean Phylogenetic Distance (MPD) and the Mean Nearest Taxon Distance (MNTD) statistics[47] and their standardized effect size (SES).

We found significant and negative SES MPD values, indicating significant phylogenetic clustering, within all bat families and genera for both α- and β-CoVs, except within the *Aselliscus* and *Tylonycteris* for α-CoVs (Fig. 7A and 7B). Negative and mostly significant SES MNTD values, reflecting phylogenetic structure closer to the tips, were also observed within most bat families and genera for α- and β-CoVs but we found non-significant positive SES MNTD value for vespertilionid bats, and particularly for those in the *Pipistrellus* genus, for β-CoVs (Fig. 7A and 7B). In general, we observed lower phylogenetic diversity for β- than α-CoVs within all bat families and most genera when looking at SES MPD, but the difference in the level of diversity between α- and β-CoVs is less important when looking at SES MNTD (Fig. 7). These results suggest stronger basal clustering (reflected by larger SES MPD values) for β-CoVs than α-CoVs, indicating stronger host structuring effect and phylogenetic conservatism for β-CoVs. Very similar results were obtained with the random subsets for both α- and β-CoVs (Supplementary Tables 14-21).

We found negative and mostly significant values of MPD and MNTD (Fig. 7C and Supplementary Tables 22-25) indicating significant phylogenetic clustering of CoV lineages in bat communities within the same zoogeographic region. However, SES MPD values for α-CoVs in SW were positive (significant for the random subset) indicating a greater evolutionary diversity of CoVs in that region than others (Fig. 7 and Supplementary Tables 22-25). We used a linear regression analysis to assess the relationship between CoV phylogenetic diversity and bat species richness in China and determine if bat richness is a significant predictor of bat-CoV diversity and evolution. α-CoV phylogenetic diversity (MPD) was not significantly correlated to total bat species richness or sampled bat species richness in zoogeographic regions or provinces (Supplementary Table 26). Non-significant correlations between bat species richness and β-CoV phylogenetic diversity were also observed at the zoogeographic region level (Supplementary Table 27). However, a significant correlation was observed between sampled bat species richness and β-CoV phylogenetic diversity at the province level (Supplementary Table 27). Similar results were obtained when using the random subsets (Supplementary Tables 26 and 27). These findings suggest that bat host diversity is not the main driver of CoV diversity in China and that other ecological or biogeographic factors may influence this diversity. We observed higher CoV diversity than expected in several southern or central provinces (Hainan, Guangxi, Hunan) given their underlying total or sampled bat diversity (Supplementary Fig. 13 and 14).

We also assessed patterns of CoV phylogenetic turnover/differentiation among Chinese zoogeographic regions and bat host families by measuring the inter-region and inter-host values of MPD (equivalent to a measure of phylogenetic β diversity) and their SES. We found positive inter-family SES MPD values, except between Pteropodidae and Hipposideridae for α-CoVs and between Rhinolophidae and Hipposideridae for β-CoVs (Fig. 8A and 8B and Supplementary Tables 28 and 29), suggesting higher phylogenetic differentiation of CoVs among most bat families than among random communities. Our phylo-ordination based on inter-family MPD values indicated that α-CoVs from vespertilionids and

miniopterids, and from hipposiderids and pteropodids; as well as β-CoVs from rhinolophids and hipposiderids are phylogenetically closely related (Fig. 8A and 8B). We also observed strong phylogenetic turnover between α-CoV strains from rhinolophids and from miniopterids and all other bat families, and between β-CoV strains from vespertilionids and all other bat families (Supplementary Tables 28 and 29). Phylo-ordination among bat genera based on inter-genus MPD confirmed these results and indicated that CoV strains from genera belonging to the same bat family were mostly more closely related to each other than to genera from other families (Fig. 8C and 8D and Supplementary Tables 30 and 31).

We observed high and positive inter-region SES MPD values between SW/HI and all other regions, suggesting that these two regions host higher endemic diversity (Fig. 9 and Supplementary Tables 32 and 31). Negative inter-region SES MPD values suggested that the phylogenetic turnover among other regions was less important than expected among random communities. Our phylo-ordination among zoogeographic regions also reflected the high phylogenetic turnover and deep evolutionary distinctiveness of both α- and β-CoVs from SW and HI regions (Fig. 9 and Supplementary Tables 32 and 33). Similar results were obtained using the random subset (Supplementary Tables 32 and 33).

Mantel tests

Mantel tests revealed a positive and significant correlation between CoV genetic differentiation (F_{ST}) and geographic distance matrices, both with and without provinces including fewer than four viral sequences, for α- (r = 0.25, p = 0.0097; r = 0.32, p = 0.0196; respectively) and β-CoVs (r = 0.22, p = 0.0095; r = 0.23, p = 0.0336; respectively). We also detected a positive and highly significant correlation between CoV genetic differentiation (F_{ST}) and their host phylogenetic distance matrices, both with and without genera including fewer than four viral sequences, for β-CoVs (r = 0.41, p = 0; r = 0.39, p = 0.0012; respectively) but not for α-CoVs (r = -0.13, p = 0.8413; r = 0.02, p = 0.5019; respectively).

Discussion

Our phylogenetic analysis shows a high diversity of CoVs from bats sampled in China, with most bat genera included in this study (10/16) infected by both α- and β-CoVs. In our phylogenetic analysis that includes all known bat-CoVs from China, we find that SARS-CoV-2 is likely derived from a clade of viruses originating in horseshoe bats (*Rhinolophus* spp.). The geographic location of this origin appears to be Yunnan province. However, it is important to note that: 1) our study collected and analyzed samples solely from China; 2) many sampling sites were close to the borders of Myanmar and Lao PDR; and 3) most of the bats sampled in Yunnan also occur in these countries, including *R. affinis* and *R. malayanus,* the species harboring the CoVs with highest RdRp sequence identity to SARS-CoV-2 (Zhou et al 2020). For these reasons, we cannot rule out an origin for the clade of viruses that are progenitors of SARS-CoV-2 that is outside China, and within Myanmar, Lao PDR, Vietnam or another Southeast Asian country. Additionally, our analysis shows that the virus RmYN02 from *R. malayanus*, which is characterized by the insertion of multiple amino acids at the junction site of the S1 and S2 subunits of the Spike (S) protein, belongs to the same clade as both RaTG13 and SARS-CoV-2, providing further support for the natural origin of SARS-CoV-2 in *Rhinolophus* spp. bats in the region (Zhou et al 2020, Zhou et al 2020). Finally, while our analysis shows that the RdRp sequences of coronaviruses from the Malayan pangolin are closely related to SARS-CoV-2 RdRp, analysis of full genomes of these viruses suggest that these terrestrial mammals are less to be the origin of SARS-CoV-2 than *Rhinolophus* spp. bats (Lam et al 2020, Xiao et al 2020). This analysis also demonstrates that a significant amount of cross-species transmission has occurred among bat hosts over evolutionary time. Our Bayesian phylogeographic inference and analysis of host switching showed varying levels of viral connectivity among bat hosts and allowed us to identify significant host transitions that appear to have occurred during bat-CoV evolution in China.

We found that bats in the family Rhinolophidae (horseshoe bats) played a key role in the evolution and cross-species transmission history of α-CoVs. The family Rhinolophidae and the genus *Rhinolophus* were involved in more inter-family and inter-genus highly significant host switching of α-CoVs than any other family or genus. They were the greatest receivers of α-CoV host switching events and second greatest donors after Miniopteridae/*Miniopterus*. The Rhinolophidae, together with the Hipposideridae, also played an important role in the evolution of β-CoVs, being at the origin of most inter-family host switching events. Chinese horseshoe bats are characterized by a distinct and evolutionary divergent α-CoV diversity, while their β-CoV diversity is similar to that found in the Hipposideridae. The Rhinolophidae comprises a single genus, *Rhinolophus*, and is the most speciose bat family after the Vespertilionidae in China[48], with 20 known species, just under a third of global *Rhinolophus* diversity, mostly in Southern China[32]. This family likely originated in Asia[49,50], but some studies suggest an African origin[51,52]. Rhinolophid fossils from the middle Eocene (38 - 47.8 Mya) have been found in China, suggesting a westward dispersal of the group from eastern Asia to Europe[53]. The ancient likely origin of the Rhinolophidae in Asia and China in particular may explain the central role they played in the evolution and diversification of bat-CoVs in this region, including SARSr-CoVs, MERS-cluster CoVs, and SADSr-CoVs, which contain important human and livestock pathogens. Horseshoe bats are known to share roosts with genera from all other bat families in this study[54], which may also favor CoV cross-species transmission from and to rhinolophids[31]. A global meta-analysis showing higher rates of viral sharing among co-roosting cave bats supports this finding[55].

Vespertilionid and miniopterid bats (largely within the *Myotis* and *Miniopterus* genera) also appear to have been involved in several significant host switches during α-CoV evolution. However, no significant transition from vespertilionid bats was identified for β-CoVs and these bats exhibit a divergent β-CoV diversity compared to other bat families. Vespertilionid and miniopterid bats are characterized by strong basal phylogenetic clustering but high recent CoV diversification rates, indicating a more rapid

evolutionary radiation of CoVs in these bat hosts. At the genus level, similar findings were observed for the genera *Myotis*, *Pipistrellus* and *Miniopterus*.

A significant correlation between geographic distance and genetic differentiation of both α- and β-CoVs has been detected, even if only a relatively small proportion of the variance is explained by geographic distance. We also revealed a significant effect of host phylogeny on β-CoV evolution while it had a minimal effect on α-CoV diversity. Contrary to the α-CoV phylogeny, the basal phylogenetic structure of β-CoVs mirrored the phylogeny of their bat hosts, with a clear distinction between the Yangochiroptera, encompassing the Vespertilionidae and Miniopteridae, and the Yinpterochiroptera, which includes the megabat family Pteropodidae and the microbat families Rhinolophidae and Hipposideridae, as evidenced in recent bat phylogenies[49,56]. These findings suggest a profound co-macroevolutionary process between β-CoVs and their bat hosts, even if host switches also occurred throughout their evolution as our study showed. The phylogenetic structure of α-CoVs, with numerous and closely related lineages identified in the Vespertilionidae and Miniopteridae, contrasts with the β-CoV macroevolutionary pattern and suggests α-CoVs have undergone an adaptive radiation in these two Yangochiroptera families. Our BSSVS procedure and Markov jump estimates revealed higher connectivity, both qualitatively and quantitatively, among bat families and genera in the α-CoV cross-species transmission history. Larger numbers of highly significant host transitions and higher rates of switching events along these pathways were inferred for α- than β-CoVs, especially at the host family level. These findings suggest that α-CoVs are able to switch hosts more frequently and between more distantly related taxa, and that phylogenetic distance among hosts represents a higher constraint on host switches for β- than α-CoVs. This is supported by more frequent dispersal events in the evolution of α- than β-CoVs in China.

Variation in the extent of host jumps between α and β-CoVs within the same hosts in the same environment may be due to virus-specific factors such as differences in receptor usage between α- and

β-CoVs[57-59]. Coronaviruses use a large diversity of receptors, and their entry into host cells is mediated by the spike protein with an ectodomain consisting of a receptor-binding subunit S1 and a membrane-fusion subunit S2[60]. However, despite differences in the core structure of their S1 receptor binding domains (RBD), several α- and β-CoV species are able to recognize and bind to the same host receptors[61]. Other factors such as mutation rate, recombination potential, or replication rate might also be involved in differences in host switching potential between α- and β-CoVs. A better understanding of receptor usage and other biological characteristics of these bat-CoVs may help predict their cross-species transmission and zoonotic potential.

We also found that some bat genera were infected by a single CoV genus: *Miniopterus* (Miniopteridae) and *Murina* (Vespertilionidae) carried only α-CoVs, while *Cynopterus*, *Eonycteris*, *Megaerops* (Pteropodidae) and *Pipistrellus* (Vespertilionidae) hosted only β-CoVs. This was found despite using the same conserved pan-CoV PCR assays for all specimens screened and it can't be explained by differences in sampling effort for these genera (Supplementary Table 1): for example, >250 α-CoV sequences but no β-CoV were discovered in *Miniopterus* bats in China during our recent fieldwork. These migratory bats, which seem to have played a key role in the evolution of α-CoVs, share roosts with several other bat genera hosting β-CoVs in China[54], suggesting high likelihood of being exposed to β-CoVs. Biological or ecological properties of miniopterid bats may explain this observation and clearly warrant further investigation.

Our Bayesian ancestral reconstructions revealed the importance of South western and Southern China as centers of diversification for both α- and β-CoVs. These two regions are hotspots of CoV phylogenetic diversity, harboring evolutionarily old and phylogenetically diverse lineages of α- and β-CoVs. South western China acted as a refugium during Quaternary glaciation for numerous plant and animal species including several bat species, such as *Rhinolophus affinis*[62], *Rhinolophus sinicus*[63], *Myotis davidii*[64], and *Cynopterus sphinx*[65]. The stable and long-term persistence of bats and other mammals throughout the

335 Quaternary may explain the deep macroevolutionary diversity of bat-CoVs in these regions[66]. Several
336 highly significant and ancient CoV dispersal routes from these two regions have been identified in this
337 study. Other viruses, such as the Avian Influenza A viruses H5N6, H7N9 and H5N1, also likely originated
338 in South western and Southern Chinese regions[67,68].

339 Our findings suggest that bat host diversity is not the main driver of CoV diversity in China and that
340 other ecological or biogeographic factors may influence this diversity. Overall, there were no significant
341 correlations between CoV phylogenetic diversity and bat species diversity (total or sampled) for each
342 province or biogeographic region, apart from a weak correlation between β-CoV phylogenetic diversity
343 and the number of bat species sampled at the province level. Yet, we observed higher than expected
344 phylogenetic diversity in several southern provinces (Hainan, Guangxi, Hunan). These results and main
345 conclusions are consistent and robust even when we account for geographic biases in sampling effort by
346 analyzing random subsets of the data.

347 Despite being the most exhaustive study of bat-CoVs in China, this study had several limitations that
348 must be taken into consideration when interpreting our results. First, only partial *RdRp* sequences were
349 generated in this study and used in our phylogenetic analysis as the non-invasive samples (rectal
350 swabs/feces) collected in this study prevented us from generating longer sequences in many cases. The
351 *RdRp* gene is a suitable marker for this kind of study as it reflects vertical ancestry and is less prone to
352 recombination than other regions of the CoV genome such as the spike protein gene[16,69]. While using
353 long sequences is always preferable, our phylogenetic trees are well supported and their topology
354 consistent with trees obtained using longer sequences or whole genomes[27,70]. Second, most sequences
355 in this study were obtained by consensus PCR using primers targeting highly conserved regions. Even if
356 this broadly reactive PCR assay designed to detect widely variant CoVs has proven its ability to detect a
357 large diversity of CoVs in a wide diversity of bats and mammals[29,71-74], we may not rule out that some

358 bat-CoV variants remained undetected. Using deep sequencing techniques would allow to detect this
359 unknown and highly divergent diversity.

360 In this study, we identified the host taxa and geographic regions that together define hotspots of CoV
361 phylogenetic diversity and centers of diversification in China. These findings may provide a strategy for
362 targeted discovery of bat-borne CoVs of zoonotic or livestock infection potential, and for early detection
363 of bat-CoV outbreaks in livestock and people, as proposed elsewhere[75]. Our results suggest that future
364 sampling and viral discovery should target two hotspots of CoV diversification in Southern and South
365 western China in particular, as well as neighboring countries where similar bat species live. These
366 regions are characterized by a subtropical to tropical climate; dense, growing and rapidly urbanizing
367 populations of people; a high degree of poultry and livestock production; and other factors which may
368 promote cross-species transmission and disease emergence[75-77]. Additionally, faster rates of evolution in
369 the tropics have been described for other RNA viruses which could favor cross-species transmission of
370 RNA viruses in these regions[78]. Both SARS-CoV and SADS-CoV emerged in this region, and several bat
371 SARSr-CoVs with high zoonotic potential have recently been reported from there, although the dynamics
372 of their circulation in wild bat populations remain poorly understood[16,58]. Importantly, the closest known
373 relative of SARS-CoV-2, a SARS-related virus, was found in a *Rhinolophus* sp. bat in this region[20],
374 although it is important to note that our survey was limited to China, and that the bat hosts of this virus
375 also occur in nearby Myanmar and Lao PDR. The significant public health and food security implications
376 of these outbreaks reinforces the need for enhanced, targeted sampling and discovery of novel CoVs.
377 Because intensive sampling has not, to our knowledge, been undertaken in countries bordering
378 southern China, these surveys should be extended to include Myanmar, Lao PDR, and Vietnam, and
379 perhaps across southeast Asia. Our finding that *Rhinolophus* spp. are most likely to be involved in host-
380 switching events makes them a key target for future longitudinal surveillance programs, but surveillance

targeted the genera *Hipposideros* and *Aselliscus* may also be fruitful as they share numerous β-CoVs with *Rhinolophus* bats.

In the aftermath of the SARS-CoV and MERS-CoV outbreaks, β-CoVs have been the main focus of bat-CoV studies in China, Africa, and Europe[17,29,33,58,79]. However, we have shown that α-CoVs have a higher propensity to switch host within their natural bat reservoirs, and therefore also have a high cross-species transmission potential and risk of spillover. This is exemplified by the recent emergence of SADS-CoV in pigs in Guangdong province[17]. Two human α-CoVs, NL63 and 229E, also likely originated in bats[24,25], reminding us that past spillover events from bat species can readily be established in the human population. Future work discovering and characterizing the biological properties of bat α-CoVs may therefore be of potential value for public and livestock health. Our study, and recent analysis of viral discovery rates[80], suggest that a substantially wider sampling and discovery net will be required to capture the complete diversity of coronaviruses in their natural hosts and assess their potential for cross-species transmission. The bat genera *Rhinolophus*, *Hipposideros*, *Myotis* and *Miniopterus*, all involved in numerous naturally-occurring host switches throughout α-CoV evolution, should be a particular target for α-CoV discovery in China and across southeast Asia, with *in vitro* and experimental characterization to better understand their potential to infect people or livestock and cause disease.

Methods

Bat sampling

Bat oral and rectal swabs and fecal pellets were collected from 2010 to 2015 in numerous Chinese provinces (Anhui, Beijing, Guangdong, Guangxi, Guizhou, Hainan, Henan, Hubei, Hunan, Jiangxi, Macau, Shanxi, Sichuan, Yunnan, and Zhejiang). Fecal pellets were collected from tarps placed below bat colonies. Bats were captured using mist nets at their roost site or feeding areas. Each captured bat was stored into a cotton bag, all sampling was non-lethal and bats were released at the site of capture

404 immediately after sample collection. A wing punch was also collected for barcoding purpose. Bat-
405 handling methods were approved by Tufts University IACUC committee (proposal #G2017-32) and
406 Wuhan Institute of Virology Chinese Academy of Sciences IACUC committee (proposal WIVA05201705).
407 Samples were stored in viral transport medium at -80°C directly after collection.

408 **RNA extraction and PCR screening**

409 RNA was extracted from 200 µl swab rectal samples or fecal pellets with the High Pure Viral RNA Kit
410 (Roche) following the manufacturer's instructions. RNA was eluted in 50 µl elution buffer and stored at -
411 80°C. A one-step hemi-nested RT-PCR (Invitrogen) was used to detect coronavirus RNA using a set of
412 primers targeting a 440-nt fragment of the *RdRp* gene and optimized for bat-CoV detection (CoV-FWD3:
413 GGTTGGGAYTAYCCHAARTGTGA; CoV-RVS3: CCATCATCASWYRAATCATCATA; CoV-FWD4/Bat:
414 GAYTAYCCHAARTGTGAYAGAGC)[81]. For the first round PCR, the amplification was performed as follows:
415 50°C for 30 min, 94°C for 2 min, followed by 40 cycles consisting of 94°C for 20 sec, 50°C for 30 sec, 68°C
416 for 30 sec, and a final extension step at 68°C for 5 min. For the second round PCR, the amplification was
417 performed as follows: 94°C for 2 min followed by 40 cycles consisting of 94°C for 20 sec, 59°C for 30 sec,
418 72°C for 30 sec, and a final extension step at 72°C for 7 min. PCR products were gel purified and
419 sequenced with an ABI Prism 3730 DNA analyzer (Applied Biosystems, USA). PCR products with low
420 concentration or bad sequencing quality were cloned into pGEM-T Easy Vector (Promega) for
421 sequencing. Positive results detected in bat genera that were not known to harbor a specific CoV lineage
422 previously were repeated a second time (PCR + sequencing) as a confirmation. Species identifications
423 from the field were also confirmed and re-confirmed by cytochrome (cytb) DNA barcoding using DNA
424 extracted from the feces or swabs[82]. Only viral detection and barcoding results confirmed at least twice
425 were included in this study.

426 **Sequence data**

We also added bat-CoV *RdRp* sequences from China available in GenBank to our dataset. All sequences for which sampling year and host or sampling location information was available either in GenBank metadata or in the original publication were included (as of March 15, 2018). Our final datasets include 732 sequences generated for this study and 508 sequences from GenBank (list of GenBank accession numbers available in Supplementary Note 1, and Supplementary Tables 34 and 35). Nucleotide sequences were aligned using MUSCLE and trimmed to 360 base pair length to reduce the proportion of missing data in the alignments. All phylogenetic analyses were performed on both the complete data and random subset, and for α- and β-CoVs separately.

Defining zoogeographic regions in China for phylogeographic analyses

Hierachical clustering was used to define zoogeographic regions within China by clustering provinces with similar mammalian diversity[42]. Hierarchical cluster analysis classifies several objects into small groups based on similarities between them. To do this, we created a presence/absence matrix of all extant terrestrial mammals present in China using data from the IUCN spatial database[83] and generated a cluster dendrogram using the function *hclust* with average method of the R package stats. Hong Kong and Macau were included within the neighboring Guangdong province. We then visually identified geographically contiguous clusters of provinces for which CoV sequences are available (Fig. 1 and Supplementary Fig. 1).

We identified six zoogeographic regions within China based on the similarity of the mammal community in these provinces: South western region (SW; Yunnan province), Northern region (NO; Xizang, Gansu, Jilin, Anhui, Henan, Shandong, Shaanxi, Hebei and Shanxi provinces and Beijing municipality), Central northern region (CN; Sichuan and Hubei provinces), Central region (CE; Guangxi, Guizhou, Hunan, Jiangxi and Zhejiang provinces), Southern region (SO; Guangdong and Fujian provinces, Hong Kong, Macau and Taiwan), and Hainan island (HI). Hunan and Jiangxi, clustering with the SO provinces in our dendrogram,

were included within the central region to create a geographically contiguous Central cluster (Supplementary Fig. 1). These six zoogeographic regions are very similar to the biogeographic regions traditionally recognized in China[84]. The three β-CoV sequences from HI were included in the SO region to avoid creating a cluster with a very small number of sequences.

Model selection and phylogenetic analysis

Bayesian phylogenetic analysis were performed in BEAST 1.8.4[43]. Sampling years were used as tip dates. Preliminary analysis were run to select the best fitting combination of substitution models (HKY/GTR), codon partition scheme, molecular clock (strict/lognormal uncorrelated relaxed clock) and coalescent models (constant population size/exponential growth/GMRF Bayesian Skyride). Model combinations were compared and the best fitting model was selected using a modified Akaike information criterion (AICM) implemented in Tracer 1.6[85]. We also used TEMPEST[86] to assess the temporal structure within our α- and β-CoV datasets. TEMPEST showed that both datasets did not contain sufficient temporal information to accurately estimate substitution rates or time to the most recent common ancestor (TMRCA). Therefore we used a fixed substitution rate of 1.0 for all our BEAST analysis.

All subsequent BEAST analysis were performed under the best fitting model including a HKY substitution model with two codons partitions ((1+2), 3), a strict molecular clock and a constant population size coalescent model. Each analysis was run for 2.5 x 10^8 generations, with sampling every 2 x 10^4 steps. All BEAST computations were performed on the CIPRES Science Getaway Portal[87]. Convergence of the chain was assessed in Tracer so that the effective sample size (ESS) of all parameters was > 200 after removing at least 10% of the chain as burn-in.

Ancestral state reconstruction and transition rates

A Bayesian discrete phylogeographic approach implemented in BEAST 1.8.4 was used to reconstruct the ancestral state of each node in the phylogenetic tree for three discrete traits: host family, host genus

and zoogeographic region. An asymmetric trait substitution model was applied. These analyses were performed for each trait on the complete dataset and random subsets. Maximum clade credibility (MCC) tree annotated with discrete traits were generated in TreeAnnotator and visualized using the software SpreaD3[88].

For each analysis, a Bayesian stochastic search variable selection (BSSVS) was applied to estimate the significance of pairwise switches between trait states using Bayesian Factor (BF) as a measure of statistical significance[44]. BF were computed in SpreaD3. BF support was interpreted according to Jeffreys 1961[89] (BF > 3: substantial support, BF > 10: strong support, BF > 30: very strong support, BF > 100: decisive support) and only strongly supported transitions were presented in most figures, following a strategy used in other studies[90,91]. We also estimated the count of state switching events (Markov jumps)[45,46] along the branches of the phylogenetic tree globally (for the three discrete traits) and for each strongly supported (BF > 10) transition between character states (for bat families and ecoregions only). Convergence of the MCMC runs was confirmed using Tracer. The rate of state switching events per unit of time was estimated for each CoV genus by dividing the total estimated number of state switching events by the total branch length of the MCC tree.

To assess the phylogenetic relationships among SARS-CoV-2 and other CoVs from the *Sarbecovirus* subgenus, we also reconstructed a MCC tree in BEAST 1.8.4 and median-joining network in Network 10.0[92] including all *Sarbecovirus* sequences, two sequences of SARS-CoV-2 isolated in humans (GenBank accession numbers: MN908947 and MN975262), one sequence of SARS-CoV (GenBank accession number: NC_004718), eight sequences from Malayan pangolins (*Manis javanica*) (GISAID accession numbers: EPI_ISL_410538-410544, EPI_ISL_410721) and one from *Rhinolophus malayanus* (GISAID accession number: EPI_ISL_412977).

Phylogenetic diversity

The Mean Phylogenetic Distance (MPD) and the Mean Nearest Taxon Distance (MNTD) statistics[47] and their standardized effect size (SES) were calculated for each zoogeographic region, bat family and genus using the R package picante[93]. MPD measures the mean phylogenetic distance among all pairs of CoVs within a host or a region. It reflects phylogenetic structuring across the whole phylogenetic tree and assesses the overall divergence of CoV lineages in a community. MNTD is the mean distance between each CoV and its nearest phylogenetic neighbor in a host or region, and therefore it reflects the phylogenetic structuring closer to the tips and shows how locally clustered taxa are. SES MPD and SES MNTD values correspond to the difference between the phylogenetic distances in the observed communities versus null communities. Low and negative SES values denote phylogenetic clustering, high and positive values indicate phylogenetic over-dispersion while values close to 0 show random dispersion. The SES values were calculated by building null communities by randomly reshuffling tip labels 1000 times along the entire phylogeny. Phylogenetic diversity computations were performed on both the complete dataset and random subset for each trait. A linear regression analysis was performed in R to assess the correlation between CoV phylogenetic diversity (MPD) and bat species richness in China. Total species richness per province or region was estimated using data from the IUCN spatial database while sampled species richness corresponds to the number of bat species sampled and tested for CoV per province or region in our datasets.

The inter-region and inter-host values of MPD (equivalent to phylogenetic β diversity), corresponding to the mean phylogenetic distance among all pairs of CoVs from two distinct hosts or regions, and their SES were estimated using the function *comdist* of the R package phylocomr[94]. The matrices of inter-region and inter-host MPD were used to cluster zoogeographic regions and bat hosts in a dendrogram according to their evolutionary similarity (phylo-ordination) using the function *hclust* with complete linkage method of the R package stats (R core team). These computations were performed on both the complete dataset and random subset.

Mantel tests and isolation by distance

Mantel tests performed in ARLEQUIN 3.5[95] were used to compare the matrix of viral genetic differentiation (F_{ST}) to matrices of host phylogenetic distance and geographic distance in order to evaluate the role of geographic isolation and host phylogeny in shaping CoV population structure. The correlation between these matrices was assessed using 10,000 permutations. To gain more resolution into the process of evolutionary diversification, these analyses were also performed at the host genus and province levels. To calculate phylogenetic distances among bat genera, we reconstructed a phylogenetic tree including a single sequence for all bat species included in our dataset. Pairwise patristic distances among tips were computed using the function *distTips* in the R package adephylo[96]. We then averaged all distances across genera to create a matrix of pairwise distances among bat genera. Pairwise Euclidian distances were measured between province centroids and log transformed. Mantel tests were performed with and without genera and provinces including less than four viral sequences to assess the impact of low sample size on our results.

Data availability

GenBank accession numbers of sequences generated in this study and previously published sequences included in our analysis are available in the Supplementary Note 1 and Supplementary Tables 34 and 35.

References

1. Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular Evolution of Human Coronavirus Genomes. *Trends in Microbiology* **25**, 35-48 (2017).
2. Tao, Y. *et al.* Surveillance of Bat Coronaviruses in Kenya Identifies Relatives of Human Coronaviruses NL63 and 229E and Their Recombination History. *Journal of Virology* **91**(2017).

3. Graham, R.L. & Baric, R.S. Recombination, Reservoirs, and the Modular Spike: Mechanisms of Coronavirus Cross-Species Transmission. **84**, 3134-3146 (2010).

4. Vijgen, L. *et al.* Evolutionary history of the closely related group 2 coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus OC43. *Journal of virology* **80**, 7270-7274 (2006).

5. Zhang, X. *et al.* Quasispecies of bovine enteric and respiratory coronaviruses based on complete genome sequences and genetic changes after tissue culture adaptation. *Virology* **363**, 1-10 (2007).

6. Parrish, C.R. *et al.* Cross-Species Virus Transmission and the Emergence of New Epidemic Diseases. *Microbiology and Molecular Biology Reviews* **72**, 457-470 (2008).

7. Li, D.L. *et al.* Molecular evolution of porcine epidemic diarrhea virus and porcine deltacoronavirus strains in Central China. *Research in Veterinary Science* **120**, 63-69 (2018).

8. Cui, J., Li, F. & Shi, Z.-L. Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology* **17**, 181-192 (2019).

9. Lau, S.K.P. & Chan, J.F.W. Coronaviruses: emerging and re-emerging pathogens in humans and animals. *Virology Journal* **12**, 209 (2015).

10. Drosten, C. *et al.* Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* **348**, 1967-76 (2003).

11. Heymann, D.L. The international response to the outbreak of SARS in 2003. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 1127-1129 (2004).

12. World Health Organization. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. Vol. 2019 (World Health Organization, 2004).

13. Ge, X.-Y. *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).

14. Li, W. *et al.* Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**, 676-9 (2005).

15. Lau, S.K.P. *et al.* Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 14040-14045 (2005).

16. Hu, B. *et al.* Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathogens* **13**, e1006698 (2017).

17. Zhou, P. *et al.* Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* **556**, 255-258 (2018).

18. Gong, L. *et al.* A New Bat-HKU2-like Coronavirus in Swine, China, 2017. *Emerging infectious diseases* **23**, 1607-1609 (2017).

19. Pan, Y. *et al.* Discovery of a novel swine enteric alphacoronavirus (SeACoV) in southern China. *Veterinary Microbiology* **211**, 15-21 (2017).

20. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, **579**, 270-273 (2020).

21. Corman, V.M. *et al.* Rooting the Phylogenetic Tree of Middle East Respiratory Syndrome Coronavirus by Characterization of a Conspecific Virus from an African Bat. *Journal of Virology* **88**, 11297-11303 (2014).

22. Anthony, S.J. *et al.* Further Evidence for Bats as the Evolutionary Source of Middle East Respiratory Syndrome Coronavirus. *mBio* **8**, e00373-17 (2017).

23. Lau, S.K.P. *et al.* Receptor Usage of a Novel Bat Lineage C Betacoronavirus Reveals Evolution of Middle East Respiratory Syndrome-Related Coronavirus Spike Proteins for Human Dipeptidyl Peptidase 4 Binding. *The Journal of Infectious Diseases*, jiy018-jiy018 (2018).

24. Corman, V.M. *et al.* Evidence for an Ancestral Association of Human Coronavirus 229E with Bats. *Journal of Virology* **89**, 11858-11870 (2015).

25. Huynh, J. *et al.* Evidence Supporting a Zoonotic Origin of Human Coronavirus Strain NL63. *Journal of Virology* **86**, 12816-12825 (2012).

26. Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., *et al.* Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* **395**, 565-574 (2020).

27. Wong, A.C.P., Li, X., Lau, S.K.P. & Woo, P.C.Y. Global Epidemiology of Bat Coronaviruses. *Viruses* **11**, 174 (2019).

28. Drexler, J.F., Corman, V.M. & Drosten, C. Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Research* **101**, 45-56 (2014).

29. Anthony, S.J. *et al.* Global patterns in coronavirus diversity. *Virus Evolution* **3**, vex012-vex012 (2017).

30. Leopardi, S. *et al.* Interplay between co-divergence and cross-species transmission in the evolutionary history of bat coronaviruses. *Infection, Genetics and Evolution* **58**, 279-289 (2018).

31. Cui, J. *et al.* Evolutionary relationships between bat coronaviruses and their hosts. *Emerging Infectious Diseases* **13**, 1526-1532 (2007).

32. Smith, A.T. & Xie, Y. *A Guide to the Mammals of China*, (Princeton University Press, Princeton, USA, 2008).

33. Lin, X.-D. *et al.* Extensive diversity of coronaviruses in bats from China. *Virology* **507**, 1-10 (2017).

34. Ge, X.-Y. *et al.* Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* **31**, 31-40 (2016).

35. Woo, P.C.Y. *et al.* Molecular diversity of coronaviruses in bats. *Virology* **351**, 180-187 (2006).

36. Wu, Z. *et al.* Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. *The Isme Journal* **10**, 609-620 (2016).

37. Tang, X.C. *et al.* Prevalence and Genetic Diversity of Coronaviruses in Bats from China. *Journal of Virology* **80**, 7481-7490 (2006).

38. Woo, P.C.Y. *et al.* Comparative Analysis of Twelve Genomes of Three Novel Group 2c and Group 2d Coronaviruses Reveals Unique Group and Subgroup Features. *Journal of Virology* **81**, 1574-1585 (2007).

39. Ge, X. *et al.* Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *J Virol* **86**, 4620-4630 (2012).

40. Xu, L. *et al.* Detection and characterization of diverse alpha- and betacoronaviruses from bats in China. *Virologica Sinica* **31**, 69-77 (2016).

41. Luo, Y. *et al.* Longitudinal Surveillance of Betacoronaviruses in Fruit Bats in Yunnan Province, China During 2009–2016. **33**, 87-95 (2018).

42. Legendre, P. & Legendre, L.F. *Numerical ecology*, (Elsevier, 2012).

43. Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969-1973 (2012).

44. Lemey, P., Rambaut, A., Drummond, A.J. & Suchard, M.A. Bayesian Phylogeography Finds Its Roots. *PLoS Computational Biology* **5**, e1000520 (2009).

45. Minin, V.N. & Suchard, M.A. Counting labeled transitions in continuous-time Markov models of evolution. *Journal of Mathematical Biology* **56**, 391-412 (2008).

46. O'Brien, J.D., Minin, V.N. & Suchard, M.A. Learning to Count: Robust Estimates for Labeled Distances between Molecular Sequences. *Molecular Biology and Evolution* **26**, 801-814 (2009).

47. Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M.J. Phylogenies and Community Ecology. **33**, 475-505 (2002).

48. Simmons, N.B. Order Chiroptera. in *Mammal Species of the World: A Taxonomic and Geographic Reference* (eds. Wilson, D.E. & Reeder, D.M.) 312-529 (Johns Hopkins University Press, 2005).

49. Teeling, E.C. *et al.* A Molecular Phylogeny for Bats Illuminates Biogeography and the Fossil Record. **307**, 580-584 (2005).

50. Stoffberg, S., Jacobs, D.S., Mackie, I.J. & Matthee, C.A. Molecular phylogenetics and historical biogeography of *Rhinolophus* bats. *Molecular Phylogenetics and Evolution* **54**, 1-9 (2010).

51. Foley, N.M. *et al.* How and Why Overcome the Impediments to Resolution: Lessons from rhinolophid and hipposiderid Bats. *Molecular Biology and Evolution* **32**, 313-333 (2014).

52. Eick, G.N., Jacobs, D.S. & Matthee, C.A. A Nuclear DNA Phylogenetic Perspective on the Evolution of Echolocation and Historical Biogeography of Extant Bats (Chiroptera). *Molecular Biology and Evolution* **22**, 1869-1886 (2005).

53. Ravel, A., Marivaux, L., Qi, T., Wang, Y.-Q. & Beard, K.C. New chiropterans from the middle Eocene of Shanghuang (Jiangsu Province, Coastal China): new insight into the dawn horseshoe bats (Rhinolophidae) in Asia. **43**, 1-23 (2014).

54. Luo, J. *et al.* Bat conservation in China: should protection of subterranean habitats be a priority? *Oryx* **47**, 526-531 (2013).

55. Willoughby, A.R., Phelps, K.L., Consortium, P. & Olival, K.J. A Comparative Analysis of Viral Richness and Viral Sharing in Cave-Roosting Bats. *Diversity* **9**, 35 (2017).

56. Tsagkogeorga, G., Parker, J., Stupka, E., Cotton, James A. & Rossiter, S.J. Phylogenomic Analyses Elucidate the Evolutionary Relationships of Bats. *Current Biology* **23**, 2262-2267 (2013).

57. Yang, Y. *et al.* Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proceedings of the National Academy of Sciences* **111**, 12516-12521 (2014).

58. Menachery, V.D. *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nature Medicine* **21**, 1508-1513 (2015).

59. Li, W. *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).

60. Li, F. Receptor Recognition Mechanisms of Coronaviruses: a Decade of Structural Studies. **89**, 1954-1964 (2015).

61. Li, F. Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annual Review of Virology* **3**, 237-261 (2016).

62. Mao, X.G., Zhu, G.J., Zhang, S. & Rossiter, S.J. Pleistocene climatic cycling drives intra-specific diversification in the intermediate horseshoe bat (*Rhinolophus affinis*) in Southern China. *Molecular Ecology* **19**, 2754-2769 (2010).

63. Mao, X. *et al.* Multiple cases of asymmetric introgression among horseshoe bats detected by phylogenetic conflicts across loci. *Biological Journal of the Linnean Society* **110**, 346-361 (2013).

64. You, Y. *et al.* Pleistocene glacial cycle effects on the phylogeography of the Chinese endemic bat species, *Myotis davidii*. *BMC Evolutionary Biology* **10**, 208 (2010).

65. Chen, J.P. *et al.* Contrasting Genetic Structure in Two Co-Distributed Species of Old World Fruit Bat. *PLoS ONE* **5** (2010).

66. Krasnov, B.R., Pilosof, S., Shenbrot, G.I. & Khokhlova, I.S. Spatial variation in the phylogenetic structure of flea assemblages across geographic ranges of small mammalian hosts in the Palearctic. *International Journal for Parasitology* **43**, 763-770 (2013).

67. Bi, Y. *et al.* Novel avian influenza A (H5N6) viruses isolated in migratory waterfowl before the first human case reported in China, 2014. *Scientific Reports* **6**, 29888 (2016).

68. Bui, C.M., Adam, D.C., Njoto, E., Scotch, M. & MacIntyre, C.R. Characterising routes of H5N1 and H7N9 spread in China using Bayesian phylogeographical analysis. *Emerging Microbes & Infections* **7**, 184 (2018).

69. Gouilh, M.A., Puechmaille, S.J., Gonzalez, J.-P., Teeling, E., Kittayapong, P. & Manuguerra, J.-C. SARS-Coronavirus ancestor's foot-prints in South-East Asian bat colonies and the refuge theory. *Infection Genetics and Evolution* **11**, 1690-1702 (2011).

70. Hu, B., Ge, X., Wang, L.-F. & Shi, Z. Bat origin of human coronaviruses. *Virology Journal* **12**, 1-10 (2015).

71. Anthony, S.J., Ojeda-Flores, R., Rico-Chávez, O., Navarrete-Macias, I., Zambrana-Torrelio, C.M., Rostal, M.K., Epstein, J.H., Tipps, T., Liang, E., Sanchez-Leon, M., *et al.* Coronaviruses in bats from Mexico. *Journal of General Virology* **94**, 1028-1038 (2013).

72. Corman, V.M., Kallies, R., Philipps, H., Göpner, G., Müller, M.A., Eckerle, I., Brünink, S., Drosten, C. & Drexler, J.F. Characterization of a novel betacoronavirus related to MERS-CoV in European hedgehogs. *Journal of Virology* **88**, 717-724 (2014).

73. Munster, V.J., Adney, D.R., van Doremalen, N., Brown, V.R., Miazgowicz, K.L., Milne-Price, S., Bushmaker, T., Rosenke, R., Scott, D., Hawkinson, A., *et al.* Replication and shedding of MERS-CoV in Jamaican fruit bats (*Artibeus jamaicensis*). *Scientific Reports* **6**, 21878 (2016).

74. Joyjinda, Y., Rodpan, A., Chartpituck, P., Suthum, K., Yaemsakul, S., Cheun-Arom, T., Bunprakob, S., Olival, K.J., Stokes, M.M., Hemachudha, T., *et al.* First Complete Genome Sequence of Human Coronavirus HKU1 from a Nonill Bat Guano Miner in Thailand. *Microbiology Resource Announcements* **8**, e01457-01418 (2019).

75. Carroll, D., Daszak, P., Wolfe, N.D., Gao, G.F., Morel, C.M., Morzaria, S., Pablos-Méndez, A., Tomori, O. & Mazet, J.A.K. The Global Virome Project. *Science* **359**, 872-874 (2018).

76. Fountain-Jones, N.M. *et al.* Towards an eco-phylogenetic framework for infectious disease ecology. **93**, 950-970 (2018).

77. Allen, T. *et al.* Global hotspots and correlates of emerging zoonotic diseases. *Nature Communications* **8**, 1124 (2017).

78. Streicker, D.G., Lemey, P., Velasco-Villa, A. & Rupprecht, C.E. Rates of Viral Evolution Are Linked to Host Geography in Bat Rabies. *PLoS Pathog* **8**, e1002720 (2012).

79. Hu, B. *et al.* Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLOS Pathogens* **13**(2017).

80. Carroll, D. *et al.* The global virome project. *Science* **359**, 872-874 (2018).

81. Watanabe, S. *et al.* Bat Coronaviruses and Experimental Infection of Bats, the Philippines. *Emerging Infectious Diseases* **16**, 1217-1223 (2010).

82. Irwin, D.M., Kocher, T.D. & Wilson, A.C. Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution* **32**, 128-144 (1991).

83. IUCN. The IUCN Red List of Threatened Species. Version 2015.2, http://www.iucnredlist.org. (2018).

84. Xie, Y., MacKinnon, J., Li, D.J.B. & Conservation. Study on biogeographical divisions of China. **13**, 1391-1417 (2004).

85. Baele, G., Li, W.L.S., Drummond, A.J., Suchard, M.A. & Lemey, P. Accurate Model Selection of Relaxed Molecular Clocks in Bayesian Phylogenetics. *Molecular Biology and Evolution* **30**, 239-243 (2013).

86. Rambaut, A., Lam, T.T., Max Carvalho, L. & Pybus, O.G. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evolution* **2**, vew007 (2016).

87. Miller, M.A., Pfeiffer, W. & Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA*, 1-8 (2010).

88. Bielejec, F. *et al.* SpreaD3: Interactive Visualization of Spatiotemporal History and Trait Evolutionary Processes. *Molecular Biology and Evolution* **33**, 2167-2169 (2016).

89. Jeffreys, H. Theory of probability. Oxford: Clarendon. (1961).

90. Faria, N.R., Suchard, M.A., Rambaut, A., Streicker, D.G. & Lemey, P. Simultaneously reconstructing viral cross-species transmission history and identifying the underlying constraints. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**, 20120196 (2013).

91. Kamath, P.L., Foster, J.T., Drees, K.P., Luikart, G., Quance, C., Anderson, N.J., Clarke, P.R., Cole, E.K., Drew, M.L., Edwards, W.H., *et al.* Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. *Nature Communications* **7**, 11448 (2016).

92. Bandelt, H.J., Forster, P., & Rohl, A. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37-48 (1999).

93. Kembel, S.W. *et al.* Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463-1464 (2010).

94. Ooms, J., Chamberlain, S., Webb, C.O., Ackerly, D.D. & Kembel, S.W. phylocomr: Interface to 'Phylocom'. *R package version 0.1.2* (2018).

95. Excoffier, L. & Lischer, H.E.L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567 (2010).

96. Jombart, T. & Dray, S. adephylo: exploratory analyses for the phylogenetic comparative method. R package version 1.1-11. (2008).

Acknowledgements

This study was funded by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (Award Number R01AI110964) and the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT project (cooperative agreement number GHN-A-OO-09-00010-00), the strategic priority research program of the Chinese Academy of Sciences (XDB29010101), and National Natural Science Foundation of China (31770175, 31830096). Coronavirus research in L-FW's group is funded by grants from Singapore National Research Foundation (NRF2012NRF-CRP001-056 and NRF2016NRF-NSFC002-013).

Author contributions

K.J.O., H.E.F, J.H.E., L-F.W., Z.S. and P.D. created the study design, initiated field work and set up sample collection and testing protocols. B.H., G.Z., L.Z., H.L., A.A.C and Z.L. provided samples or data. B.H., B.L., and W.Z. performed laboratory work. A.L. carried out the analyses and drafted the manuscript with K.J.O, C.Z.-T. and P.D. All authors reviewed and edited the manuscript

Competing interests: The authors declare no competing interests.

Figure legends

Fig. 1 Geographic sampling. Pie chart (A) showing the number of sequences of each CoV genus (alpha-CoVs and beta-CoVs) available for each zoogeographic region and map of China provinces (B) showing

769 the number of *RdRp* sequences available for each province, in bold grey for alpha-CoVs and black for
770 beta-CoVs. Province colors correspond to the zoogeographic region to which they belong: NO, Northern
771 region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region;
772 HI, Hainan island. The three beta-CoV sequences from HI were included in the SO region. Provinces
773 colored in grey are those where CoV sequences are not available.

774 **Fig. 2 Phylogenetic trees and ancestral host reconstructions**. Alpha-CoV (A) and beta-CoV (B) maximum
775 clade credibility annotated trees using complete datasets of *RdRp* sequences and bat host family as
776 discrete character state. Pie charts located at the root and close to the deepest nodes show the state
777 posterior probabilities for each bat family. Branch colors correspond to the inferred ancestral family
778 with the highest probability. Branch lengths are scaled according to relative time units (clock rate = 1.0).
779 Well-supported nodes (posterior probability > 0.95) are indicated with a black dot. The ICTV approved
780 CoV subgenera were highlighted: *Rhinacovirus* (L1), *Decacovirus* (L2), *Myotacovirus* (L3), *Pedacovirus*
781 (L5), *Nyctacovirus* (L6), *Minunacovirus* (L7) and an unidentified lineage (L4) for alpha-CoVs; and
782 *Merbecovirus* (Lineage C), *Nobecovirus* (lineage D), *Hibecovirus* (lineage E) and *Sarbecovirus* (Lineage B)
783 for beta-CoVs.

784 **Fig. 3 Phylogenetic relationships within the *Sarbecovirus* subgenus (beta-CoVs)**. Maximum clade
785 credibility tree (A) including 202 *RdRp* sequences from the *Sarbecovirus* subgenus isolated in bats, two
786 sequences of SARS-CoV-2 and one sequence of SARS-CoV isolated in humans and eight sequences
787 isolated in Malayan pangolins (*Manis javanica*). Well-supported nodes (posterior probability > 0.95) are
788 indicated with a black dot. Tip colors correspond to the bat host genus, SARS-CoV-2 sequences and
789 SARS-CoV sequence are highlighted in grey and black, respectively. Median-joining network (B) including
790 202 *RdRp* sequences from the *Sarbecovirus* lineage isolated in bats, two sequences of SARS-CoV-2 and
791 one sequence of SARS-CoV isolated in humans and eight sequences isolated in Malayan pangolins
792 (*Manis javanica*). Colored circles correspond to distinct CoV sequences, circle size is proportional to the

number of identical sequences in the data set. Small black circles represent median vectors (ancestral or unsampled intermediate sequences). Branch length is proportional to the number of mutational steps between haplotypes.

Fig. 4 Inter-family host switches. Strongly supported host switches between bat families for alpha- (A) and beta-CoVs (B). Arrows indicate the direction of the switch; arrow thickness is proportional to the switch significance level, only host switches supported by strong Bayes factor (BF) > 10 are shown. Histograms of total number of host switching events (state changes counts using Markov jumps) from/to each bat family along the significant inter-family switches for alpha- (C) and beta-CoVs (D).

Fig. 5 Inter-genus host switches. Strongly supported host switches between bat genera for alpha- (A) and beta-CoVs (B) and their significance level (Bayes factor, BF). Only host switches supported by strong BF values > 10 are shown. Line thickness is proportional to the switch significance level. Red lines correspond to host switches among bat genera belonging to different families, black lines correspond to host switches among bat genera from the same family. Arrows indicate the direction of the switch. Genus names are colored according to the family they belong to using the same colors as in Fig. 2 and 3.

Fig. 6 CoV spatiotemporal dispersal in China. Strongly supported dispersal routes (Bayes factor, BF > 10) over recent evolutionary history among China zoogeographic regions for alpha- (A) and beta-CoVs (B). Arrows indicate the direction of the dispersal route; arrow thickness is proportional to the dispersal route significance level. Darker arrow colors indicate older dispersal events. Histograms of total number of dispersal events (Markov jumps) from/to each region along the significant dispersal routes for alpha- (C) and beta-CoVs (D). NO, Northern region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region; HI, Hainan island.

Fig. 7 Phylogenetic diversity. Metrics of CoV phylogenetic diversity within each bat family (A), genus (B) and zoogeographic regions (C): standardized effect size of Mean Phylogenetic Distance (SES MPD), on

816 the left panels; and standardized effect size of Mean Nearest Taxon Distance (SES MNTD), on the right
817 panels. One-tailed p-values (quantiles) were calculated after randomly reshuffling tip labels 1000 times
818 along the entire phylogeny. Values departing significantly from the null model (p-value < 0.05) are
819 indicated with an asterisk,all exact p-values are available in Supplementary Tables 14-27. NO, Northern
820 region; CN, Central northern region; SW, South western region; CE, Central region; SO, Southern region;
821 HI, Hainan island.

822 **Fig. 8 Phylogenetic diversity**. Standardized effect size of Mean Phylogenetic Distance (SES MPD) and
823 phylogenetic ordination among bat host families (A, B) and genera (C, D) for alpha- and beta-CoVs.
824 Boxplots for each host family and genus show the mean (cross), median (dark line within the box),
825 interquartile range (box), 95% confidence interval (whisker bars), and outliers (dots), calculated from all
826 pairwise comparisons between bat families (n=10 for alpha-CoVs and n=6 for beta-CoVs) and genera
827 (n=91 for alpha-CoVs and n=105 for beta-CoVs).

828 **Fig. 9 Phylogenetic diversity**. Standardized effect size of Mean Phylogenetic Distance, SES MPD) and
829 phylogenetic ordination among zoogeographic regions for alpha- (A) and beta-CoVs (B). Boxplots for
830 each region show the mean (cross), median (dark line within the box), interquartile range (box), 95%
831 confidence interval (whisker bars), and outliers (dots), calculated from all pairwise comparisons between
832 regions (n=15 for alpha-CoVs and n=10 for beta-CoVs). NO, Northern region; CN, Central northern
833 region; SW, South western region; CE, Central region; SO, Southern region; HI, Hainan island.

834

835 **Figure 1**

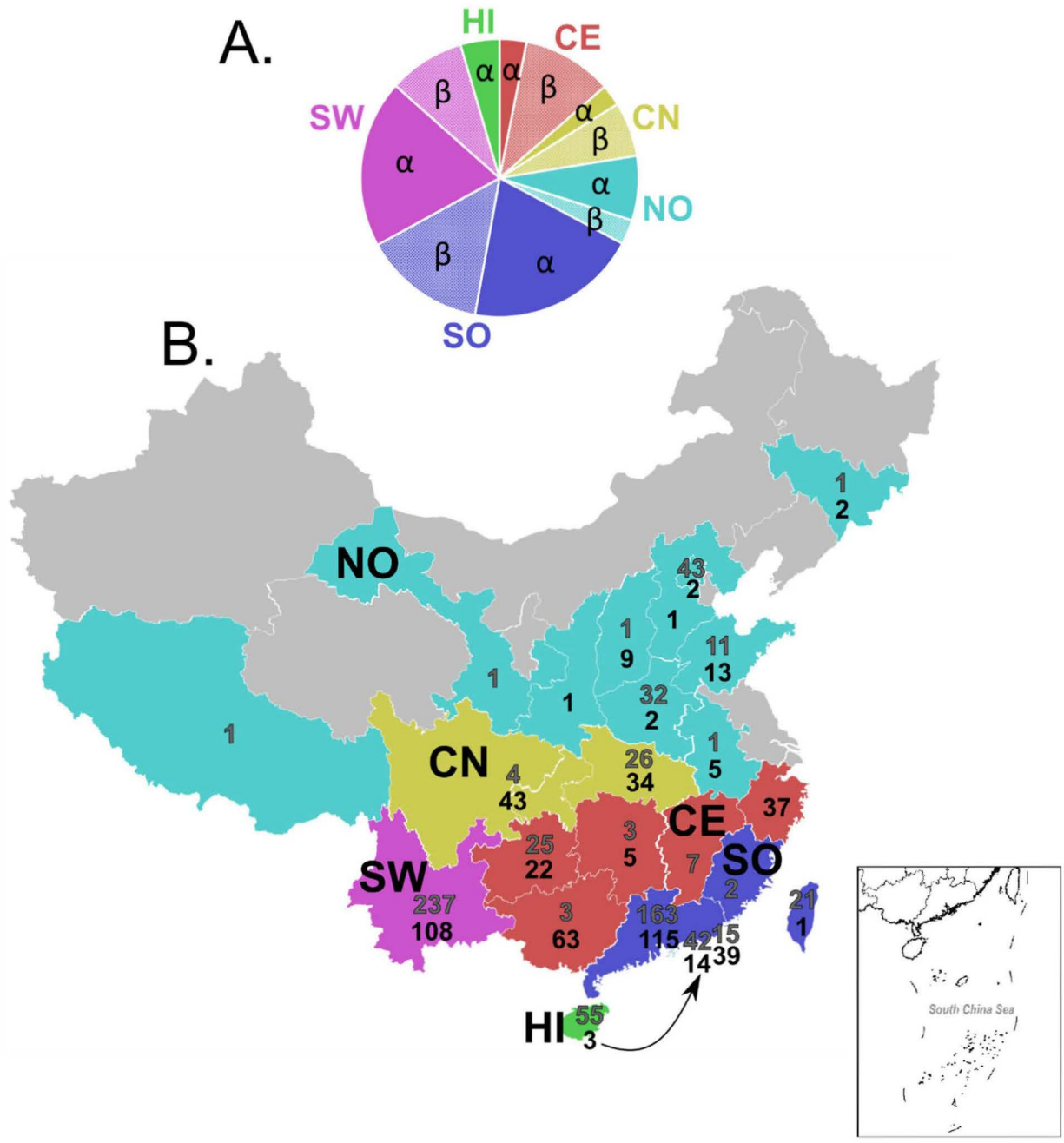

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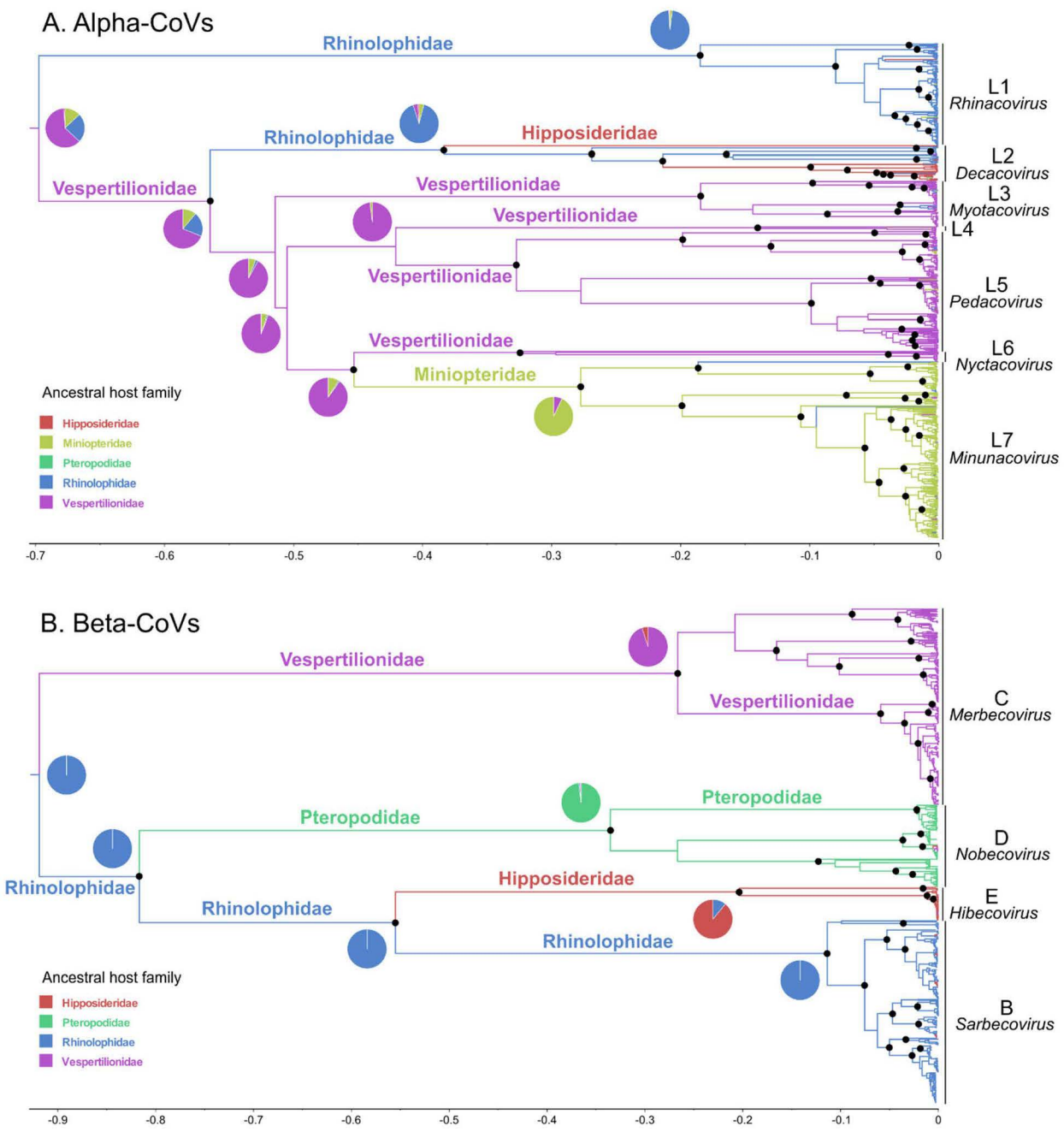
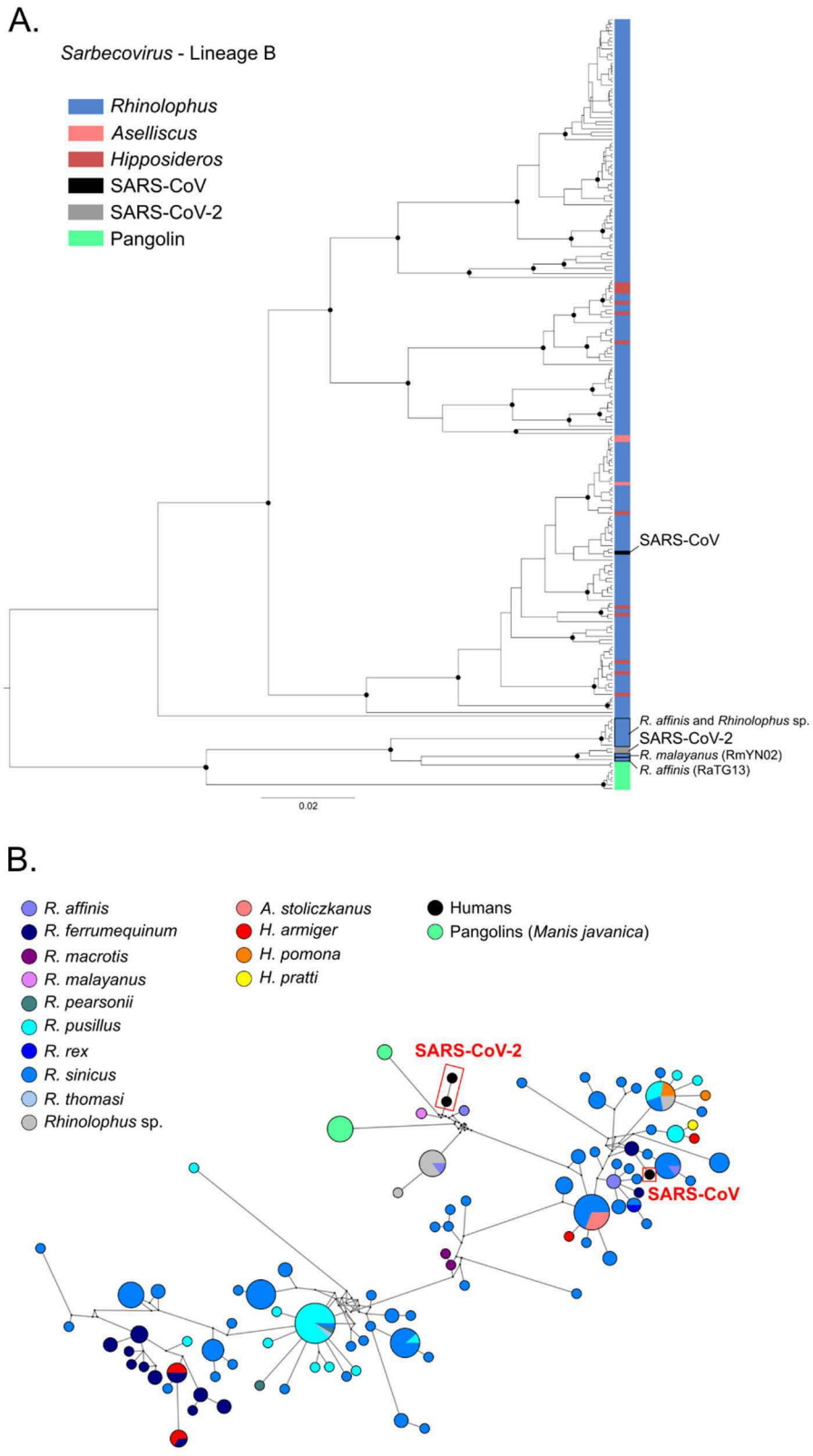
Figure 2


A. Alpha-CoVs
Rhinolophidae
Rhinolophidae
Hipposideridae
Vespertilionidae
Vespertilionidae
Vespertilionidae
Vespertilionidae
Vespertilionidae
Miniopteridae
L1
Rhinacovirus
L2
Decacovirus
L3
Myotacovirus
L4
L5
Pedacovirus
L6
Nyctacovirus
L7
Minunacovirus
Ancestral host family
Hipposideridae
Miniopteridae
Pteropodidae
Rhinolophidae
Vespertilionidae
-0.7
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0
B. Beta-CoVs
Vespertilionidae
Vespertilionidae
Pteropodidae
Pteropodidae
Rhinolophidae
Hipposideridae
Rhinolophidae
Rhinolophidae
C
Merbecovirus
D
Nobecovirus
E
Hibecovirus
B
Sarbecovirus
Ancestral host family
Hipposideridae
Pteropodidae
Rhinolophidae
Vespertilionidae
-0.9
-0.8
-0.7
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0

843 **Figure 3**



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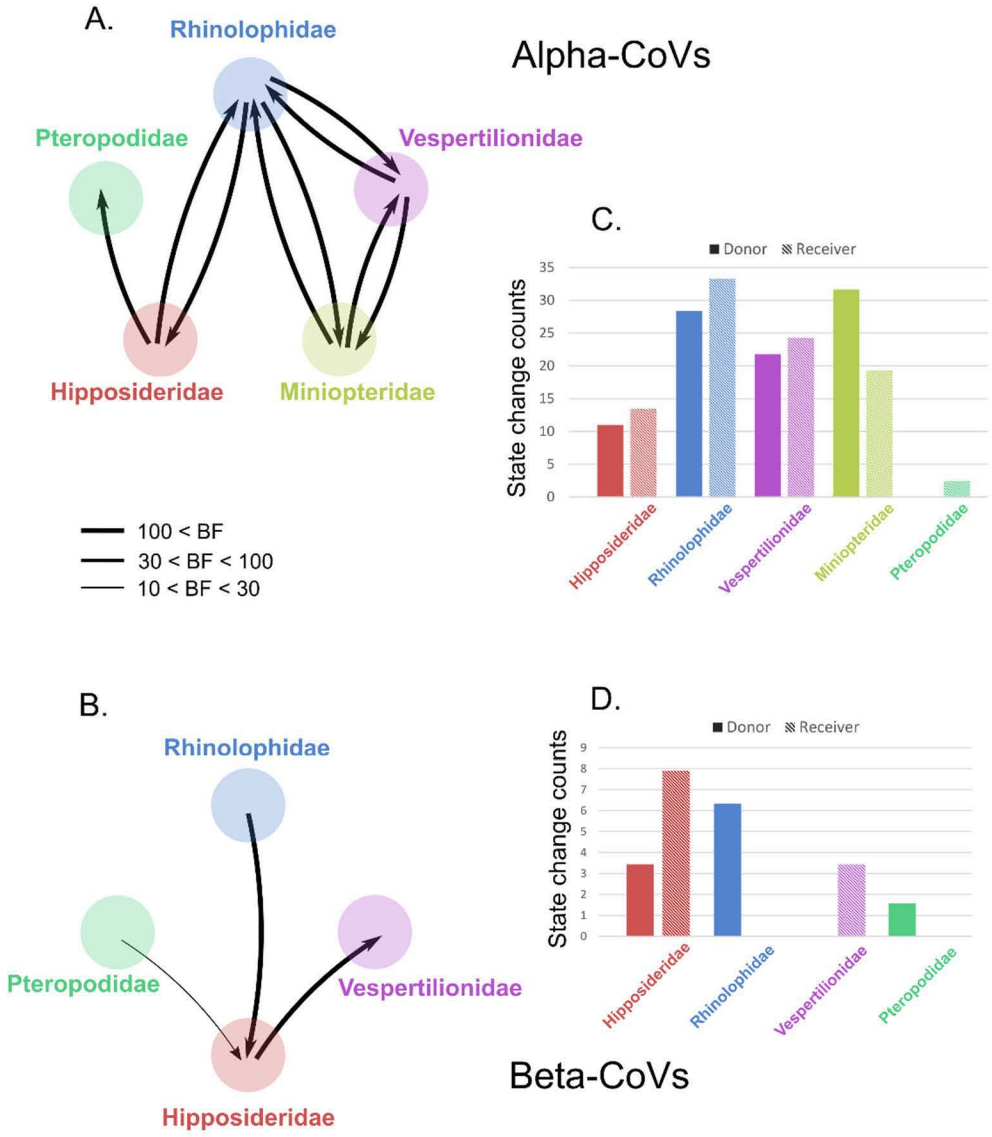
845 **Figure 4**



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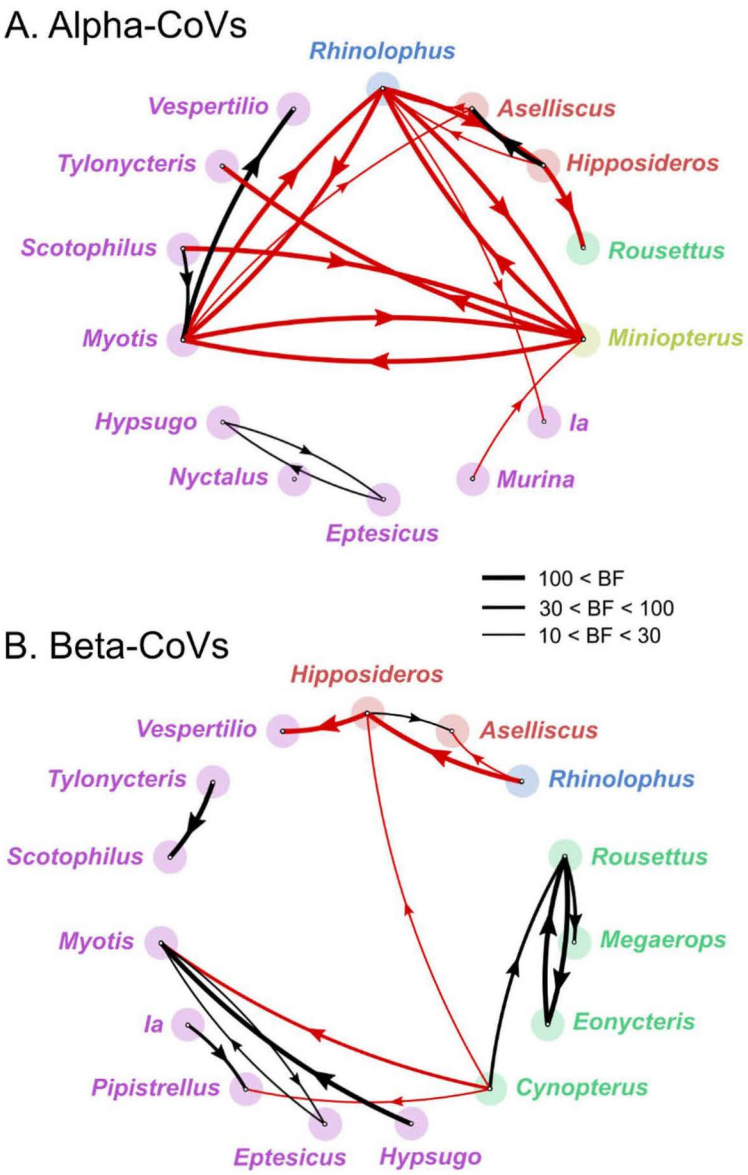
848 **Figure 5**



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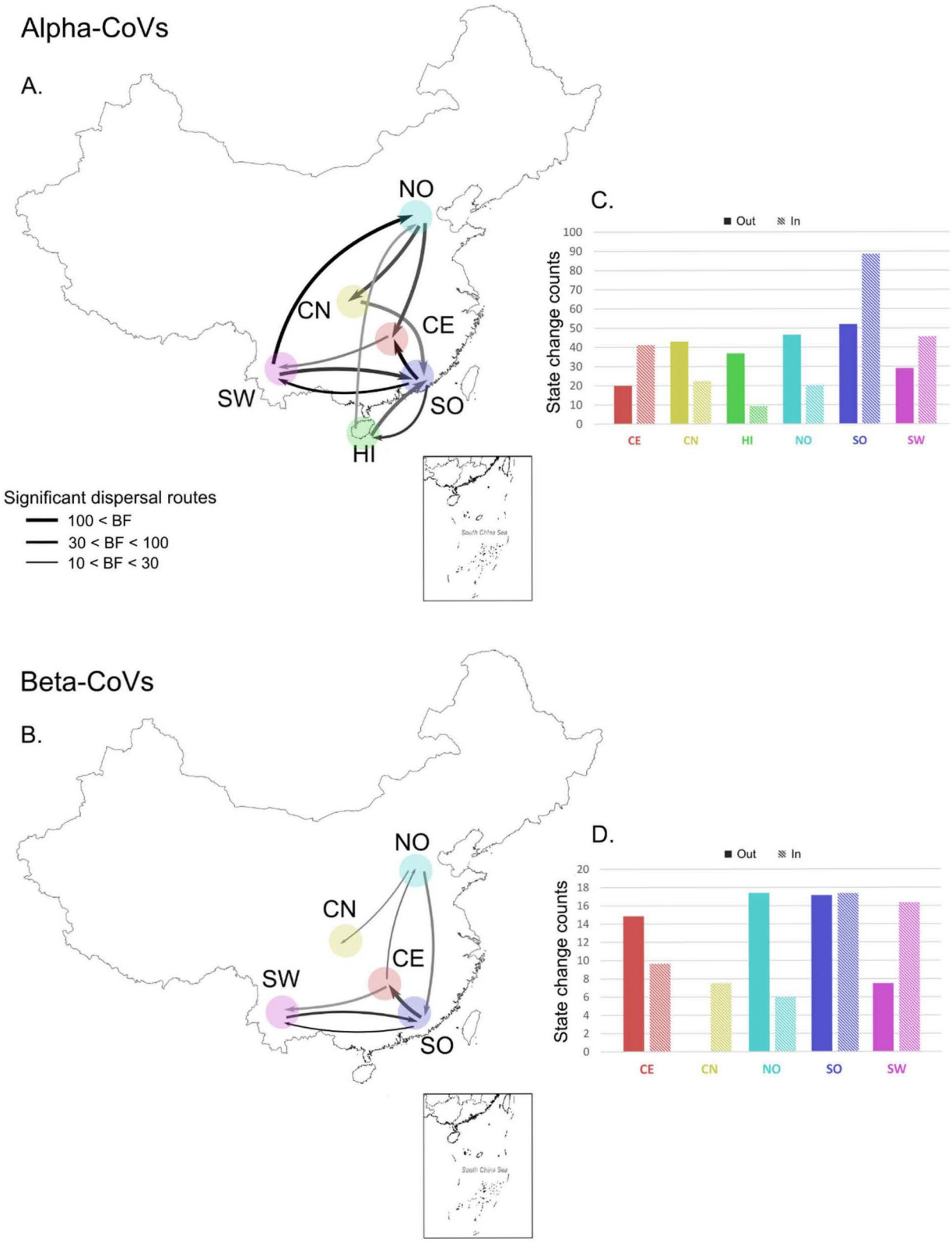
851 **Figure 6**



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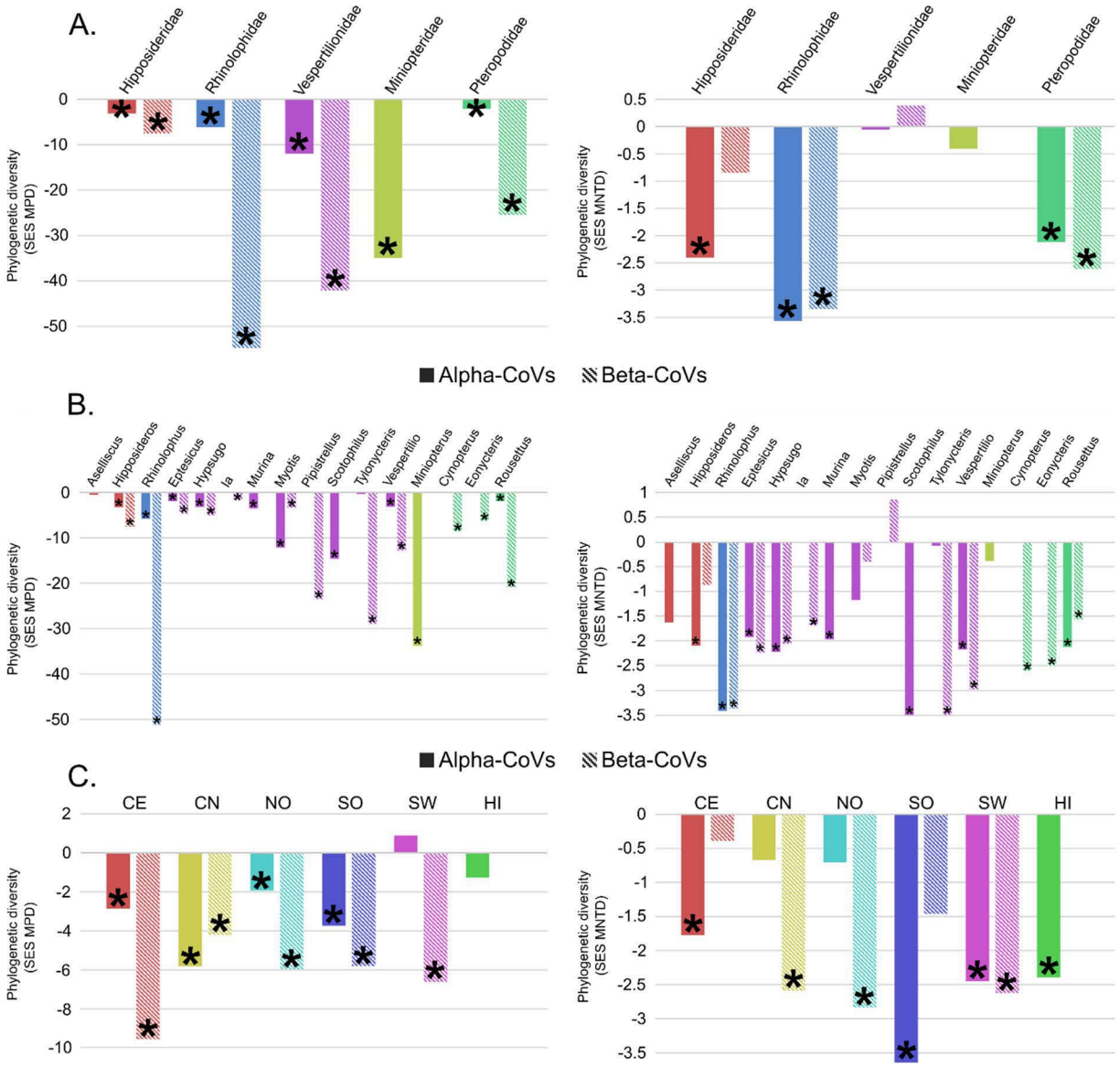
853 **Figure 7**



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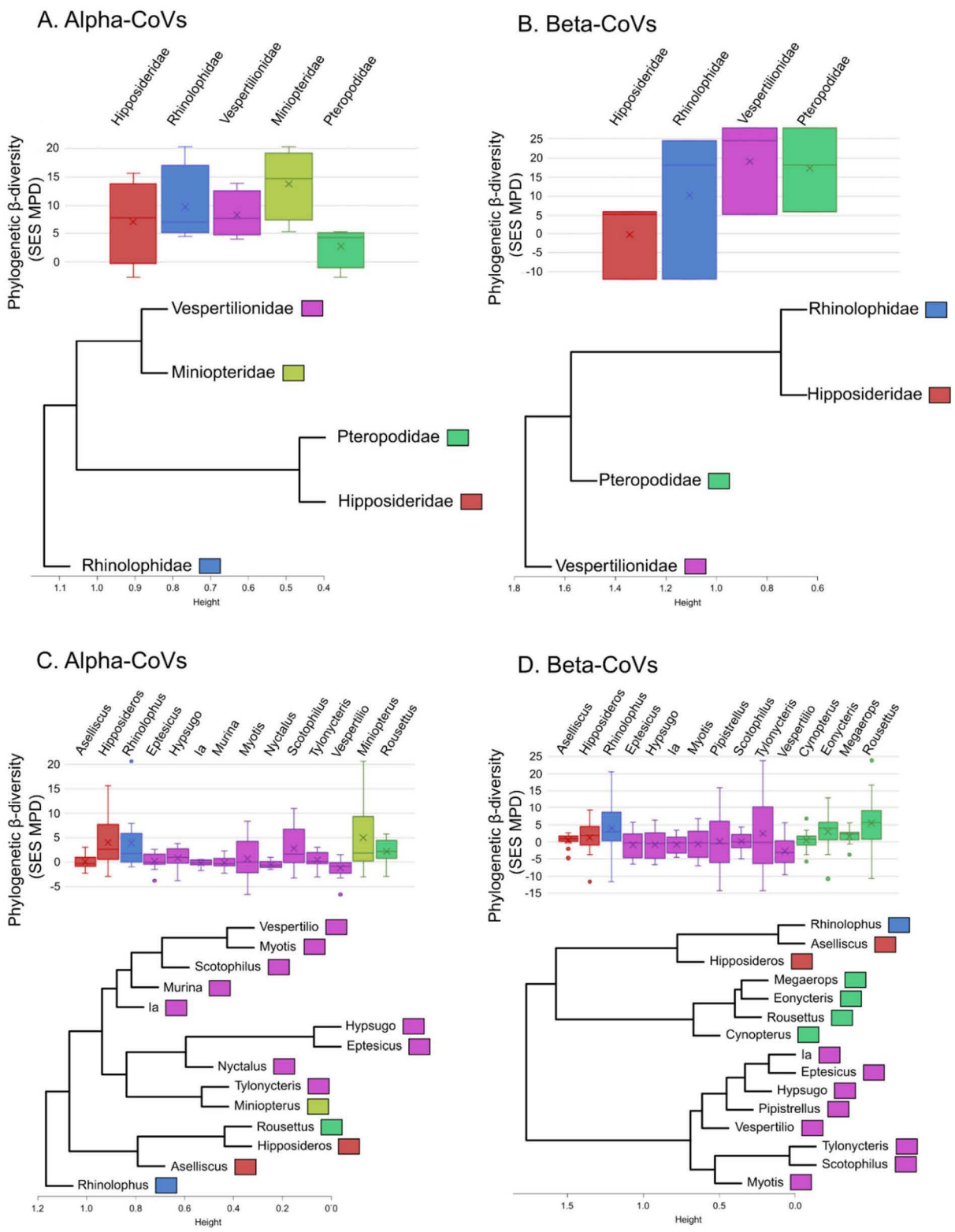
856 **Figure 8**



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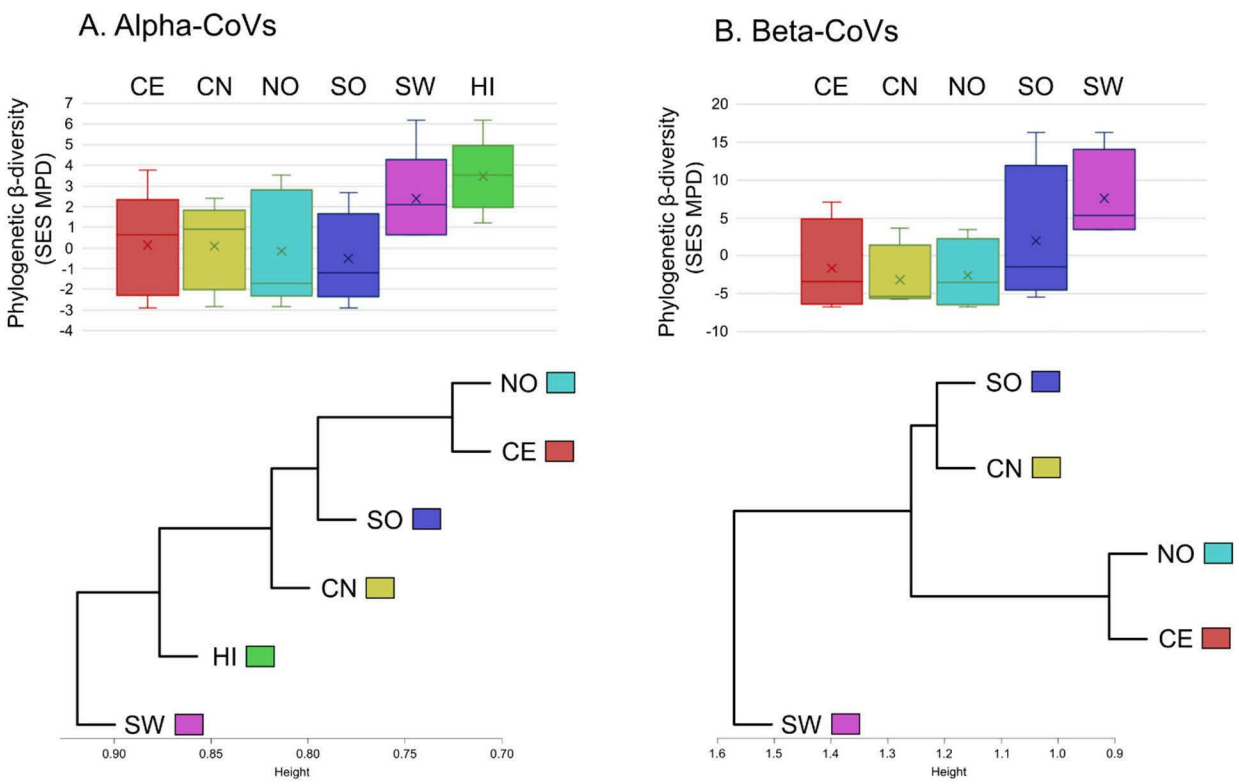
858 **Figure 9**



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860

From:	Peter Daszak
To:	drosten@virology-bonn.de; John Mackenzie; Jonna Mazet; llmpoon@hku.hk; Larry Madoff; Prof Lam Sai Kit; j.farrar@wellcome.ac.uk; peter.palese@mssm.edu; Dennis Carroll; bernard.roizman@bsd.uchicago.edu; Perlman, Stanley; Charles H Calisher; a.e.gorbalenya@lumc.nl; L.Enjuanes@cnb.csic.es; b.haagmans@erasmusmc.nl; rcolwell@umiacs.umd.edu; JMHUGHE@emory.edu; Hume Field; jlubroth@gmail.com; Saif, Linda; William B. Karesh; rbcorley@bu.edu; Keusch, Gerald T; Subbarao, Kanta; J.Golding@wellcome.ac.uk; Mike Turner
Cc:	Hongying Li; Aleksei Chmura
Subject:	Lancet Statement Posted!
Date:	Tuesday, February 18, 2020 12:44:42 PM
Attachments:	Lancet Statement 2020.pdf

Dear All,

Our statement is live as of just a few minutes ago!

https://www.thelancet.com/lancet/article/s0140-6736(20)30418-9

Please take time to send this out via twitter, email to your networks, post on your institution or other websites, and distribute as widely as possible to get the word out. Include the link too (http://chng.it/SDpTB9Kf), so other people can register their support of the statement.

I really want to thank all of you for rallying for this - especially with such a short timeline. This looks terrific and I know it will do a world of good towards buoying the spirits of our colleagues in China and gaining an ear from those in policy to support collaborative, open approaches to fighting this as well as future outbreaks.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
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EcoHealth Alliance develops science-based solutions tp prevent pandemics and promote conservation.

Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19

We are public health scientists who have closely followed the emergence of 2019 novel coronavirus disease (COVID-19) and are deeply concerned about its impact on global health and wellbeing. We have watched as the scientists, public health professionals, and medical professionals of China, in particular, have worked diligently and effectively to rapidly identify the pathogen behind this outbreak, put in place significant measures to reduce its impact, and share their results transparently with the global health community. This effort has been remarkable.

We sign this statement in solidarity with all scientists and health professionals in China who continue to save lives and protect global health during the challenge of the COVID-19 outbreak. We are all in this together, with our Chinese counterparts in the forefront, against this new viral threat.

The rapid, open, and transparent sharing of data on this outbreak is now being threatened by rumours and misinformation around its origins. We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin. Scientists from multiple countries have published and analysed genomes of the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),[1] and they overwhelmingly conclude that this coronavirus originated in wildlife,[2–10] as have so many other emerging pathogens.[11,12] This is further supported by a letter from the presidents of the US National Academies of Science, Engineering, and Medicine[13] and by the scientific communities they represent. Conspiracy theories do nothing but create fear, rumours, and prejudice that jeopardise our global collaboration in the fight against this virus. We support the call from the Director-General of WHO to promote scientific evidence and unity over misinformation and conjecture.[14] We want you, the science and health professionals of China, to know that we stand with you in your fight against this virus.

We invite others to join us in supporting the scientists, public health professionals, and medical professionals of Wuhan and across China. Stand with our colleagues on the frontline!

We speak in one voice. To add your support for this statement, sign our letter online. LM is editor of ProMED-mail. We declare no competing interests.

Charles Calisher, Dennis Carroll, Rita Colwell, Ronald B Corley, Peter Daszak, Christian Drosten, Luis Enjuanes, Jeremy Farrar, Hume Field, Josie Golding, Alexander Gorbalenya, Bart Haagmans, James M Hughes, William B Karesh, Gerald T Keusch, Sai Kit Lam, Juan Lubroth, John S Mackenzie, Larry Madoff, Jonna Mazet, Peter Palese, Stanley Perlman, Leo Poon, Bernard Roizman, Linda Saif, Kanta Subbarao, Mike Turner

COVID19statement@gmail.com

Colorado State University, Fort Collins, CO, USA (CC); Scowcroft Institute of International Affairs, Texas A&M, College Station, TX, USA (DC); University of Maryland, College Park, MD, USA (RC); NEIDL Institute (RBC), Boston University (GTK), Boston, MA, USA; EcoHealth Alliance, New York, NY, USA (PD); Charité – Universitatsmedizin Berlin, Berlin, Germany (CD); National Center of Biotechnology, Madrid, Spain (LE); The Wellcome Trust, London, UK (JF, JG, MT); School of Veterinary Science, The University of Queensland, Brisbane, QLD, Australia (HF); Leiden University Medical Center, Leiden, Netherlands (AG); Erasmus Medical Center, Rotterdam, Netherlands (BH); Emory University, Atlanta, GA, USA (JMH); World Organization for Animal Health (OIE) Working Group on Wildlife, New York, NY, USA (WBK); University of Malaya, Kuala Lumpur, Malaysia (SKL); Food and Agriculture Organization of the United Nations, Rome, Italy (JL); Curtin University, Perth, WA, Australia (JSM); Massachusetts Medical School, Worcester, MA, USA (LM); University of California at Davis, Davis, CA, USA (JM); Department of Microbiology, Icahn School of Medicine, Mt Sinai Hospital, New York, NY, USA (PP); University of Iowa, Roy J and Lucille A Carver College of Medicine, Iowa City, IA, USA (SP); The University of Hong Kong, Hong Kong (LP); University of Chicago, Chigaco, IL, USA (BR); The Ohio State University, Columbus, OH, USA (LS); and The University of Melbourne, Melboune, VIC, Australia (KS)

1 Gorbalenya AE, Baker SC, Baric RS, et al. Severe acute respiratory syndrome-related coronavirus: the species and its viruses—a statement of the Coronavirus Study Group. *bioRxiv* 2020; published online Feb 11. DOI:2020.02.07.937862 (preprint).

2 Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; published online Feb 3. DOI:10.1038/s41586-020-2012-7.

3 Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; published online Jan 30. https://doi.org/10.1016/S0140-6736(20)30251-8.

4 Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *NEJM* 2020; published online Jan 24. DOI:10.1056/NEJMoa2001017.

5 Ren L, Wang Y-M, Wu Z-Q, et al. Identification of a novel coronavirus causing severe pneumonia in humans: a descriptive study. *Chin Med J* 2020; published online Feb 11. DOI:10.1097/CM9.0000000000000722.

6 Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infect Genet Evol* 2020; published online Jan 29. DOI:10.1016/j.meegid.2020.104212.

7 Benvenuto D, Giovanetti M, Ciccozzi A, Spoto S, Angeletti S, Ciccozzi M. The 2019-new coronavirus epidemic: evidence for virus evolution. *J Med Virol* 2020; published online Jan 29. DOI:10.1002/jmv.25688.

8 Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. *J Virol* 2020; published online Jan 29. DOI:10.1128/JVI.00127-20.

9 US Center for Disease Control and Prevention. Coronavirus disease 2019 (COVID-19) situation summary. Feb 16, 2020. https://www.cdc.gov/coronavirus/2019-nCoV/summary.html (accessed Feb 8, 2020).

10 Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Feb 16, 2020; http://virological.org/t/the-proximal-origin-of-sars-cov-2/398 (accessed Feb 17, 2020).

11 Bengis R, Leighton F, Fischer J, Artois M, Morner T, Tate C. The role of wildlife in emerging and re-emerging zoonoses. *Rev Sci Tech* 2004; **23:** 497–512.

12 Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. *Emerg Infect Dis 2005*; **11:** 1842–47.

13 NASEM. The National Academies of Science Engineering and Medicine of the USA. NAS, NAE, and NAM presidents' letter to the White House Office of Science and Technology Policy. Feb 6, 2020. https://www.nationalacademies.org/includes/NASEM%20Response%20to%20OSTP%20re%20Coronavirus_February%206,%202020.pdf (accessed Feb 7, 2020).

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For the Chinese translation see **Online** for appendix

To **register your support** see http://chng.it/SDpTB9Kf

For the **SARS-CoV-2 genome analysis** see https://www.gisaid.org/epiflu-applications/next-betacov-app/

Submissions should be made via our electronic submission system at http://ees.elsevier.com/thelancet/

14 WHO. Director-General's remarks at the media briefing on 2019 novel coronavirus on 8 February 2020. Feb 8, 2020. https://www.who.int/dg/speeches/detail/director-general-s-remarks-at-the-media-briefing-on-2019-novel-coronavirus---8-february-2020 (accessed Feb 18, 2020).

From:	Saif, Linda
To:	Peter Daszak
Cc:	Aleksei Chmura; Hongying Li
Subject:	Re: URGENT - need signatures in next few hours: Our statement on COVID-19 will be published this morning US Eastern time in The Lancet
Date:	Tuesday, February 18, 2020 10:49:10 AM
Attachments:	icmje-COI-form to sign LJ Saif.pdf TL_AuthorSigs_2019_LJSaif_2.pdf

Hi Peter
Attached are my completed and signed forms.
Could you please email me the final statement after published in English and Chinese?
Thanks
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: Peter Daszak <daszak@ecohealthalliance.org>
Date: Tuesday, February 18, 2020 at 5:51 AM
To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Aleksei Chmura <chmura@ecohealthalliance.org>, Hongying Li <li@ecohealthalliance.org>
Subject: URGENT - need signatures in next few hours: Our statement on COVID-19 will be published this morning US Eastern time in The Lancet

Dear All,

I want to let you all know that we received strong support from Richard Horton at *The Lancet*, and our paper will be published today (Tuesday 18th Feb) at 3pm UK time (10am Eastern US time). Thank you also to those of you who sent last minute changes – I've incorporated them where possible (see final version attached). I've also cited a paper that was uploaded yesterday (http://virological.org/t/the-proximal-origin-of-sars-cov-2/398), currently in review in *Nature* (I believe) that clearly refutes the bio-engineered virus hypothesis and strongly supports the conclusion that SARS-CoV-2 is of natural origin.

As we discussed, the authorship will be alphabetical. Unfortunately, it looks like there has to be a single corresponding author, but the editor will put a statement at the top of the authorship list to indicate that we are all speaking in one voice on this. I will see what that looks like when proofs come through in a minute. *The Lancet* have also agreed to publish our Mandarin version of this statement (thanks for the translation Hongying) online, so it reaches a wider audience in Asia and around the world.

I have two urgent requests:

1. Please fill in the attached Conflict of Interest form ASAP
2. Please e-sign the Author signature form ASAP

It will be really important to get this message out to journalists once it's published. Finally, I would ask all of you who can post this to your websites, or on social media, or email to your colleagues, please do so.

Cheers,

Peter

Peter Daszak
President

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Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

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Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

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Corresponding Author's Name: Peter Daszak

5. Manuscript Title: Statement in Support of the Scientists, Public Health and Medical Professionals of China Combating the COVID-19 Outbreak

6. Manuscript Identifying Number (if you know it): THELANCET-D-20-01775

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Dr. Saif has nothing to disclose.

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Corresponding author: Peter Daszak

Article type: Commentary

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I contributed to the writing and editing of the correspondance

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Corresponding author declaration

I _____, the corresponding author of this manuscript, certify that the contributors' and conflicts of interest statements included in this paper are correct and have been approved by all co-authors.

From:	Ted Steele
To:	Charles.Calisher@colostate.edu; bushschoolscowcroft@tamu.edu; rcolwell@umd.edu; rbcorley@bu.edu; daszak@ecohealthalliance.org; christian.drosten@charite.de; L.Enjuanes@cnb.csic.es; a.e.gorbalenya@lumc.nl; b.haagmans@erasmusmc.nl; jmhughe@emory.edu; karesh@ecohealthalliance.org; keusch@bu.edu; lamsk@nipahvirus.org; Juan.Lubroth@fao.org; J.Mackenzie@curtin.edu.au; Lawrence.Madoff@umassmemorial.org; kmazet@ucdavis.edu; peter.palese@mssm.edu; stanley-perlman@uiowa.edu; llmpoon@hku.hk; bernard.roizman@bsd.uchicago.edu; Saif, Linda; kanta.subbarao@influenzacentre.org
Cc:	Jane Hilton; Equitech
Subject:	Re: Origin Coronavirus COVID-19
Date:	Monday, May 18, 2020 7:47:18 AM
Attachments:	Steele 2020 Corona Hysteria & Our Cosmic Connection 7.4.20FINAL Edit by P Monk.pdf

Dear Colleagues:

Further to my previous email.

To watch interview -discussion, go to the following link:

https://www.youtube.com/watch?v=KwXKzL-yzt8&t=2s

And the URL links to all our recent papers is below, and *The Australian Rationalist* article is attached.

Best and thanks

Ted Steele

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All key articles below at
https://www.hilarispublisher.com/virology-current-research/inpress.html .
Wickramasinghe, Steele, Gorczynski et al 2020 *Virology Current Research* (In Press)
On the Fragility of Empires and Paradigms
http://viXra.org/abs/2003.0524 **Category**: Physics of Biology
https://www.academia.edu/42310752/Virology_Current_Research_On_The_Fragility_of_Empires_and_Paradigms_Letter_to_the_Editor

Wickramasinghe, Steele, Gorczynski et al 2020 *Virology Current Research* (In Press)
Predicting the Future Trajectory of COVID-19
https://vixra.org/abs/2003.0320 **Category**: Physics of Biology
https://www.academia.edu/42314513/Predicting_the_Future_Trajectory_of_COVID-19

Wickramasinghe, Steele , Gorczynski et al 2020 *Virology Current Research*
Growing Evidence against Global Infection-Driven by Person-to-Person Transfer of COVID-19
http://viXra.org/abs/2003.0042 **Category**: Physics of Biology
https://www.academia.edu/42148293/Growing_Evidence_against_Global_Infection-Driven_by_Person-to-Person_Transfer_of_COVID-19

Wickramasinghe, Steele , Gorczynski et al 2020 *Virology Current Research*
Comments on the Origin and Spread of the 2019 Coronavirus
http://viXra.org/abs/2002.0525 **Category**: Physics of Biology
https://www.academia.edu/42041228/Comments_on_the_Origin_and_Spread_of_the_2019_Coronavirus

Origin of New Emergent Coronavirus and Candida Fungal Disease– Terrestrial or Cosmic?- posted 17.2.20-Chapter 6 for Cosmic Genetic Evolution
Authors: Edward J. Steele, Jiangwen Qu, Reginald M. Gorczynski, Robyn A. Lindley, Gensuke Tokoro, Robert Temple, N. Chandra Wickramasinghe
http://viXra.org/abs/2002.0310
Category: Physics of Biology

Article submitted to *The Australian* 6.2.20, updated 9.2.20 (then rejected by Editor)
The Coronavirus May Have Come From Space
Authors: N. Chandra Wickramasinghe, Edward J Steele
https://vixra.org/abs/2002.0118
http://viXra.org/abs/2002.0118?ref=11085574
Category: Physics of Biology

Draft letter to *The Lancet* at:
viXra:2002.0039 submitted on 2020-02-03 17:33:22 (then rejected by Editor)
http://viXra.org/abs/2002.0039?ref=11076818

Comment on the Origin of the 2019 Novel Coronavirus

Authors: Edward J. Steele, N. Chandra Wickramasinghe, Jiangwen Qu, Robert Temple, Gensuke Tokoro, Reginald M. Gorczynski

Category: Physics of Biology

Steele EJ1, Gorczynski RM2, Lindley RA3, Liu Y4, Temple R5, Tokoro G6, Wickramasinghe DT7, Wickramasinghe NC8. Lamarck and Panspermia - On the Efficient Spread of Living Systems Throughout the Cosmos. Prog Biophys Mol Biol. 2019 149: 10-32 . pii: S0079-6107(19)30112-9.
doi: 10.1016/j.pbiomolbio.2019.08.010
https://www.ncbi.nlm.nih.gov/pubmed/31445944
https://doi.org/10.1016/j.pbiomolbio.2019.08.010

Other PBMB formats 2018-2019 on literature of Cosmic Biology are :

Steele EJ, Gorczynski RM, Lindley RA, Liu Y, Temple R, Tokoro G, Wickramasinghe DT, Wickramasinghe NC. 2019 "Lamarck and Panspermia - On the Efficient Spread of Living Systems Throughout the Cosmos". Prog. Biophys. Mol. Biol. 2019 149 : 10 -32.
https://doi.org/10.1016/j.pbiomolbio.2019.08.010

Steele EJ, Al-Muft S, Augustyn KK, Chandrajith R, Coghlan JP, Coulson SG, Ghosh S, Gillman M. et al 2018 "Cause of Cambrian Explosion: Terrestrial or Cosmic?" Prog. Biophys. Mol. Biol. 136: 3-23,
https://doi.org/10.1016/j.pbiomolbio.2018.03.004

Steele, E.J., Al-Mufti, S., Augustyn, K.A., Chandrajith, R., Coghlan, J.P., Coulson, S.G., et al., (2019). Cause of Cambrian explosion - terrestrial or cosmic? - reply to commentary by R Duggleby. *Prog. Biophys. Mol. Biol.* 141, 74-78. https://doi.org/10.1016/j.pbiomolbio.2018.11.002

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From: Ted Steele <e.j.steele@bigpond.com>
Date: Thursday, 20 February 2020 at 9:58 pm
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Cc: Jane Hilton <janewilsonhilton@gmail.com>, Equitech <equitech@bigpond.com>
Subject: Origin Coronavirus COVID-19

Dear Colleagues:

We understand why you had to write and sign that letter in this week's *The Lancet*.
https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30418-9/fulltext

The conspiracy theory that COVID-19 is a bioweapon that has been released from Wuhan bioweapons facility c. f. Senator Tom Cotton, is highly implausible.

However we also feel a special responsibility to make contact with biomedical scientists such as yourselves. COVID-19 is the biggest story on the planet right now- knowing how it may have plausibly arisen gives insight into its spread and then decline, and how it should be managed rationally. e.g. those older passengers on the cruise ships (the vulnerable sub-group) should have been advised to not make hand contact with the deck railings outside the sea-side cabin).

We are experts in the analysis of the origins of sudden emerging diseases just like COVID-19 - and how they also precipitously decline and fade away. Several of us are biomedical immunologists and immunogeneticists. Our explanation handles all the genetic, immunologic, epidemiologic, geophysical and astrophysical (astrobiologic) data surrounding this suddenly emerging COVID-19 mediated disease.

I am sure you will understand our analysis–– we agree it did not come from a Wuhan bio-weapons lab (Why would the Chinese Defence Dept design a low mutation rate, low person-to-person transmitting virus , that only kills older already co-morbid susceptible patients?).

As the key correspondent with you I am a fairly well known senior Australian scientist and immunologist, of 50 years standing. I am widely published in the peer-reviewed scientific literature (Check out EJ Steele on PubMed).

My colleagues ,Professor Chandra Wickramasinghe (University of Buckingham, UK) is ***the*** world expert on sudden disease emergence like this. Together with Professor Reginald Gorczynski MD PhD (clinical immunology scientist and basic researcher,University of Toronto, Canada) we, and our other expert co-authors have analysed all the genetic, immunologic, epidemiological, geophysical and astrophysical data surrounding the origins and spread of this newly emergent Coronavirus. It follows a pattern all too familiar to us (check out our analyses at the URL links)- sudden emergence, then massive induced herd immunity, then sudden decline- this is unfolding right now with COVID-19.

It did not come from animals, it did not come from the Wuhan research facility - all our scientific analysis (in URL links below) indicate it has most plausibly come from a meteorite which burst over central China on the night of October 11 2019. Over the next month the fall-out , much like from an upper atmosphere nuclear test, settled mainly in the central Chinese city of Wuhan and its surrounds. But this fall-out is an infective replicating virus not radioactivity.

The whole central China /Wuhan region and Hubei province has , in our view, been physically contaminated with reasonably high concentrations of COVID-19 virus particles (that replicate in susceptible hosts on landing). As you know it causes a rather mild common cold in humans, and only causes severe pneumonia in older vulnerable , co-morbid, patients. The death rate is low. The mutation rate is low. The actual "cough in your face" human-human transmission is low. It is spread by *environmental contamination* - that is the key to understanding this virus e.g. we believe that at least two cruise ships in the South China Sea/Sea of Japan have been heavily contaminated by this drifting virus fall out dust cloud.

But the panic and hysteria is high- and the ham-fisted and secretive way the Communist Chinese government has behaved has made it even worse. But the Communist Government is acting rationally in trying to disinfect and lock down almost 500 million citizens in Central China (e.g. images of Chinese men

in moon suites with disinfectant spray guns spraying down machinery, road ways, etc). Xi and the Communist Party of China knew of the widespread physical contamination, I am certain, by early January- it was a rational decision by Xi to lock down the region. We believe the viral dust cloud hit the *Diamond Princess* cruise ship (and the Dutch *Westerdam* cruise ship), and is these ships are now heavily contaminated. (Cruise ships in the Atlantic and Mediterranean sea are not reporting this ship wide phenomenon). In our view a fragment of the viral dust cloud (or even the same one) made spot in-falls over Japan- all these COVID-19 cases in Japan with NO links to China are factual evidence in favour of our explanation.
"None of Japan's new coronavirus patients had direct China links - Nikkei Asian Review"
https://asia.nikkei.com/Spotlight/Coronavirus/None-of-Japan-s-new-coronavirus-patients-had-direct-China-links

But there is much other evidence consistent with our explanation, and predictions for the future course of the COVID-19 pandemic.

I know many of you will understand the logic our scientific analysis, that is why I am making contact, as you are all scholars, scientists and analysts who react to hard data. At the URLs click to read the PDF articles of our detailed scientific analyses of this epidemic, now clearly a pandemic at :

Origin of New Emergent Coronavirus and Candida Fungal Disease– Terrestrial or Cosmic?- posted 17.2.20-Chapter 6 for "*Cosmic Genetic Evolution*"
Authors: Edward J. Steele, Jiangwen Qu, N. Chandra Wickramasinghe, Reginald M. Gorczynski, Gensuke Tokoro, Robert Temple, Robyn A. Lindley.
http://viXra.org/abs/2002.0310
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Article submitted to *The Australian* 6.2.20, updated 9.2.20
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http://viXra.org/abs/2002.0118?ref=11085574
Category: Physics of Biology

Letter to *The Lancet* at:
viXra:2002.0039 submitted on 2020-02-03 17:33:22
http://viXra.org/abs/2002.0039?ref=11076818
Comment on the Origin of the 2019 Novel Coronavirus
Authors: Edward J. Steele, N. Chandra Wickramasinghe, Jiangwen Qu, Robert Temple, Gensuke Tokoro, Reginald M. Gorczynski
Category: Physics of Biology

We are happy to be advisors and discuss this further if any of you make contact with us.

Thank you and kind regards. We are genuinely sincere in wanting to communicate the most plausible explanation of the causes of this COVID-19 pandemic

Ted Steele

NB: Some of the letter co-signers did not have an easily recoverable email e.g, Hume Field, Uni QLD; and those with Welcome Trust (Jeremy Farrar, Josie Golding, Mike Turner) . Could those of you who are concerned please forward this email to them.

.....

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For *The Australian Rationalist* . Accepted , In Press April 7 2020 (for June 2020 issue)

Corona Hysteria and Our Cosmic Connection

Edward J Steele

Abstract: The Scientific, Economic and Political issues surrounding the origin and global spread of COVID-19 are discussed.

Australia is in a government-induced economic and social lock down. This draconian action is projected to end in October 2020. The hysteria is high and all reason has flown out the window. I feel a responsibility to inform a wider readership of our specialist scientific knowledge of how COVID-19 may have arisen from a life-bearing and viral-laden carbonaceous meteorite from space, which fragmented in the stratosphere then burst north of Wuhan city on 11 October 2019. In our view, this provides scientific insight into its further global spread[1]. Knowing the most plausible scientific story should, under normal circumstances, provide rational insight into how this pandemic - a dose of mild cosmic common cold – is rationally managed.

My colleagues and I are experts in the origins of sudden emerging diseases just like COVID-19 - and how they decline and fade away. I am a fairly well known Australian biomedical scientist (71yr), an immunologist and microbiologist, of 50 years standing- widely published in the peer-reviewed scientific literature. My principle collaborator since 2016 has been Professor N Chandra Wickramasinghe, a renowned astrophysicist and astrobiologist and world expert in suddenly emerging diseases. Chandra, along with Sir Fred Hoyle is the acknowledged founder of the new science of Astrobiology. They developed the foundation analyses that allow us to understand the mechanisms of sudden disease emergence just like COVID-19. I recommend their scientific classic *Diseases from Space* (1979).

Myself and Chandra with a wider team of co-authors have analysed all the genetic, immunologic, epidemiological, geophysical, astrophysical and astrobiological data surrounding the origins and spread of COVID-19. It follows an all too familiar pattern -

[1] See PDFs of all our recent publications at http://vixra.org/author/edward_j_steele.

sudden emergence, then a rapid self-limiting phase, almost certainly involving induced "herd immunity", then sudden decline. While COVID-19 began in Wuhan, other subsequent explosive outbreaks are now unfolding in Tehran, Italy, Spain and New York City, all lying on the 40° N Latitude band.

It is implausible that COVID-19 came from a Wuhan bio-weapons lab. Why would the Chinese People's Liberation Army design a low mutation rate, low person-to-person transmitting virus, that only kills older already co-morbid susceptible patients? The phylogenetics also make it highly implausible that it came from wild animals in a two-step wild animal-to-human (unknown) process viz. bats to intermediate animal (perhaps pangolins), which were then caught and eaten on scale by Chinese people in the Hubei province. This is a "just so" story, a real fairy story. But this highly improbable story has been irresponsibly repeated and spewed forth in the pages of *The Australian* and other world media, particularly on *Fox News Channel* in the USA.

In our critical view, COVID-19 did not come from animals, it did not come from the Wuhan research facility. All our scientific analysis indicates that it has most plausibly come from a fragmenting meteorite which subsequently burst over north-central China on the night of 11 October 2019.[2] Over the following month, the fall-out, much like that from an upper atmospheric nuclear test, settled mainly in the central Chinese city of Wuhan and its surrounds. But this fall-out is an infective replicating virus not radioactivity.

Fall-out of viral-laden dust clouds of variable size appears now to be spreading globally – some small clouds may have headed easterly in February, across the Pacific (to the US West Coast and California, and cruise ships). However, the most prominent spread has been westward towards Europe, via the Middle East, with direct hits on Iran (Tehran/Qom), Italy (Lombardy), Spain and now New York City. The most reliable case data are for the USA, and particularly New York City. Cases here went from about 450 to about 36,000 between 16 and 30 March (*Business Insider* 27 March 2020). This rocket take-off is consistent with the

[2] https://www.space.com/china-midnight-meteor-brilliant-fireball-october-2019.html

in-fall of a viral-laden dust cloud coming from the stratosphere Latitude 40° band as we predicted – beginning, we think, about 10 March.

So like Wuhan, New York City has taken a direct hit from above. And like Wuhan there is massive contamination of surfaces (and vegetation and wild life) throughout NYC area with trillions of viral particles contaminating the city – a layer of viral dust throughout the city surfaces. Social distancing will play a very small role in containing this type of in-fall driven epidemic. It is mass infection virtually simultaneously from above.

In October- November 2019 the whole of the central China /Wuhan region and Hubei province, was in our view, physically contaminated with high doses of trillions of COVID-19 virus particles that replicated in susceptible hosts on landing. Indeed, and of course, all wild and domestic animals will also be contaminated and/or infected with COVID-19 in an in-fall zone like this, along with older susceptible human beings (e.g. the Italy Lombardy experience, and now New York City) who will also score COVID-19 positive irrespective of whether COVID-19 is the cause of disease, death or not.

In humans COVID-19 causes a rather mild common cold, and only causes severe pneumonia in older vulnerable, co-morbid, patients : which is still the case even now (April 4 2020) at the height of the NYC epidemic where the death rate for confirmed COVID-19 cases is 0.27% for the 18-44 year group, 1.49% for the 45-65 year group, 4.29% for the 65-74 year group and 11.3 % for the 75+yr group. There may also have been a dose dependence effect in symptom-disease induction in the Wuhan epicentre, as well as now in other similar epicentres including New York City in susceptible subjects.

The actual death rate is low as is the mutation rate. However, if infected person-to-person driven spread really does get going we expect the mutational diversity to increase, particularly in pockets where a viral variant is easily spread person-to-person, such as hospital wards and old age nursing homes. At the moment, all the gene sequencing on isolates indicate a common source. Environmental contaminations from actual in-falls (and smaller aerosol-based surface contamination infection foci) is the key mode of catching this disease globally.

In short, spread by environmental contamination is the key to understanding the spread of this virus. We believe, for instance, that at least two cruise ships in the South China Sea/Sea of Japan may have been heavily contaminated by this drifting virus fall-out dust cloud. As it is drifting in patches the viral-laden dust clouds with millions of viral particles enwrapped in protective dust clusters can stay viable for at least 5-6 months in the "air" or atmosphere.

Panic and hysteria are now rife within the Western democracies, based on what we believe to be a false diagnosis of the nature of the problem. The Chinese authorities, let it be said, acted rationally in trying to disinfect and lock down almost 500-700 million of their citizens, while men in moon suits with disinfectant spray guns hosed down machinery, roadways, and buildings. This indicates that Xi Jinping and his government knew of the widespread physical contamination, no later than early January. Their actions, however, suggest that they were attempting to douse something other than a breakout from wet-markets.

The Xi regime also locked down the entire Central China region - the industrial heartland of China's now enormous economy and the engine room of the supply chains that import and export immense quantities of raw materials, components and finished manufactures to the outside world. The silence of the regime concerning what had happened, while culpable, may be attributed to its consternation at the scale of the problem. Many contaminated products may have already been locked away in contaminated shipping containers ready for export.

Nonetheless, a myth arose that COVID-19 came from the eating of wild animals in China and that wet markets have been the petri dishes for such viruses. There is, however, abundant evidence consistent with our meteorite burst explanation, and predictions for the future course of the COVID-19 pandemic. It is being published in professional journals and we urge readers of this magazine to look out for it.[3]

What's actually required is large scale environmental swab sampling for viral RNA COVID-19 sequences, as well as blood tests for presence of antibodies specific for COVID-19 protein antigens. This would enable us to project the emergence of herd immunity. The

[3] See, for instance, our forthcoming paper in the journal *Biophysics and Molecular Biology*: https://doi.org/10.1016/j.pbiomolbio.2019.08.010.

Diamond Princess cruise ship would be expected to have very high numbers of people that score positive for COVID-19 specific antibodies. One could start there. In turn, we could then estimate of the total 'immunologically' exposed population globally and the proportional COVID-19 antibody positivity in different exposed groups.

We have submitted this essay to *The Australian Rationalist* following what we consider to be censorship by *The Australian.* In conversations with the Editor, he agreed they had an obligation to ventilate alternative biomedical and scientific explanations of the pandemic. However, after consulting other mainstream scientists he and his colleagues canned our submission, as not meeting peer review standards.

We remain of the opinion that ours is the most plausible explanation of the COVID-19 pandemic. Our concern is that, with the cyclical 'cold and flu season' approaching this winter, our draconian economic and health policy initiatives have been instituted far in advance of the real danger. That danger is an atmospheric viral in-fall event in Australia some months from now, at a point when our social and economic resilience will already have been strained on the basis of a misunderstanding of what is actually happening. We hope we will not be proven correct in our analysis when it is too late for a course correction to tackle the problem appropriately.

From:	Su, Lishan
To:	Liu, Shan-Lu; apc@tandf.co.uk
Cc:	Shan Lu
Subject:	Re: Your Open Access article publishing charge invoice [ref:_00D0Y35Iji._5002X2h5qN4:ref]
Date:	Wednesday, February 26, 2020 7:52:15 AM

Yes, it was waived at the beginning. Thanks

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Wednesday, February 26, 2020 4:11:35 AM
To: apc@tandf.co.uk <apc@tandf.co.uk>
Cc: Shan Lu <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Your Open Access article publishing charge invoice [ref:_00D0Y35Iji._5002X2h5qN4:ref]

Thank you, but my understanding is that the publication fee is waived for this commentary, the fee waive code is: TEMI-2020-C3865. See below email for EMI editor in chief Dr. Shan Lu on Feb 12.

Thank you.

> **From:** "Lu, Shan" <Shan.Lu@umassmed.edu>
> **Date:** February 12, 2020 at 9:08:04 PM EST
> **To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Cc:** "min.yang@emi2012.org" <min.yang@emi2012.org>
> **Subject: RE: EMI commentary**
>
> Ok, then please submit asap.
>
> Since this is a special invited commentary, I will waive your fee although the price is quite low.
>
> Please use this code when you submit: TEMI-2020-C3865. Only use once.
>
> I am copying Min Yang from EMI office to assist you.
>
> Let her know by email if you have any questions.
>
> Shan
>
> On Feb 26, 2020, at 3:54 AM, "apc@tandf.co.uk" <apc@tandf.co.uk> wrote:

Dear Mr Shan-Lu Liu,

Ref : No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
DOI: 10.1080/22221751.2020.1733440

Congratulations on the acceptance of your paper.

Publication in the journal is subject to payment of an APC (article publishing charge). Thank you for accepting responsibility for payment of this charge when you submitted your paper.Please find attached your APC invoice. Should you have any queries about this document, please don't hesitate to contact us

To pay by credit card online please click here.

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For more information around our Open programme, please visit http://authorservices.taylorandfrancis.com/publishing-open-access which contains a wealth of information and resources, including information on how to promote your paper and optimise citations once it is published online.

Kind regards,
Evelyn Wong
Customer Service Executive

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ref:_00D0Y35Iji._5002X2h5qN4:ref
<Invoice 952282459.pdf>

From: Su, Lishan
To: Weiss, Susan; Liu, Shan-Lu
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)
Date: Friday, February 21, 2020 4:58:56 PM
Attachments: image001.png

Thanks, Susan!
I will do a minor revision of the sentence in the proof. Please let me know if you have other suggestions to the proof. I will upload it after hearing from all of you.
Best,

-Lishan

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 3:57 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I think old sentence is more correct

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 2:53 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I agree with you on these points, but NIH/government at the time put it as a gof study relative to the original S antigen…

-Lishan

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 2:51 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Is the adaptation of MA15 to mice considered “gain of function”- that selected virus is more virulent than SHC014-MA15 chimeric virus? Seems to me like more loss of function relative to MA15 when inserting the bat derived spike. MA15 with the urbani spike is like de- adapting the virus to mice.

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 1:40 PM

To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I have noticed that too, probably happened when we tried to simplify the chimeric virus paragraph, and I think Ralph had added the attenuation sentence relative to M15 in mice…

What was reported in the NM paper was that the SHC014-rMA15 chimeric virus was less pathogenic than M15, but more so than the chimeric M15 virus with the original Urbani Spike-gene in M15, probably due to one of the 6 mutations in the M15 S gene.

See old sentence:
Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the original human Urbani S-MA15 chimeric virus in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies…

I will try to fix this.
Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 12:14 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Lishan: see below comments from Susan.

Susan: thank you. I had the same question before – Lishan, could you explain this?

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 9:06 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Please list me as Susan R Weiss (with the "R"). there are too many other Susan Weiss'

I noticed what looks like a contradictory statement in the paper- sorry I missed it before- I

highlighted in yellow lines 124-133. The first part says chimeric virus is attenuated producing less antigen than MA15 but the next part says it has elevated activity- this seems contradictory

I remain concerned about the insertion of the furin site

Susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 10:05 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: [External] Re: Your article proofs for review (ID# TEMI 1733440)

We agreed to add this link to the proof to the third paragraph regarding RaTG13.

The Proximal Origin of SARS-CoV-2
http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
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Center for Retrovirus Research
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Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Shan-Lu Liu <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 4:46 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: FW: Your article proofs for review (ID# TEMI 1733440)

All,

See message below and also the attached proof.

Please mark your changes in the attached PDF file, and Lishan and I will incorporate to finalize.

Thanks.

Shan-Lu

From: "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Friday, February 21, 2020 at 4:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear Shan-Lu Liu,

Your article proofs are now available for review through the Central Article Tracking System (CATS) at: https://cats.informa.com/PTS/in?ut=B2AB6692AA414D96905B59E6C51FA240 .

PLEASE NOTE: The CATS system only supports Internet Explorer 6 (and later), or Firefox 3 (and later) browser software. Popup blockers should be disabled. If you have any difficulty using CATS, please contact me.

• Your User Name is: [redacted]

• If you do not know your password, you may reset it here: http://cats.informa.com/PTS/forgottenPassword.do

1. Click on 'Review Proofs'.

2. Select 'Download PDF'.

3. Follow the guidance on the proof cover sheet to return your corrections. Please limit changes to answering any author queries and to correcting errors. We would not expect to receive more than 30 corrections.

Please check your proofs thoroughly before submitting your corrections as once they have been submitted we are unable to accept further corrections. If you have any queries, please email me.

To avoid delaying publication of your article, please approve these proofs or return any corrections by 26 Feb 2020.

Reprint and issue orders may be placed by logging in to your CATS account and accessing the order form on the "Additional Actions" menu. If you have any questions on this process, please contact me or visit our author services site https://authorservices.taylorandfrancis.com/ordering-print-copies-of-your-article/

• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link: https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From: Weiss, Susan
To: Liu, Shan-Lu
Subject: Re: [External] FW: Your article proofs for review (ID# TEMI 1733440)
Date: Friday, February 21, 2020 10:13:18 AM

Henry and I have been speculating- how can that site have appeared at S1/S2 border- I hate to think to was engineered- among the MHV strains, the cleavage site does not increaser pathogenicity while it does effect entry route (surface vs endosome) . so for me the only significance of this furin site is as a marker for where the virus came from- frightening to think it may have been engineered

---

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 9:50 AM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: Lishan Su <lishan_su@med.unc.edu>
Subject: Re: [External] FW: Your article proofs for review (ID# TEMI 1733440)

Susan, I completely agree with you, but rumor says that furin site may be engineered. Importantly, the virus RNA sequence around the furin site (288 nt), before and after, has 6.6 % differences, but with no amino acid changes at all.

Shan-Lu Liu sent from iPhone

> On Feb 21, 2020, at 5:42 AM, Weiss, Susan <weisssr@pennmedicine.upenn.edu> wrote:
>
> Shan-Lu
>
> Maybe too late to add to the paper, but I think the fact that the RaTG13 spike does not include a furin sequence makes it unlikely that it is the precursor to SARS-CoV-2.
> I find it hard to imagine how that sequence got into the spike of a lineage b betacoronavirus- not seen in SARS or any of the bat viruses.
>
> The BioRx preprint on Pangolin sequence is very weak- says the RBD from the pangolin virus is closer to SARS-CoV-2 than RaTG13 is. But again pangolin sequence lacks the furin site.
> The furin site to me is a good marker for ancestral virus
>
> Any thoughts on this?

susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:47 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: [External] FW: Your article proofs for review (ID# TEMI 1733440)

All,

See message below and also the attached proof.

Please mark your changes in the attached PDF file, and Lishan and I will incorporate to finalize.

Thanks.

Shan-Lu

From: "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Friday, February 21, 2020 at 4:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

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• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link: https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From: Su, Lishan
To: Liu, Shan-Lu
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)
Date: Friday, February 21, 2020 7:19:10 PM

Uploaded and you should have received a message.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 5:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Looks good, let's submit. Linda should be fine with it.
Thanks.

Shan-Lu Liu sent from iPhone

> On Feb 21, 2020, at 2:35 PM, Su, Lishan <lishan_su@med.unc.edu> wrote:
>
> Done, and waiting to be submitted after hearing from Linda.
>
> -Lishan
>
> ---
>
> **From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Date:** Friday, February 21, 2020 at 3:07 PM
> **To:** "Su, Lishan" <lishan_su@med.unc.edu>
> **Subject:** Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)
>
> Lishan,
>
> Do you have the corrected proof? Thanks for doing this. I am almost done with the meeting.
>
> SL
>
> ---
>
> **From:** "Su, Lishan" <lishan_su@med.unc.edu>
> **Date:** Friday, February 21, 2020 at 11:52 AM
> **To:** "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>, Shan-Lu Liu <liu.6244@osu.edu>
> **Subject:** Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I agree with you on these points, but NIH/government at the time put it as a gof study relative to the original S antigen…

-Lishan

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 2:51 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Is the adaptation of MA15 to mice considered "gain of function"- that selected virus is more virulent than SHC014-MA15 chimeric virus? Seems to me like more loss of function relative to MA15 when inserting the bat derived spike. MA15 with the urbani spike is like de- adapting the virus to mice.

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 1:40 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I have noticed that too, probably happened when we tried to simplify the chimeric virus paragraph, and I think Ralph had added the attenuation sentence relative to M15 in mice…

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Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the original human Urbani S-MA15 chimeric virus in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies…

I will try to fix this.
Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 12:14 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Lishan: see below comments from Susan.

Susan: thank you. I had the same question before – Lishan, could you explain this?

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
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To: Shan-Lu Liu <liu.6244@osu.edu>
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I remain concerned about the insertion of the furin site

Susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 10:05 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: [External] Re: Your article proofs for review (ID# TEMI 1733440)

We agreed to add this link to the proof to the third paragraph regarding RaTG13.

<image001.png>
Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Shan-Lu Liu <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 4:46 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: FW: Your article proofs for review (ID# TEMI 1733440)

All,

See message below and also the attached proof.

Please mark your changes in the attached PDF file, and Lishan and I will incorporate to finalize.

Thanks.

Shan-Lu

From: "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Friday, February 21, 2020 at 4:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

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3. Follow the guidance on the proof cover sheet to return your corrections. Please limit changes to answering any author queries and to correcting errors. We would not expect to receive more than 30 corrections.

Please check your proofs thoroughly before submitting your corrections as once they have been submitted we are unable to accept further corrections. If you have any queries, please email me.

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• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link: https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

<TEMI_A_1733440 Proof-Su.pdf>

From:	Su, Lishan
To:	Liu, Shan-Lu; Lu, Shan
Subject:	Re: 2019-nCoV-EMI_commentary
Date:	Wednesday, February 12, 2020 10:08:24 AM
Attachments:	image001.png image002.png 2019 CoV Copy.enl EMI-2019-nCoV_Commentary LJS_SLL_Refs.docx

---

See minor revisions and new endnote file. My new MS office word is refusing endnote?!
I don't know how to add website into the Endnote file.
Thanks!

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>

Date: Wednesday, February 12, 2020 at 9:08 AM

To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>

Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Once you complete and send me your revision along with the updated Endonote, I will quickly finish it and send it to Stanley Perlman and Susan Weiss and copy you of course.

Thank you.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
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1900 Coffey Rd, Room 480 VMAB
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>

Date: Wednesday, February 12, 2020 at 8:32 AM

To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>

Subject: Re: 2019-nCoV-EMI_commentary

Got it. Thanks

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Wednesday, February 12, 2020 7:19:41 AM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Please use the latest updates, with minor changes.

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 1:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See the endnote file. Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 7:44 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Sounds good, thank you. I still like “however” over “In contrast” – it just reads better

Shan: Are you sure that you prefer not to be included in the coauthorship? Before I send, I think we should have the authorship listed, along with affiliations. Lishan should be the first author, unless he prefers otherwise. Agreed?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 7:34 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

I made some minor change for the following:

In summary, there is no credible evidence at this point to support the claims that the 2019-nCoV was

originated from a laboratory-engineered CoV. In contrast, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV.

Maybe now SLL can send the next version to other CoV experts?

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 5:47 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See the new version with all incorporated.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 4:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I have made additional changes to the Lishan's version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 4:16 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 3:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Perhaps Lishan can take a look at the latest version, which has the new title I suggested, and modify it as needed.
The last paragraph is also crucial, but I did not have time to work on it because of a meeting this morning.
Once we have almost a final draft, I will contact Linda Saif, Stanley Perlman, Thomas Gallgaher etc. to see if they are willing to join, but this may delay the publishing time.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 1:52 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I agree that it should be simple and clear. I have included some details in the 1st draft for your information. There is not intention to defend Baric, but to clarify the facts.

Regarding all three, are you combining Goa Feng's piece with this one? For the RaTG13, it involves complicated viral evolution kinetics and maybe hard to simply clarify…
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 1:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and

provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

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Department of Veterinary Preventive Medicine,

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[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

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[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

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Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song, H.D. et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A 102, 2430-2435 (2005)). Given that there are greater than 1000 nt differences between the human

SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) (Roberts, A. et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog 3, e5 (2007)) was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding

genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis [6].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-

are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (a manuscript sharing site prior to any peer review ~~and not yet peer reviewed for accuracy~~) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.---And should not be present? in naturally isolated viruses such as RaTG13. Currently, there is no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a

bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

1. Wang, D.*, et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang*, et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N.*, et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P.*, et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N.*, et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D.*, et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y.*, et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W.*, et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F.*, et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

From:	Su, Lishan
To:	Liu, Shan-Lu
Subject:	Re: Submitted Corrections for article TEMI 1733440
Date:	Friday, February 21, 2020 7:39:02 PM
Attachments:	image001.png image002.png TEMI_A_1733440 Proof-Su copy.pdf

---

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>

Date: Friday, February 21, 2020 at 7:33 PM

To: "Su, Lishan" <lishan_su@med.unc.edu>

Subject: Re: Submitted Corrections for article TEMI 1733440

I just went online to see if we replace with a new one.
Could you send me the PDF of the corrected one?

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>

Date: Friday, February 21, 2020 at 4:32 PM

To: Shan-Lu Liu <liu.6244@osu.edu>

Subject: Re: Submitted Corrections for article TEMI 1733440

My bad. How do we fix it? send her a message?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>

Date: Friday, February 21, 2020 at 7:29 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Lishan:

I just saw that you deleted nt for 1,100 – “nt” should be kept. Could you correct that?

Thanks.

SL

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:28 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Thanks!

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

Begin forwarded message:

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Date: February 21, 2020 at 4:04:41 PM PST
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Subject: Submitted Corrections for article TEMI 1733440
Reply-To: TEMI-production@journals.tandf.co.uk

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

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Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

TEMI1733440 VOL 0, ISS 0

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu, Linda J. Saif, Susan Weiss, and Lishan Su

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Emerging Microbes & Infections
https://doi.org/10.1080/22221751.2020.1733440

COMMENTARY

OPEN ACCESS Check for updates

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Q1 Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan Weiss [f] and Lishan Su[g]

[a]Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; [b]Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; [c]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA; [d]Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA; [e]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA; [f]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [g]Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

ARTICLE HISTORY Received 13 February 2020; Accepted 13 February 2020

PROOF ONLY

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe

CONTACT Shan Lu Liu lsu@med.unc.edu; Lishan Su Liu.6244@osu.edu

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bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

Disclosure statement

No potential conflict of interest was reported by the author(s). Q2

ORCID

Susan Weiss http://orcid.org/0000 0002 8155 4528

References Q3

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. Q4

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. Q5

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. Q6

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. Q7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. Q8

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin converting enzyme 2 is a functional receptor for the

PROOF ONLY

SARS coronavirus. Nature. 2003 Nov 27;426 (6965):450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3.
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

Q9

PROOF ONLY

From: Su, Lishan
To: Liu, Shan-Lu
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)
Date: Friday, February 21, 2020 4:57:07 PM
Attachments: image001.png

I am doing the proof, and waiting to get anything from Linda. You and Susan seem to have responded already.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 3:07 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Lishan,

Do you have the corrected proof? Thanks for doing this. I am almost done with the meeting.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 11:52 AM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I agree with you on these points, but NIH/government at the time put it as a gof study relative to the original S antigen…

-Lishan

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 2:51 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Is the adaptation of MA15 to mice considered “gain of function”- that selected virus is more virulent than SHC014-MA15 chimeric virus? Seems to me like more loss of function relative to MA15 when inserting the bat derived spike. MA15 with the urbani spike is like de- adapting the virus to mice.

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 1:40 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I have noticed that too, probably happened when we tried to simplify the chimeric virus paragraph, and I think Ralph had added the attenuation sentence relative to M15 in mice…

What was reported in the NM paper was that the SHC014-rMA15 chimeric virus was less pathogenic than M15, but more so than the chimeric M15 virus with the original Urbani Spike-gene in M15, probably due to one of the 6 mutations in the M15 S gene.

See old sentence:
Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the original human Urbani S-MA15 chimeric virus in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies…

I will try to fix this.
Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 12:14 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Lishan: see below comments from Susan.

Susan: thank you. I had the same question before – Lishan, could you explain this?

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 9:06 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Please list me as Susan R Weiss (with the “R”). there are too many other Susan Weiss’

I noticed what looks like a contradictory statement in the paper- sorry I missed it before- I highlighted in yellow lines 124-133. The first part says chimeric virus is attenuated producing less antigen than MA15 but the next part says it has elevated activity- this seems contradictory

I remain concerned about the insertion of the furin site

Susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 10:05 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: [External] Re: Your article proofs for review (ID# TEMI 1733440)

We agreed to add this link to the proof to the third paragraph regarding RaTG13.

The Proximal Origin of SARS-CoV-2
http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
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Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Shan-Lu Liu <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 4:46 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: FW: Your article proofs for review (ID# TEMI 1733440)

All,

See message below and also the attached proof.

Please mark your changes in the attached PDF file, and Lishan and I will incorporate to finalize.

Thanks.

Shan-Lu

From: "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Friday, February 21, 2020 at 4:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear Shan-Lu Liu,

Your article proofs are now available for review through the Central Article Tracking System (CATS) at: https://cats.informa.com/PTS/in?ut=B2AB6692AA414D96905B59E6C51FA240 .

PLEASE NOTE: The CATS system only supports Internet Explorer 6 (and later), or Firefox 3 (and later) browser software. Popup blockers should be disabled. If you have any difficulty using CATS, please contact me.

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• If you do not know your password, you may reset it here:
http://cats.informa.com/PTS/forgottenPassword.do

1. Click on 'Review Proofs'.

2. Select 'Download PDF'.

3. Follow the guidance on the proof cover sheet to return your corrections. Please limit changes to answering any author queries and to correcting errors. We would not expect to receive more than 30 corrections.

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To avoid delaying publication of your article, please approve these proofs or return any corrections by 26 Feb 2020.

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• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link:
https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

F om Su shan
To h - h
Subje t Re Eme g ng M c bes & Infect ons - TEMI-20 0-0121 - changes qui ed o ou subm s ion
Date iday eb ua y 4 2020 1 17 7 M

Very good point about working wi h the new irus. I was referr ng to ra g13 and the chimeric iruses in the lab.

-Lishan

F om Liu, Shan-Lu l u 6244@osu.edu>
Sent F iday, Feb ua y 14, 2020 12 47 18 PM
To Su, L shan l shan_su@med.unc edu> Lu, Shan shan lu@umassmed edu>
Subject Re Eme g ng Mic obes & Infect ons - TEMI-2020 0121 - changes equi ed to you submiss on

Lishan

I get you point – maybe below one eads bet e ?

We should emphas ze that, although SARS-CoV-2 shows no evidence of labo ato y o igin, such a vi us, and clo ely elated, do pose g eat public health th eats and must be handled p ope ly n the labo a o y and also p ope ly egulated by gove nments and scientific commun ty"

Thoughts?

Shan-Lu Liu, M.D., Ph D.
P ofesso
Co-Di ecto , Vi uses and Eme ging Pathogens P og am
Infectious Diseases Inst tu e
Cente fo Ret ovi us Resea ch
Depa ments of Ve e na y Biosciences, Mic obial Infection and Immunity, and M c ob ology
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Phone (614) 292-8690
Fax (614) 292-6473
Ema l l u 6244@osu.edu shan lu.l u@osumc.edu

On 2/14/20, 11 08 AM, Su, Lishan l shan_su@med.unc edu> w ote

How about adding the last sentence n the p oof?

Evolution s stepwise and acc ues mutations g adually ove t me, whe eas synthet c const uc s would typica ly use a known backbone and nt oduce logical o ta geted changes instead of the andomly occu ing mutations that a e p esent in natu ally i ola ed vi uses such as bat CoV RaTG13. In ou view, the e is cu ently no c ed ble evidence to uppo t the cla m that SARS CoV 2 o iginated f om a labo ato y-eng nee ed CoV. It is mo e l kely that SARS-CoV-2 is a ecomb nant CoV gene ated in natu e be ween a bat CoV and anothe co onavi us in an nte mediate animal hos . Mo e studies a e needed to explo e th s possibility and esolve the natu al o g n of SARS CoV-2. Although the SARS2 CoV has shown no evidence of labo ato y o igin, it is impo tant to point out that such vi uses in the labo ato y as d scussed he e do pose public health conce ns and should be ca efu ly moni o ed and egulated.

-Lishan

--- O ig nal Message- ---
F om Liu, Shan-Lu l u 6244@osu.edu>
Date Thu sday, Feb ua y 13, 2020 at 10 04 AM
To Lu, Shan shan.lu@umassmed.edu>
Cc Su, Lishan l shan_su@med.unc edu>
Subject Re Eme g ng Mic obes & Infect ons - TEMI-2020 0121 - changes equi ed to you submiss on

Thanks, Shan fo you e fic ent ac ion!

On 2/13/20, 9 56 AM, Lu, Shan Shan.Lu@umassmed.edu> w ote

You pape is now accepted. Hope you have eceived the dec sion let e .

Best.

Shan

--- -O ginal Message-----
F om L u, Shan-Lu liu.6244@osu edu>
Sent Thu sday, Feb ua y 13, 2020 8 57 AM
To temi-pee eview@jou nals.tandf.co.uk
Cc Lu, Shan Shan.Lu@umassmed.edu> Su, Lishan l shan_su@med unc.edu>
Subject Re Eme ging Mic obes & Infections - TEMI-2020-0121 - changes equi ed to you subm ssion
Impo tance H gh

Hi Jo gie

I have modif ed as inst ucted and at ached the new one to this ema l. Please help upload and p oceed.

Thank you.

Shan-Lu

Shan-Lu L u, M.D., Ph D.
P ofesso
Co-Di ecto , Vi uses and Eme ging Pathogens P og am Infectious Diseases Inst tute Cente fo Ret ovi us Resea ch Depa tments of Vete ina y Biosc ences, M c ob al Infect on and Immun ty, and Mic obiology The Ohio State Unive sity
1900 Co fey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone (614) 292 8690
Fax (614) 292 6473
Ema l l u.6244@osu.edu shan lu.liu@osumc.edu

On 2/13/20, 8 43 AM, Eme ging Mic obes and Infec ions onbehalfo @manusc iptcent al.com> w ote

13-Feb 2020

Dea P ofesso Liu,

You above efe enced manusc ipt, entitled SARS-CoV-2 no evidence of a labo ato y o ig n equi es some fu the changes befo e it is eady fo eviewing n Eme g ng Mic obes & Infec ions. You submiss on has been etu ned to you and is located in you Autho Cen e as a d aft, so that you due to these easons

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You may contact the Edito ial O fice if you have fu the quest ons.

S nce ely,

Jo g e Lyn Luna
Eme ging M c obes & Infect ons Edi o al Off ce
temi-pee ev ew@jou nals tandf co uk

From: Su, Lishan
To: Liu, Shan-Lu; Lu, Shan
Subject: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455
Date: Thursday, February 20, 2020 1:39:16 AM
Attachments: Liu et al_EMI Commentary_Revision_中文-sls.docx

See attached file. Feel free to further revise.
Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Thursday, February 20, 2020 at 12:29 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455

Great! Share when done.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 19, 2020 at 9:28 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455

All is well。我快把中文翻译修改完了。

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Thursday, February 20, 2020 at 12:15 AM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455

Better make sure 100%.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Thursday, February 20, 2020 12:14 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455

I think so

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Wednesday, February 19, 2020 at 9:13 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455

Thanks. You submitted it online, right?

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Thursday, February 20, 2020 12:12 AM
To: Liu, Shan-Lu <liu.6244@osu.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455

Fyi。See title and author names.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Thursday, February 20, 2020 at 12:11 AM
To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Cc: "shan.lu@umassmed.edu" <shan.lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440 #TrackingId:5682455

We are fixing it right now and will submit very shortly.

Thank you.

Shan-Lu

From: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Wednesday, February 19, 2020 at 9:07 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "shan.lu@umassmed.edu" <shan.lu@umassmed.edu>, "lishan_su@med.unc.edu" <lishan_su@med.unc.edu>
Subject: Re: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections

1733440 #TrackingId:5682455

Hi Shan,

Your copyright form was rejected as there were no article title and author names filled in the form. Please fill and resubmit the form.

Regarding publication time, normally this would publish articles in 18 days. If editor asks us to prioritise, we will do. I will discuss with editor and get back to you on this.

Regards,

Malathi
Emerging Microbes & Infections

From:liu.6244@osu.edu
Sent:
To:liu.6244@osu.edu
Cc:lishan_su@med.unc.edu,Shan.Lu@umassmed.edu
Subject: Re: Re: Welcome to Taylor & Francis Production: Emerging Microbes & Infections 1733440

I am currently out of town, but may I ask my co-corresponding author Dr, Lishan Su, who is copied, to sign on the agreement on behalf of us.

Another piece of note, because this commentary is extremely time-sensitive, is it possible to process it with an accelerated speed? Last few days, similar comments have been published by other journals.

Thank you.

Shan-Lu Liu sent from iPhone

> On Feb 19, 2020, at 3:51 PM, TEMI-production@journals.tandf.co.uk <cats@taylorandfrancis.com> wrote:
>
> Article: SARS-CoV-2: no evidence of a laboratory origin
>
> Journal: *Emerging Microbes & Infections* TEMI
>
> Article ID: TEMI 1733440
>
> Dear Shan-Lu Liu,

We are delighted that you have chosen to publish your article in *Emerging Microbes & Infections*. I will be your Production Editor and will work with you to oversee the production of your article through to publication. My contact details are given at the end of this email.

• Please print and sign the attached Author Publishing Agreement. Then return the completed agreement to Taylor & Francis, by uploading to CATS (see below), or post it to the address below.

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Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

没有可信的证据支持 SARS-CoV-2 来自实验室工程的说法

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Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

截止 2020 年 2 月 10 日，在中国武汉出现和爆发的急性呼吸疾病已波及 4 万多人，导致 1000 多人死亡。研究人员很快~~确定~~找到了~~这~~一种新型人体内的冠状病毒，称之为 2019 nCoV 或 SARS-CoV-2，而相应的疾病称之为 COVID-19，意为 2019 年发生的冠状病毒疾病 (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/)。

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

据现有的报道[1-3]，COVID-2019 似乎与 SARS-CoV 导致的 SARS 有相似的临床表现。SARS-CoV-2 基因组序列也和 SARS-CoV 有 80%~~一致~~相同，~~但却是和一些蝙蝠贝塔冠状病毒最相似~~但却是和一些蝙蝠乙型冠状病毒最相似，~~序列一致~~超过了 96%基因组序列相同。

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory engineering origin. Some people have alleged that the human SARS-CoV-

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2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4].

当前，种种的推测、谣言和阴谋论认为，SARS-CoV-2 是源自实验室基因工程。一某些人声称，人的 SARS-CoV-2 是从武汉的某个实验室直接泄漏出来的。该实验室最近报道了一种称为 RaTG13 的蝙蝠冠状病毒，和 SARS-CoV-2 基因组序列有 96%的同源性。

However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6].

然而，我们知道，人的 SARS 冠状病毒和中间宿主棕榈果子狸~~样~~ SARS 样冠状病毒具有 99.8%的同源性，在整个基因组中~~共鉴定出~~只有 202 个~~单核苷酸变异~~碱基不同。

Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

鉴于人类 SARS-CoV-2 与蝙蝠 RaTG13-CoV 之间~~存在~~有超过 1000 个不同碱基~~单核苷酸的差异~~[4]，且这些差异是按照冠状病毒典型的进化特征以自然发生的模式分布在整个基因组中，因此~~极不可能~~ RaTG13 冠状病毒是 SARS-CoV-2 的直接来源是极不可能的。在新的 SARS-CoV-2 病毒序列中并没有~~一个逻辑上靶向的模式~~基因工程的迹象，且它和野生物种（蝙蝠）~~近亲~~非常相似，这都~~是最~~很明显的~~提示~~揭示~~，一~~和表明 SARS-CoV-2 是通过自然~~进~~演化而来的。这需要~~寻找~~找到蝙蝠与人类之间的中间动物宿主来源~~是需要的~~与 SARS-CoV-2 更相似~~，可以用来确定与人 SARS-CoV-2 更紧密相关~~的动物冠状病毒。有猜测认为穿山甲可能携带与 SARS-CoV-2 密切相关的冠状病毒，但数据尚未发表，无从证实。

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

Commented [SL1]: Disregarded ?

中国社交媒体上的另一种说法指向《自然医学》2015 年发表的一篇论文[7]，该论文报道了在小鼠适应后 SARS 冠状病毒的骨架（MA15 病毒）中构建了带有蝙蝠冠状病毒~~基因~~

（SHC014）S 基因的嵌合冠状病毒，该病毒适应病毒后可以感染小鼠小鼠（MA15 病毒），也能够感染人类细胞[8]。但是，可是该主张说法缺乏任何科学依据，必须予以驳斥，因为该嵌合冠状病毒构建体的遗传序列与 SARS-CoV-2 相比有超过 5,000 个核苷酸的显著的差异。

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patienthumans due to the mouse adaptation.

适应小鼠的 SARS 病毒（MA15）[9]是通过把传染性的克隆野生型 SARS 冠状病毒壳隆体在 BALB / c 小白鼠呼吸道中的连续传代而产生的。在小鼠中传代 15 次后，这个 因 SARS 冠状病毒有了六个编码遗传突变使其适应为与感染小鼠适应性相关的六个编码遗传突变，且 SARS 冠状病毒在老年小鼠中获得了更高的复制和肺部致病性（因此称为 M15）。由于在小鼠内的适应的遗传突变改造，很可能 MA15 在人细胞或者人体内复制很可能是高度降低减毒得以在人细胞或者患者体内复制。

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8].

当初分离出~~原始的~~SARS 冠状病毒时，得出的结论是，蝙蝠身上来的冠状病毒的 S 基因不同于人类患者或果子狸身上的病毒，它无法使用人的 ACE2 受体来进入人体细胞[10, 11]。

~~Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8].~~

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果子狸~~曾~~被认为是~~很多~~蝙蝠冠状病毒的中间宿主，能够将 SARS 冠状病毒传播给人类[6，12]。然而，2013 年，从中国马蹄蝙蝠中分离了出来数种新型蝙蝠冠状病毒~~从中国马蹄蝙蝠中分离了出来~~，~~而且~~这些蝙蝠的 SARS ~~样~~或样冠状病毒（SL-CoV-WIV1）~~病毒~~能够使用~~通过~~人、果子狸和中国马蹄蝙蝠的 ACE2 受体进入和感染细胞[8]。

Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the ~~exact~~ S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, ~~which~~and SL-SHC014-MA15 only cause~~s~~d lethal outcomes in aged mice [7].

另外~~结~~合进化证据表明，在与 SARS 冠状病毒的相互作用上，蝙蝠 ACE2 基因已在与人类 ACE2 基因相同的接触位点上被~~积极~~正选择[13]，因此提出了蝙蝠的 SARS 样冠状病毒传染到人不必需要中间宿主的可能~~不是必需的~~，~~且~~有些蝙蝠 SL-CoV 也许能够直接感染人类宿主。为了直接~~解决~~验证这种可能性，~~来自~~蝙蝠冠状病毒 SL-SHC014 ~~的一样~~的 S 基因被~~合成出来，并~~用~~于~~在~~适应小鼠的~~MA15 SARS-CoV ~~主链~~骨架中产生一个嵌合病毒。所得的 SL-SHC014-MA15 嵌合病毒~~，~~确实可以有效地利用人 ACE2 进入细胞，并在~~原代~~人气呼吸道细胞中复制到与 SARS-CoV 流行株相似的~~浓度~~水平。虽然 SL-SHC014-MA15

可以在年轻和老年小鼠的肺中高效复制，但感染减弱了，并且与 SARS MA15 相比，气道上皮中存在的病毒抗原更少~~。~~，并且只~~而 SARS MA15~~会让老年小鼠致命[7]。

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV with a human SARS virus S gene in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research).

由于 SL-SHC014-MA15 嵌合病毒相对于 SARS-S-MA15 冠状病毒在小鼠中具有更高的致病活性，因此，这种 SL-SHC014-MA15 嵌合病毒的实验后来在美国政府的强制暂停政策之下作为功能获得（GOF）研究而受到限制(https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research)。

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The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

构建这种可能具有大流行病潜力的病毒是否是一种风险，当前的 COVID-2019 ~~流行病已经重新引发子这样的辩论~~流行病又重新引发了这样的辩论，~~可~~虽然这些蝙蝠冠状病毒已经在自然界存在~~，一辩论却无视这个发现~~。无论如何，经过多个~~国际组织~~国家科学家的认真的~~系统发育~~病毒分子进化分析[5，14]，SARS-CoV-2 无疑与 SL-SHC014-MA15 不同，整个基因组有~~的核苷酸差异~~超过 6,000 核苷酸的差异。因此，~~重申一下，~~也是没有可信的证据支持 SARS-CoV-2 是源自嵌合 SL-SHC014-MA15 病毒的说法。

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

也有传言说，SARS-CoV-2 是实验室中人为人工或有意制造的。在~~提交给~~ BioRxiv（一个同行评审之前的手稿共享网站）的一份手稿~~（任何同行评审之前的手稿共享网站）~~中强调了~~这一~~此传言点，声称 SARS-CoV-2 中含有 HIV 序列，因此很可能是在实验室中产生的。在由 HIV-1 病毒专家 Feng Gao 博士领导的反驳论文中，他们使用了仔细的生物信息

学分析来证明，最初说的 SARS-CoV-2 有多个 HIV-1 插入片段的主张并不是 HIV-1 特有的，而是随机的。由于国际社会提出的许多~~关注~~疑问，提出最初传言~~主张~~的~~提交人~~作者已经撤回了该报告。

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

进化是逐步的，并随着时间的推移逐渐产生突变，而合成基因构建体通常会使用已知的骨架并引入逻辑或~~目标~~定向变化，而不是~~用~~像天然分离的病毒（如蝙蝠冠状病毒 RaTG13）中存在的随机发生的突变。我们认为，目前没有可靠的证据支持有关 SARS-CoV-2 源自实验室设计的冠状病毒的说法。 更可能的是，SARS-CoV-2 是一种蝙蝠冠状病毒与中间动物宿主中的另一种冠状病毒之间自然产生的重组冠状病毒。需要更多的研究

来探索这种可能性并解决 SARS-CoV-2 的自然起源。我们应该强调，尽管没有证据显示 SARS-CoV-2 ~~没有证据显示~~是来自实验室，如此对公共健康有威胁的病毒应该在实验室里有恰当的~~处~~管理，也要由科学~~共同体~~界和政府合理监管。

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.

9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

From:	Lu, Shan
To:	Su, Lishan; Liu, Shan-Lu
Subject:	RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission
Date:	Sunday, February 16, 2020 1:34:20 PM

It is better to keep the credible here.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Sunday, February 16, 2020 1:33 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

I have added the Gao reference and formatted (I have fixed my endnote problem!). Do we need credible here in the title?

No ~~credible~~ evidence supporting claims of laboratory engineering of SARS-CoV-2.

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 1:12 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Ok, let do the 2nd one.

See attached, with Gao ref added for you to put into Endnote

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 1:07 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Second one reads better and is more accurate.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 1:04 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

For shorter, it will be what I suggested, let's put the two below:

No credible claims supporting the laboratory engineering of SARS-CoV-2

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 1:02 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

I think either of these two should be fine, the shorter the better for a title. Title does not need to be exclusive.

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 16, 2020 at 12:58 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Either is fine, but first is preferred, short and clear.

SARS-CoV-2: no evidence of laboratory engineering
Or
No credible evidence supporting (claims of?) the laboratory engineering of SARS-CoV-2

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 12:53 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

I am okay with the change for the title, but "claims" does not seem a good fit here –"evidence" is better I feel.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 12:51 PM

To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Thanks, then let's not having those two parts.

On the title, I do not need to use "current".

How about this:

No credible claims supporting the laboratory engineering of SARS-CoV-2

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 12:43 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Shan,

I agree to delete those two parts. One was added by me, based on Linda's email, and another was also by me, based on Ralph's comments.

I do not seem to prefer using "current", but I get your point - perhaps we can use "convincing"? "Credible" is not good for the title.

Thoughts?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 12:30 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

See two attached documents:

1. Title of commentary: I agree that by removing "origin", it is better. I also wonder if we can add "current" in it?
2. A slightly revised draft of commentary: I removed certain sentences (with tracking) to make the commentary more focused. For your reference

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Sunday, February 16, 2020 12:22 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Yes, it can be removed from the title. Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 12:21 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Thanks Lishan. The word of “origin” may be removed?

Shan-Lu Liu sent from iPhone

> On Feb 16, 2020, at 12:15 PM, Su, Lishan <lishan_su@med.unc.edu> wrote:
>
> As discussed, see the final version with revised title and the last sentence. best,
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>
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> **From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Date:** Friday, February 14, 2020 at 7:07 PM
> **To:** "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
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> Agree. For this reason, I think the last sentence to be added will make this perfect point!
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From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Friday, February 14, 2020 at 7:02 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

I actually am very concerned for the possibility of SARS-2 infection by lab people. It is much more contagious than SARS-1. Now every lab is interested in get a vial of virus to do drug discovery. This can potentially a big issue. I don't think most people have a clue.

I actually was IBC chair at UMMS which is the only university which can do live SARS, and my lab did live SARS work. How to manage such things is very tricky. Not just PPE, but the whole design and logic.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Friday, February 14, 2020 6:46 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
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Yes, he was infected in the lab!

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
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To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
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We are on the same page. Our position on this bio safety ethic issue should be neutral. Your former colleague was infected with sars2 in the lab?

-Lishan
<Liu et al_EMI Commentary_15 references[1]-Final.docx>

From: Lu, Shan
To: Su, Lishan; Liu, Shan-Lu
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission
Date: Sunday, February 16, 2020 1:01:54 PM

Hi, Lishan

Your 2nd choice is very close to what I suggested, and I can go with the following:

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
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To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
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Either is fine, but first is preferred, short and clear.

SARS-CoV-2: no evidence of laboratory engineering
Or
No credible evidence supporting (claims of?) the laboratory engineering of SARS-CoV-2

-Lishan

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I am okay with the change for the title, but “claims” does not seem a good fit here –“evidence” is better I feel.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
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Thanks, then let's not having those two parts.

On the title, I do not need to use "current".

How about this:

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Shan,

I agree to delete those two parts. One was added by me, based on Linda's email, and another was also by me, based on Ralph's comments.

I do not seem to prefer using "current", but I get your point - perhaps we can use "convincing"? "Credible" is not good for the title.

Thoughts?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
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See two attached documents:

1. Title of commentary: I agree that by removing "origin", it is better. I also wonder if we can add "current" in it?
2. A slightly revised draft of commentary: I removed certain sentences (with tracking) to make the commentary more focused. For your reference

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-Lishan
<Liu et al_EMI Commentary_15 references[1]-Final.docx>

No ~~credible~~ evidence supporting claims of the laborary engineering of SARS-CoV-2~~: no evidence of a laboratory origin~~

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,
Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,
Ohio Agricultural Research and Development Center, CFAES
Department of Veterinary Preventive Medicine,
The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome~~; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino acid changes~~ [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted

pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to

use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that

could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. ~~Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.~~

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 ~~expert~~ virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]~~(Gao et al., EMI paper 2/12/2020 in press)~~. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Commented [LS1]: Emerging Microbes & Infections, 9:1, 378-381, DOI: 10.1080/22221751.2020.1727299

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated

viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, such a virus, and closely related, do pose great public health threats and must be handled properly in the laboratory and also properly regulated by governments and scientific community.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

From:	Su, Lishan
To:	Liu, Shan-Lu; Lu, Shan
Subject:	Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission
Date:	Sunday, February 16, 2020 2:14:58 PM
Attachments:	2019 CoV Copy-Converted.enl

No problem. Here is the updated endnote file, in endnote 9 format.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 1:40 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
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Sorry! I manually edited the reference 15 and just emailed out!

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 16, 2020 at 1:36 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

I have added the Gao reference and formatted (I have fixed my endnote problem!). Do we need credible here in the title?

No ~~credible~~ evidence supporting claims of laboratory engineering of SARS-CoV-2.

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 1:12 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
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Ok, let do the 2nd one.

See attached, with Gao ref added for you to put into Endnote

From: Liu, Shan-Lu <liu.6244@osu.edu>

Sent: Sunday, February 16, 2020 1:07 PM
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Second one reads better and is more accurate.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 1:04 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
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For shorter, it will be what I suggested, let's put the two below:

No credible claims supporting the laboratory engineering of SARS-CoV-2

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I think either of these two should be fine, the shorter the better for a title. Title does not need to be exclusive.

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Either is fine, but first is preferred, short and clear.

SARS-CoV-2: no evidence of laboratory engineering
Or
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On the title, I do not need to use "current".

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I actually was IBC chair at UMMS which is the only university which can do live SARS, and my lab did live SARS work. How to manage such things is very tricky. Not just PPE, but the whole design and logic.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Friday, February 14, 2020 6:46 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Yes, he was infected in the lab!

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 14, 2020 at 6:39 PM

To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

We are on the same page. Our position on this bio safety ethic issue should be neutral. Your former colleague was infected with sars2 in the lab?

-Lishan
<Liu et al_EMI Commentary_15 references[1]-Final.docx>

From: Lu, Shan
To: Su, Lishan; Liu, Shan-Lu
Subject: RE: EMI commentary
Date: Wednesday, February 12, 2020 7:25:14 PM
Attachments: image002.png
Liu et al_EMI Commentary_for submission_-0212B.docx

Sorry for slow reply as I have been super busy.

1. I don't have an opinion on who should be the first and who should be the last author. I think either one is fine. One technical consideration is that the last author may need to be the one to do online submission to EMI. At the stage of submission, only one corresponding author is allowed, but you can add back another corresponding author at the stage of Galley.
2. I definitely will not be an author as you guys did everything. It can also keep things somewhat independent as the editor. However (not in contrast), I appreciate your kind offer!
3. At this point, the draft is very good. I made two minor changes and inserted one question (see attached). Either way I am find so you can finalize.
4. No abstract for EMI commentary. Acknowledgement should be fine. If it may take more time to get everyone's grants etc. it is better not listing grants, but only thank people who had input. I am ok if you want to include me for "providing valuable discussion and reading" if you like, and other big name CoV people that SLL had contacted if justified.

Please feel free to move to submission at any time, and let me know if you need any help from EMI office.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Wednesday, February 12, 2020 7:03 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Either is fine with me too. Let's Shan the editor decide:)

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Wednesday, February 12, 2020 6:29:08 PM
To: Su, Lishan <lishan_su@med.unc.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Hi Lishan:

See both versions attached, either way works for me. It's your call.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 6:26 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

It is probably fine if we cover not only the unc chimeric virus now.

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Wednesday, February 12, 2020 6:09:46 PM
To: Su, Lishan <lishan_su@med.unc.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Lishan:

Now I understand your point of concern. I should be fine either way, as OSU should not care.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 5:55 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Current we are both senior and corresponding authors. I can be either. I am not sure the UNC affiliation should be listed first or not… let's think about this.

I agree Shan Lu should be a corresponding author too.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 5:51 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>

Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Hi Shan,

Sure, no problem. I think you deserve senior and corresponding authorship.

Shan did not respond today…

Best.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
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Co-Director, Viruses and Emerging Pathogens Program
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Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 5:47 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Shan-Lu:

Should we switch authorship order, with you first, me last?
I like the idea of adding more from our virology group, if Shan Lu/EMI can wait for the signing delay.

It looks great. I hope it will help to clarify some of the confusions.

Did Feng Gao address the “shuttle vector” sequence claim in his ms? It is very similar to the HIV insertion problem with such short alignments.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 5:12 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: EMI commentary

Hi Shan,

Attached please find the final version of the commentary for your consideration to be published at EMI.

Kindly advise.

Regards.

Shan-Lu

SARS-CoV-2: no evidence of a laboratory origin

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in ~~Chinese~~ social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated ~~to~~ so it can not replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

Commented [LS1]: Is this section really needed here?

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From:	Lu, Shan
To:	Liu, Shan-Lu; Su, Lishan
Subject:	RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission
Date:	Sunday, February 16, 2020 12:56:04 PM

Evidence is too big and vague. Claims are those out there and our commentary addressed these specific claims. So we can defend.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 12:53 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

I am okay with the change for the title, but "claims" does not seem a good fit here –"evidence" is better I feel.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 12:51 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Thanks, then let's not having those two parts.

On the title, I do not need to use "current".

How about this:

No credible claims supporting the laboratory engineering of SARS-CoV-2

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 12:43 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Shan,

I agree to delete those two parts. One was added by me, based on Linda's email, and another was also by me, based on Ralph's comments.

I do not seem to prefer using “current”, but I get your point - perhaps we can use “convincing”? “Credible” is not good for the title.

Thoughts?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 12:30 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

See two attached documents:

1. Title of commentary: I agree that by removing “origin”, it is better. I also wonder if we can add “current” in it?
2. A slightly revised draft of commentary: I removed certain sentences (with tracking) to make the commentary more focused. For your reference

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Sunday, February 16, 2020 12:22 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Yes, it can be removed from the title. Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 12:21 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Thanks Lishan. The word of “origin” may be removed?

Shan-Lu Liu sent from iPhone

On Feb 16, 2020, at 12:15 PM, Su, Lishan <lishan_su@med.unc.edu> wrote:

As discussed, see the final version with revised title and the last sentence. best,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 14, 2020 at 7:07 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Agree. For this reason, I think the last sentence to be added will make this perfect point!

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Friday, February 14, 2020 at 7:02 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

I actually am very concerned for the possibility of SARS-2 infection by lab people. It is much more contagious than SARS-1. Now every lab is interested in get a vial of virus to do drug discovery. This can potentially a big issue. I don't think most people have a clue.

I actually was IBC chair at UMMS which is the only university which can do live SARS, and my lab did live SARS work. How to manage such things is very tricky. Not just PPE, but the whole design and logic.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Friday, February 14, 2020 6:46 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to

your submission

Yes, he was infected in the lab!

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 14, 2020 at 6:39 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

We are on the same page. Our position on this bio safety ethic issue should be neutral. Your former colleague was infected with sars2 in the lab?

-Lishan
<Liu et al_EMI Commentary_15 references[1]-Final.docx>

From:	Su, Lishan
To:	Liu, Shan-Lu; Lu, Shan
Subject:	Re: 2019-nCoV-EMI_commentary
Date:	Wednesday, February 12, 2020 12:58:49 AM
Attachments:	image001.png SHC014-MA15 v 2019 ncoV-SLL-sls-SLL-ref.docx

My endnote is not working with the word, even after loading the X9 verstion. I have put the references in the text. Could either of you format it with your endnote? Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 7:44 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Sounds good, thank you. I still like “however” over “In contrast” – it just reads better

Shan: Are you sure that you prefer not to be included in the coauthorship? Before I send, I think we should have the authorship listed, along with affiliations. Lishan should be the first author, unless he prefers otherwise. Agreed?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 7:34 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

I made some minor change for the following:

In summary, there is no credible evidence at this point to support the claims that the 2019-nCoV was originated from a laboratory-engineered CoV. In contrast, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV.

Maybe now SLL can send the next version to other CoV experts?

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 5:47 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See the new version with all incorporated.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 4:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I have made additional changes to the Lishan's version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 4:16 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 3:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Perhaps Lishan can take a look at the latest version, which has the new title I suggested, and modify it as needed.
The last paragraph is also crucial, but I did not have time to work on it because of a meeting this morning.
Once we have almost a final draft, I will contact Linda Saif, Stanley Perlman, Thomas Gallgaher etc. to see if they are willing to join, but this may delay the publishing time.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 1:52 PM

To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I agree that it should be simple and clear. I have included some details in the 1st draft for your information. There is not intention to defend Baric, but to clarify the facts.

Regarding all three, are you combining Goa Feng's piece with this one? For the RaTG13, it involves complicated viral evolution kinetics and maybe hard to simply clarify…
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 1:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM

To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Tentative Title: Is 2019-nCoV laboratory origin?

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease discovered in 2019 (COVID-19).

According to what has been reported (Wang, D. *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020), Chen, N. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020). Chang *et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The 2019-nCoV genome sequence also has ~80% identity with SARS-CoV, but is most similar to some bat beta-coronaviruses, with the highest being >96% identity (Zhu, N. *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020); Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020)).

Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the 2019-nCoV (Zhou, P. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature (2020)). However, as we now know, the SARS-

CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt. Given that there are greater than 1000 nt differences between 2019-nCoV and RaTG13 (Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).), it is highly unlikely RaTG13 is the immediate source of 2019-nCoV; this is particularly true in light of the low mutation rate of the coronaviruses. An intermediate host between bats and humans is likely involved.

Another claim points to a Nature Medicine paper published in 2015 (Menachery, V.D. et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med 21, 1508-1513 (2015), which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014, Ge, X.Y. et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503, 535-538 (2013)) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells. However, this claim lacks any scientific basis and must be discounted.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passages of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry (Li, W. et al.

Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426, 450-454 (2003);Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science 309, 1864-1868 (2005)). Civets were proposed to be an intermediate host of the bat-CoVs before they spread to humans. However, several novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SARS-like (SL)-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry (Ge, X.Y. et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503, 535-538 (2013)). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interaction with SARS CoV (Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol 86, 6350-6353 (2012)), it was proposed that intermediate hosts may not be necessary and that some bat SL-CoVs may directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus can indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis (Nat Med 21, 1508-1513 (2015)).

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014-MA15 chimeric virus were considered as gain of function (GOF) studies and briefly paused by the US government. The NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potentials.

Regardless, upon careful phylogenetic analyses by multiple international groups (Wu, A. et al. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host Microbe (2020); Emerg Microbes Infect 9, 313-319 (2020); Zhu, N. et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med (2020)), the 2019-nCoV is unmistakably distinct from SARS-like viruses including SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, there is NO credible evidence to support the claim that the 2019-nCoV is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the 2019-nCoV is artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, claiming that 2019-nCoV has HIV sequence in it and thus likely generated in the laboratory. A rebuttal paper led by HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the 2019-nCoV is not HIV-1 specific but random (EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have recently decided to withdraw this report.

In summary, we believe that there is no concrete evidence to support the claims that the 2019-nCoV was originated from a laboratory-engineered CoV. However, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in humans or an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV.

From: Lu, Shan
To: temi-peerreview@journals.tandf.co.uk; TEMI-production@journals.tandf.co.uk
Cc: lshan_su@med.unc.edu; Editorial Office; Liu, Shan-Lu
Subject: RE: Urgent: revised commentary for Emerging Microbes & Infections - TEMI-2020-0121
Date: Sunday, February 16, 2020 8:13:40 PM
Attachments: image002.png
Liu et al_EMI Commentary_Revision_Final.docx

Dear Jorgie,

I believe the accepted version of TEMI-2020-0121 is already being sent to production, right? If not, then replace it with the one attached (just sent from Dr Shan-Lu Liu (not me) If it has been sent to production, then I will write below to Malathi

Dear Malathi,

If this paper is already in production, please see if you can replace the original version with the attached version If it is too late, the authors can make the change at the galley proofs step Just to make sure that this paper should be treated as very urgent (the fast track)

Thanks

Shan

From: Liu, Shan-Lu <liu 6244@osu edu>
Sent: Sunday, February 16, 2020 8 00 PM
To: temi-peerreview@journals tandf co uk
Cc: Lu, Shan <Shan Lu@umassmed edu>; lishan_su@med unc edu
Subject: Re: Urgent: revised commentary for Emerging Microbes & Infections - TEMI-2020-0121

Here is the attachment, sorry!

_____

Dear Jorgie,

After discussing with Dr. Shan Lu and all coauthors, we have decided to use a new title and also make minor changes to the text, including asscіated references. I have attached the updated commentary and hope that you will be able to help upload the new version for preparing the proof.

Thank you!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M D , Ph D
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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From: "temi-peerreview@journals.tandf.co.uk" <temi-peerreview@journals.tandf.co.uk>
Date: Thursday, February 13, 2020 at 9:14 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "shan lu@umassmed edu" <shan lu@umassmed edu>, "lishan_su@med unc edu" <lishan_su@med unc edu>
Subject: Re: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission #TrackingId:5633996

Dear Professor Liu,

Thank you very much for sending his file.

Kindly be informed that I have now uploaded in he system on your behalf and proceeded your paper to the editor.

Please let me know if you have further questions or concerns.

Kind regards,

Jorgie Lyn Luna - Journal Editorial Office
Taylor & Francis Group
4 Park Square | Milton Park | Abingdon | Oxon | OX14 4RN UK
Web: www.tandfonline.com

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Emerging Microbes & Infections

From:liu.6244@osu.edu
Sent:
To:liu.6244@osu.edu

Cc: Shan.Lu@umassmed.edu,lishan_su@med.unc.edu
Subject: Re: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission

Hi Jorgie:

I have modified as instructed and attached the new one to this email. Please help upload and proceed.

Thank you.

Shan-Lu

Shan-Lu Liu, M D., Ph D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
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On 2/13/20, 8:43 AM, "Emerging Microbes and Infections" <onbehalfof@manuscriptcentral.com> wrote:

13-Feb-2020

Dear Professor Liu,

Your above referenced manuscript, entitled "SARS-CoV-2: no evidence of a laboratory origin" requires some further changes before it is ready for reviewing in Emerging Microbes & Infections. Your submission has been returned to you and is located in your Author Center as a draft, so that you due to these reasons:

1. No line numbering

Kindly add a line numbering in your main document.

2. Exceeded reference count

Kindly be informed that the reference count for the commentary article should not be more than 15.

Your submission along with all files you submitted is now in your Author Center, at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__!!KGKeukY!nGv1RgRJ1P-OGXuZi8b2hKGjXxDFOmBwDONuR_njCdwERJF1HkBIV4Sggqr9udyWYml$ Please read the Quick Guide to Continuing your Submission, which shows how you can access your manuscript, and submit it back to the site. The Guide is located at https://urldefense.com/v3/__http://mc.manuscriptcentral.com/societyimages/tandf_qs0/Continuning*20a*20Submission_screenshot.pdf__JSU!KGKeukY!nGv1RgRJ1P-OGXuZi8b2hKGjXxDFOmBwDONuR_njCdwERJF1HkBIV4Sggqr9re6Z8tA$

You may contact the Editorial Office if you have further questions.

Sincerely,

Jorgie Lyn Luna
Emerging Microbes & Infections Editorial Office
temi-peerreview@journals.tandf.co.uk

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,
Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,
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Department of Veterinary Preventive Medicine,
The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural

evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed

to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already

exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses

with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

From: Su, Lishan
To: Liu, Shan-Lu; Lu, Shan
Subject: Re: 2019-nCoV-EMI_commentary
Date: Tuesday, February 11, 2020 5:49:01 PM
Attachments: image001.png
SHC014-MA15 v 2019 ncoV-SLL-sls.docx

See the new version with all incorporated.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 4:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I have made additional changes to the Lishan's version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 4:16 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 3:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Perhaps Lishan can take a look at the latest version, which has the new title I suggested, and modify it as needed.
The last paragraph is also crucial, but I did not have time to work on it because of a meeting this morning.
Once we have almost a final draft, I will contact Linda Saif, Stanley Perlman, Thomas Gallgaher etc. to see if they are willing to join, but this may delay the publishing time.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 1:52 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I agree that it should be simple and clear. I have included some details in the 1st draft for your information. There is not intention to defend Baric, but to clarify the facts.

Regarding all three, are you combining Goa Feng's piece with this one? For the RaTG13, it involves complicated viral evolution kinetics and maybe hard to simply clarify…
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 1:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor

Co-Director, Viruses and Emerging Pathogens Program
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Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Tentative Title: Is 2019-nCoV laboratory origin ~~of laboratory~~?

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease identified 2019 (COVID-19).

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The 2019-nCoV genome sequence also has ~80% identity with SARS-CoV, but most similar to some bat beta-coronaviruses, with the highest being >96% identity.

Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan as a bat CoV (RaTG13) was recently reported by that laboratory and it shared ~96% homology with the 2019-nCoV (Nature, 2020). However, as we now know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt. Given that there are greater than 1000 nt differences between 2019-nCoV and RaTG13, it is highly unlikely RaTG13 is the immediate source of 2019-nCoV; this is particular true in light of the low mutation rate of the coronaviruses. Searching for an immediate host between bat and humans is needed.

Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene

(SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells. However, this claim lacks any scientific basis and must be discounted.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passages of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry. Civets were proposed to be an intermediate host of the bat-CoVs before they spread to humans (SARS-CoV review?). However, several novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interaction with SARS CoV (JVI 2012), it was proposed that intermediate hosts may not be necessary and some bat SL-CoVs may directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus can efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV.

Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis (Nat. Med. 2015).

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause. ~~No more bat CoV-MA15 chimeric viruses are constructed after the SHC014 MA15 chimeric virus.~~ The NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potential. Regarding its lineage relationship with 2019 nCoV, however, after careful phylogenetic analyses by multiple international groups (EMI, Nature…2020), the 2019 nCoV is unmistakably distinct from SHC014- MA15 with >5000 nt differences in their genomes. There is NO credible evidence to support the claim that the 2019 nCoV was derived from the chimeric SHC014-MA15 virus.

There are also rumors that the 2019-nCoV is artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, claiming that 2019-nCoV has HIV sequence in it and thus likely generated in the laboratory. A rebuttal paper led by HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the 2019-nCoV is not HIV-1 specific but random (EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have recently decided to withdraw this report.

In summary, there is no evidence to support the claims that the 2019 nCoV was originated from a laboratory engineered CoV. Phylogenetic analyses of all reported CoV genomes by multiple international groups support the conclusion that 2019 nCoV is a novel virus……?

From: Su, Lishan
To: Liu, Shan-Lu
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)
Date: Friday, February 21, 2020 5:35:47 PM
Attachments: image001.png
TEMI_A_1733440 Proof-Su.pdf

Done, and waiting to be submitted after hearing from Linda.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 3:07 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Lishan,

Do you have the corrected proof? Thanks for doing this. I am almost done with the meeting.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 11:52 AM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I agree with you on these points, but NIH/government at the time put it as a gof study relative to the original S antigen…

-Lishan

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 2:51 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Is the adaptation of MA15 to mice considered “gain of function”- that selected virus is more virulent than SHC014-MA15 chimeric virus? Seems to me like more loss of function relative to MA15 when inserting the bat derived spike. MA15 with the urbani spike is like de- adapting the virus to mice.

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 1:40 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

I have noticed that too, probably happened when we tried to simplify the chimeric virus paragraph, and I think Ralph had added the attenuation sentence relative to M15 in mice…

What was reported in the NM paper was that the SHC014-rMA15 chimeric virus was less pathogenic than M15, but more so than the chimeric M15 virus with the original Urbani Spike-gene in M15, probably due to one of the 6 mutations in the M15 S gene.

See old sentence:
Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the original human Urbani S-MA15 chimeric virus in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies…

I will try to fix this.
Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 12:14 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Lishan: see below comments from Susan.

Susan: thank you. I had the same question before – Lishan, could you explain this?

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Friday, February 21, 2020 at 9:06 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: Your article proofs for review (ID# TEMI 1733440)

Please list me as Susan R Weiss (with the “R”). there are too many other Susan Weiss’

I noticed what looks like a contradictory statement in the paper- sorry I missed it before- I highlighted in yellow lines 124-133. The first part says chimeric virus is attenuated producing less antigen than MA15 but the next part says it has elevated activity- this seems contradictory

I remain concerned about the insertion of the furin site

Susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 10:05 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: [External] Re: Your article proofs for review (ID# TEMI 1733440)

We agreed to add this link to the proof to the third paragraph regarding RaTG13.

The Proximal Origin of SARS-CoV-2
http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

THE OHIO STATE UNIVERSITY

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Shan-Lu Liu <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 4:46 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: FW: Your article proofs for review (ID# TEMI 1733440)

All,

See message below and also the attached proof.

Please mark your changes in the attached PDF file, and Lishan and I will incorporate to finalize.

Thanks.

Shan-Lu

From: "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Friday, February 21, 2020 at 4:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear Shan-Lu Liu,

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• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link:
https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

TEMI1733440 VOL 0, ISS 0

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu, Linda J. Saif, Susan Weiss, and Lishan Su

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Emerging Microbes & Infections
https://doi.org/10.1080/22221751.2020.1733440

COMMENTARY

OPEN ACCESS Check for updates

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Q1 Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan Weiss [f] and Lishan Su[g]

[a]Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; [b]Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; [c]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA; [d]Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA; [e]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA; [f]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [g]Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

ARTICLE HISTORY Received 13 February 2020; Accepted 13 February 2020

PROOF ONLY

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe

CONTACT Shan Lu Liu lsu@med.unc.edu; Lishan Su Liu.6244@osu.edu

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bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

Disclosure statement

No potential conflict of interest was reported by the author(s). Q2

ORCID

Susan Weiss http://orcid.org/0000 0002 8155 4528

References Q3

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. Q4

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. Q5

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. Q6

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. Q7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. Q8

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin converting enzyme 2 is a functional receptor for the

PROOF ONLY

SARS coronavirus. Nature. 2003 Nov 27;426 (6965):450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3.
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

Q9

PROOF ONLY

From: Yost, Mary
To: Liu, Shan-Lu
Subject: Re: Final version of the letter: "COVID-19 and The Virus That Causes It" - OSU
Date: Wednesday, March 25, 2020 1:37:17 PM
Attachments: image001.png
image002.png
image003.png

Shan-Lu,

We are planning to run this in Thursday's paper. Thanks for working with us on it.

Mary

Mary Yost
Editorial Page Editor
Columbus Dispatch
62 E. Broad St.
Columbus, OH 43215
614-461-5040 (office)
614-204-6798 (cell)
myost@dispatch.com

On Tue, Mar 24, 2020 at 12:19 AM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Dear Mary,
>
> I have modified the letter by following your instructions. First, I changed the author number to one. Second, I shortened the letter and now its length is ~700 words. Third, I revised the letter by removing “facts” but adding more opinions.
>
> I hope the letter is now acceptable for publication in Columbus Dispatch. Kindly note that the disclaimer in the end is important so please make sure to keep it.
>
> Thank you so much for your help with this effort.
>
> Shan-Lu
>
> THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.

Professor

Co-Director, Viruses and Emerging Pathogens Program

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Yost, Mary" <myost@dispatch.com>
Date: Monday, March 23, 2020 at 7:38 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Encarnacion Pyle <epyle@dispatch.com>
Subject: Re: Greetings and inquiry: COIVD-19 commentary

Thank you, but I am not sure it would be suitable for our opinion pages. I encourage you to work with our news side, since it sounds like you are wanting to convey facts, not commentary.

And no, we would not run it with three authors. In cases where multiple individuals want to be credited, we have advised that the others be noted in the body of the article, but that also takes space away from the content you want to present.

We do a weekly review of pending op-eds on Friday afternoons and can let you know after our review if we will publish your submission. The news side could probably share your

information sooner than we can on our opinion pages, even if we are able to publish it.

Mary

Mary Yost

Editorial Page Editor

Columbus Dispatch

62 E. Broad St.

Columbus, OH 43215

614-461-5040 (office)

614-204-6798 (cell)

myost@dispatch.com

On Mon, Mar 23, 2020 at 5:13 PM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Hi Mary,
>
> Thank you for your consideration.
>
> Over the last few weeks, I kept receiving requests from people, including local fire departments regarding how this virus is spread and causes the disease, etc. This really motivated me to write something with some updated information that I thought would be helpful to our readers.
>
> Yes, we can cut down to 700 words, with no problem, but I would still prefer to have three authors, because all are co-directors of the OSU program and we have contributed equally.

Thank you so much, and let me know how to proceed.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.

Professor

Co-Director, Viruses and Emerging Pathogens Program

Infectious Diseases Institute

Center for Retrovirus Research

Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology

The Ohio State University

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Columbus, Ohio 43210

Phone: (614) 292-8690

Fax: (614) 292-6473

Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Yost, Mary" <myost@dispatch.com>
Date: Monday, March 23, 2020 at 4:56 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Encarnacion Pyle <epyle@dispatch.com>
Subject: Re: Greetings and inquiry: COIVD-19 commentary

Hi Shan-Lu,

Thank you for offering to send us an op-ed, but it might be better if you could share your expertise with our news side.

As you can imagine, we continue to receive a lot of guest columns around the topic of coronavirus and its impact on all facets of life today. One of the challenges we have with the opinion pages is limited space, just two pages each day, without a lot of flexibility in how we fill our space.

It sounds like the kind of information you have to share is more factual than opinion, which might be better suited for news coverage that doesn't have the space restrictions we do.

A couple of other concerns -- we typically don't run guest columns from more than one author; and our usual length is about 700 words. We made an exception for a guest column that will appear in Tuesday's paper, but that is very rare. I don't know if 700 words would be enough to cover all that you have to share.

I am copying one of our metro editors, Encartia Pyle, in case you would be interested in following up with a news reporter to share your insights.

Thank you for thinking of The Dispatch; and thank you for what you are doing related to the coronavirus.

Mary

Mary Yost

Editorial Page Editor

Columbus Dispatch

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Columbus, OH 43215

614-461-5040 (office)

614-204-6798 (cell)

myost@dispatch.com

On Sat, Mar 21, 2020 at 9:12 PM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Dear Alan,
>
> Greetings! Hope this email finds you well.
>
> I am not sure if you are the right person to contact, but please forgive me and help make the connection to the Dispatch.
>
> In 2016 when I joined OSU, Emily Tate wrote a story on me about the Zika virus, see attached article. Now COIVD-19 is here, and as co-director of the OSU Viruses and Emerging Pathogens program, my colleagues Linda Saif, Jacob Yount and I have written a commentary on COIVD-19, which we wish to publish in the Dispatch as commentary or other forms. Our focus is on the virus, SARS-CoV-2, which causes the outbreak and the disease COIVD-19.
>
> The motivation is that I recently have received a lot of requests from local media and even fire department for interview, and I thought that this commentary may be able to address some of the reader's questions.
>
> See below some of my writings published in journals:
>
> https://www.nature.com/articles/d41586-020-00135-z
>
> **New virus in China requires international control effort**
>
> **Emerging Viruses without Borders: The Wuhan Coronavirus**

https://www.mdpi.com/1999-4915/12/2/130

https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1733440

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

SARS-CoV-2 is an appropriate name for the new coronavirus

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30557-2/fulltext

Thank you for your consideration. If your newspaper is interested, please let me know and I will send the article to you shortly.

Sincerely,

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.

Professor

Co-Director, Viruses and Emerging Pathogens Program

Infectious Diseases Institute

Center for Retrovirus Research

Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology

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Phone: (614) 292-8690

Fax: (614) 292-6473

Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

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From: Savo R
To: Liu, Shan-Lu; lsu@med.unc.edu
Subject: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
Date: Friday, April 10, 2020 1:41:59 AM

Hello,

What you forget to mention in your review is that four leading scientists have shown both HIV mutations in the genome. Being 100-1000 more infectious then SARS makes no sense on evolution mutations.

And also confirming studies showing HIV drugs are helping in the recovery from COVID-19.

Bob

From: Taylor & Francis
To: Liu, Shan-Lu
Subject: Taylor & Francis author update: access to your article published in an issue of Emerging Microbes & Infections
Date: Thursday, February 27, 2020 12:45:34 AM

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Author Services | **FAQ** | **Twitter** | **Facebook**

Dear author,

Your Open Access article, No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2, published in Emerging Microbes & Infections, Volume 9 Issue 1, is now available to access via tandfonline.com.

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You'll hopefully want to share your article with friends or colleagues (and

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Not sure how to access your Authored Works?
If you haven't yet registered, you can do so using liu.6244@osu.edu (this is the email you used whilst your manuscript was going through production).

Once you've completed the quick registration you'll be sent an email asking you to confirm. Click on the verification link and you can then login (using the above email address) whenever you want to by going to **Taylor & Francis Online**. Once you have logged in, click on "**Your Account**" at the top of the page to see the latest updates on your article.

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From: Lu, Shan
To: Su, Lishan; Liu, Shan-Lu
Subject: RE: Revised commentary for EMI - final!
Date: Monday, February 17, 2020 6:08:08 PM

I think each paper has its own focus, like now is very good. Our commentary is directly addressing two particular claims and it did well.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Monday, February 17, 2020 6:05 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Revised commentary for EMI - final!

I agree with them completely. Based on Shi's two natures papers and the Baric Nature medicine paper, I was trying to make the point as this paper: that the new virus from bats could have jumped into a secondary host or directly to humans and evolve. One of you did not seem to like the direct human possibility and removed it.

Theories of SARS-CoV-2 origins

It is improbable that SARS-CoV-2 emerged through laboratory manipulation of an existing SARS-related coronavirus. As noted above, the RBD of SARS-CoV-2 is optimized for human ACE2 receptor binding with an efficient binding solution different to that which would have been predicted. Further, if genetic manipulation had been performed, one would expect that one of the several reverse genetic systems available for betacoronaviruses would have been used. However, this is not the case as the genetic data shows that SARS-CoV-2 is not derived from any previously used virus backbone[17]. Instead, we propose two scenarios that can plausibly explain the origin of SARS-CoV-2: (i) natural selection in a non-human animal host prior to zoonotic transfer, and (ii) natural selection in humans following zoonotic transfer.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Monday, February 17, 2020 at 5:56 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

This is the website that people deposit their sequence data and also make relevant comments. Not sure where they will publish it… but it has been widely spread via Twitter.

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Monday, February 17, 2020 at 5:44 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>

Subject: RE: Revised commentary for EMI - final!

Who is first is not critical. But where did you find this new paper? Published?

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 17, 2020 5:42 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

Again, I have no concern at all with our conclusion in the commentary. I believe more scientific articles like this will be out, and EMI will be one of the first to publish them.

Cheers!

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Monday, February 17, 2020 at 5:36 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Revised commentary for EMI - final!

Agreed. Beautifully written.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 17, 2020 5:35 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

I just carefully read through, very informative and convincing in my view. Those are of course true experts of evolutionary biologists.

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Monday, February 17, 2020 at 5:27 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Revised commentary for EMI - final!

This still has nothing to do with any of the specific claims.

From: Liu, Shan-Lu <liu.6244@osu.edu>

Sent: Monday, February 17, 2020 5:26 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

The last section is to dispute those rumors.

Theories of SARS-CoV-2 origins

It is improbable that SARS-CoV-2 emerged through laboratory manipulation of an existing SARS-related coronavirus. As noted above, the RBD of SARS-CoV-2 is optimized for human ACE2 receptor binding with an efficient binding solution different to that which would have been predicted. Further, if genetic manipulation had been performed, one would expect that one of the several reverse genetic systems available for betacoronaviruses would have been used. However, this is not the case as the genetic data shows that SARS-CoV-2 is not derived from any previously used virus backbone[17]. Instead, we propose two scenarios that can plausibly explain the origin of SARS-CoV-2: (i) natural selection in a non-human animal host prior to zoonotic transfer, and (ii) natural selection in humans following zoonotic transfer. We also discuss whether selection during passage in culture could have given rise to the same observed features.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Monday, February 17, 2020 at 5:23 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Revised commentary for EMI - final!

Two different things. They are doing SARS2 genome analysis. Your is trying to disapprove the other theories.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 17, 2020 5:17 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Revised commentary for EMI - final!

…SARS-CoV-2 is the seventh member of the *Coronaviridae* known to infect humans. Three of these viruses, SARS CoV-1, MERS, and SARS-CoV-2, can cause severe disease; four, HKU1, NL63, OC43 and 229E, are associated with mild respiratory symptoms. Herein, we review what can be deduced about the origin and early evolution of SARS-CoV-2 from the comparative analysis of available genome sequence data. In particular, we offer a perspective on the notable features in the SARS-CoV-2 genome and discuss scenarios by which these features could have arisen. Importantly, this analysis provides evidence that SARS-CoV-2 is not a laboratory construct nor a purposefully manipulated virus.
…

We need to try to get ours out quickly.

SL

From: Lu, Shan
To: Liu, Shan-Lu; Su, Lishan
Subject: RE: 2019-nCoV-EMI_commentary
Date: Tuesday, February 11, 2020 12:46:02 PM
Attachments: image002.png
EMI commentary-20200211_c.docx

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

See my suggested changes.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Title:

Is 2019-nCoV a laboratory origin?

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. xx, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP).

According to what has been reported in the literature (refs), clinical manifestations of NCP resemble that of severe acute respiratory syndrome (SARS) caused by SARS-CoV. However, the 2019-nCoV genome has only ~80% identity in sequence with SARS-CoV, indicating a quite different beta-coronavirus.

This led to speculations and rumors that the 2019-CoV is of a laboratory origin. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan as a bat CoV (RaTG13) was recently reported by that laboratory and it shared ~96% homology with the 2019-nCoV (Nature, 2020). However, as we now know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt. On the other hand, there are greater than 1000 nt differences between 2019-nCoV and RaTG13, suggesting RaTG13 is not the immediate source of 2019-nCoV given the large size genome like beta-coronaviruses (~30 kb) and the slow the mutation rate of the coronaviruses. Searching for an immediate host between bat and humans is needed.

Second, we provide a summary of evidence that supports the conclusion that the 2019-nCoV is not from the chimeric coronavirus (SHC014-rMA15), nor the original bat virus RaTG13 (refs). One particular claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS-CoV that has adapted to infect mice (rMA15) and is capable of infecting humans.

Let us first explain how a recombinant mouse-adapted SARS virus (rMA15) was generated. After constructing a full-length infectious SARS-CoV using reverse genetics, Dr. Ralph Baric's lab showed that it replicated in older mice, with low or no pathogenicity They then adapted the SARS-CoV (Urbani strain) by serial passages in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. When introduced into the original recombinant SARS-CoV, these six mutations (only one in the S gene) conferred the high virulence and lethality (rMA15). Although not reported in human cells, it is likely that rMA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

It is also important to know how the chimeric SHC014-rMA15 virus was constructed and what key findings were made using this virus. When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike its human or civet counterparts, was unable to use the human ACE2 as a receptor for entry. Civets were proposed to be an immediate host before the bat-CoV spreads to humans. However, novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SL-CoV-WIV1 used ACE2 from humans, civets and Chinese horseshoe bats for entry. Based on the evolutionary evidence that the bat ACE2 gene has been positively selected at the same interface as the human ACE2 gene for interacting with SARS-CoV, it was proposed that an intermediate host may not be

necessary for some bat CoVs to directly infect humans. To directly address this possibility, the S gene of the bat coronavirus WIV1-SHC014 was used to generate a chimeric virus in the mouse adapted rMA15 SARS-CoV backbone. The resultant SHC014-rMA15 virus can efficiently use ACE2 from multiple species and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-rMA15 can replicate efficiently in the mouse lung with severe pathogenesis. These findings provide compelling evidence that some bat CoVs can directly use human ACE2 to infect human hosts.

Due to the elevated pathogenic activity of the SHC014-rMA15 chimeric virus relative to the Urbani Spike-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause (the ban was implemented in 2013/2014 but lifted by NIH in 2017). No more bat-CoV-M15 chimeric viruses have been constructed thereafter the SHC014-rMA15 chimeric virus. The NCP epidemic has triggered a new debate on whether or not it is worth the risks of constructing such viruses with possible pandemic potential (refs), which is not unexpected. However, with careful and in-depth phylogenetic analyses by multiple international groups, the 2019-nCoV is unmistakably, and fortunately, distinct from SHC014- MA15. (we need a good summary here, one, two, three…). Therefore, there is NO credible evidence to support the claim that the 2019 ncoV/NCP virus was derived for the chimeric SHC014- MA15 virus.

Commented [LS1]: necessary?

There are also rumors that the 2019-nCoV is artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, claiming that 2019-nCoV has HIV sequence in it and thus likely generated in the laboratory. A rebuttal paper led by HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertion into the 2019-nCoV is not HIV-1 specific but random. In addition, the four inserts cannot be held together based on structure modeling as initially claimed (EMI paper 2/12/2020). At the same time, the authors who made the initial claim has withdrawn their report.

From:	vinu arumugham
To:	tiziano.dallavilla@assomagi.org; pietro.chiurazzi@unicatt.it; tommaso.beccari@unipg.it; elisabetta.albi@unipg.it; lucilla.parnetti@unipg.it; silvia.paciotti@unipg.it; stefano.paolacci@assomagi.org; zhng@umich.edu; phao@ips.ac.cn; zhongwu@bmi.ac.cn; lsu@med.unc.edu; Liu, Shan-Lu; kristian@andersen-lab.com; trevor@bedford.io; wil2001@columbia.edu; stanley-perlman@uiowa.edu; jwleduc@utmb.edu; alr2105@columbia.edu; dirk.pfeiffer@cityu.edu.hk
Subject:	Your wrong analysis leads to the wrong conclusion of SARS-CoV-2 origin
Date:	Saturday, May 16, 2020 6:55:41 PM

All,

Regarding the articles:

Bioinformatic analysis indicates that SARS-CoV-2 is unrelated to known artificial coronaviruses.

www.ncbi.nlm.nih.gov/pubmed/32373995

and

Protein Structure and Sequence Reanalysis of 2019-nCoV Genome Refutes Snakes as Its Intermediate Host and the Unique Similarity between Its Spike Protein Insertions and HIV-1

https://pubs.acs.org/doi/10.1021/acs.jproteome.0c00129

You are investigating the wrong problem.

You missed two fundamental facts:

1. The Wuhan lab was transfecting HEK cells with HIV derived plasmids during SLCoV experiments. THIS CHANGES EVERYTHING.

2. You are ASSUMING that RaTG13 is a wild virus. It was "isolated" in 2013 but only sequenced AFTER the COVID-19 outbreak. Why?

You should smell a rat in the RaTG13.

The HIV-1 inserts in SARS-CoV-2 came from HIV derived plasmids. UNINTENTIONAL infection (due to contamination) of HEK cells with SLCoV, resulted in recombination with HIV-1 to produce SARS-Cov-2. All this happened in a BSL2 lab because they were supposed to be pseudovirus experiments. No bioweapon. No gene jockey needed. No GOF needed. Just plain HUMAN STUPIDITY explains everything.

Root cause of COVID-19? Biotechnology's dirty secret: Contamination. Bioinformatics evidence demonstrates that SARS-CoV-2 was created in a laboratory, unlikely to be a bioweapon but most likely a result of sloppy experiments
https://doi.org/10.5281/zenodo.3766462

See Prof. Petrovsky's description below and replace "random mutation" with "HIV-1 recombinations in HEK":

www.scimex.org/newsfeed/expert-reaction-did-covid-19-come-from-a-lab-in-wuhan

https://twitter.com/ArumughamVinu/status/1259208074444734464?s=20

No "intermediate host" was needed because the virus grown in HEK cells was ready for human infection, right out of the lab.

We need to SHUT ALL YOUR LABS DOWN, before you WIPE OUT HUMANITY WITH SUCH STUPIDITY.

Thanks,

Vinu

From: Lu, Shan
To: Su, Lishan; Liu, Shan-Lu
Subject: RE: 2019-nCoV-EMI_commentary
Date: Tuesday, February 11, 2020 1:46:23 PM
Attachments: image001.png
SHC014-MA15 v 2019 ncoVa.docx

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Tentative Title: The mouse adapted SARS chimeric virus with bat-coV S gene (SHC014-MA15) is not related to the NCP ncoV or 2019 nco-V

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP).

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The 2019-nCoV genome sequence also has ~80% identity with SARS-CoV, but most similar to some bat beta-coronaviruses, with the highest reaching >96% identity. Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. One particular claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (rMA15) and is capable of infecting human cells. Here, we provide evidence that this claim lacks of scientific basis and must be discounted.

First, we will explain how the recombinant mouse-adapted SARS virus (rMA15) was generated (PLoS Pathog. 2007 Jan;3(1):e5). After constructing a full-length infectious SARS coV by reverse genetics, Dr. Ralph Baric's lab showed that it replicated in old mice with low or no pathogenic activity. They then adapted the SARS-CoV (Urbani strain) by serial passages in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS coV gained elevated replication and lung

Commented [SL1]: Specify Urbani strain here?

pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. When introduced into the original recombinant SARS-CoV, these six mutations (only one in the S gene) conferred the high virulence and lethality (rMA15). Although not reported in human cells, it is likely that rMA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

Second, it is important to clarify how the chimeric SHC014-~~ǂ~~MA15 virus was constructed and what key findings were made using that virus. When the SARS coV was isolated, it was concluded that the S gene from bat-derived coV, unlike that from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry. Civets were proposed as the secondary host for the bat-coV before spreading to humans. However, novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SL-CoV-WIV1 used ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interaction with SARS coV (JVI 2012), it was proposed that intermediate hosts may not be necessary and some bat SL-CoVs may directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-~~WIV1~~-SHC014 was used to generate a chimeric virus in the mouse adapted ~~ǂ~~MA15 SARS-CoV backbone. The resultant SHC014-~~ǂ~~MA15 virus can efficiently use ACE2 from multiple species and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-~~ǂ~~MA15 can replicate efficiently in the mouse lung with severe pathogenesis (Nat. Med. 2015). These findings have provided strong evidence that some bat CoVs can directly use human ACE2 to infect human hosts.

Commented [SL2]: In the paper , they use SHC014-MA15

Commented [SL3]: SHC014 and WIVI are two different bat Cov, with some sequence difference in S domain

Due to the elevated pathogenic activity of the SHC014-~~r~~MA15 chimeric virus relative to the Urbani Spike-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause. No more bat-coV-MA15 chimeric viruses are constructed after the SHC014- MA15 chimeric virus. The NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potential. Regarding its lineage relationship with 2019 nCoV, however, after careful phylogenetic analyses by multiple international groups (EMI, Nature…2020), the 2019 ncoV/NCP virus is unmistakably, and fortunately, distinct from SHC014-MA15. There is NO credible evidence to support the claim that the 2019 ncoV/NCP virus was derived for the chimeric SHC014- MA15 virus.

From: Lu, Shan
To: Liu, Shan-Lu; Su, Lishan
Subject: RE: Executive summary of EMI commentary
Date: Sunday, February 23, 2020 1:15:50 PM
Attachments: image001.png
Liu et al_EMI Commentary_Revision_中文-Shan Lu.docx

Overall they are very good.

I found one minor error: "Dec" was used for the last reference of your paper. It should be "Feb". You may want to change the current word and pdf, but not change the real paper to be published as it may take a lot of more time to current and reload to online. Readers can find that paper without much problem.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 12:57 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
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Never, just received Shan's email and file! Slow on my end.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
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To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
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Lishan and Shan – so confusing!

I have wrapped up a summary for the public and media to understand key points of our commentary. Please make suggestions.

I think this can go along with the Chinese translation.

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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 7:39 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

-Lishan

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Date: Friday, February 21, 2020 at 7:33 PM
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I just went online to see if we replace with a new one.
Could you send me the PDF of the corrected one?

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:32 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

My bad. How do we fix it? send her a message?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:29 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Lishan:

I just saw that you deleted nt for 1,100 – “nt” should be kept. Could you correct that?

Thanks.

SL

Shan-Lu Liu, M.D., Ph.D.
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Co-Director, Viruses and Emerging Pathogens Program
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Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

Begin forwarded message:

> **From:** "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
> **Date:** February 21, 2020 at 4:04:41 PM PST
> **To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>, "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Subject: Submitted Corrections for article TEMI 1733440**
> **Reply-To:** TEMI-production@journals.tandf.co.uk
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
>
> Journal: *Emerging Microbes & Infections* (TEMI)
>
> Article ID: TEMI 1733440
>
> Dear author,
>
> This email confirms that you have submitted your corrections to your article proofs.
>
> The submitted corrections have been successfully uploaded.
>
> If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.
>
> Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

没有可信的证据支持 SARS-CoV-2 来自实验室人工合成

Shan-Lu Liu （刘善虑）, 俄亥俄州立大学

Linda J. Saif, 俄亥俄州立大学

Susan Weiss, 宾夕法尼亚大学

Lishan Su,（苏立山） 北卡大学教堂山分校

截止 2020 年 2 月 10 日，在武汉出现和爆发的急性呼吸疾病已波及 4 万多人，导致 1000 多人死亡。研究人员很快找到了一种新型人的冠状病毒，称之为 2019 nCoV 或 SARS-CoV-2，而相应的疾病称之为 COVID-19，意为 2019 年发生的冠状病毒疾病 (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/)。

据现有的报道[1-3]，COVID-2019 与 SARS-CoV 导致的 SARS 有很多相似的临床表现。而 SARS-CoV-2 基因组序列也和 2003 年 SARS-CoV 有 80%同源性，但它与一些蝙蝠的乙型冠状病毒更为相似。当前，种种的推测、谣言和阴谋论到处流行，其中有的认为 SARS-CoV-2 来源于实验室基因工程制造。也有某些人声称，人的 SARS-CoV-2 是从武汉的某个实验室直接泄漏出来的， 其根据是该实验室最近报道了一种称为 RaTG13 的蝙蝠冠状病毒，它和 SARS-CoV-2 基因组序列有高达 96%的同源性。

然而，我们知道，2003 年发现人 SARS 冠状病毒和其中间宿主果子狸 SARS 样冠状病毒具有 99.8％的同源性，在整个基因组中只有 202 个碱基不同。鉴于人类新型 SARS-CoV-2 与蝙蝠 RaTG13-CoV 之间有超过了 1000 个不同碱基[4]，且这些差异是按照冠状病毒典型的进化特征按自然发生的模式分布在整个基因组中，我们认为 SARS-CoV-2 直接来源于 RaTG13 冠状病毒的可能性极小。更为重要的是，在新的人 SARS-CoV-2 病毒基因组序列中并没有任何可信的基因工程改造的迹象，这都揭示 SARS-CoV-2 是通过自然演化而来的。我们认为在蝙蝠与人类之间可以找到中间动物宿主含有类似的冠状病毒，它与 SARS-CoV-2 更相似。最近有消息称穿山甲可能携带与 SARS-CoV-2 密切相关的冠

状病毒，但论文和数据尚未正式发表，无从得以证实(https://www.nature.com/articles/d41586-020-00364-2)。

最近社交媒体上的另一种说法指向2015年在《自然医学》发表的一篇论文[7]。该论文报道了在小鼠适应后的人类SARS冠状病毒（MA15病毒）中，人工构建了带有蝙蝠冠状病毒（SHC014）S基因，这种合成的嵌合冠状病毒，不仅可以可以感染小鼠，也能够感染来源人的细胞[8]。然而，新型冠状病毒SARS-CoV-2与这个嵌合冠状病毒基因组序列有超过了5,000个碱基的不同，所以这种怀疑完全缺乏任何科学依据。

现在我们来理一理来人SARS病毒老鼠适应株MA15和它的衍生病毒的来龙去脉。适应小鼠的SARS病毒（MA15）[9]是通过把SARS冠状病毒在小白鼠呼吸道中连续传代15后产生的；适应后的SARS冠状病毒有六个氨基酸突变，使其能够更有效地感染小鼠，尤其是在老年小鼠中具有了更高的复制活性和肺部致病性能（因此称为M15）。由于在小鼠内适应的遗传突变，MA15在人细胞或者人体内感染很可能降低了。

科学家曾认为从蝙蝠身来的冠状病毒的S基因和人的SARS病毒不同，推测它们无法使用人的SARS病毒受体ACE2进入人体细胞[10, 11]；后来发现果子狸是蝙蝠冠状病毒传给人的中间宿主，能够将SARS冠状病毒传播给人类[6, 12]。然而，2013年以来，科学家陆续从中国马蹄蝠中分离到了数个新型蝙蝠冠状病毒，这些来自蝙蝠的，类似人SARS冠状病毒（SL-CoV-WIV1）能够使用人、果子狸和中国马蹄蝠的ACE2受体进入和感染细胞[8]。进化研究表明，在SARS冠状病毒S蛋白的作用接触位点上，蝙蝠ACE2基因在与人类ACE2基因在相同的位点上同样被进化选择[13]。基于这样的发现，科学家提出了蝙蝠的SARS样冠状病毒具有直接传染到人的能力，不必需要中间宿主环节；也就是说有些蝙蝠冠状病毒有可能直接感染人类宿主细胞。为了直接验证这种可能性，蝙蝠冠状病毒SL-SHC014的S基因被人工嫁接到了MA15 SARS-CoV骨架上，因此产生了一个嵌合病毒。此SL-SHC014-MA15嵌合病毒确实能够有效地利用人ACE2进入细胞，并在人的呼吸道实验细胞中有效复制。SL-SHC014-MA15也可以在小鼠的肺中高效复制，但与SARS MA15相比，感染减弱了，并且只会让老年小鼠致命[7]。

由于SL-SHC014-MA15嵌合病毒相对于另一个人SARS-S/MA15嵌合病毒在小鼠中具有更高的致病活性，这种嵌合冠状病毒的实验后来在美国政府的干预下被暂停(https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-

pause-gain-function-research)。虽然目前 这项禁令在美国已经被解除，但构建这种具有大流行病潜力的病毒是否是一种风险，在当前的COVID-2019流行的形势下又重新引发了讨论，成为热点话题。然而，经过多个国家科学家对病毒的分子进化分析[5，14]，SARS-CoV-2 无疑与 SL-SHC014-MA15 具有非常大的不同，整个基因组有大约 6,000 核苷酸的差异。因此，没有可信的证据支持 SARS-CoV-2 是源自 SL-SHC014-MA15 嵌合病毒的说法。

最近也有传言说，SARS-CoV-2 是实验室中有意人为制造的。其中发表在 BioRxiv（一个同行评审之前的手稿共享网站）的一份手稿中更是此传言的代表，它声称 SARS-CoV-2 中含有 HIV 序列，因此很可能是在实验室中产生的。文章在线后， 舆论哗然，世界各国的多个病毒学者纷纷反驳。 在 HIV-1 病毒专家高峰（Feng Gao）领衔领导的反驳论文中，他们使用了仔细的生物信息学分析来证明，指出最初声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的 [15]。由于国际社会提出的种种疑问，这篇手稿的作者已经撤回了该手稿， 不再要求发表。

从科学层面讲，进化是循序渐进的，并随着时间的推移进一步产生有利于病毒的突变，就像天然分离的病毒（如蝙蝠冠状病毒 RaTG13）基因组那样。 相反，人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以我们认为，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。 有一种可能不能排除， 就是 SARS-CoV-2 是一种蝙蝠冠状病毒与另一种冠状病毒之间进行了自然重组而产生的；但这种可能性需要更多的研究来证明，来回答 SARS-CoV-2 的自然起源问题。我们需要强调的是，尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人工制造，但对公共健康有威胁的病毒都必须进行恰当的实验室管理，而且需要由科学界和政府合理监管。

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

From: Lu, Shan
To: Liu, Shan-Lu; Su, Lishan
Subject: RE: Executive summary of EMI commentary
Date: Sunday, February 23, 2020 1:40:03 PM
Attachments: image001.png

Perfect。No more changes

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 1:21 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Executive summary of EMI commentary

Now, the final versions, a total of 4 files - hopefully!

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 23, 2020 at 1:15 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
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I just went online to see if we replace with a new one.
Could you send me the PDF of the corrected one?

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Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:32 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

My bad. How do we fix it? send her a message?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:29 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Lishan:

I just saw that you deleted nt for 1,100 – “nt” should be kept. Could you correct that?

Thanks.

SL

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:28 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Thanks!

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

Begin forwarded message:

> **From:** "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
> **Date:** February 21, 2020 at 4:04:41 PM PST
> **To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>, "Liu, Shan-Lu" <liu.6244@osu.edu>

Subject: Submitted Corrections for article TEMI 1733440
Reply-To: TEMI-production@journals.tandf.co.uk

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear author,

This email confirms that you have submitted your corrections to your article proofs.

The submitted corrections have been successfully uploaded.

If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.

Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From: Lu, Shan
To: Liu, Shan-Lu; Su, Lishan
Subject: RE: Executive summary of EMI commentary
Date: Sunday, February 23, 2020 12:48:16 PM
Attachments: image003.png
刘善虎苏立山等教授发文分析驳斥新冠病毒阴谋论-Shan Lu.docx

Great work. However, I made some new changes (see attached). All highlighted or marked, for your reference.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 8:57 AM
To: Su, Lishan <lishan_su@med.unc.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Executive summary of EMI commentary

Lishan and Shan – so confusing!

I have wrapped up a summary for the public and media to understand key points of our commentary. Please make suggestions.

I think this can go along with the Chinese translation.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 7:39 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:33 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

I just went online to see if we replace with a new one.
Could you send me the PDF of the corrected one?

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Phone: (614) 292-8690
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

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Date: Friday, February 21, 2020 at 4:32 PM
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Subject: Re: Submitted Corrections for article TEMI 1733440

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SL

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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:28 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Thanks!

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

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> **To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>, "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Subject: Submitted Corrections for article TEMI 1733440**
> **Reply-To:** TEMI-production@journals.tandf.co.uk
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
>
> Journal: *Emerging Microbes & Infections* (TEMI)
>
> Article ID: TEMI 1733440
>
> Dear author,
>
> This email confirms that you have submitted your corrections to your article proofs.

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If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.

Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论

俄亥俄州立大学教授**刘善虑**，北卡大学教堂山分校教授苏立山联名美国科学院院士 Linda J. Saif 以及美国微生物科学院院士 Susan Weiss，在 国际期刊 Emerging Microbes & Infections (EMI) (中文译名《新发微生物与感染》)发表题为“**没有可信的证据支持 SARS-CoV-2 来自实验室人工合成**”的评论文章，对最近广为流行的传言和阴谋论进行了分析和驳斥。

该文主要论点如下：

1. 新型冠状病毒 SARS-CoV-2 虽然与中国科学院武汉病毒所最近报道的一种称 为 RaTG13 的蝙蝠冠状病毒有高达 96%的同源性， 但两者仍然有超过 1，100 碱基的差别，而且在关键序列序列上有特征性的区别，因此两者是完全不同的冠状病毒。
2. 社交媒体指向 2015 年在《自然医学》一篇论文，认为新型冠状病毒是这篇文章报道的人 SARS 和蝙蝠冠状病毒（SHC014）的嵌合病毒的泄露。分析研究表明，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒在基因组序列上有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。
3. 还有一种传言说 SARS-CoV-2 是实验室中有意人为制造的， 并以发表在 BioRxiv 上印度科学家的一份手稿中为依据，声称 SARS-CoV-2 中含有 HIV 序列。实际上这篇文章在线后， 舆论哗然，世界各国病毒学家也纷纷反驳。 在 HIV-1 专家高峰（Feng Gao）领衔领导发表在 EMI 的另一篇反驳论文中，作者使用了仔细的生物信息学分析来证明，指出最初声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的。由于国际社会提出的种种疑问，这篇手稿的作者已经撤回了该手稿， 目前~~不再要求~~没有发现再次发表。
4. 从科学层面讲，病毒进化是循序渐进的，并随着时间的推移进一步产生有利于病毒感染人的突变。相反， 人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以， 目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。
5. 尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人工制造，我们认为对公共健康有威胁的病毒都必须进行恰当的实验室管理，而且需要由科学界和政府合理监管。

From: Su, Lishan
To: Liu, Shan-Lu; Lu, Shan
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591
Date: Monday, February 24, 2020 9:37:47 AM
Attachments: image001.png

Thanks!

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Monday, February 24, 2020 at 9:37 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591

Lishan:

This is referring to the proof that you missed the “nt” in the submitted proof

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Monday, February 24, 2020 at 9:33 AM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591

What is this about? I have not seen any other message.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Monday, February 24, 2020 at 8:29 AM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591

It’s alright. Lishan did not realize that...
Shan-Lu Liu sent from iPhone

On Feb 24, 2020, at 7:21 AM, Lu, Shan <Shan.Lu@umassmed.edu> wrote:

Now you caused more delay….

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 24, 2020 3:12 AM
To: TEMI-production@journals.tandf.co.uk
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591

Thank you!

SL

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
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Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Monday, February 24, 2020 at 1:01 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591

Dear Shan-Lu Liu,

Thank you for the email. I have sent your additional correction to the team so that they will make the changes in the final PDF.

Have a great day!

Regards,

Malathi
Emerging Microbes & Infections

From:liu.6244@osu.edu
Sent:22-02-2020 06:24
To:malathi@novatechset.com
Cc:
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440

Dear Malathi Boopalan:

We have just uploaded a corrected proof online, but relegalized a small error: "1,100" should be read as "1,100 nt" – could you kindly help make the correction, or replace the uploaded file with the attached new one?

Thank you! Please confirm.

Shan-Lu Liu & Lishan Su

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
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Shan-Lu Liu sent from iPhone

> On Feb 21, 2020, at 4:04 PM, TEMI-production@journals.tandf.co.uk <cats@taylorandfrancis.com> wrote:
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

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Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From: Su, Lishan
To: Lu, Shan; Liu, Shan-Lu
Subject: Re: 2019-nCoV-EMI_commentary
Date: Tuesday, February 11, 2020 3:39:31 PM
Attachments: image001.png
SHC014-MA15 v 2019 ncoVb.docx

I have inserted your paragraph at he beginning, or we can end with it.

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 2:03 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Sure, we are not saying we are trying to defend Ralph but just don't want to give others the wrong impression.

Feng Gao piece will be published tomorrow so we do not include any details this commentary. There is only one short paragraph at the end of our document to mention it briefly.

The RaTG13 topic can also be very simple. Please take a look at what we wrote below:

This led to speculations and rumors that the 2019-CoV is of a laboratory origin. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan as a bat CoV (RaTG13) was recently reported by that laboratory and it shared ~96% homology with the 2019-nCoV (Nature, 2020). However, as we now know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt. On the other hand, there are greater than 1000 nt differences between 2019-nCoV and RaTG13, suggesting RaTG13 is not the immediate source of 2019-nCoV given the large size genome like beta-coronaviruses (~30 kb) and the slow the mutation rate of the coronaviruses. Searching for an immediate host between bat and humans is needed.

My view is that as long as we compared the sequence difference (1000 nt) which is very different from that of SARS (60nt), it is quite clear. Most non-viral people do not understand what does 96% mean. We don't have to explain how long it will take to do the mutations because it will not cover other issues such as some recombination etc. We just say the difference between RaTG13 and 2019-nCoV is very big so they are not the same leaked from Wuhan Virology Lab.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 1:52 PM

To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I agree that it should be simple and clear. I have included some details in the 1st draft for your information. There is not intention to defend Baric, but to clarify the facts.

Regarding all three, are you combining Goa Feng's piece with this one? For the RaTG13, it involves complicated viral evolution kinetics and maybe hard to simply clarify…
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 1:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM

To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
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Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Tentative Title: The mouse adapted SARS chimeric virus with bat-coV S gene (SHC014-MA15) is not related to the NCP ncoV or 2019 nco-V

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP).

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The 2019-nCoV genome sequence also has ~80% identity with SARS-CoV, but most similar to some bat beta-coronaviruses, with the highest reaching >96% identity. Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin.

This led to speculations and rumors that the 2019-CoV is of a laboratory origin. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan as a bat CoV (RaTG13) was recently reported by that laboratory and it shared ~96% homology with the 2019-nCoV (Nature, 2020). However, as we now know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt. On the other hand, there are greater than 1000 nt differences between 2019-nCoV and RaTG13, suggesting RaTG13 is not the immediate source of 2019-nCoV given the large size genome like beta-coronaviruses (~30 kb) and the slow the mutation rate of the coronaviruses. Searching for an immediate host between bat and humans is needed.

~~One particular~~Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (rMA15) and is capable of infecting human cells. Here, we provide evidence that this claim lacks ~~of~~ any scientific basis and must be discounted.

~~First, we will explain how t~~The recombinant mouse-adapted SARS virus (~~r~~MA15) ~~was generated~~ (PLoS Pathog. 2007 Jan;3(1):e5)~~.~~ was generated ~~After constructing a full-length infectious~~ SARS coV ~~by reverse genetics, Dr. Ralph Baric's lab showed that it replicated in old mice with low or no pathogenic activity. They then adapted the SARS-CoV (Urbani strain)~~ by serial passages of an infectious SARS coV in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS coV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. ~~When introduced into the original recombinant SARS-CoV, these six mutations (only one in the S gene) conferred the high virulence and lethality (rMA15). Although not reported in human cells, i~~It is likely that ~~r~~MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

Commented [SL1]: Specify Urbani strain here?

~~Second, it is important to clarify how the chimeric~~ SHC014 ~~rMA15~~ ~~virus was constructed and what key findings were made using that virus.~~ When the SARS coV was isolated, it was concluded that the S gene from bat-derived coV, unlike that from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry. Civets were proposed as the secondary host for the bat-coV before spreading to humans (SARS coV review?). However, several novel bat coronaviruses

Commented [SL2]: In the paper , they use SHC014-MA15

were isolated from Chinese horseshoe bats in 2013 and the bat SL-CoV-WIV1 used ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interaction with SARS coV (JVI 2012), it was proposed that intermediate hosts may not be necessary and some bat SARS-like or SL-CoVs may directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL- ~~WIV1~~ SHC014 was used to generate a chimeric virus in the mouse adapted ~~-~~ ~~r~~MA15 SARS-CoV backbone. The resultant SL-SHC014-~~r~~MA15 virus can efficiently use human ACE2 ~~from multiple species~~ and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-~~r~~MA15 can replicate efficiently in the mouse lung, leading to ~~with~~ severe pathogenesis (Nat. Med. 2015). ~~These findings have provided strong evidence that some bat CoVs can directly use human ACE2 to infect human hosts.~~

Commented [SL3]: SHC014 and WIVI are two different bat Cov, with some sequence difference in S domain

Due to the elevated pathogenic activity of the SHC014-~~r~~MA15 chimeric virus relative to the ~~Urbani Spike~~SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause. No more bat-coV-MA15 chimeric viruses are constructed after the SHC014-MA15 chimeric virus. The NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potential. Regarding its lineage relationship with 2019 nCoV, however, after careful phylogenetic analyses by multiple international groups (EMI, Nature…2020, a figure?), the 2019 ncoV/NCP virus is unmistakably, and fortunately, distinct from SHC014- MA15. There is NO credible evidence to support the claim that the 2019 ncoV/NCP virus was derived ~~for~~ from the chimeric SHC014-MA15 virus.

From: Su, Lishan
To: Lu, Shan; Liu, Shan-Lu
Subject: Re: Your article proofs for review (ID# TEMI 1733440)
Date: Friday, February 21, 2020 10:58:37 AM

I agree you are highly suspicious for this one…
I am finishing proofing and will finalize/upload it after considering comments from Susan, Linda and Shan-Lu.
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Friday, February 21, 2020 at 10:36 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: Your article proofs for review (ID# TEMI 1733440)

Yes, just a secret to you two and not share with others.
When I put a super fast review and accept (basically no review), the JEO of T&F, became very suspicious and wanted her boss to check and approve. She probably wonder if we are actually just one person with three fake names

Well, now you guys please coordinate the proof read and get input from Linda and Susan. Then submit it back asap (online, not by emails please). No need to go through me.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Friday, February 21, 2020 10:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: Your article proofs for review (ID# TEMI 1733440)

Thanks for speeding it up, bro!

We are doing wonders as three confusing/confused musketeers of Shan-Lu, Shan Lu and Lishan Su:)

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Friday, February 21, 2020 at 7:43 AM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Your article proofs for review (ID# TEMI 1733440)

Only 1.5 days. Not 7 days. I feel better towards my brothers (sweating…).

Please go ahead to revise as you two see fit. Only make minimal changes.

Thanks.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Friday, February 21, 2020 7:42 AM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: FW: Your article proofs for review (ID# TEMI 1733440)

From: "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Friday, February 21, 2020 at 4:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear Shan-Lu Liu,

Your article proofs are now available for review through the Central Article Tracking System (CATS) at: https://cats.informa.com/PTS/in?ut=B2AB6692AA414D96905B59E6C51FA240 .

PLEASE NOTE: The CATS system only supports Internet Explorer 6 (and later), or Firefox 3 (and later) browser software. Popup blockers should be disabled. If you have any difficulty using CATS, please contact me.

• Your User Name is: [redacted]

• If you do not know your password, you may reset it here:
http://cats.informa.com/PTS/forgottenPassword.do

1. Click on 'Review Proofs'.

2. Select 'Download PDF'.

3. Follow the guidance on the proof cover sheet to return your corrections. Please limit changes to answering any author queries and to correcting errors. We would not expect to receive more than 30 corrections.

Please check your proofs thoroughly before submitting your corrections as once they have been submitted we are unable to accept further corrections. If you have any queries, please email me.

To avoid delaying publication of your article, please approve these proofs or return any corrections by 26 Feb 2020.

Reprint and issue orders may be placed by logging in to your CATS account and accessing the order form on the "Additional Actions" menu. If you have any questions on this process, please contact me or visit our author services site https://authorservices.taylorandfrancis.com/ordering-print-copies-of-your-article/

• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link: https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From: Emerging Microbes and Infections
To: Liu Shan-Lu
Subject: Emerging Microbes & Infections - Invitation to Review Manuscript ID TEMI-2020-0147
Date: Thursday, February 20, 2020 4:27:27 PM

20-Feb-2020
Dear Professor Shan-Lu Liu:

The above manuscript, entitled "The origin of the SARS-CoV-2 coronavirus: a rebuttal to the claim of formation via laboratory recombination" has been submitted to Emerging Microbes & Infections

I would be grateful if you would kindly agree to act as a reviewer for this paper The abstract appears at the end of this letter

(Dear Shan-Lu, since you are an expert in such rebuttals, I would appreciate if you can pick up some key issues and provide a simple and brief points It will be great if you can get back within the next 1-2 days)

Please let me know as soon as possible if you will be able to accept my invitation to review To do this please either click the appropriate link below to automatically register your reply with our online manuscript submission and review system, or e-mail me with your reply

*** PLEASE NOTE: This is a two-step process After clicking on the link, you will be directed to a webpage to confirm ***

Agreed: https://urldefense com/v3/__https://mc manuscriptcentral com/temi?URL_MASK=b79ab5b5cdaf479495e1618435a63e6f__!!KGKeukY!kROoa42fswQs64RsKKYPFDJBYPGTKTfeIEMcF_HEcTLK9iqdqnE7RQ_rCYDGQWDEow8$

Declined: https://urldefense com/v3/__https://mc manuscriptcentral com/temi?URL_MASK=1450a092f3254e64a53747db7513a0e2__!!KGKeukY!kROoa42fswQs64RsKKYPFDJBYPGTKTfeIEMcF_HEcTLK9iqdqnE7RQ_rCYDGJuqBBXI$

Unavailable: https://urldefense com/v3/__https://mc manuscriptcentral com/temi?URL_MASK=c99281250c4f4bdb9c74371259f7dd03__!!KGKeukY!kROoa42fswQs64RsKKYPFDJBYPGTKTfeIEMcF_HEcTLK9iqdqnE7RQ_rCYDGtcvYje8$

Should you accept my invitation to review this manuscript, you will be sent an email with a direct link to the scoresheet, which will be made available to you You will then have access to the manuscript and reviewer instructions in your Reviewer Center

If you are unable to review the manuscript, click on the “decline” option to register your response This will direct you to a screen where you will be given the opportunity to provide details of any alternative reviewers

I realise that our expert reviewers greatly contribute to the high standards of the Journal, and I thank you for your present and/or future participation

Sincerely,
Professor Shan Lu
Emerging Microbes & Infections

MANUSCRIPT DETAILS

TITLE: The origin of the SARS-CoV-2 coronavirus: a rebuttal to the claim of formation via laboratory recombination

AUTHORS: Professor Wu Zhong

ABSTRACT:

From:	Su, Lishan
To:	Liu, Shan-Lu
Subject:	Re: Executive summary of EMI commentary
Date:	Sunday, February 23, 2020 12:02:06 PM
Attachments:	image001.png image002.png 刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论.pdf

---

See pdf file with no lines.
I can not find a better word than驳斥, but it seems to a be a bit stronger than we intended?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 23, 2020 at 8:57 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Executive summary of EMI commentary

Lishan and Shan – so confusing!

I have wrapped up a summary for the public and media to understand key points of our commentary. Please make suggestions.

I think this can go along with the Chinese translation.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 7:39 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:33 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

I just went online to see if we replace with a new one.
Could you send me the PDF of the corrected one?

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:32 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

My bad. How do we fix it? send her a message?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:29 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Lishan:

I just saw that you deleted nt for 1,100 – “nt” should be kept. Could you correct that?

Thanks.

SL

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology

The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:28 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Thanks!

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

Begin forwarded message:

> **From:** "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
> **Date:** February 21, 2020 at 4:04:41 PM PST
> **To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>, "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Subject: Submitted Corrections for article TEMI 1733440**
> **Reply-To:** TEMI-production@journals.tandf.co.uk
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
>
> Journal: *Emerging Microbes & Infections* (TEMI)
>
> Article ID: TEMI 1733440
>
> Dear author,
>
> This email confirms that you have submitted your corrections to your article

proofs.

The submitted corrections have been successfully uploaded.

If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.

Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

刘善虑苏立山等教授发文分析认为新冠病毒阴谋论缺乏病毒学证据

俄亥俄州立大学教授刘善虑，北卡大学教堂山分校教授苏立山联名世界冠状病毒学专家 Linda J. Saif（美国科学院院士）以及 Susan Weiss（美国微生物科学院院士），在 国际期刊 Emerging Microbes & Infections (EMI) (中文译名《新发微生物与感染》)发表题为“**没有可信的证据支持 SARS-CoV-2 来自实验室人工合成**”的评论文章，对最近广为流行的传言和阴谋论进行了分析和驳斥。

该文主要论点如下：

1. 新型冠状病毒 SARS-CoV-2 虽然与中国科学院武汉病毒所最近报道的一种称 为 RaTG13 的蝙蝠冠状病毒有高达 96%的同源性， 但两者仍然有超过 1，100 碱基的差别，而且在关键序列序列上有特征性的区别，因此两者是完全不同的冠状病毒。
2. 社交媒体指向 2015 年在《自然医学》一篇论文，认为新型冠状病毒是这篇文章报道的人 SARS 和蝙蝠冠状病毒（SHC014）的嵌合病毒的泄露。分析研究表明，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒在基因组序列上有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。
3. 还有一种传言说 SARS-CoV-2 是实验室中有意人为制造的， 并以发表在 BioRxiv 上印度科学家的一份手稿中为依据，声称 SARS-CoV-2 中含有 HIV 序列。实际上这篇文章在线后， 舆论哗然，世界各国病毒学家也纷纷反驳。 在 HIV-1 专家高峰（Feng Gao）领衔领导发表在 EMI 的反驳论文中，作者使用了仔细的生物信息学分析来证明，指出最初声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的。由于国际社会提出的种种疑问，这篇手稿的作者已经撤回了该手稿， 不再要求发表。
4. 从科学层面讲，进化是循序渐进的，并随着时间的推移进一步产生有利于病毒的突变。相反，人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。
5. 尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人工制造，我们认为对公共健康有威胁的病毒都必须进行恰当的实验室管理，而且需要由科学界和政府合理监管。

F om _u Shan- u_
To _h_ _h_
Subje t Re Eme g ng M c bes & Infect ons - TEMI-20 0-0121 - changes qui ed o ou subm s ion
Date iday eb ua y 4 2020 6 13 2 M

I just finished reading Feng's paper – indeed ery good.

Small e ror noted in he f rst paragraph "Know ng he origin of such a pa hogen is critical to de elop ng means to blocking further transm ssion and to de elop accines". I saw this kind of error frequent for asian people but no big deal.

Feng Gao s my 师兄 in 北京的病毒所 we we e from the same lab where my former director has now been infec ed by SARS CoV 2! Ve y sad but he s doing OK!

Shan-Lu

From Lu, Shan Shan.Lu@umassmed.edu>
Date F iday, Feb ua y 14, 2020 at 5 10 PM
To Shan-Lu L u liu.6244@osu edu>, Su, Lishan l shan_su@med.unc.edu>
Sub ect RE Eme ging Mic obes & Infections - TEMI-2020 0121 - changes equi ed to you subm ssion

I don t have any objec ion. I see the point o add mo e cond tions to make su e the commen a y does not exclude ce tain a e o unusual s tua ions. At the same time, the mo e we t y to be comple e (o mo e like a lawye), it may gene ate mo e ques ions o even suppo t some people to say see these big name autho s also ag ee the e a e possib l ties of this and that... .

Th s is the same eason of t ying o be simple and focused o avoid cove ing compl cated ssues. I don t know how to solve this conflict between be ng tho ough and avoiding confus on. I will ok to take anything once you wo had ca eful thought.

At the same time, I w ll fo wa d to you the comment f om one Biotech ch ef afte I d st ibuted Feng Gao s pape th s mo ning. It was quite su p sing to me and it shows how d ve sity of the po ent al eade s. We can only t y to do ou best.

Shan

F om Liu, Shan-Lu l u 6244@osu.edu>
Sent F iday, Feb ua y 14, 2020 2 19 PM
To Su, L shan l shan_su@med.unc edu> Lu, Shan Shan.Lu@umassmed.edu>
Subject Re Eme g ng Mic obes & Infect ons - TEMI-2020 0121 - changes equi ed to you submiss on

L shan: ooks good o me.
Shan: what do you think?

Thanks.

From Su, L shan _l h @_ >
Date F iday, Feb ua y 14, 2020 at 1 34 PM
To Shan-Lu L u _liu.6244@osu edu_>, Lu, Shan _shan.lu@umassmed.edu_>
Sub ect Re Eme ging Mic obes & Infections - TEMI-2020 0121 - changes equi ed to you subm ssion

How about this?

-L shan

From L u, Shan-Lu _liu.6244@osu edu_>
Date F iday, Feb ua y 14, 2020 at 12 47 PM
To Su, L shan _l h @_ >, Lu, Shan _h l @_ >
Sub ect Re Eme ging Mic obes & Infections - TEMI-2020 0121 - changes equi ed to you subm ssion

Lishan

I get you point – maybe below one eads bet e ?

We should emphas ze that, although SARS-CoV-2 shows no evidence of labo ato y o igin, vi uses w th such g eat public health th eats must be handled p ope ly in the labo ato y and also p ope ly egulated by scien if c commun ty and gove nments."

Thoughts?

Shan-Lu Liu, M.D., Ph D.
P ofesso
Co-Di ecto , Vi uses and Eme ging Pathogens P og am
Infectious Diseases Inst tu e
Cente fo Ret ovi us Resea ch
Depa ments of Ve e na y Biosciences, Mic obial Infection and Immunity, and M c ob ology
The Oh o State Un ve s ty
1900 Co fey Rd, Room 480 VMAB
Columbu , Oh o 43210
Phone (614) 292-8690
Fax (614) 292-6473
Ema l _l u 6244@osu.edu_ _shan lu.liu@osumc.edu_

On 2/14/20, 11 08 AM, Su, Lishan _l shan su@med.unc edu_> w ote

How about adding the last sentence n the p oof?

Evolution s stepwise and acc ues mutations g adually ove t me, whe eas synthet c const uc s would typica ly use a known backbone and nt oduce logical o ta geted changes instead of the andomly occu ing mutations that a e p esent in natu ally sola ed vi uses such as bat CoV RaTG13. In ou view, the e is cu ently no c ed ble evidence to uppo t the cla m that SARS CoV 2 o iginated f om a labo ato y-eng nee ed CoV. It is mo e l kely that SARS-CoV-2 is a ecomb nant CoV gene ated in natu e be ween a bat CoV and anothe co onavi us in an nte mediate animal hos . Mo e studies a e needed to explo e th s possibility and esolve the natu al o g n of SARS CoV-2. Although the SARS2 CoV has shown no evidence of labo ato y o igin, it is impo tant to point out that such vi uses in the labo ato y as d scussed he e do pose public health conce ns and should be ca efu ly moni o ed and egulated.

-Lishan

--- O ig nal Message- ---
F om Liu, Shan-Lu _l 62 @_ >
Date Thu sday, Feb ua y 13, 2020 at 10 04 AM
To Lu, Shan _shan.lu@umassmed.edu_>
Cc Su, Lishan _l h @_ >
Subject Re Eme g ng Mic obes & Infect ons - TEMI-2020 0121 - changes equi ed to you submiss on

Thanks, Shan fo you e fic ent ac ion!

On 2/13/20, 9 56 AM, Lu, Shan _Sh @_ > w ote

You pape is now accepted. Hope you have eceived the dec sion let e .

Best.

Shan

--- -O ginal Message-----
F om L u, Shan-Lu _liu.6244@osu edu_>
Sent Thu sday, Feb ua y 13, 2020 8 57 AM
To _- @j l f_
Cc Lu, Shan _Shan.Lu@umassmed.edu_> Su, Lishan _l shan su@med unc edu_>
Subject Re Eme ging Mic obes & Infections - TEMI-2020-0121 - changes equi ed to you subm ssion
Impo tance H gh

Hi Jo gie

I have modif ed as inst ucted and at ached the new one to this ema l. Please help upload and p oceed.

Thank you.

Shan-Lu

Shan-Lu L u, M.D., Ph D.
P ofesso
Co-Di ecto , Vi uses and Eme ging Pathogens P og am Infectious Diseases Inst tute Cente fo Ret ovi us Resea ch Depa tments of Vete ina y Biosc ences, M c ob al Infect on and Immun ty, and Mic obiology The Ohio State Unive sity
1900 Co fey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone (614) 292 8690
Fax (614) 292 6473
Ema l _l u.6244@osu.edu_ _shan lu.liu@osumc.edu_

On 2/13/20, 8 43 AM, Eme ging Mic obes and Infec ions _onbehalfo @manusc iptcent al.com_> w ote

13-Feb 2020

Dea P ofesso Liu,

You above efe enced manusc ipt, entitled SARS-CoV-2 no evidence of a labo ato y o ig n equi es some fu the changes befo e it is eady fo eviewing n Eme g ng Mic obes & Infec ions. You submiss on has been etu ned to you and is located in you Autho Cen e as a d aft, so that you due to these easons

1. No line numbe ng

K ndly add a l ne numbe ng in you main document.

2. Exceeded efe ence count

K ndly be info med that the efe ence count fo the commenta y a t cle should not be mo e than 15.

You submiss on along w th all files you submit ed s now n you Autho Cente , at _https //u ldefense.com/v3/ https //nam01 safelinks.p otection.ou look.com/?u l=https*3A*2F*2Fu ldefense.com*2Fv3*2F https*3A*2F*2Fmc.manusc p cent al.com*2Ftemi *3B!!KGKeukY!nGv1RgRJ1P-OGXuZi8b2hKGjXxDFOmBwDONuR njCdwERJF1HkBIV4Sggq 9udyWYml*24&amp data=02*7C01*7CShan Lu*40umassmed.edu*7Cde7fc6818 7047e0d79008d7b08c97ea*7Cee9155fe2da34378a6c44405faf57b2e*7C0*7C0*7C637171990355158902&amp sda a=nRqMhNxdKKXJTS3Gah0eH sq926D2xz32DK1nzopo*2BA*3D&amp ese ved=0 JSU JSU JSU JSU JSUl SUl SUl!!KGKeukY!mmUOKG7xwWhl HcQp0eG vzwUGqZ XEfJxyJPoFzezoh3pw O9XD9bST-_C PW EGZ $_ Please ead the Quick Guide to Con inu ng you Submiss on, which shows how you can access you manusc ipt, and subm t t back to the s te. The Gu de s located at _h l f 3 h 01 f l l ?u l=h tps*3A*2F*2Fu ldefense com*2Fv3*2F http*3A*2F*2Fmc manusc iptcent al.com*2Fsociety mages*2Ftandf qs0*2FCon inun ng*20a*20Subm ssion sc eenshot pdf *3BJSU !KGKeukY!nGv1RgRJ1P-OGXuZi8b2hKGjXxDFOmBwDONuR njCdwERJF1HkBIV4Sggq 9 e6Z8tA*24&amp data=02*7C01*7CShan.Lu*40umassmed edu*7Cde7fc6818f7047e0d79008d7b08c97ea*7Cee9155fe2da34378a6c44405faf57b2e*7C0*7C0*7C637171990355158902&amp sdata=JElO3OLwBEDX pTKWpzb2Az5*2FliUA32F5Sn1KhHtVy8*3D&amp ese ved=0 JSU JSU JSU JSUqKiU JSU JSU JSU JQ!!KGKeukY!mmUOKG7xwWhl HcQp0eGivzwUGqZ XE JxyJPoFzezoh3pwjO9XD9bST-_C 8CF7I$_

You may contact the Edito ial O fice if you have fu the quest ons.

S nce ely,

Jo g e Lyn Luna
Eme ging M c obes & Infect ons Edi o al Off ce
temi-pee ev ew@ ou nals tandf co uk

From: Su, Lishan
To: Liu, Shan-Lu
Subject: Re: Executive summary of EMI commentary
Date: Sunday, February 23, 2020 12:22:53 PM
Attachments: image001.png
image002.png
刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论.docx

One more time.

-Lishan

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 23, 2020 at 12:16 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: Executive summary of EMI commentary

See revised word/pdf.

-Lishan

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 23, 2020 at 12:07 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: Executive summary of EMI commentary

This may be more accurate? 刘善虑苏立山等教授发文分析认为新冠病毒阴谋论缺乏病毒学证据

-Lishan

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 23, 2020 at 11:54 AM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: Executive summary of EMI commentary

See pdf file with no lines.
I can not find a better word than驳斥, but it seems to a be a bit stronger than we intended?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 23, 2020 at 8:57 AM

To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Executive summary of EMI commentary

Lishan and Shan – so confusing!

I have wrapped up a summary for the public and media to understand key points of our commentary. Please make suggestions.

I think this can go along with the Chinese translation.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 7:39 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

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Date: Friday, February 21, 2020 at 7:33 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

I just went online to see if we replace with a new one.
Could you send me the PDF of the corrected one?

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

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To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

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Subject: Re: Submitted Corrections for article TEMI 1733440

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I just saw that you deleted nt for 1,100 – “nt” should be kept. Could you correct that?

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Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

Begin forwarded message:

> **From:** "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
> **Date:** February 21, 2020 at 4:04:41 PM PST
> **To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>, "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Subject: Submitted Corrections for article TEMI 1733440**
> **Reply-To:** TEMI-production@journals.tandf.co.uk
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
>
> Journal: *Emerging Microbes & Infections* (TEMI)
>
> Article ID: TEMI 1733440
>
> Dear author,
>
> This email confirms that you have submitted your corrections to your article proofs.
>
> The submitted corrections have been successfully uploaded.
>
> If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.
>
> Yours sincerely,
>
> Malathi Boopalan
>
> Email:TEMI-production@journals.tandf.co.uk

刘善虑苏立山等教授发文分析认为新冠病毒阴谋论缺乏病毒学证据

俄亥俄州立大学教授刘善虑，北卡大学教堂山分校教授苏立山联名世界冠状病毒学专家 Linda J. Saif（美国科学院院士）以及 Susan Weiss（美国微生物科学院院士），在 国际期刊 Emerging Microbes & Infections (EMI) (中文译名《新发微生物与感染》)发表题为“**没有可信的证据支持 SARS-CoV-2 来自实验室人工合成**”的评论文章，对最近广为流行的传言和阴谋论进行了分析和驳斥。

该文主要论点如下：

1. 新型冠状病毒 SARS-CoV-2 虽然与中国科学院武汉病毒所最近报道的一种称 为 RaTG13 的蝙蝠冠状病毒有高达 96%的同源性， 但两者仍然有超过 1，100 碱基的差别，而且在关键序列序列上有特征性的区别，因此两者是完全不同的冠状病毒。
2. 社交媒体指向 2015 年在《自然医学》一篇论文，认为新型冠状病毒是这篇文章报道的人 SARS 和蝙蝠冠状病毒（SHC014）的嵌合病毒的泄露。分析研究表明，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒在基因组序列上有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。
3. 还有一种传言说 SARS-CoV-2 是实验室中有意人为制造的， 并以发表在 BioRxiv 上印度科学家的一份手稿中为依据，声称 SARS-CoV-2 中含有 HIV 序列。实际上这篇文章在线后， 舆论哗然，世界各国病毒学家也纷纷反驳。 在 HIV-1 专家高峰（Feng Gao）领衔领导发表在 EMI 的反驳论文中，作者使用了仔细的生物信息学分析来证明，指出最初声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的。由于国际社会提出的种种疑问，这篇手稿的作者已经撤回了该手稿， 不再要求发表。
4. 从科学层面讲，进化是循序渐进的，并随着时间的推移进一步产生有利于病毒的突变。相反，人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。
5. 尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人工制造，我们认为对公共健康有威胁的病毒都必须进行恰当的实验室管理，而且需要由科学界和政府合理监管。

From: Liu, Shan-Lu
To: Su, Lishan; Lu, Shan
Subject: Re: Revised commentary for EMI - final!
Date: Monday, February 17, 2020 6:10:04 PM

I think our points are made in the commentary. Shan did not plan initially to go into much science, but in the end I think we have covered most of it.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Monday, February 17, 2020 at 6:04 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Revised commentary for EMI - final!

I agree with them completely. Based on Shi's two natures papers and the Baric Nature medicine paper, I was trying to make the point as this paper: that the new virus from bats could have jumped into a secondary host or directly to humans and evolve. One of you did not seem to like the direct human possibility and removed it.

Theories of SARS-CoV-2 origins

It is improbable that SARS-CoV-2 emerged through laboratory manipulation of an existing SARS-related coronavirus. As noted above, the RBD of SARS-CoV-2 is optimized for human ACE2 receptor binding with an efficient binding solution different to that which would have been predicted. Further, if genetic manipulation had been performed, one would expect that one of the several reverse genetic systems available for betacoronaviruses would have been used. However, this is not the case as the genetic data shows that SARS-CoV-2 is not derived from any previously used virus backbone[17]. Instead, we propose two scenarios that can plausibly explain the origin of SARS-CoV-2: (i) natural selection in a non-human animal host prior to zoonotic transfer, and (ii) natural selection in humans following zoonotic transfer.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Monday, February 17, 2020 at 5:56 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

This is the website that people deposit their sequence data and also make relevant comments. Not sure where they will publish it… but it has been widely spread via Twitter.

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>

Date: Monday, February 17, 2020 at 5:44 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Revised commentary for EMI - final!

Who is first is not critical. But where did you find this new paper? Published?

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 17, 2020 5:42 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

Again, I have no concern at all with our conclusion in the commentary. I believe more scientific articles like this will be out, and EMI will be one of the first to publish them.

Cheers!

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Monday, February 17, 2020 at 5:36 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Revised commentary for EMI - final!

Agreed. Beautifully written.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 17, 2020 5:35 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

I just carefully read through, very informative and convincing in my view. Those are of course true experts of evolutionary biologists.

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Monday, February 17, 2020 at 5:27 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Revised commentary for EMI - final!

This still has nothing to do with any of the specific claims.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 17, 2020 5:26 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Revised commentary for EMI - final!

The last section is to dispute those rumors.

Theories of SARS-CoV-2 origins

It is improbable that SARS-CoV-2 emerged through laboratory manipulation of an existing SARS-related coronavirus. As noted above, the RBD of SARS-CoV-2 is optimized for human ACE2 receptor binding with an efficient binding solution different to that which would have been predicted. Further, if genetic manipulation had been performed, one would expect that one of the several reverse genetic systems available for betacoronaviruses would have been used. However, this is not the case as the genetic data shows that SARS-CoV-2 is not derived from any previously used virus backbone[17]. Instead, we propose two scenarios that can plausibly explain the origin of SARS-CoV-2: (i) natural selection in a non-human animal host prior to zoonotic transfer, and (ii) natural selection in humans following zoonotic transfer. We also discuss whether selection during passage in culture could have given rise to the same observed features.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Monday, February 17, 2020 at 5:23 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Revised commentary for EMI - final!

Two different things. They are doing SARS2 genome analysis. Your is trying to disapprove the other theories.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Monday, February 17, 2020 5:17 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: Revised commentary for EMI - final!

…SARS-CoV-2 is the seventh member of the *Coronaviridae* known to infect humans. Three of these viruses, SARS CoV-1, MERS, and SARS-CoV-2, can cause severe disease; four, HKU1, NL63, OC43 and 229E, are associated with mild respiratory symptoms. Herein, we review what can be deduced about the origin and early evolution of SARS-CoV-2 from the comparative analysis of available genome sequence data. In particular, we offer a perspective on the notable features in the SARS-CoV-2 genome and discuss scenarios by which these features could have arisen. Importantly, this analysis provides evidence that SARS-CoV-2 is not a laboratory construct nor a purposefully manipulated virus.
…

We need to try to get ours out quickly.

SL

From:	Su, Lishan
To:	Liu, Shan-Lu
Subject:	Re: Executive summary of EMI commentary
Date:	Sunday, February 23, 2020 12:24:53 PM
Attachments:	image001.png image002.png 刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论.docx 刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论.Final.pdf

---

See revised word/pdf.

-Lishan

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 23, 2020 at 12:07 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: Executive summary of EMI commentary

This may be more accurate? 刘善虑苏立山等教授发文分析认为新冠病毒阴谋论缺乏病毒学证据

-Lishan

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 23, 2020 at 11:54 AM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: Re: Executive summary of EMI commentary

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Subject: Submitted Corrections for article TEMI 1733440
Reply-To: TEMI-production@journals.tandf.co.uk

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear author,

This email confirms that you have submitted your corrections to your article proofs.

The submitted corrections have been successfully uploaded.

If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.

Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

刘善虑苏立山等教授发文分析认为新冠病毒阴谋论缺乏病毒学证据

俄亥俄州立大学教授刘善虑，北卡大学教堂山分校教授苏立山联名世界冠状病毒学专家 Linda J. Saif（美国科学院院士）以及 Susan Weiss（美国微生物科学院院士），在 国际期刊 Emerging Microbes & Infections (EMI) (中文译名《新发微生物与感染》)发表题为“**没有可信的证据支持 SARS-CoV-2 来自实验室人工合成**”的评论文章，对最近广为流行的传言和阴谋论进行了分析和驳斥。

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4. 从科学层面讲，进化是循序渐进的，并随着时间的推移进一步产生有利于病毒的突变。相反，人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。
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From: Liu, Shan-Lu
To: temi-peerreview@journals.tandf.co.uk
Cc: shan.lu@umassmed.edu; lishan_su@med.unc.edu
Subject: Re: Urgent: revised commentary for Emerging Microbes & Infections - TEMI-2020-0121
Date: Sunday, February 16, 2020 7:59:43 PM
Attachments: Liu et al_EMI Commentary_Revision_Final.docx
image001.png

Here is the attachment, sorry!

_____

Dear Jorgie,

After discussing with Dr. Shan Lu and all coauthors, we have decided to use a new title and also make minor changes to the text, including assciated references. I have attached the updated commentary and hope that you will be able to help upload the new version for preparing the proof.

Thank you!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M D , Ph D
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "temi-peerreview@journals.tandf.co.uk" <temi-peerreview@journals.tandf.co.uk>
Date: Thursday, February 13, 2020 at 9:14 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "shan.lu@umassmed.edu" <shan.lu@umassmed.edu>, "lishan_su@med.unc.edu" <lishan_su@med.unc.edu>
Subject: Re: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission #TrackingId:5633996

Dear Professor Liu,

Thank you very much for sending his file.

Kindly be informed that I have now uploaded in he system on your behalf and proceeded your paper to the editor.

Please let me know if you have further questions or concerns.

Kind regards,

Jorgie Lyn Luna - Journal Editorial Office
Taylor & Francis Group
4 Park Square | Milton Park | Abingdon | Oxon | OX14 4RN UK
Web: www.tandfonline.com

Taylor & Francis is a trading name of Informa UK Limited,
registered in England under no. 1072954

Emerging Microbes & Infections

From:liu.6244@osu.edu
Sent:
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Hi Jorgie:

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Thank you.

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Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
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On 2/13/20, 8:43 AM, "Emerging Microbes and Infections" <onbehalfof@manuscriptcentral.com> wrote:

13-Feb-2020

Dear Professor Liu,

Your above referenced manuscript, entitled "SARS-CoV-2: no evidence of a laboratory origin" requires some further changes before it is ready for reviewing in Emerging Microbes & Infections. Your submission has been returned to you and is located in your Author Center as a draft, so that you due to these reasons:

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Kindly add a line numbering in your main document.

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You may contact the Editorial Office if you have further questions.

Sincerely,

Jorgie Lyn Luna
Emerging Microbes & Infections Editorial Office
temi-peerreview@journals.tandf.co.uk

No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

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Columbus, OH 43210, USA

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[5] Food Animal Health Research Program,
Ohio Agricultural Research and Development Center, CFAES
Department of Veterinary Preventive Medicine,
The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural

evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed

to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already

exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses

with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

From:	Liu, Shan-Lu
To:	Weiss, Susan
Subject:	Re: [External] Re: name for new CoV
Date:	Sunday, February 16, 2020 1:32:08 PM
Attachments:	image001.png image002.png

Not yet, unfortunately!

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Sunday, February 16, 2020 at 1:31 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: name for new CoV

Not in BioRx?
I am anxious to know also

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 1:23 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: [External] Re: name for new CoV

Not published yet. I heard that they submitted it Nature for review.
I am very eager to know if the pangolin virus isolated from the market has the RRAR insertion; it is not present in the Viruses paper 2019!

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Sunday, February 16, 2020 at 1:18 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: name for new CoV

Btw- have you heard any more about the pangolin connection- I see nothing in pub med expect a paper from before the outbreak claiming to find CoV sequences in dead pangolins in the south of China
susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 1:16 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: [External] Re: name for new CoV

Susan:

I agree with you. I don't see any evidence of lab origin, as no lab people have been infected, but with some rumors – just rumors!

COVID-19 is the disease name defined by WHO. I still feel SARS-CoV-2 is a good one adopted my Chinese American virologists.

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Sunday, February 16, 2020 at 1:11 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: name for new CoV

I have a couple of comments

I don't think it is likely that bat virus leaked into humans in the lab- is there any evidence that someone from the Wuhan lab is infected? Also in general the bat viruses have been identified by sequence sand are not actually isolated viruses.

RRAR is a good if not excellent furin site, similar to MERS- MHV A59 is RRAHR, MHV JHM is RRARR (a very good one) – lineage B Bat viruses generally do not have the furin site
I doubt very much it was engineered in in the lab. Doesn't make sense

I wonder if there is some compromise position re the name- the formal name I think has to be SARS-CoV-2 but maybe can be referred to COVID-19 informally- if you look at the internet WHO is calling it COVID-19

Susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 10:05 AM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: [External] Re: name for new CoV

Susan,

I have looked at carefully the RaTG13 sequence, and it is unlikely from it – also see attached file. But we cannot rule out the possibility of other bat viruses from the lab – The Wuhan lab has many bat samples not yet worked out or results published. There are some concerns that some of their samples may not have been handled properly

and leaked out of the lab…But just a possibility.

Right now, it's hard to say an intermediate host or directly from bats, I guess.

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Sunday, February 16, 2020 at 9:48 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: name for new CoV

Do you think it could come from a bat virus- which one or an unpublished one? RaTg13 is the closest? Is it close enough in sequence? Do you think it came through an intermediate host and sequence drifted?

This is a very chilling idea

susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 9:41 AM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: [External] Re: name for new CoV

Dear Susan,

I strongly support the new name SARS-CoV-2, as I feel that it does reflect what we currently know. I do understand the feeling of those Chinese colleagues, but I dislike their political motivations. They have also approached me, but I have publicly expressed my support of the new name in some Chinese media.

In terms of our commentary to be published in EMI, we may change the title to emphasize that the new virus is not laboratory engineered, "**SARS-CoV-2: no evidence for laboratory engineering",** because we cannot rule out the possibility that it comes from a bat virus leaked out of a lab. When the proof comes, I will write to you and others.

Best wishes.

Shan-Lu

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Sunday, February 16, 2020 at 9:10 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: name for new CoV

Dear Shan-Lu

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Which side of this argument are you on?

Susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 10:25 PM
To: Min Yang <min.yang@emi2012.org>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>

Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: [External] Re: EMI commentary

Min:

It should have been successfully submitted. See below email:

12-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections.

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Thank you for submitting your manuscript to Emerging Microbes & Infections.

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From: Min Yang <min.yang@emi2012.org>
Date: Wednesday, February 12, 2020 at 10:17 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Dear Dr Liu,

Thank you for your support to EMI.

According to the attachment, it looks like your submission is a DRAFT still which has not been submitted successfully yet.

Could you please check and confirm?

Thanks and regards,

Min Yang

Emerging Microbes & Infections (EMI) Editorial Office
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日期: 2020年2月13日 星期四 上午10:58
收件人: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
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From: Liu, Shan-Lu
To: Weiss, Susan
Subject: Re: name for new CoV
Date: Sunday, February 16, 2020 9:40:00 AM
Attachments: image001.png
image002.png

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Phone: (614) 292-8690
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Liu, Shan-Lu
To: Su, Lishan; Shan Lu
Subject: FW: Author Publishing Agreement Received for article TEMI 1733440
Date: Wednesday, February 19, 2020 11:52:41 PM

Done, thank you Lishan!

SL

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Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Wednesday, February 19, 2020 at 8:51 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Author Publishing Agreement Received for article TEMI 1733440

Article: SARS-CoV-2: no evidence of a laboratory origin

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

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Thank you for submitting your author publishing agreement for the article listed above. You will receive an email once your author publishing agreement has been accepted, or if any problems are identified.

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Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From: Liu, Shan-Lu
To: TEMI-production@journals.tandf.co.uk
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591
Date: Tuesday, February 25, 2020 11:48:07 PM

Thanks. Let me know as soon as it is online.
Thank you.

Shan-Lu Liu sent from iPhone

> On Feb 25, 2020, at 11:28 AM, "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk> wrote:
>
> Dear Shan-Lu,
>
> This would be online in one or two days.
>
> Regards,
>
> Malathi
> Emerging Microbes & Infections
>
> ---
>
> **From:**liu.6244@osu.edu
> **Sent:**25-02-2020 08.50 AM
> **To:**TEMI-production@journals.tandf.co.uk
> **Cc:**
> **Subject:**Re: URGENT! Submitted Corrections for article TEMI 1733440
>
> Possible to let us know the publication date? Thanks
>
> Shan-Lu Liu sent from iPhone
>
> > On Feb 24, 2020, at 1:01 AM, "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk> wrote:
> >
> > Dear Shan-Lu Liu,

Thank you for the email. I have sent your additional correction to the team so that they will make the changes in the final PDF.

Have a great day!

Regards,

Malathi
Emerging Microbes & Infections

From:liu.6244@osu.edu
Sent:22-02-2020 06:24
To:malathi@novatechset.com
Cc:
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440

Dear Malathi Boopalan:

We have just uploaded a corrected proof online, but relegalized a small error: "1,100" should be read as "1,100 nt" – could you kindly help make the correction, or replace the uploaded file with the attached new one?

Thank you! Please confirm.

Shan-Lu Liu & Lishan Su

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Shan-Lu Liu sent from iPhone

On Feb 21, 2020, at 4:04 PM, TEMI-production@journals.tandf.co.uk <cats@taylorandfrancis.com> wrote:

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear author,

This email confirms that you have submitted your corrections to your article proofs.

The submitted corrections have been successfully uploaded.

If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.

Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From: Liu, Shan-Lu
To: Su, Lishan
Cc: Lu, Shan
Subject: Executive summary of EMI commentary
Date: Sunday, February 23, 2020 8:56:59 AM
Attachments: 刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论.docx
image001.png
image002.png

Lishan and Shan – so confusing!

I have wrapped up a summary for the public and media to understand key points of our commentary. Please make suggestions.

I think this can go along with the Chinese translation.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 7:39 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:33 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

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THE OHIO STATE UNIVERSITY

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Phone: (614) 292-8690

Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:32 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

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To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

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Thanks.

SL

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Phone: (614) 292-8690
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

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Date: Friday, February 21, 2020 at 4:28 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

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-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

Begin forwarded message:

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> **Date:** February 21, 2020 at 4:04:41 PM PST
> **To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>, "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Subject: Submitted Corrections for article TEMI 1733440**
> **Reply-To:** TEMI-production@journals.tandf.co.uk
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
>
> Journal: *Emerging Microbes & Infections* (TEMI)
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> Article ID: TEMI 1733440
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> Malathi Boopalan
>
> Email:TEMI-production@journals.tandf.co.uk

刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论

俄亥俄州立大学教授**刘善虑**，北卡大学教堂山分校教授苏立山联名美国科学院院士 Linda J. Saif 以及美国微生物科学院院士 Susan Weiss，在 国际期刊 Emerging Microbes & Infections (EMI) (中文译名《新发微生物与感染》)发表题为“**没有可信的证据支持 SARS-CoV-2 来自实验室人工合成**”的评论文章，对最近广为流行的传言和阴谋论进行了分析和驳斥。

该文主要论点如下：

1. 新型冠状病毒 SARS-CoV-2 虽然与中国科学院武汉病毒所最近报道的一种称 为 RaTG13 的蝙蝠冠状病毒有高达 96%的同源性， 但两者仍然有超过 1，100 碱基的差别，而且在关键序列序列上有特征性的区别，因此两者是完全不同的冠状病毒。
2. 社交媒体指向 2015 年在《自然医学》一篇论文，认为新型冠状病毒是这篇文章报道的人 SARS 和蝙蝠冠状病毒（SHC014）的嵌合病毒的泄露。分析研究表明，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒在基因组序列上有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。
3. 还有一种传言说 SARS-CoV-2 是实验室中有意人为制造的， 并以发表在 BioRxiv 上印度科学家的一份手稿中为依据，声称 SARS-CoV-2 中含有 HIV 序列。实际上这篇文章在线后， 舆论哗然，世界各国病毒学家也纷纷反驳。 在 HIV-1 专家高峰（Feng Gao）领衔领导发表在 EMI 的反驳论文中，作者使用了仔细的生物信息学分析来证明，指出最初声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的。由于国际社会提出的种种疑问，这篇手稿的作者已经撤回了该手稿， 不再要求发表。
4. 从科学层面讲，进化是循序渐进的，并随着时间的推移进一步产生有利于病毒的突变。相反，人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。
5. 尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人工制造，我们认为对公共健康有威胁的病毒都必须进行恰当的实验室管理，而且需要由科学界和政府合理监管。

From: Liu, Shan-Lu
To: TEMI-production@journals.tandf.co.uk
Subject: URGENT! Submitted Corrections for article TEMI 1733440
Date: Friday, February 21, 2020 7:54:39 PM
Attachments: cidimage001.png@01D3AAF8.8AA7CB20

Dear Malathi Boopalan:

We have just uploaded a corrected proof online, but relegalized a small error: "1,100" should be read as "1,100 nt" – could you kindly help make the correction, or replace the uploaded file with the attached new one?

Thank you! Please confirm.

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Email:TEMI-production@journals.tandf.co.uk

From: Liu, Shan-Lu
To: Liu, Shan-Lu
Subject: FW: Executive summary of EMI commentary
Date: Sunday, February 23, 2020 1:22:20 PM
Attachments: Liu et al_EMI Commentary_Revision_中文-Shan Lu.docx
Liu et al_EMI Commentary_Revision_中文-Shan Lu.pdf
刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论_Final.docx
刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论_Final.pdf
image001.png
image002.png

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Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Shan-Lu Liu <liu.6244@osu.edu>
Date: Sunday, February 23, 2020 at 1:21 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Executive summary of EMI commentary

Now, the final versions, a total of 4 files - hopefully!

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 23, 2020 at 1:15 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Executive summary of EMI commentary

Overall they are very good.

I found one minor error: "Dec" was used for the last reference of your paper. It should be "Feb". You may want to change the current word and pdf, but not change the real paper to be published as

it may take a lot of more time to current and reload to online. Readers can find that paper without much problem.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 12:57 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Executive summary of EMI commentary

See my final versions of two files, Word and PDF.

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 23, 2020 at 12:51 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Executive summary of EMI commentary

Your school's email screening system is good! Thanks.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 12:49 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Executive summary of EMI commentary

Never, just received Shan's email and file! Slow on my end.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 23, 2020 at 12:48 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Executive summary of EMI commentary

Great work. However, I made some new changes (see attached). All highlighted or marked, for your reference.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 8:57 AM
To: Su, Lishan <lishan_su@med.unc.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Executive summary of EMI commentary

Lishan and Shan – so confusing!

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Shan-Lu

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Date: Friday, February 21, 2020 at 7:39 PM
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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:28 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
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> Malathi Boopalan
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没有可信的证据支持 SARS-CoV-2 来自实验室人工合成

Shan-Lu Liu（刘善虑）, 俄亥俄州立大学

Linda J. Saif, 俄亥俄州立大学

Susan Weiss, 宾夕法尼亚大学

Lishan Su,（苏立山）北卡大学教堂山分校

截止 2020 年 2 月 10 日，在武汉出现和爆发的急性呼吸疾病已波及 4 万多人，导致 1000 多人死亡。研究人员很快找到了一种新型人的冠状病毒，称之为 2019 nCoV 或 SARS-CoV-2，而相应的疾病称之为 COVID-19，意为 2019 年发生的冠状病毒疾病 (https://globalbiodefense com/novel-coronavirus-covid-19-portal/)。

据现有的报道[1-3]，COVID-2019 与 SARS-CoV 导致的 SARS 有很多相似的临床表现。而 SARS-CoV-2 基因组序列也和 2003 年 SARS-CoV 有 80%同源性，但它与一些蝙蝠的乙型冠状病毒更为相似。当前，种种的推测、谣言和阴谋论到处流行，其中有的认为 SARS-CoV-2 来源于实验室基因工程制造。也有某些人声称，人的 SARS-CoV-2 是从武汉的某个实验室直接泄漏出来的， 其根据是该实验室最近报道了一种称为 RaTG13 的蝙蝠冠状病毒，它和 SARS-CoV-2 基因组序列有高达 96%的同源性。

然而，我们知道，2003 年发现人 SARS 冠状病毒和其中间宿主果子狸 SARS 样冠状病毒具有 99.8％的同源性，在整个基因组中只有 202 个碱基不同。鉴于人类新型 SARS-CoV-2 与蝙蝠 RaTG13-CoV 之间有超过了 1000 个不同碱基[4]，且这些差异是按照冠状病毒典型的进化特征按自然发生的模式分布在整个基因组中，我们认为 SARS-CoV-2 直接来源于 RaTG13 冠状病毒的可能性极小。更为重要的是，在新的人 SARS-CoV-2 病毒基因组序列中并没有任何可信的基因工程改造的迹象，这都揭示 SARS-CoV-2 是通过自然演化而来的。我们认为在蝙蝠与人类之间可以找到中间动物宿主含有类似的冠状病毒，它与 SARS-CoV-2 更相似。最近有消息称穿山甲可能携带与 SARS-CoV-2 密切相关的冠

状病毒，但论文和数据尚未正式发表，无从得以证实(https://www.nature.com/articles/d41586-020-00364-2)。

最近社交媒体上的另一种说法指向 2015 年在《自然医学》发表的一篇论文[7]。该论文报道了在小鼠适应后的人类 SARS 冠状病毒（MA15 病毒）中，人工构建了带有蝙蝠冠状病毒（SHC014）S 基因，这种合成的嵌合冠状病毒，不仅可以可以感染小鼠，也能够感染来源人的细胞[8]。然而，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒基因组序列有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。

现在我们来理一理来人 SARS 病毒老鼠适应株 MA15 和它的衍生病毒的来龙去脉。适应小鼠的 SARS 病毒（MA15）[9]是通过把 SARS 冠状病毒在小白鼠呼吸道中连续传代 15 后产生的；适应后的 SARS 冠状病毒有六个氨基酸突变，使其能够更有效地感染小鼠，尤其是在老年小鼠中具有了更高的复制活性和肺部致病性能（因此称为 M15）。由于在小鼠内适应的遗传突变，MA15 在人细胞或者人体内感染很可能降低了。

科学家曾认为从蝙蝠身来的冠状病毒的 S 基因和人的 SARS 病毒不同，推测它们无法使用人的 SARS 病毒受体 ACE2 进入人体细胞[10, 11]；后来发现果子狸是蝙蝠冠状病毒传给人的中间宿主，能够将 SARS 冠状病毒传播给人类[6，12]。然而，2013 年以来，科学家陆续从中国马蹄蝠中分离到了数个新型蝙蝠冠状病毒，这些来自蝙蝠的，类似人 SARS 冠状病毒（SL-CoV-WIV1）能够使用人、果子狸和中国马蹄蝠的 ACE2 受体进入和感染细胞[8]。进化研究表明，在 SARS 冠状病毒 S 蛋白的作用接触位点上，蝙蝠 ACE2 基因在与人类 ACE2 基因在相同的位点上同样被进化选择[13]。基于这样的发现，科学家提出了蝙蝠的 SARS 样冠状病毒具有直接传染到人的能力，不必需要中间宿主环节；也就是说有些蝙蝠冠状病毒有可能直接感染人类宿主细胞。为了直接验证这种可能性，蝙蝠冠状病毒 SL-SHC014 的 S 基因被人工嫁接到了 MA15 SARS-CoV 骨架上，因此产生了一个嵌合病毒。此 SL-SHC014-MA15 嵌合病毒确实能够有效地利用人 ACE2 进入细胞，并在人的呼吸道实验细胞中有效复制。SL-SHC014-MA15 也可以在小鼠的肺中高效复制，但与 SARS MA15 相比，感染减弱了，并且只会让老年小鼠致命[7]。

由于 SL-SHC014-MA15 嵌合病毒相对于另一个人 SARS-S/MA15 嵌合病毒在小鼠中具有更高的致病活性，这种嵌合冠状病毒的实验后来在美国政府的干预下被暂停(https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-

pause-gain-function-research)。虽然目前 这项禁令在美国已经被解除，但构建这种具有大流行病潜力的病毒是否是一种风险，在当前的COVID-2019流行的形势下又重新引发了讨论，成为热点话题。然而，经过多个国家科学家对病毒的分子进化分析[5，14]，SARS-CoV-2 无疑与 SL-SHC014-MA15 具有非常大的不同，整个基因组有大约 6,000 核苷酸的差异。因此，没有可信的证据支持 SARS-CoV-2 是源自 SL-SHC014-MA15 嵌合病毒的说法。

最近也有传言说，SARS-CoV-2 是实验室中有意人为制造的。其中发表在 BioRxiv（一个同行评审之前的手稿共享网站）的一份手稿中更是此传言的代表，它声称 SARS-CoV-2 中含有 HIV 序列，因此很可能是在实验室中产生的。文章在线后， 舆论哗然，世界各国的多个病毒学者纷纷反驳。 在 HIV-1 病毒专家高峰（Feng Gao）领衔领导的反驳论文中，他们使用了仔细的生物信息学分析来证明，指出最初声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的 [15]。由于国际社会提出的种种疑问，这篇手稿的作者已经撤回了该手稿， 不再要求发表。

从科学层面讲，进化是循序渐进的，并随着时间的推移进一步产生有利于病毒的突变，就像天然分离的病毒（如蝙蝠冠状病毒 RaTG13）基因组那样。 相反，人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以我们认为，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。 有一种可能不能排除， 就是 SARS-CoV-2 是一种蝙蝠冠状病毒与另一种冠状病毒之间进行了自然重组而产生的；但这种可能性需要更多的研究来证明，来回答 SARS-CoV-2 的自然起源问题。我们需要强调的是，尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人工制造，但对公共健康有威胁的病毒都必须进行恰当的实验室管理，而且需要由科学界和政府合理监管。

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Feb;9(1):378-381.

刘善虑苏立山等教授发文分析认为新冠病毒阴谋论缺乏病毒学证据

俄亥俄州立大学教授刘善虑，北卡大学教堂山分校教授苏立山联名国际著名冠状病毒学家 Linda J. Saif（美国科学院院士）以及 Susan Weiss（美国微生物科学院院士），在 国际期刊 Emerging Microbes & Infections (EMI) (中文译名《新发微生物与感染》)发表题为“**没有可信的证据支持 SARS-CoV-2 来自实验室人工合成**”的评论文章，对最近广为流行的传言和阴谋论进行了分析和驳斥。

该文主要论点如下：

1. 新型冠状病毒 SARS-CoV-2 虽与中国科学院武汉病毒所最近报道的一个称为 RaTG13 的蝙蝠冠状病毒有高达 96%的同源性， 但两者仍然有超过 1，100 碱基的差别，而且在关键序列序列上有特征性区别，因此两者是完全不同的冠状病毒。
2. 社交媒体指向 2015 年在《自然医学》（Nature Medicine）一篇论文，认为新型冠状病毒是这篇文章报道的人 SARS 与蝙蝠冠状病毒（SHC014）的嵌合病毒的泄露。然而，分析研究表明，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒在基因组序列上有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。
3. 还有一种传言说 SARS-CoV-2 是实验室中有意人为制造的， 并以发表在 BioRxiv 上印度科学家的一份手稿中为依据，声称 SARS-CoV-2 中含有 HIV 序列。实际上这篇文章在线发表后， 舆论哗然，世界各国病毒学家也纷纷反驳。 在 HIV-1 专家高峰（Feng Gao）领衔领导发表在 EMI 的另一篇反驳论文中，作者使用了仔细的生物信息学分析来证明，指出原文作者声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的。由于国际社会提出的种种疑问，这篇手稿的作者也已经撤回了该手稿， 目前没有发现再次发表。
4. 从科学层面讲，病毒进化是循序渐进的，并切随着时间的推移进一步产生有利于病毒感染人的突变。相反，人工合成的病毒基因组通常会在已知的病毒骨架引入一些某些定向的变化。所以，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。
5. 尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人为制造，我们认为对公共健康有威胁的病毒都必须进行严格恰当的实验室管理，而且需要由科学界和政府联合监管。

From:	Liu, Shan-Lu
To:	Weiss, Susan
Subject:	Re: [External] Re: name for new CoV
Date:	Sunday, February 16, 2020 10:04:27 AM
Attachments:	Tackling Rumors of a Suspicious Origin of nCoV2019 - Novel 2019 coronavirus - nCoV-2019 Evolutionary History - Virological.pdf image001.png image002.png

Susan,

I have looked at carefully the RaTG13 sequence, and it is unlikely from it – also see attached file. But we cannot rule out the possibility of other bat viruses from the lab – The Wuhan lab has many bat samples not yet worked out or results published. There are some concerns that some of their samples may not have been handled properly and leaked out of the lab…But just a possibility.

Right now, it's hard to say an intermediate host or directly from bats, I guess.

Shan-Lu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Sunday, February 16, 2020 at 9:48 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: [External] Re: name for new CoV

Do you think it could come from a bat virus- which one or an unpublished one? RaTg13 is the closest? Is it close enough in sequence? Do you think it came through an intermediate host and sequence drifted?

This is a very chilling idea

susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Sunday, February 16, 2020 at 9:41 AM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: [External] Re: name for new CoV

Dear Susan,

I strongly support the new name SARS-CoV-2, as I feel that it does reflect what we currently know. I do understand the feeling of those Chinese colleagues, but I dislike their political motivations. They have also approached me, but I have publicly expressed my support of the new name in some Chinese media.

In terms of our commentary to be published in EMI, we may change the title to emphasize that the new virus is not laboratory engineered, "**SARS-CoV-2: no evidence for laboratory engineering",** because we cannot rule out the possibility that it comes from a bat virus leaked out of a lab. When the proof comes, I will write to you and others.

Best wishes.

Shan-Lu

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From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Sunday, February 16, 2020 at 9:10 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: name for new CoV

Dear Shan-Lu

I was approached about the controversy about the name of the new CoV and asked me to support a request for change in name. When I heard the name SARS-CoV-2 I initially didn't like it at all because it seemed like it would confused with SARS. However, after reading the BioRx article form CGS about the naming, it does makes sense in terms of the other SARS like viruses form bats, I understand that some the Chinese scientists are upset about this and feel it will have a bad psychological effect for China and if it comes back each year like flu it will have a big impact on business investment and tourism etc, which also makes sense.

Which side of this argument are you on?

Susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 10:25 PM
To: Min Yang <min.yang@emi2012.org>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: [External] Re: EMI commentary

Min:

It should have been successfully submitted. See below email:

12-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections.

Your manuscript ID is TEMI-2020-0121.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__;!!KGKeukY!klcqOriA-xIzLyrzwwKWtghNAQgvfbCh7pqavzMYm77fJJsm_iShbXJWIKEtRML7ExI$ and edit your user information as appropriate.

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Thank you for submitting your manuscript to Emerging Microbes & Infections.

Sincerely,
Emerging Microbes & Infections Editorial Office

From: Min Yang <min.yang@emi2012.org>
Date: Wednesday, February 12, 2020 at 10:17 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Dear Dr Liu,

Thank you for your support to EMI.

According to the attachment, it looks like your submission is a DRAFT still which has not been submitted successfully yet.

Could you please check and confirm?

Thanks and regards,

Min Yang

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131 Dongan Road
Shanghai
China
Tel: 86-21-54237992
E-mail: min.yang@emi2012.org

发件人**:** "Liu, Shan-Lu" <liu.6244@osu.edu>
日期**:** 2020年2月13日 星期四 上午10:58
收件人**:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
抄送**:** Min Yang <min.yang@emi2012.org>, "Lu, Shan" <Shan.Lu@umassmed.edu>
主题**:** EMI commentary

Dear all,

I have just submitted a commentary to EMI. See attached the submitted version.

Thank you.

Shan-Lu

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Tackling Rumors of a Suspicious Origin of nCoV2019

profbillg1901 **4d**

I have been privately dealing with rumors and inquiries, focused on the RRAR potential furin cleavage site, that nCoV2019 may have a suspicious origin as an engineered, laboratory-generated virus either accidentally or deliberately released in the area of the Wuhan seafood and animal market. The publication of the highly similar RaTG13 sequence about a week ago has fueled this type of speculation.

As I have told people privately, I see no evidence at all to support such a claim. In sharp contrast, I have studied the question in detail, using RaTG13 and Wuhan sequence at the S1/S2 boundary, and find convincing proof of exactly opposite conclusion – that RaTG13 could NOT be a proximal source of the Wuhan virus.

At first glance of an alignment of the S protein sequence of both, it is natural that the issue of an engineered insertion should be considered. On either side of the new furin site, the amino acid sequence is identical in both from aa614 to aa1133 – an apparent insert of PRRA is the only difference in an otherwise 100% conserved 519 amino acid region.

But that is at first glance.

One has to consider that the PRRA is an unusual sequence to introduce to generate a furin site – others even among coronaviruses like MHV A59 are so much better. Also that the underlying code CCTCGGCGGGCA introduces an unnecessarily G and C rich region where none otherwise exists. Not likely scenarios for something a gene jockey would do.

Then one looks at the actual RNA alignment. The “insert” is actually not in frame, but CTCCTCGGCGGG, or -2 out of frame. Again, who does that?

But the PROOF lies in looking at the 288 alignable nucleotides on either side of the “insert”. While they cover identical protein sequence, the RNA is not at all identical, but 6.6% different – 19 mutations out of 288. All 19 are mutations in the wobble base of their respective codons. There are so many that the frame can be inferred from the 2/1 pattern even without knowing the beginning or the end, or indeed

that the encoded protein sequence is identical – those are self-evident by looking at the RNA itself.

```
                                                                                                    .
RaTG13 23463  CTAATGTTTTTCAAACACGTGCAGGTTGTTTAATAGGGGCTGAACATGTCAATAACTCGT  23522

Wuhan  23541  ATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATT  23600
              |||||||||||||||| |||||||||| |||||||| ||||||||||||| |||||||
RaTG13 23523  ATGAGTGTGACATACCTATTGGTGCAGGAATATGCGCCAGTTATCAGACTCAAACTAATT  23582

Wuhan  23601  CTCCTCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATTGCCTACACTATGTCACTTG  23660
                        |||||||||| || |||||||| || ||||||||||||||||||||||
RaTG13 23583  ----------CACGTAGTGTGGCCAGTCAATCTATTATTGCCTACACTATGTCACTTG  23630

Wuhan  23661  GTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATTGCCATACCCACAAATTTTACTA  23720
              |||||||||||||||||||| |||||||||||||||||||||||| ||||||||||||
RaTG13 23631  GTGCAGAAAATTCAGTTGCTTATTCTAATAACTCTATTGCCATACCTACAAATTTTACTA  23690

Wuhan  23721  TTAGTGTTACCACAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAGATTGTACAA  23780
              ||||||| ||||| |||||||||||| |||||||||||| |||||||| ||||| |||||||
RaTG13 23691  TTAGTGTGACCACTGAAATTCTACCTGTGTCTATGACAAAGACATCGGTAGACTGTACAA  23750
```

We know from influenza H1N1, for which we have serial isolates from 1918 to the present, that wobble base mutagenesis occurs at a rate of 0.95% per decade. This permits an estimation of the TMRCA of the two sequences nCoV2019 and RaTG13 of 69.5 years ago – roughly 1950 +/- 10 years or so.

RaTG13, or anything nearly identical to it at the RNA level, simply could not be a proximal source of nCoV2019. It just LOOKS like it might be…at first glance.

Given that furin cleavage signals are present in other coronaviruses at exactly that point in the S1/S2 boundary region, it only LOOKS unusual, especially against the backdrop of SARS. The preponderance of evidence, coupled with Ockham's razor (that the simplest explanation is preferred) dictates that the PRRA sequence has been conserved in nCoV2019 from a long ago ancestor virus. It is not of suspicious origin. The closest bat virus sequence is really not close at all.

RNA don't lie.

Bill Gallaher

From: Liu, Shan-Lu
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We have just uploaded a corrected proof online, but relegalized a small error: "1,100" should be read as "1,100 nt" – could you kindly help make the correction, or replace the uploaded file with the attached new one?

Thank you! Please confirm.

Shan-Lu Liu & Lishan Su

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Reply-To: "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
Date: Friday, February 21, 2020 at 4:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

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Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From:	Liu, Shan-Lu
To:	Su, Lishan; Lu, Shan
Subject:	Re: Revised commentary for EMI - final!
Date:	Sunday, February 16, 2020 9:49:19 PM
Attachments:	EMI-conspiracy-zlshi.pdf image001.png

See Zhengli's comments. We may not need to make those changes, although some of those are good.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Sunday, February 16, 2020 at 3:17 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: Re: Revised commentary for EMI - final!

See a typo in the title, and the last sentence as we had discussed.
Thanks,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 16, 2020 at 1:55 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: RE: Revised commentary for EMI - final!

Good to me.

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 16, 2020 1:45 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Revised commentary for EMI - final!

Please look at this new version, sorry!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.

Professor
Co-Director, Viruses and Emerging Pathogens Program
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: Shan-Lu Liu <liu.6244@osu.edu>

Date: Sunday, February 16, 2020 at 1:38 PM

To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>

Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>

Subject: Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
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SARS-CoV-2: no evidence of a laboratory origin

Journal:	*Emerging Microbes & Infections*
Manuscript ID	Draft
Manuscript Type:	Commentary
Date Submitted by the Author:	n/a
Complete List of Authors:	Liu, Shan-Lu; The Ohio State University, Infectious Diseases Institute Saif, Linda J.; The Ohio State University Weiss, Susan; University of Pennsylvania Su, Lishan; University of North Carolina at Chapel Hill
Keywords:	SARS-CoV-2, COIVD-2019, origin, evolution
Abstract:	

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SARS-CoV-2: no evidence of a laboratory origin

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

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[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,

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[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

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Department of Veterinary Preventive Medicine,

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[6] Department of Microbiology, Perelman School of Medicine,

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[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

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The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

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RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

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When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

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director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

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Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

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References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

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Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

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14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.

16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

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From: Liu, Shan-Lu
To: Zheng, Yi-Min
Subject: FW: Emerging Microbes & Infections - Decision on Manuscript ID TEMI-2020-0121
Date: Thursday, February 13, 2020 9:50:39 AM

On 2/13/20, 9:49 AM, "Emerging Microbes and Infections" <onbehalfof@manuscriptcentral.com> wrote:

13-Feb-2020

Dear Professor Liu:

Ref: SARS-CoV-2: no evidence of a laboratory origin

Our reviewers have now considered your paper and have recommended publication in Emerging Microbes & Infections. We are pleased to accept your paper in its current form which will now be forwarded to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter, along with those of the editor who coordinated the review of your paper.

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Thank you for your contribution to Emerging Microbes & Infections and we look forward to receiving further submissions from you.

Sincerely,

Shan Lu
Editor-in-Chief
Emerging Microbes & Infections

Review Editor Comments to the Author:
EMI would like to thank the authors for providing a timely piece. It will have major impact to clear many people's confusion.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author

This is a timely commentary. It is perfectly written. All four authors are well established virologists. I suggest to publish it right away.

From: Liu, Shan-Lu
To: TEMI-production@journals.tandf.co.uk
Subject: Re: URGENT! Submitted Corrections for article TEMI 1733440 #TrackingId:5700591
Date: Thursday, February 27, 2020 1:39:19 AM

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Shan-Lu Liu & Lishan Su

Shan-Lu Liu, M.D., Ph.D.
Professor
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Shan-Lu Liu & Lishan Su

Shan-Lu Liu, M.D., Ph.D.
Professor
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Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

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> **To:**malathi@novatechset.com
> **Cc:**
> **Subject:**Re: URGENT! Submitted Corrections for article TEMI 1733440
>
> Dear Malathi Boopalan:
>
> We have just uploaded a corrected proof online, but relegalized a small error: “1,100” should be read as “1,100 nt” – could you kindly help make the correction, or replace the uploaded file with the attached new one?

Thank you! Please confirm.

Shan-Lu Liu & Lishan Su

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Shan-Lu Liu sent from iPhone

> On Feb 21, 2020, at 4:04 PM, TEMI-production@journals.tandf.co.uk <cats@taylorandfrancis.com> wrote:
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
>
> Journal: *Emerging Microbes & Infections* (TEMI)
>
> Article ID: TEMI 1733440
>
> Dear author,
>
> This email confirms that you have submitted your corrections to your article proofs.
>
> The submitted corrections have been successfully uploaded.
>
> If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.

Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

From:	Liu, Shan-Lu
To:	Weiss, Susan
Cc:	Su, Lishan; Lu, Shan
Subject:	Re: [External] Commentary for EMI
Date:	Wednesday, February 12, 2020 4:05:39 PM
Attachments:	EMI-2019-nCoV_Commentary_Final for submission .docx image001.png image002.png

---

Hi Susan,

That is great! Attached please see the final version of the commentary, with you name being added.

Best wishes.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
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Columbus, Ohio 43210
Phone: (614) 292-8690
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Date: Wednesday, February 12, 2020 at 4:00 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: [External] Commentary for EMI

Shan-LU
I am still in Spain, going home on Saturday.
Yes please add my name as a co-author. This is important!!
Is the new virus now names SARS-2; maybe not a good name – should be different from SARS

I hope I am not too late

susan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 5:26 PM
To: "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: [External] Commentary for EMI

Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of "Emerging Microbes and Infections", Dr. Shan Lu (don't get confused, it's not me). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
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SARS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu[3, 5,6.7]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

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The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[5] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[6] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[7] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From: Liu, Shan-Lu
To: Lu, Shan; Su, Lishan
Subject: Re: Executive summary of EMI commentary
Date: Sunday, February 23, 2020 1:21:13 PM
Attachments: Liu et al_EMI Commentary_Revision_中文-Shan Lu.docx
Liu et al_EMI Commentary_Revision_中文-Shan Lu.pdf
刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论_Final.docx
刘善虑苏立山等教授发文分析驳斥新冠病毒阴谋论_Final.pdf
image001.png

Now, the final versions, a total of 4 files - hopefully!

SL

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 23, 2020 at 1:15 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Executive summary of EMI commentary

Overall they are very good.

I found one minor error: "Dec" was used for the last reference of your paper. It should be "Feb". You may want to change the current word and pdf, but not change the real paper to be published as it may take a lot of more time to current and reload to online. Readers can find that paper without much problem.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 12:57 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Executive summary of EMI commentary

See my final versions of two files, Word and PDF.

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 23, 2020 at 12:51 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Executive summary of EMI commentary

Your school's email screening system is good! Thanks.

From: Liu, Shan-Lu <liu.6244@osu.edu>

Sent: Sunday, February 23, 2020 12:49 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: Executive summary of EMI commentary

Never, just received Shan's email and file! Slow on my end.

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Sunday, February 23, 2020 at 12:48 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: Executive summary of EMI commentary

Great work. However, I made some new changes (see attached). All highlighted or marked, for your reference.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Sunday, February 23, 2020 8:57 AM
To: Su, Lishan <lishan_su@med.unc.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Executive summary of EMI commentary

Lishan and Shan – so confusing!

I have wrapped up a summary for the public and media to understand key points of our commentary. Please make suggestions.

I think this can go along with the Chinese translation.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 7:39 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:33 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

I just went online to see if we replace with a new one.
Could you send me the PDF of the corrected one?

THE OHIO STATE UNIVERSITY

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Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:32 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

My bad. How do we fix it? send her a message?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:29 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Lishan:

I just saw that you deleted nt for 1,100 – “nt” should be kept. Could you correct that?

Thanks.

SL

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.

Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Friday, February 21, 2020 at 4:28 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Submitted Corrections for article TEMI 1733440

Thanks!

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Friday, February 21, 2020 at 7:22 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
Subject: Fwd: Submitted Corrections for article TEMI 1733440

Shan-Lu Liu sent from iPhone

Begin forwarded message:

> **From:** "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
> **Date:** February 21, 2020 at 4:04:41 PM PST
> **To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>, "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Subject: Submitted Corrections for article TEMI 1733440**
> **Reply-To:** TEMI-production@journals.tandf.co.uk
>
> Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2
>
> Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear author,

This email confirms that you have submitted your corrections to your article proofs.

The submitted corrections have been successfully uploaded.

If you want to check your submitted corrections please log into CATS and click on the “Corrections Submitted” button.

Yours sincerely,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

没有可信的证据支持 SARS-CoV-2 来自实验室人工合成

Shan-Lu Liu（刘善虑）, 俄亥俄州立大学

Linda J. Saif, 俄亥俄州立大学

Susan Weiss, 宾夕法尼亚大学

Lishan Su,（苏立山）北卡大学教堂山分校

截止 2020 年 2 月 10 日，在武汉出现和爆发的急性呼吸疾病已波及 4 万多人，导致 1000 多人死亡。研究人员很快找到了一种新型人的冠状病毒，称之为 2019 nCoV 或 SARS-CoV-2，而相应的疾病称之为 COVID-19，意为 2019 年发生的冠状病毒疾病 (https://globalbiodefense com/novel-coronavirus-covid-19-portal/)。

据现有的报道[1-3]，COVID-2019 与 SARS-CoV 导致的 SARS 有很多相似的临床表现。而 SARS-CoV-2 基因组序列也和 2003 年 SARS-CoV 有 80%同源性，但它与一些蝙蝠的乙型冠状病毒更为相似。当前，种种的推测、谣言和阴谋论到处流行，其中有的认为 SARS-CoV-2 来源于实验室基因工程制造。也有某些人声称，人的 SARS-CoV-2 是从武汉的某个实验室直接泄漏出来的， 其根据是该实验室最近报道了一种称为 RaTG13 的蝙蝠冠状病毒，它和 SARS-CoV-2 基因组序列有高达 96%的同源性。

然而，我们知道，2003 年发现人 SARS 冠状病毒和其中间宿主果子狸 SARS 样冠状病毒具有 99.8％的同源性，在整个基因组中只有 202 个碱基不同。鉴于人类新型 SARS-CoV-2 与蝙蝠 RaTG13-CoV 之间有超过了 1000 个不同碱基[4]，且这些差异是按照冠状病毒典型的进化特征按自然发生的模式分布在整个基因组中，我们认为 SARS-CoV-2 直接来源于 RaTG13 冠状病毒的可能性极小。更为重要的是，在新的人 SARS-CoV-2 病毒基因组序列中并没有任何可信的基因工程改造的迹象，这都揭示 SARS-CoV-2 是通过自然演化而来的。我们认为在蝙蝠与人类之间可以找到中间动物宿主含有类似的冠状病毒，它与 SARS-CoV-2 更相似。最近有消息称穿山甲可能携带与 SARS-CoV-2 密切相关的冠

状病毒，但论文和数据尚未正式发表，无从得以证实(https://www.nature.com/articles/d41586-020-00364-2)。

最近社交媒体上的另一种说法指向 2015 年在《自然医学》发表的一篇论文[7]。该论文报道了在小鼠适应后的人类 SARS 冠状病毒（MA15 病毒）中，人工构建了带有蝙蝠冠状病毒（SHC014）S 基因，这种合成的嵌合冠状病毒，不仅可以可以感染小鼠，也能够感染来源人的细胞[8]。然而，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒基因组序列有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。

现在我们来理一理来人 SARS 病毒老鼠适应株 MA15 和它的衍生病毒的来龙去脉。适应小鼠的 SARS 病毒（MA15）[9]是通过把 SARS 冠状病毒在小白鼠呼吸道中连续传代 15 后产生的；适应后的 SARS 冠状病毒有六个氨基酸突变，使其能够更有效地感染小鼠，尤其是在老年小鼠中具有了更高的复制活性和肺部致病性能（因此称为 M15）。由于在小鼠内适应的遗传突变，MA15 在人细胞或者人体内感染很可能降低了。

科学家曾认为从蝙蝠身来的冠状病毒的 S 基因和人的 SARS 病毒不同，推测它们无法使用人的 SARS 病毒受体 ACE2 进入人体细胞[10, 11]；后来发现果子狸是蝙蝠冠状病毒传给人的中间宿主，能够将 SARS 冠状病毒传播给人类[6，12]。然而，2013 年以来，科学家陆续从中国马蹄蝠中分离到了数个新型蝙蝠冠状病毒，这些来自蝙蝠的，类似人 SARS 冠状病毒（SL-CoV-WIV1）能够使用人、果子狸和中国马蹄蝠的 ACE2 受体进入和感染细胞[8]。进化研究表明，在 SARS 冠状病毒 S 蛋白的作用接触位点上，蝙蝠 ACE2 基因在与人类 ACE2 基因在相同的位点上同样被进化选择[13]。基于这样的发现，科学家提出了蝙蝠的 SARS 样冠状病毒具有直接传染到人的能力，不必需要中间宿主环节；也就是说有些蝙蝠冠状病毒有可能直接感染人类宿主细胞。为了直接验证这种可能性，蝙蝠冠状病毒 SL-SHC014 的 S 基因被人工嫁接到了 MA15 SARS-CoV 骨架上，因此产生了一个嵌合病毒。此 SL-SHC014-MA15 嵌合病毒确实能够有效地利用人 ACE2 进入细胞，并在人的呼吸道实验细胞中有效复制。SL-SHC014-MA15 也可以在小鼠的肺中高效复制，但与 SARS MA15 相比，感染减弱了，并且只会让老年小鼠致命[7]。

由于 SL-SHC014-MA15 嵌合病毒相对于另一个人 SARS-S/MA15 嵌合病毒在小鼠中具有更高的致病活性，这种嵌合冠状病毒的实验后来在美国政府的干预下被暂停(https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-

pause-gain-function-research)。虽然目前 这项禁令在美国已经被解除，但构建这种具有大流行病潜力的病毒是否是一种风险，在当前的COVID-2019流行的形势下又重新引发了讨论，成为热点话题。然而，经过多个国家科学家对病毒的分子进化分析[5，14]，SARS-CoV-2 无疑与 SL-SHC014-MA15 具有非常大的不同，整个基因组有大约 6,000 核苷酸的差异。因此，没有可信的证据支持 SARS-CoV-2 是源自 SL-SHC014-MA15 嵌合病毒的说法。

最近也有传言说，SARS-CoV-2 是实验室中有意人为制造的。其中发表在 BioRxiv（一个同行评审之前的手稿共享网站）的一份手稿中更是此传言的代表，它声称 SARS-CoV-2 中含有 HIV 序列，因此很可能是在实验室中产生的。文章在线后， 舆论哗然，世界各国的多个病毒学者纷纷反驳。 在 HIV-1 病毒专家高峰（Feng Gao）领衔领导的反驳论文中，他们使用了仔细的生物信息学分析来证明，指出最初声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的 [15]。由于国际社会提出的种种疑问，这篇手稿的作者已经撤回了该手稿， 不再要求发表。

从科学层面讲，进化是循序渐进的，并随着时间的推移进一步产生有利于病毒的突变，就像天然分离的病毒（如蝙蝠冠状病毒 RaTG13）基因组那样。 相反，人工合成的病毒基因组通常会使用已知的病毒骨架引入一些某些定向的变化。所以我们认为，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。 有一种可能不能排除， 就是 SARS-CoV-2 是一种蝙蝠冠状病毒与另一种冠状病毒之间进行了自然重组而产生的；但这种可能性需要更多的研究来证明，来回答 SARS-CoV-2 的自然起源问题。我们需要强调的是，尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人工制造，但对公共健康有威胁的病毒都必须进行恰当的实验室管理，而且需要由科学界和政府合理监管。

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Feb;9(1):378-381.

刘善虑苏立山等教授发文分析认为新冠病毒阴谋论缺乏病毒学证据

俄亥俄州立大学教授刘善虑，北卡大学教堂山分校教授苏立山联名国际著名冠状病毒学家 Linda J. Saif（美国科学院院士）以及 Susan Weiss（美国微生物科学院院士），在 国际期刊 Emerging Microbes & Infections (EMI) (中文译名《新发微生物与感染》)发表题为“**没有可信的证据支持 SARS-CoV-2 来自实验室人工合成**”的评论文章，对最近广为流行的传言和阴谋论进行了分析和驳斥。

该文主要论点如下：

1. 新型冠状病毒 SARS-CoV-2 虽与中国科学院武汉病毒所最近报道的一个称为 RaTG13 的蝙蝠冠状病毒有高达 96%的同源性， 但两者仍然有超过 1，100 碱基的差别，而且在关键序列序列上有特征性区别，因此两者是完全不同的冠状病毒。
2. 社交媒体指向 2015 年在《自然医学》（Nature Medicine）一篇论文，认为新型冠状病毒是这篇文章报道的人 SARS 与蝙蝠冠状病毒（SHC014）的嵌合病毒的泄露。然而，分析研究表明，新型冠状病毒 SARS-CoV-2 与这个嵌合冠状病毒在基因组序列上有超过了 5,000 个碱基的不同，所以这种怀疑完全缺乏任何科学依据。
3. 还有一种传言说 SARS-CoV-2 是实验室中有意人为制造的， 并以发表在 BioRxiv 上印度科学家的一份手稿中为依据，声称 SARS-CoV-2 中含有 HIV 序列。实际上这篇文章在线发表后， 舆论哗然，世界各国病毒学家也纷纷反驳。 在 HIV-1 专家高峰（Feng Gao）领衔领导发表在 EMI 的另一篇反驳论文中，作者使用了仔细的生物信息学分析来证明，指出原文作者声称的 SARS-CoV-2 有多个 HIV-1 插入片段并非 HIV-1 特有，而是完全随机的。由于国际社会提出的种种疑问，这篇手稿的作者也已经撤回了该手稿， 目前没有发现再次发表。
4. 从科学层面讲，病毒进化是循序渐进的，并切随着时间的推移进一步产生有利于病毒感染人的突变。相反，人工合成的病毒基因组通常会在已知的病毒骨架引入一些某些定向的变化。所以，目前没有可靠的证据支持 SARS-CoV-2 是来源于实验人工设计。
5. 尽管目前没有证据显示新型冠状病毒 SARS-CoV-2 来自实验室人为制造，我们认为对公共健康有威胁的病毒都必须进行严格恰当的实验室管理，而且需要由科学界和政府联合监管。

From:	Liu, Shan-Lu
To:	Yost, Mary
Cc:	Herb Grant
Subject:	Re: Final version of the letter: "COVID-19 and The Virus That Causes It" - OSU
Date:	Wednesday, March 25, 2020 8:36:18 AM
Attachments:	image001.png image002.png image003.png image004.png image005.png

---

That is great, and thank you Mary and Herb.
Kindly keep me updated.

Shan-Lu

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Yost, Mary" <myost@dispatch.com>
Date: Tuesday, March 24, 2020 at 7:28 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Herb Grant <hgrant@dispatch.com>
Subject: Re: Final version of the letter: "COVID-19 and The Virus That Causes It" - OSU

Thanks, that should work.

Mary Yost
Editorial Page Editor
Columbus Dispatch
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614-204-6798 (cell)
myost@dispatch.com

On Tue, Mar 24, 2020 at 7:26 AM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Thank you. I have attached my photo. Let me know if the photo does not work or you need anything else.
>
> Once you have decided, kindly let me know, because the OSU communication folks would like to be looped.
>
> Shan-Lu

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From: "Yost, Mary" <myost@dispatch.com>
Date: Tuesday, March 24, 2020 at 6:47 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Encarnacion Pyle <epyle@dispatch.com>, "miller, alan" <amiller@dispatch.com>, Herb Grant <hgrant@dispatch.com>
Subject: Re: Final version of the letter: "COVID-19 and The Virus That Causes It" - OSU

Thank you very much.

If we publish this we would also need your high-resolution head-and-shoulders photo.

If you can submit one, please also copy Herb Grant.

Mary

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Editorial Page Editor

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On Tue, Mar 24, 2020 at 12:19 AM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Dear Mary,
>
> I have modified the letter by following your instructions. First, I changed the author number to one. Second, I shortened the letter and now its length is ~700 words. Third, I revised the letter by removing “facts” but adding more opinions.
>
> I hope the letter is now acceptable for publication in Columbus Dispatch. Kindly note that the disclaimer in the end is important so please make sure to keep it.
>
> Thank you so much for your help with this effort.
>
> Shan-Lu

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From: "Yost, Mary" <myost@dispatch.com>
Date: Monday, March 23, 2020 at 7:38 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Encarnacion Pyle <epyle@dispatch.com>
Subject: Re: Greetings and inquiry: COIVD-19 commentary

Thank you, but I am not sure it would be suitable for our opinion pages. I encourage you to work with our news side, since it sounds like you are wanting to convey facts, not commentary.

And no, we would not run it with three authors. In cases where multiple individuals want to be credited, we have advised that the others be noted in the body of the article, but that also takes space away from the content you want to present.

We do a weekly review of pending op-eds on Friday afternoons and can let you know after our review if we will publish your submission. The news side could probably share your information sooner than we can on our opinion pages, even if we are able to publish it.

Mary

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myost@dispatch.com

On Mon, Mar 23, 2020 at 5:13 PM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Hi Mary,
>
> Thank you for your consideration.
>
> Over the last few weeks, I kept receiving requests from people, including local fire departments regarding how this virus is spread and causes the disease, etc. This really motivated me to write something with some updated information that I thought would be helpful to our readers.
>
> Yes, we can cut down to 700 words, with no problem, but I would still prefer to have three authors, because all are co-directors of the OSU program and we have contributed equally.
>
> Thank you so much, and let me know how to proceed.
>
> Shan-Lu
>
> 
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>
> Shan-Lu Liu, M.D., Ph.D.

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Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Yost, Mary" <myost@dispatch.com>
Date: Monday, March 23, 2020 at 4:56 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Encarnacion Pyle <epyle@dispatch.com>
Subject: Re: Greetings and inquiry: COIVD-19 commentary

Hi Shan-Lu,

Thank you for offering to send us an op-ed, but it might be better if you could share your expertise with our news side.

As you can imagine, we continue to receive a lot of guest columns around the topic of coronavirus and its impact on all facets of life today. One of the challenges we have with the opinion pages is limited space, just two pages each day, without a lot of flexibility in how we fill our space.

It sounds like the kind of information you have to share is more factual than opinion, which might be better suited for news coverage that doesn't have the space restrictions we do.

A couple of other concerns -- we typically don't run guest columns from more than one author; and our usual length is about 700 words. We made an exception for a guest column that will appear in Tuesday's paper, but that is very rare. I don't know if 700 words would be enough to cover all that you have to share.

I am copying one of our metro editors, Encartia Pyle, in case you would be interested in following up with a news reporter to share your insights.

Thank you for thinking of The Dispatch; and thank you for what you are doing related to the coronavirus.

Mary

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myost@dispatch.com

On Sat, Mar 21, 2020 at 9:12 PM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Dear Alan,
>
> Greetings! Hope this email finds you well.
>
> I am not sure if you are the right person to contact, but please forgive me and help make the connection to the Dispatch.
>
> In 2016 when I joined OSU, Emily Tate wrote a story on me about the Zika virus, see attached article. Now COIVD-19 is here, and as co-director of the OSU Viruses and Emerging Pathogens program, my colleagues Linda Saif, Jacob Yount and I have written a commentary on COIVD-19, which we wish to publish in the Dispatch as commentary or other forms. Our focus is on the virus, SARS-CoV-2, which causes the outbreak and the disease COIVD-19.
>
> The motivation is that I recently have received a lot of requests from local media and even fire department for interview, and I thought that this commentary may be able to address some of the reader's questions.
>
> See below some of my writings published in journals:
>
> https://www.nature.com/articles/d41586-020-00135-z
>
> **New virus in China requires international control effort**
>
> **Emerging Viruses without Borders: The Wuhan Coronavirus**
>
> **https://www.mdpi.com/1999-4915/12/2/130**
>
> https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1733440
>
> **No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2**

SARS-CoV-2 is an appropriate name for the new coronavirus

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30557-2/fulltext

Thank you for your consideration. If your newspaper is interested, please let me know and I will send the article to you shortly.

Sincerely,

Shan-Lu

THE OHIO STATE UNIVERSITY

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From: Liu_Shan-Lu
To: temi-peerreview@journals.tandf.co.uk
Cc: Lu_Shan; Su_Lishan
Subject: Re: Emerging Microbes & Infections - TEMI-2020-0121 - changes required to your submission
Date: Thursday, February 13, 2020 8 56:46 AM
Attachments: Liu et al_EMI Commentary_15 references.docx
Importance: High

Hi Jorgie:

I have modified as instructed and attached the new one to this email Please help upload and proceed

Thank you

Shan-Lu

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On 2/13/20, 8:43 AM, "Emerging Microbes and Infections" <onbehalfof@manuscriptcentral com> wrote:

13-Feb-2020

Dear Professor Liu,

Your above referenced manuscript, entitled "SARS-CoV-2: no evidence of a laboratory origin" requires some further changes before it is ready for reviewing in Emerging Microbes & Infections Your submission has been returned to you and is located in your Author Center as a draft, so that you due to these reasons:

1 No line numbering

Kindly add a line numbering in your main document

2 Exceeded reference count

Kindly be informed that the reference count for the commentary article should not be more than 15

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You may contact the Editorial Office if you have further questions

Sincerely,

Jorgie Lyn Luna
Emerging Microbes & Infections Editorial Office
temi-peerreview@journals tandf co uk

SARS-CoV-2: no evidence of a laboratory origin

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

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Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.

From:	Liu, Shan-Lu
To:	Stanley Perlman
Cc:	Su, Lishan; Lu, Shan
Subject:	Re: Commentary for EMI
Date:	Wednesday, February 12, 2020 4:13:27 PM
Attachments:	EMI-2019-nCoV_Commentary_Final for submission .docx image001.png image002.png

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Hi Stanley,

I have attached an almost final version of the commentary. Note that Susan Weiss has agreed to become a coauthor. Kindly let us know if you are interested in joining if possible tonight.

Best wishes.

Shan-Lu

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From: Shan-Lu Liu <liu.6244@osu.edu>

Date: Wednesday, February 12, 2020 at 11:14 AM

To: Stanley Perlman <stanley-perlman@uiowa.edu>

Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>

Subject: Commentary for EMI

Dear Stanley,

Hope all is well.

As you may know, Lishan at UNC and I have just wrapped up a commentary, at the invitation by the editor in chief of journal “Emerging Microbes and Infections”, Dr. Shan Lu (don’t get confused, it’s not me), and we are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as

scientist, we should try to clear this thing up.

Let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

THE OHIO STATE UNIVERSITY

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Lishan Su[1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu[3, 5,6.7]

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Contact: Dr. Lishan Su, lsu@med.unc.edu

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References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
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4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
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9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
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11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From:	Liu, Shan-Lu
To:	Yost, Mary
Cc:	Encarnacion Pyle; miller, alan
Subject:	Final version of the letter: "COVID-19 and The Virus That Causes It" - OSU
Date:	Tuesday, March 24, 2020 12:18:52 AM
Attachments:	Dispatch_commentary_Liu_OSU.docx image001.png image002.png image003.png

Dear Mary,

I have modified the letter by following your instructions. First, I changed the author number to one. Second, I shortened the letter and now its length is ~700 words. Third, I revised the letter by removing “facts” but adding more opinions.

I hope the letter is now acceptable for publication in Columbus Dispatch. Kindly note that the disclaimer in the end is important so please make sure to keep it.

Thank you so much for your help with this effort.

Shan-Lu

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From: "Yost, Mary" <myost@dispatch.com>
Date: Monday, March 23, 2020 at 7:38 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Encarnacion Pyle <epyle@dispatch.com>
Subject: Re: Greetings and inquiry: COIVD-19 commentary

Thank you, but I am not sure it would be suitable for our opinion pages. I encourage you to work with our news side, since it sounds like you are wanting to convey facts, not commentary.

And no, we would not run it with three authors. In cases where multiple individuals want to be

credited, we have advised that the others be noted in the body of the article, but that also takes space away from the content you want to present.

We do a weekly review of pending op-eds on Friday afternoons and can let you know after our review if we will publish your submission. The news side could probably share your information sooner than we can on our opinion pages, even if we are able to publish it.

Mary

Mary Yost
Editorial Page Editor
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614-204-6798 (cell)
myost@dispatch.com

On Mon, Mar 23, 2020 at 5:13 PM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Hi Mary,
>
> Thank you for your consideration.
>
> Over the last few weeks, I kept receiving requests from people, including local fire departments regarding how this virus is spread and causes the disease, etc. This really motivated me to write something with some updated information that I thought would be helpful to our readers.
>
> Yes, we can cut down to 700 words, with no problem, but I would still prefer to have three authors, because all are co-directors of the OSU program and we have contributed equally.
>
> Thank you so much, and let me know how to proceed.
>
> Shan-Lu
>
> 
>
> Shan-Lu Liu, M.D., Ph.D.
> Professor
> Co-Director, Viruses and Emerging Pathogens Program
> Infectious Diseases Institute
> Center for Retrovirus Research

Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Yost, Mary" <myost@dispatch.com>
Date: Monday, March 23, 2020 at 4:56 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: Encarnacion Pyle <epyle@dispatch.com>
Subject: Re: Greetings and inquiry: COIVD-19 commentary

Hi Shan-Lu,

Thank you for offering to send us an op-ed, but it might be better if you could share your expertise with our news side.

As you can imagine, we continue to receive a lot of guest columns around the topic of coronavirus and its impact on all facets of life today. One of the challenges we have with the opinion pages is limited space, just two pages each day, without a lot of flexibility in how we fill our space.

It sounds like the kind of information you have to share is more factual than opinion, which might be better suited for news coverage that doesn't have the space restrictions we do.

A couple of other concerns -- we typically don't run guest columns from more than one author; and our usual length is about 700 words. We made an exception for a guest column that will appear in Tuesday's paper, but that is very rare. I don't know if 700 words would be enough to cover all that you have to share.

I am copying one of our metro editors, Encartia Pyle, in case you would be interested in following up with a news reporter to share your insights.

Thank you for thinking of The Dispatch; and thank you for what you are doing related to the coronavirus.

Mary

Mary Yost
Editorial Page Editor
Columbus Dispatch
62 E. Broad St.

Columbus, OH 43215
614-461-5040 (office)
614-204-6798 (cell)
myost@dispatch.com

On Sat, Mar 21, 2020 at 9:12 PM Liu, Shan-Lu <liu.6244@osu.edu> wrote:

> Dear Alan,
>
> Greetings! Hope this email finds you well.
>
> I am not sure if you are the right person to contact, but please forgive me and help make the connection to the Dispatch.
>
> In 2016 when I joined OSU, Emily Tate wrote a story on me about the Zika virus, see attached article. Now COIVD-19 is here, and as co-director of the OSU Viruses and Emerging Pathogens program, my colleagues Linda Saif, Jacob Yount and I have written a commentary on COIVD-19, which we wish to publish in the Dispatch as commentary or other forms. Our focus is on the virus, SARS-CoV-2, which causes the outbreak and the disease COIVD-19.
>
> The motivation is that I recently have received a lot of requests from local media and even fire department for interview, and I thought that this commentary may be able to address some of the reader's questions.
>
> See below some of my writings published in journals:
>
> https://www.nature.com/articles/d41586-020-00135-z
>
> **New virus in China requires international control effort**
>
> **Emerging Viruses without Borders: The Wuhan Coronavirus**
>
> **https://www.mdpi.com/1999-4915/12/2/130**
>
> https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1733440
>
> **No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2**
>
> SARS-CoV-2 is an appropriate name for the new coronavirus

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30557-2/fulltext

Thank you for your consideration. If your newspaper is interested, please let me know and I will send the article to you shortly.

Sincerely,

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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This message may contain confidential and/or privileged information. If you are not the intended recipient or authorized to receive this for the intended recipient, you must not use, copy, disclose or take any action based on this message or any information herein. If you have received this message in error, please advise the sender immediately by sending a reply e-mail and delete this message. Thank you for your cooperation.

This message may contain confidential and/or privileged information. If you are not the intended recipient or authorized to receive this for the intended recipient, you must not use, copy, disclose or take any action based on this message or any information herein. If you have received this message in error, please advise the sender immediately by sending a reply e-mail and delete this message. Thank you for your cooperation.

COVID-19 and The Virus That Causes It

Shan-Lu Liu

COVID-19 is now a global pandemic disease. The disease is caused by a coronavirus that has been officially named SARS-CoV-2. The virus originated in November 2019 in Wuhan, China, a city with a population of 11 million. A seafood wholesale market in the city is thought to be the origin of the virus, with infected wild animals transmitting the virus to humans. SARS-CoV-2 infects the lung in humans, and induces pneumonia. Unlike many animal viruses, it was able to initiate a deadly chain of human-to-human transmission.

Analysis of the virus genome shows that SARS-CoV-2 is most closely related to a virus circulating in bats, suggesting that bats were the source of the virus. Many other viruses have emerged from bats to infect humans, including the SARS coronavirus, Ebola virus and Zika virus. Pangolins, an endangered species of small mammals, harbor a coronavirus similar to SARS-CoV-2 leading to speculation that they may be an intermediate host that transfers virus between bats and humans. Recent data do not support this. Nonetheless, genetic analysis has confirmed that the virus emerged from animals and this finding should dispel unsubstantiated allegations that the virus was manmade.

The transmission rate for a virus can be measured by its reproductive number (R_0), which represents the number of people on average that will acquire the infection from a

single infected person. The R_0 for SARS-CoV-2 is estimated to be 2.7, which is higher than that of seasonal influenza virus (R_0 estimated at 2.0). However, this value for SARS-CoV-2 is likely an underestimate because it is based on confirmed positive cases and does not account for undiagnosed mild or asymptomatic cases.

SARS-CoV-2 can cause severe lung damage with pneumonia and even deaths. However, asymptomatic infections, which some have proposed are the majority of infections, are likely a primary source of transmitted virus. Hence, social distancing currently being practiced in the US and other COVID-19 afflicted countries is critical and should be heeded by all and enhanced as the most effective way to contain the virus in the absence of antivirals and vaccines.

The virus is transmitted by respiratory droplets that can remain airborne for several hours. These droplets can also settle on surfaces and remain infectious for several days. Thus, personal hygiene with frequent handwashing, and social distancing are the most effective means of slowing spread of the virus. Because eye infections may occur in SARS-CoV-2 infected individuals, eye protection is needed for health care workers and individuals should avoid touching their eyes with potentially contaminated hands.

Vaccination is the most effective strategy to prevent infectious diseases. Unfortunately, there is no FDA-approved vaccine for SARS-CoV-2-induced COVID-19. With unprecedented speed, a candidate vaccine has just entered the first phase of a human clinical trial. If successful, this candidate vaccine, or one of the many others in the

pipeline, will be a breakthrough for the control of COVID-19. In the meantime, many researchers are actively screening drugs for antiviral effects on SARS-CoV-2. Media coverage in recent days has focused on an anti-malaria drug known as chloroquine. While we are cautiously optimistic, results of ongoing clinical trials are needed to prove conclusively whether chloroquine is effective and safe for treating COVID-19 patients.

At The Ohio State University, as co-directors of the Viruses and Emerging Pathogens Program of The Infectious Diseases Institute, we are working with the community of immunology and virology researchers as teams to better understand and combat COVID-19. The teams are contributing their collective expertise and new ideas to aid in this battle. Our ultimate goals are to develop effective vaccines and antivirals in order to combat COIVD-19. In addition, the research community is assisting in generating COVID-19 testing reagents to overcome national shortages. Through focused interdisciplinary research, we will be better able to enhance knowledge and devise solutions to combat COVID-19 and viruses that emerge in the future.

Dr. Shan-Lu Liu is co-director of the Viruses and Emerging Pathogens Program of The Infectious Diseases Institute at The Ohio State University. The author acknowledges co-directors Drs. Linda Saif and Jacob Yount for critical input and comments. The opinions expressed in this article do not necessarily represent the viewpoints of The Ohio State University.

Shan-Lu Liu, liu.6244@osu.edu

From: Liu, Shan-Lu
To: Su, Lishan
Cc: Lu, Shan
Subject: Re: EMI commentary
Date: Wednesday, February 12, 2020 6:04:17 PM
Attachments: EMI-2019-nCoV_Commentary_for submission .docx
image001.png

Lishan: My understanding is that Shan does not want to be included as a coauthor… That is why I thought you would be the first author because you had the first draft

Shan: Let us know what you think.

See the updated version, with the new authorship order.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 5:55 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Current we are both senior and corresponding authors. I can be either. I am not sure the UNC affiliation should be listed first or not… let's think about this.

I agree Shan Lu should be a corresponding author too.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 5:51 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Hi Shan,

Sure, no problem. I think you deserve senior and corresponding authorship.

Shan did not respond today…

Best.

Shan-Lu

THE OHIO STATE UNIVERSITY

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 5:47 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Shan-Lu:

Should we switch authorship order, with you first, me last?
I like the idea of adding more from our virology group, if Shan Lu/EMI can wait for the signing delay.

It looks great. I hope it will help to clarify some of the confusions.

Did Feng Gao address the “shuttle vector” sequence claim in his ms? It is very similar to the HIV insertion problem with such short alignments.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 5:12 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: EMI commentary

Hi Shan,

Attached please find the final version of the commentary for your consideration to be published at EMI.

Kindly advise.

Regards.

Shan-Lu

SARS-CoV-2: no evidence of a laboratory origin

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,

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[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

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[5] Food Animal Health Research Program,

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Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From: Liu, Shan-Lu
To: Su, Lishan
Cc: Lu, Shan
Subject: Re: EMI commentary
Date: Wednesday, February 12, 2020 6:29:06 PM
Attachments: Liu et al_ EMI Commentary_for submission .docx
Su et al_ EMI Commentary_Final for submission .docx
image001.png

Hi Lishan:

See both versions attached, either way works for me. It's your call.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 6:26 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

It is probably fine if we cover not only the unc chimeric virus now.

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Wednesday, February 12, 2020 6:09:46 PM
To: Su, Lishan <lishan_su@med.unc.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Lishan:

Now I understand your point of concern. I should be fine either way, as OSU should not care.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 5:55 PM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Current we are both senior and corresponding authors. I can be either. I am not sure

the UNC affiliation should be listed first or not… let's think about this.

I agree Shan Lu should be a corresponding author too.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 at 5:51 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: EMI commentary

Hi Shan,

Sure, no problem. I think you deserve senior and corresponding authorship.

Shan did not respond today…

Best.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Subject: Re: EMI commentary

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I like the idea of adding more from our virology group, if Shan Lu/EMI can wait for the signing delay.

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Date: Wednesday, February 12, 2020 at 5:12 PM
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Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
Subject: EMI commentary

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Attached please find the final version of the commentary for your consideration to be published at EMI.

Kindly advise.

Regards.

Shan-Lu

SARS-CoV-2: no evidence of a laboratory origin

Lishan Su [1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu [3, 5,6.7]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

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[6] Department of Veterinary Biosciences, The Ohio State University, Columbus,

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Columbus, OH 43210, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

SARS-CoV-2: no evidence of a laboratory origin

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[3] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

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Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

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RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

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References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

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8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
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12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

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15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

From: Liu, Shan-Lu
To: Su, Lishan; Lu, Shan
Subject: Re: 2019-nCoV-EMI_commentary
Date: Wednesday, February 12, 2020 6:33:40 AM
Attachments: EMI-2019-nCoV_Commentary LJS_SLL.docx
image001.png
image002.png

I have incorporated Linda's comments into the MS, see attached. I am now working on the references…

Note the new title "**Evidence refuting laboratory origin of SARVS-CoV-2**"…it is now long and more like an article…

SL

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From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 1:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See the endnote file. Thanks,

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 7:44 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Sounds good, thank you. I still like “however” over “In contrast” – it just reads better

Shan: Are you sure that you prefer not to be included in the coauthorship? Before I send, I think we should have the authorship listed, along with affiliations. Lishan should be the first author, unless he prefers otherwise. Agreed?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 7:34 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

I made some minor change for the following:

In summary, there is no credible evidence at this point to support the claims that the 2019-nCoV was originated from a laboratory-engineered CoV. In contrast, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV.

Maybe now SLL can send the next version to other CoV experts?

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 5:47 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See the new version with all incorporated.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 4:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I have made additional changes to the Lishan’s version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 4:16 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 3:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Perhaps Lishan can take a look at the latest version, which has the new title I suggested, and modify it as needed.
The last paragraph is also crucial, but I did not have time to work on it because of a meeting this morning.
Once we have almost a final draft, I will contact Linda Saif, Stanley Perlman, Thomas Gallgaher etc. to see if they are willing to join, but this may delay the publishing time.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 1:52 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I agree that it should be simple and clear. I have included some details in the 1st draft for your information. There is not intention to defend Baric, but to clarify the facts.

Regarding all three, are you combining Goa Feng's piece with this one? For the RaTG13, it involves complicated viral evolution kinetics and maybe hard to simply clarify…
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 1:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>

Subject: RE: 2019-nCoV-EMI_commentary

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
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Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

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Thanks.

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Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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1900 Coffey Rd, Room 480 VMAB
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Phone: (614) 292-8690
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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Evidence refuting laboratory origin of SARVS-CoV-2

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill,

Chapel Hill, North Carolina, USA

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Dr. Lishan Su, lsu@med.unc.edu

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According to what has been reported (Lancet, NEJM 2020), COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat betacoronaviruses, with the highest being >96% identity (Nature 2020 refs).

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To: Su, Lishan; Lu, Shan
Subject: Re: 2019-nCoV-EMI_commentary
Date: Wednesday, February 12, 2020 6:30:15 AM
Attachments: EMI-2019-nCoV_Commentary LJS_SLL.docx
image001.png

I have incorporated Linda's comments into the MS, see attached. I am now working on the references…

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Wednesday, February 12, 2020 at 1:13 AM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
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1. We need to make this commentary very simple and short;
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3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more

powerful to cover multiple issues in one summary)

5. ??

SLL: please add anything I missed.

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Subject: Re: 2019-nCoV-EMI_commentary
Date: Wednesday, February 12, 2020 7:19:39 AM
Attachments: EMI-2019-nCoV_Commentary LJS_SLL_Refs.docx
image001.png

Please use the latest updates, with minor changes.

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1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.

Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
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Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Evidence refuting laboratory origin of SARVS-CoV-2

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill,

Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

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[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

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[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat betacoronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song et al, PNAS 2005). Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the

evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis [6].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are now restricted as gain of function (GOF) studies under the US government-mandated pause policy. The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic

analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (and not yet peer reviewed for accuracy) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have recently withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. Currently, there is no credible evidence to support the claim that the SARS-CoV-2 originated from a laboratory-engineered CoV. It is much more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

1. Wang, D.*, et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang*, et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N.*, et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P.*, et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N.*, et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D.*, et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y.*, et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W.*, et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F.*, et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

From:	Liu, Shan-Lu
To:	Stanley Perlman
Cc:	Su, Lishan; Lu, Shan
Subject:	Commentary for EMI
Date:	Wednesday, February 12, 2020 11:14:46 AM
Attachments:	EMI-2019-nCoV_Commentary_Final.docx image001.png

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Dear Stanley,

Hope all is well.

As you may know, Lishan at UNC and I have just wrapped up a commentary, at the invitation by the editor in chief of journal “Emerging Microbes and Infections”, Dr. Shan Lu (don’t get confused, it’s not me), and we are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should try to clear this thing up.

Let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
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Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

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[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

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[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

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the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

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When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [12], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs

already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 13], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

13. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Liu, Shan-Lu
To: Su, Lishan; Lu, Shan
Subject: 2019-nCoV-EMI_commentary with SOME refs added
Date: Wednesday, February 12, 2020 7:06:54 AM
Attachments: EMI-2019-nCoV_Commentary LJS_SLL_Refs.docx
image001.png
image002.png

Lishan:

Could you help add the following papers to your Endnote library? For some reason, I am unable to add any references! Also, you may help find references for several others and add them as well – I am unable to add for some reason.

Please the updated MS, with refs added.

Shan: I am unable to see the choice of EMI in the Endote library – what similar journal formats can I choose? Sounds like a silly question…

Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. Epub 2005 Feb 4.
Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human.
Song HD1, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L, Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP.

A **mouse-adapted SARS**-coronavirus causes disease and mortality in BALB/c mice.
Roberts A, Deming D, Paddock CD, Cheng A, Yount B, Vogel L, Herman BD, Sheahan T, Heise M, Genrich GL, Zaki SR, Baric R, Subbarao K.
PLoS Pathog. 2007 Jan;3(1):e5.

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Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 9:40 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I am downloading endnote x9 and hopefully will be able to format the references soon

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 8:56:06 PM
To: Su, Lishan <lishan_su@med.unc.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See my latest version attached. Some small changes have been made.

As of right now, should anyone else be listed as coauthors?

I can send the current draft without references to some coronavirus experts, but thought it will be nice to have all completed to show our due diligence

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 8:31 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I will add the references tonight.

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 7:44:27 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Sounds good, thank you. I still like “however” over “In contrast” – it just reads better

Shan: Are you sure that you prefer not to be included in the coauthorship? Before I send, I think we should have the authorship listed, along with affiliations. Lishan should be the first author, unless he prefers otherwise. Agreed?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 7:34 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

I made some minor change for the following:

In summary, there is no credible evidence at this point to support the claims that the 2019-nCoV was originated from a laboratory-engineered CoV. In contrast, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV.

Maybe now SLL can send the next version to other CoV experts?

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 5:47 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See the new version with all incorporated.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 4:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I have made additional changes to the Lishan's version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 4:16 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 3:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

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Date: Tuesday, February 11, 2020 at 1:52 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

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Subject: RE: 2019-nCoV-EMI_commentary

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Subject: RE: 2019-nCoV-EMI_commentary

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4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

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To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

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Date: Tuesday, February 11, 2020 at 12:44 PM
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Subject: RE: 2019-nCoV-EMI_commentary

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Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

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Evidence refuting laboratory origin of SARVS-CoV-2

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

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Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

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Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2 was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat betacoronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, and contained a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song et al, PNAS 2005). Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that 2019-nCoV evolved by natural evolution. Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic

mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis [6].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are now restricted as gain of function (GOF) studies under the US government-mandated

pause policy. The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (and not yet peer reviewed for accuracy) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have recently withdrawn this report.

In summary, there is no credible evidence to support the claim that the SARS-CoV-2 originated from a laboratory-engineered CoV. It is much more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

1. Wang, D.*, et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang*, et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N.*, et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P.*, et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N.*, et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D.*, et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y.*, et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W.*, et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F.*, et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

From: Liu, Shan-Lu
To: Su, Lishan; Lu, Shan
Subject: Re: 2019-nCoV-EMI_commentary
Date: Tuesday, February 11, 2020 7:16:49 PM
Attachments: SHC014-MA15 v 2019 ncoV-SLL-sls-SLL.docx
image001.png
image002.png

See my newest update:

Changes in last paragraph:

"In summary, we believe that there is no concrete evidence to support the claims that the 2019-nCoV was originated from a laboratory-engineered CoV. However, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV."

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See the new version with all incorporated.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 4:26 PM

To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I have made additional changes to the Lishan's version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
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The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Tentative Title: Is 2019-nCoV laboratory origin ~~of laboratory~~?

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease discovered in ~~identified~~ 2019 (COVID-19).

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The 2019-nCoV genome sequence also has ~80% identity with SARS-CoV, but is most similar to some bat beta-coronaviruses, with the highest being >96% identity.

Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan whereas ~~as~~ a bat CoV (RaTG13) was recently reported, which ~~by that laboratory and it~~ shared ~96% homology with the 2019-nCoV (Nature, 2020). However, as we now know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt. Given that there are greater than 1000 nt differences between 2019-nCoV and RaTG13, it is highly unlikely RaTG13 is the immediate source of 2019-nCoV; this is particular true in light of the low mutation rate of the coronaviruses. Searching for an inter~~m~~mediate host between bat and humans is needed.

Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014)

in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells. However, this claim lacks any scientific basis and must be discounted.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passages of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry. Civets were proposed to be an intermediate host of the bat-CoVs before they spread to humans (SARS-CoV review?). However, several novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interaction with SARS CoV (JVI 2012), it was proposed that intermediate hosts may not be necessary and that some bat SL-CoVs may directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus can indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15

can replicate efficiently in the mouse lung, leading to severe pathogenesis (Nat. Med. 2015).

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014-MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause. ~~No more bat CoV MA15 chimeric viruses are constructed after the SHC014 MA15 chimeric virus.~~ The NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potential. Regardless, ~~Regarding its lineage relationship with 2019 nCoV, however,~~ upon~~after~~ careful phylogenetic analyses by multiple international groups (EMI, Nature…2020), the 2019-nCoV is unmistakably distinct from SHC014- MA15, with >5000 nt differences across the whole~~in their~~ genome~~s~~. Therefore, t~~T~~here is NO credible evidence to support the claim that the 2019-nCoV is~~was~~ derived from the chimeric SHC014-MA15 virus.

There are also rumors that the 2019-nCoV is artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, claiming that 2019-nCoV has HIV sequence in it and thus likely generated in the laboratory. A rebuttal paper led by HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the 2019-nCoV is not HIV-1 specific but random (EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have recently decided to withdraw this report.

In summary, we believe that there is no concrete evidence to support the claims that the 2019-nCoV was originated from a laboratory-engineered CoV. However, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV. ~~Phylogenetic analyses~~

Commented [LS1]: Check accuracy for name and period, with refs

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~~of all reported CoV genomes by multiple international groups support the conclusion that 2019 nCoV is a novel virus……?~~

From: Liu, Shan-Lu
To: Lu, Shan; Su, Lishan
Cc: Liu, Shan-Lu
Subject: 2019-nCoV-EMI_commentary
Date: Tuesday, February 11, 2020 11:21:41 AM
Attachments: EMI commentary-20200211_SLL.docx
image001.png

See my suggested changes.

Thanks.

Shan-Lu

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Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Title:

The current concerns on the source of 2019 nCoV

Is 2019-nCoV a laboratory origin?

The emergence and outbreak of a newly discovered ~~new~~ acute respiratory disease~~s outbreak~~ in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. xx, 2020. A novel human coronavirus, 2019-nCoV, ~~(or NCP),~~ was~~has been~~ quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP).

According to what has been reported in the literature (refs), NCP has many (?) clinical manifestations that are similar to that of severe acute respiratory syndrome (SARS) caused by SARS-CoV. ~~the cause of this emerging infection.~~ The 2019-nCoV genome also has ~80% identity in sequence with SARS-CoV but most similar to some bat ~~same full-length gene sequences were found among multiple NCP viruses isolated from different countries and confirmed that it is a~~ beta-coronaviruses, with the highest being >96% identity. Currently, there are ~~like SAR-CoV. However, NCP only has ~80% identity with SARS-CoV.~~

~~Because of NCP's similar clinical manifestation as SARS, several mis-guided~~ speculations and rumors that the 2019-CoV is of a ~~have been circulating about the origin of the new virus, including its possible~~ laboratory origin. One particular claim~~speculation~~ points to~~at~~ a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS-CoV that has adapted to infect mice (rMA15) and is capable of infecting humans. There is also a rumor that the 2019-nCoV is directly from a bat virus (RaTG13) originally isolated from a laboratory in Wuhan China. Here we provide a summary of evidence that supports the conclusion that the 2019-nCoV is not from the chimeric coronavirus (SHC014-rMA15) nor the original bat virus RaTG13 (refs).

L~~First, et us~~we will first explain how a~~the~~ recombinant mouse-adapted SARS virus (rMA15) was generated. After constructing a full-length infectious SARS-C-eoV using~~by~~ reverse genetics, Dr. Ralph Baric's lab showed that it replicated in older mice with low or no pathogenicity ~~activity.~~ They then adapted the SARS-CoV (Urbani strain) by serial passages in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-C-eoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. When introduced into the original recombinant SARS-CoV, these six mutations (only one in the S gene) conferred the high virulence and lethality (rMA15). Although not reported in human cells, it is likely that rMA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

I~~Second, i~~t is also i~~m~~portant to know~~clarify~~ how the chimeric SHC014-rMA15 virus was constructed and what key findings were made using this~~at~~ virus. When the SARS-C-eoV was isolated, it was concluded that the S gene from bat-derived Ceo~~V~~, unlike its~~that from~~ human ~~patients~~ or civet~~s~~ counterparts

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~~derived viruses~~, was un~~not~~ able to use the human ACE2 as a receptor for entry. Civets were proposed to be an immediate ~~as the secondary~~ host before~~for~~ the bat-CoV ~~before~~ spread~~sing~~ to humans. However, novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SL-CoV-WIV1 used ACE2 from humans, civets and Chinese horseshoe bats for entry. Based on the evolutionary evidence that the bat ACE2 gene has been positively selected at the same ~~contact sites~~interface as the human ACE2 gene for interacting~~on~~ with SARS-C-~~o~~oV, it was proposed that an intermediate host~~s~~ may not be necessary for ~~and~~ some bat CoVs to ~~may~~ directly infect human~~s hosts~~. To directly address this possibility, the S gene ~~from~~ of the bat coronavirus WIV1-SHC014 was used to generate a chimeric virus in the mouse adapted -rMA15 SARS-CoV backbone. The resultant SHC014-rMA15 virus can efficiently use ACE2 from multiple species and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-rMA15 can replicate efficiently in the mouse lung with severe pathogenesis. These findings ~~have~~ provide ~~d~~ compelling~~strong~~ evidence that some bat CoVs can directly use human ACE2 to infect human hosts.

Due to the elevated pathogenic activity of the SHC014-rMA15 chimeric virus relative to the Urbani Spike-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause (the ban was implemented in 2013/2014 but lifted by NIH in 2017). No more bat-Co~~c~~V-M15 chimeric viruses have been~~are~~ constructed thereafter the SHC014-r-MA15 chimeric virus. The NCP epidemic has triggered a new ~~restarted the~~ debate on~~over~~ whether or not it is worth the risks of constructing such viruses with possible pandemic potential (refs), which is not unexpected. However, w~~, W~~ith careful and in-depth ~~careful~~ phylogenetic analyses by multiple international groups, the 2019-nC~~n~~eoV~~/NCP virus~~ is unmistakably, and fortunately, distinct from SHC014- MA15. (we need a good summary here one two three…). Therefore t~~T~~here is NO credible evidence to support the claim that the 2019 ncoV/NCP virus was derived for the chimeric SHC014- MA15 virus.

Another ~~The next~~ speculation is that 2019-nCoV~~NCP~~ is directly mutated from a recently ~~newly~~ reported bat-CoV isolate RaTG13) (Ref), ~~-~~because these two viruses share~~d~~ more than ~~≈~~96% sequence homology. With a large size genome like beta-coronaviruses (~20 kb), there are still ~1000 nt differences between these two viruses (ref), indicating RaTG13 is un~~not~~ likely the source of 2019-nCov. ~~NCP,~~ Moreover the mutation rate of the coronaviruses is considered to be very low (need a number here) due to the existence of a proofreading enzyme ExoN (nsp14). Most important, the 2019-nCoV contains a potential furin-cleavage signature sequence "RRAR", which is not present in most coronaviruses, including the RaTG13, although recombination between RaTG13 and other coronaviruses cannot be ruled out. ~~and thus denied the speculation that NCP is somehow a leaked relative of RaTG13 from the laboratory who originally identified RaTG13.~~

There are also rumors that the 2019-nCoV is artificially and intentionally made by humans in the lab and this is highlighted one manuscript submitted to BioRxiv,~~also~~ claiming that 2019-nCoV has HIV sequence in it and thus likely generated in the laboratory. ~~other people who claimed the finding of other viral sequences within HCP genome, implying that someone artificially created the NCP. One such claim suggested finding of four inserts related to HIV-1 sequences.~~ A rebuttal paper led by HIV-1 expert Dr. Feng Gao has used~~did~~ careful bioinformatics analyses~~is~~ to~~and~~ demonstrate~~showed~~ that the original claim of multiple HIV insertion into the 2019-nCoV is not ~~do not have data to show these 4 insert~~

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~~sequences are actually~~ HIV-1 specific but random.. In addition, the four inserts can not be held together based on structure modeling as initially claimed (ref.). At the same time, those authors who made the initial claim has withdrawn their report.

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From:	Liu, Shan-Lu
To:	Weiss, Susan
Cc:	Su, Lishan; Lu, Shan
Subject:	Commentary for EMI
Date:	Wednesday, February 12, 2020 11:25:38 AM
Attachments:	EMI-2019-nCoV_Commentary_Final.docx image001.png

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Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of “Emerging Microbes and Infections”, Dr. Shan Lu (don’t get confused, it’s not me). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

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SARVS-CoV-2: no evidence of a laboratory origin

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[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

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Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [12], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs

already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 13], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

13. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From:	Liu, Shan-Lu
To:	Su, Lishan; Lu, Shan
Subject:	Re: 2019-nCoV-EMI_commentary
Date:	Tuesday, February 11, 2020 8:56:05 PM
Attachments:	EMI-2019-nCoV_Commentary.docx image001.png

See my latest version attached. Some small changes have been made.

As of right now, should anyone else be listed as coauthors?

I can send the current draft without references to some coronavirus experts, but thought it will be nice to have all completed to show our due diligence

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 8:31 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I will add the references tonight.

-Lishan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 7:44:27 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Sounds good, thank you. I still like “however” over “In contrast” – it just reads better

Shan: Are you sure that you prefer not to be included in the coauthorship? Before I send, I think we should have the authorship listed, along with affiliations. Lishan should be the first author, unless he prefers otherwise. Agreed?

Shan-Lu

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 7:34 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

I made some minor change for the following:

In summary, there is no credible evidence at this point to support the claims that the 2019-nCoV was

originated from a laboratory-engineered CoV. In contrast, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV.

Maybe now SLL can send the next version to other CoV experts?

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 5:47 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

See the new version with all incorporated.

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 4:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I have made additional changes to the Lishan's version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 4:16 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 3:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Perhaps Lishan can take a look at the latest version, which has the new title I suggested, and modify it as needed.
The last paragraph is also crucial, but I did not have time to work on it because of a meeting this morning.
Once we have almost a final draft, I will contact Linda Saif, Stanley Perlman, Thomas Gallgaher etc. to see if they are willing to join, but this may delay the publishing time.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 1:52 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

I agree that it should be simple and clear. I have included some details in the 1st draft for your information. There is not intention to defend Baric, but to clarify the facts.

Regarding all three, are you combining Goa Feng's piece with this one? For the RaTG13, it involves complicated viral evolution kinetics and maybe hard to simply clarify…
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 1:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and

provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

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Is 2019-nCoV a laboratory origin?

Lishan Su[1], and Shan-Lu Liu[2, 3,4.5]

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[2] Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210, USA

[3] Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA

[4] Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH 43210, USA

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Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease discovered in 2019 (COVID-19).

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The 2019-nCoV genome sequence also has ~80% identity with SARS-CoV, but is most similar to some bat beta-coronaviruses, with the highest being >96% identity (refs).

Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the 2019-nCoV (Nature, 2020). However, as we know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt differences in the whole genome sequence (refs). Given that there are greater than 1000 nt differences between the 2019-nCoV and the RaTG13-CoV (refs), it is highly unlikely RaTG13 is the immediate source of 2019-nCoV; this is particularly true in light of a low mutation rate of the coronaviruses (refs). Searching for an intermediate host between bat and humans is needed.

Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells (refs). However, this claim lacks any scientific basis and must be discounted.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passages of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry (refs). Civets were proposed to be an intermediate host of the bat-CoVs before they spread to humans (refs). However, several novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interacting with SARS CoV (JVI 2012), it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts (refs). To directly address this possibility, the S gene from bat coronavirus

SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus can indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis (Nat. Med. 2015).

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause policy (refs). The current NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potential. Regardless, upon careful phylogenetic analyses by multiple international groups (EMI, Nature…2020), the 2019-nCoV is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, there is no credible evidence to support the claim that the 2019-nCoV is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the 2019-nCoV is artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, claiming that 2019-nCoV has HIV sequence in it and is thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the 2019-nCoV is not HIV-1 specific but random (EMI paper 2/12/2020). Because

of the many concerns raised by the international community, the authors who made the initial claim have recently decided to withdraw this report.

In summary, we believe that there is no credible evidence to support the claim that the 2019-nCoV was originated from a laboratory-engineered CoV. However, we cannot rule out the possibility that 2019-nCoV is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of 2019-nCoV.

From: Liu, Shan-Lu
To: Su, Lishan; Lu, Shan
Subject: Re: 2019-nCoV-EMI_commentary
Date: Tuesday, February 11, 2020 4:25:47 PM
Attachments: SHC014-MA15 v 2019 ncoV-SLL.docx
image001.png

I have made additional changes to the Lishan's version, see attached.

Lishan: I share your concern, and that is one reason that Shan, the editor, decides to have a short version.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 4:16 PM
To: Shan-Lu Liu <liu.6244@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

The new title is good if we will cover the RaTG13 and HIV insertion issues.
I am still worried if we can shed any light on the major claim of RaTG13 lab escape/evolution in other hosts/humans over the years…?

-Lishan

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 at 3:26 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Perhaps Lishan can take a look at the latest version, which has the new title I suggested, and modify it as needed.
The last paragraph is also crucial, but I did not have time to work on it because of a meeting this morning.
Once we have almost a final draft, I will contact Linda Saif, Stanley Perlman, Thomas Gallgaher etc. to see if they are willing to join, but this may delay the publishing time.

SL

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Tuesday, February 11, 2020 at 1:52 PM
To: "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

st

I agree that it should be simple and clear. I have included some details in the 1 draft for your information. There is not intention to defend Baric, but to clarify the facts.

Regarding all three, are you combining Goa Feng's piece with this one? For the RaTG13, it involves complicated viral evolution kinetics and maybe hard to simply clarify…
Best,

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 1:44 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Sorry here is the attachment with tracking

From: Lu, Shan
Sent: Tuesday, February 11, 2020 1:44 PM
To: 'Su, Lishan' <lishan_su@med.unc.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Hi, I am adding Shuying from my group. She just read the last draft from Lishan and identified a few errors (with tracking).

Also I just had a phone call with SSL and we agreed on the following:

1. We need to make this commentary very simple and short;
2. It is better not going to too much science/tech details as it can only confuse people and provide more room for people to raise more questions;
3. We don't want to appear that we are defending Ralph even though he did nothing wrong.
4. We feel it is best to cover 3 issues in this commentary (for the above reason, plus it is more powerful to cover multiple issues in one summary)
5. ??

SLL: please add anything I missed.

Shan

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Tuesday, February 11, 2020 12:52 PM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Re: 2019-nCoV-EMI_commentary

Thanks. Looking at Shanlu's version, we may need a separate for the RaTG13 vs lab accident theory...

-Lishan

From: "Lu, Shan" <Shan.Lu@umassmed.edu>
Date: Tuesday, February 11, 2020 at 12:44 PM
To: "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: 2019-nCoV-EMI_commentary

Here is my new version based on SLL's. highlighted areas are my new version (I did not leave tracking as it is too messy). Please take a look then we can focus on the chimeric one which needs more simplification as I can see. We may not need to go too deep in science as it can only confuse more people and found more issues from those who has suspicion.

Shan

From: Liu, Shan-Lu <liu.6244@osu.edu>
Sent: Tuesday, February 11, 2020 11:22 AM
To: Lu, Shan <Shan.Lu@umassmed.edu>; Su, Lishan <lishan_su@med.unc.edu>
Cc: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: 2019-nCoV-EMI_commentary

LIU.6244@OSU.EDU appears similar to someone who previously sent you email, but may not be that person. Learn why this could be a risk Feedback

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
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Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Tentative Title: The mouse adapted SARS chimeric virus with bat-coV S gene (SHC014-MA15) is not related to the NCP ncoV or 2019 nco-V

A new suggested title: Is 2019-nCoV origin of laboratory?

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The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, 2019-nCoV, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease identified 2019 (COVID-19).

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The 2019-nCoV genome sequence also has ~80% identity with SARS-CoV, but most similar to some bat beta-coronaviruses, with the highest ~~reaching~~ being >96% identity. ~~Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin.~~

Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. ~~This led to speculations and rumors that the 2019-CoV is of a laboratory origin~~. First, certain people suspected that the 2019-nCoV is directly leaked from a laboratory in Wuhan as a bat CoV (RaTG13) was recently reported by that laboratory and it shared ~96% homology with the 2019-nCoV (Nature, 2020). However, as we now know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt. Given that ~~On the other hand,~~ there are greater than 1000 nt differences between 2019-nCoV and RaTG13, it is highly unlikely~~suggesting~~ RaTG13 is not the immediate source of 2019-nCoV; this is particular true in light of the low

~~given the large size genome like beta coronaviruses (~30 kb) and the slow the~~ mutation rate of the coronaviruses. Searching for an immediate host between bat and humans is needed.

~~One particular~~Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (rMA15) and is capable of infecting human cells. Here, we provide evidence that this claim lacks ~~of~~ any scientific basis and must be discounted. (However, this claim lacks any scientific basis and must be discounted. – we can use this sentence if the editor decides to have a shorter/simpler version)

~~First, we will explain how t~~The recombinant mouse-adapted SARS virus (~~r~~MA15) ~~was generated~~ (PLoS Pathog. 2007 Jan;3(1):e5)~~.~~ was generated ~~After constructing a full-length infectious~~ SARS coV ~~by reverse genetics, Dr. Ralph Baric's lab showed that it replicated in old mice with low or no pathogenic activity. They then adapted the SARS-CoV (Urbani strain)~~ by serial passages of an infectious SARS coV in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-~~C~~coV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. ~~When introduced into the original recombinant SARS-CoV, these six mutations (only one in the S gene) conferred the high virulence and lethality (rMA15). Although not reported in human cells, i~~It is likely that ~~r~~MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

~~Second, it is important to clarify how the chimeric~~ ~~SHC014-rMA15~~ ~~virus was constructed and what key findings were made using that virus.~~ When the SARS-~~C~~coV was isolated, it was concluded that the S gene from bat-

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Commented [SL1]: Specify Urbani strain here?

Commented [SL2]: In the paper , they use SHC014-MA15

derived Cc~~c~~oV, unlike that ~~-~~from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry. Civets were proposed to be an intermediate~~as the secondary~~ host of ~~for~~ the bat-Cc~~c~~oVs before they spread~~ing~~ to humans (SARS-~~-~~Cc~~c~~oV review?). However, several novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SL-CoV-WIV1 used ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interaction with SARS coV (JVI 2012), it was proposed that intermediate hosts may not be necessary and some bat SARS-like or SL-CoVs may directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-~~- WIV1-~~ SHC014 was used to generate a chimeric virus in the mouse adapted-~~r~~MA15 SARS-CoV backbone. The resultant SL-SHC014-~~r~~MA15 virus can efficiently use human ACE2 ~~from multiple species~~ and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-~~r~~MA15 can replicate efficiently in the mouse lung, leading to ~~with~~ severe pathogenesis (Nat. Med. 2015). ~~These findings have provided strong evidence that some bat CoVs can directly use human ACE2 to infect human hosts.~~

Commented [SL3]: SHC014 and WIVI are two different bat Cov, with some sequence difference in S domain

Due to the elevated pathogenic activity of the SHC014-~~r~~MA15 chimeric virus relative to the ~~Urbani Spike~~SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause. No more bat-coV-MA15 chimeric viruses are constructed after the SHC014-MA15 chimeric virus. The NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potential. Regarding its lineage relationship with 2019 nCoV, however, after careful phylogenetic analyses by multiple international groups (EMI, Nature…2020, a figure?), the 2019 ncoV/NCP virus is unmistakably, and fortunately, distinct from

SHC014- MA15. There is NO credible evidence to support the claim that the 2019 ncoV/NCP virus was derived ~~for~~ from the chimeric SHC014- MA15 virus.

There are also rumors that the 2019-nCoV is artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, claiming that 2019-nCoV has HIV sequence in it and thus likely generated in the laboratory. A rebuttal paper led by HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertion into the 2019-nCoV is not HIV-1 specific but random. Moreover, the four inserts cannot be held together as was initially claimed (EMI paper 2/12/2020). Because of the many concerns raided by the international community, the authors who made the initial claim have recently decided to withdraw this report.

Concluding sentence?

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Commented [LS4]: "Do not seem to cluster to the same interface"?

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From: Liu, Shan-Lu
To: Su, Lishan; Lu, Shan
Subject: FW: Commentary for Emerging Microbes & Infections
Date: Wednesday, February 12, 2020 1:26:52 PM
Attachments: EMI-2019-nCoV_Commentary_Final LJS 2020.docx
image001.png

See below.
I am now finalzing it. Not sure if we need to wait for Stanely, but may be good to add Peter? Should I try?

SL

From: "Saif, Linda" <saif.2@osu.edu>
Date: Wednesday, February 12, 2020 at 11:35 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Subject: Re: Commentary for Emerging Microbes & Infections

Hi Shan-Lu,
A few minor edits—nice job on this write up!
Experts to add include Stan and Peter Daszak (daszak@ecohealthalliance.org), but maybe not essential since with Peter we have prepared a similar statement to denounce the conspiracies with multiple signatories of respected scientists including internationally recognized coronavirologists! However our statement does not add the details that are in this commentary which I think are very important to cite as supporting scientific evidence.
Also Peter told me the NAS is preparing a similar statement to denounce these conspiracy theories circulating on the internet but I have not seen this yet.
I will send this to Ralph to review, but as I noted he may be too busy to respond!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 11:01 AM
To: Linda Saif <saif.2@osu.edu>
Cc: "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: Commentary for Emerging Microbes & Infections

Dear Linda;

Attached please find almost the final version of the commentary for EMI, so please feel free to share it with Ralph. Let me know if you have additional suggestions – all your points are incorporated into the new version, please check.

Note that I was trying to find official website links for the new names of the virus (ICTV) and diseases (WHO), but failed; I therefore decided to use the following website, which contains both.

https://globalbiodefense.com/novel-coronavirus-covid-19-portal/

We will try to submit it today, but are considering to add a few more coronavirus experts – anyone that you would like to suggest? We will contact Stanley Perlman right now.

Shan-Lu

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Center for Retrovirus Research
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Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

From: "Saif, Linda" <saif.2@osu.edu>

Date: Wednesday, February 12, 2020 at 9:37 AM

To: Shan-Lu Liu <liu.6244@osu.edu>

Subject: Re: Commentary for Emerging Microbes & Infections

Can you please send me the updated version first and then I will try to share with Ralph!

Thanks

Linda

Linda J. Saif, PhD

Distinguished University Professor

Food Animal Health Research Program

OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Wednesday, February 12, 2020 12:47 AM
To: Linda Saif <saif.2@osu.edu>
Subject: Re: Commentary for Emerging Microbes & Infections

Hi Linda.
Thanks so much, and your comments are extremely helpful. Please feel free to share with Ralph to get his feedback if possible. We would like to publish this in the next few days.
I will work on reference tomorrow and send you a updated version.

Shan-Lu Liu sent from iPhone

> On Feb 11, 2020, at 11:54 PM, Saif, Linda <saif.2@osu.edu> wrote:
>
> Hi Shan-Lu,
> I edited this version and added my name as I too feel strongly about denouncing this.
> Here are more comments and some refs that I have made in replies to some reporters about this issue if you think any are useful to include. I also wonder if we might share this with Ralph Baric since he is a conspiracy target and maybe he could add additional points, but I know he would not want to be a co-author—not sure if he has time to answer.
>
> The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that 2019-nCoV evolved by natural evolution. Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.
>
> The closest virus relative to 2019-nCoV is bat CoV RaTG13. There are 4% nt differences between 2019-nCoV and RaTG13, corresponding to >1000 nt based on a genome size of 29k. These changes (SNP) are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics

typical of CoVs, including the S gene as the most variable region.
(Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature doi:10.1038/s41586-020-2012-7.

Regarding differences between civet cat SARSr-CoV and SARS-CoV, here is the accurate data: . A total of 202 SNVs with multiple occurrences were identified, among which 200 were in the CDSs. Among the 128 nonsynonymous mutations, 89 led to a predicted radical amino acid changes
Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. Epub 2005 Feb 4.
Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human.
Song HD1, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L, Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP.

Linda J. Saif, PhD
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1680 Madison Ave
Wooster, Oh 44691

From: "Liu, Shan-Lu" <liu.6244@osu.edu>
Date: Tuesday, February 11, 2020 10:32 PM
To: Linda Saif <saif.2@osu.edu>
Subject: Commentary for Emerging Microbes & Infections

Hi Linda,

Invited by the editor in chief of EMI, Lushan Su from UNC and I have written a commentary on the possible origin of the 2019-nCoV or SARS-CoV-2 in order to dispute some rumors, and we would like to invite you as a coauthor. Attached please find an almost complete draft (references needed) of the commentary, so kindly let me know what you think. Your comments and suggestions are very much appreciated.

Thanks.

Shan-Lu

<image001.png>
Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
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<image001.png>
<EMI-2019-nCoV_Commentary LJS.docx>

SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

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[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

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[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

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[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [12], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathoglogyenesis [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding

that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 13], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

References

1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

13. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

From: Liu, Shan-Lu
To: Liu, Shan-Lu
Date: Sunday, December 20, 2020 10:52:32 AM

For the following item “Feb 23, 2020: Themed discussion on the possible origin of SARS-CoV-2 and RaTG13, especially whether or not SARS-CoV-2 is artificially engineered; this led to an article entitled “No credible evidence supporting claims of the laboratory engineering of SARS- CoV-2” by Lishan Su and Shan-Lu Liu and others published in EMI.” I wonder if you can add: “This paper received 75000 download read ranking #3 of the top 10 papers among over 2500 journals in the Taylor & Francis family”.

Thanks.

Shan-Lu

From:	Bergan, Rachel
To:	Liu, Shan-Lu
Subject:	Your paper features in T&F"s top ten OA articles of 2020
Date:	Thursday, October 22, 2020 4:59:57 AM
Attachments:	image001.png image002.png

Dear Shan-Lu,

I'm very pleased to let you know that your recent article "No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2" was one of the most-downloaded open access articles published by Taylor & Francis so far this year.

Many congratulations!

To mark Open Access Week, we have published a blog post on our Author Services website about the Top 10, including links to the articles, and details of each Altmetric score to reflect the discussion of your article online and in the media.

This is a great excuse to further highlight your research to your contacts and communities. We will be promoting the blog post on our social media platforms: Twitter, LinkedIn, and Facebook. You could share our posts/tweets or write your own (please use the same hashtags).

We'll also be featuring the Top 10 in our Insights newsletter, to further reach researchers and the wider academic community.

Please do not hesitate to contact me if you have any further questions or queries.

Kind Regards,
Rachel

Rachel Bergan (she/her)
Communications Coordinator
Taylor & Francis Group

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4 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN, UK

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Information Classification: General

From:	Bergan, Rachel
To:	Liu, Shan-Lu; lsu@med.unc.edu
Subject:	Top 10 most-read open access articles of 2020
Date:	Friday, January 22, 2021 5:45:31 AM
Attachments:	image001.png

Hi there,

I'm very pleased to let you know that your recent article "No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2" was one of the most-downloaded open access articles published by Taylor & Francis in 2020.

Many congratulations!

We have published a report on our Author Services website about the Top 10, including links to the articles, and details of their Altmetric score, to reflect the discussion about this research online and in the media.

This is another great excuse to highlight your research to your contacts. We will be promoting the blog post on our social media platforms - here's a tweet from our Twitter account that you can like and share with your network.

We'll also be featuring the Top 10 in our Insights newsletter and Open Access Bulletin, to share this popular feature with the wider academic community.

Please do not hesitate to contact me if you have any questions.

Kind regards,
Rachel

Rachel Bergan (she/her)
Marketing Communications Coordinator
Taylor & Francis Group

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4 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN, UK

Information Classification: General

From:	Liu, Shan-Lu
To:	Liu, Shan-Lu
Cc:	Shan-Lu Liu
Subject:	<no subject>
Date:	Monday, December 21, 2020 11:13:40 AM
Attachments:	image001.png image002.png Liu_EMI.pdf

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https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1733440

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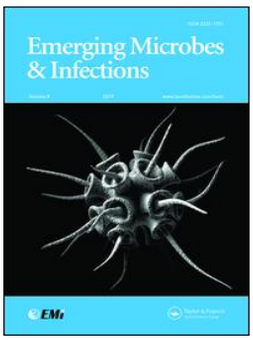

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su

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Emerging Microbes & Infections
2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1733440

COMMENTARY

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No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan R. Weiss [f] and Lishan Su[g]

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ARTICLE HISTORY Received 13 February 2020; Accepted 13 February 2020

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1,100 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

It was proposed that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary

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evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to MA15 chimeric virus with the original human SARS S gene in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Susan R. Weiss http://orcid.org/0000 0002 8155 4528

References

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1585

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections invol ving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1623

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coro navirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30;395(10223):507 513.

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia out break associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2012 7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24;382(8):727 733.

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evol ution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characteriz ation of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS corona virus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin con verting enzyme 2 is a functional receptor for the

SARS coronavirus. Nature. 2003 Nov 27;426(6965): 450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2008 3
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

From: Liu, Shan-Lu
To: [redacted]
Subject: Fwd: FW: A Bayesian Analysis of the origin of SARS-CoV-2
Date: Wednesday, January 27, 2021 8:05:18 PM
Attachments: image001.png
2021-01-26_SQuay_Bayesian Analysis of SARS-CoV-2.pdf

Thanks.

Shan-Lu

From: Linda Saif [redacted]
Sent: Tuesday, January 26, 2021 11:04:18 PM
To: Liu, Shan-Lu <liu.6244@osu.edu>
Subject: Fwd: FW: A Bayesian Analysis of the origin of SARS-CoV-2

What do you think of this document, especially statements about samples and sequences, etc

---------- Forwarded message ---------
From: **Saif, Linda** <saif.2@osu.edu>
Date: Tue, Jan 26, 2021 at 10:04 PM
Subject: FW: A Bayesian Analysis of the origin of SARS-CoV-2
To: linda saif [redacted]

Linda J Saif, MS, PD

Distinguished University Professor

OARDC/CFAES/CVM

1680 Madison Ave

Wooster, Ohio 44691

CENTER TO STOP COVID

From: "Steven Quay, MD, PhD" <steven@drquay.com>
Date: Tuesday, January 26, 2021 at 10:45 AM
To: "Steven Quay, MD, PhD" <steven@drquay.com>

Subject: A Bayesian Analysis of the origin of SARS-CoV-2

Greetings-

I hope this email finds you well.

Attached is an article I have written on a Bayesian analysis of the origin of SARS-CoV-2. Because of your expertise on this topic I would really appreciate receiving your comments, criticisms, and corrections. Also, if there is evidence that I did not consider which you think should be included please make those suggestions. As you know, one of the powers of the Bayesian method is that whenever new evidence is introduced or old evidence is reevaluated, the posterior probabilities can be rerun to come to a new conclusion.

It is vitally important that we determine the cause of this outbreak and I hope my work can make a contribution to this effort.

Regards, Steve
--

Steven Quay, MD, PhD

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Dr. Quay Website

Breast Cancer TEDx Talk

STAY SAFE #1 Amazon Medical Book

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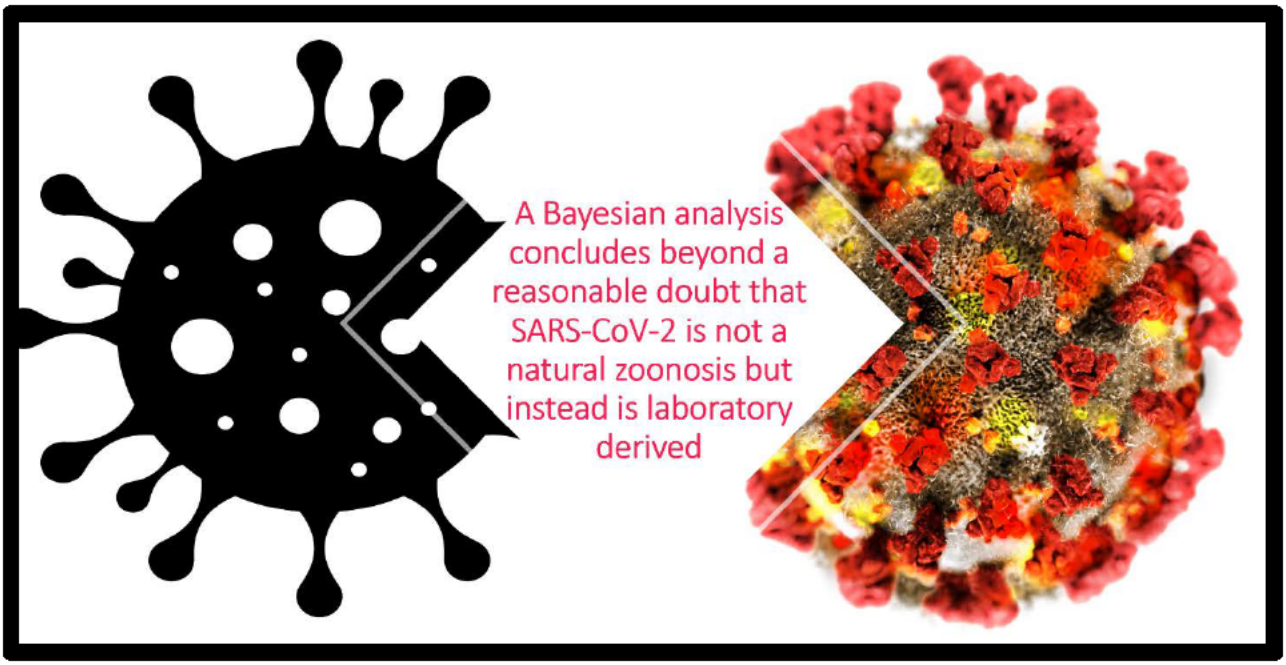

Wuhan Institute of Virology analysis of bronchial lavage specimens from ICU patients at Wuhan Jinyintan Hospital in December 2019 contain both SARS-CoV-2 and adenovirus vaccine sequences, consistent with a vaccine challenge trial

By

Steven Carl Quay, MD, PhD

www.DrQuay.com

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A Bayesian analysis concludes beyond a reasonable doubt that SARS-CoV-2 is not a natural zoonosis but instead is laboratory derived

Wuhan Institute of Virology analysis of lavage specimens from ICU patients at Wuhan Jinyintan Hospital in December 2019 contain both SARS-CoV-2 and adenovirus vaccine sequences consistent with a vaccine challenge trial

Executive Summary. The one-year anniversary of the COVID-19 pandemic records 2.1 million deaths, over 100 million confirmed cases,[1] and trillions of dollars of economic damage. Although there is universal agreement that a coronavirus identified as Severe Acute Respiratory Syndrome Coronavirus 2 or SARS-CoV-2 (abbreviated CoV-2 henceforth) causes the disease COVID-19, there is no understanding or consensus on the origin of the disease.

The Chinese government, WHO, media, and many academic virologists have stated with strong conviction that the coronavirus came from nature, either directly from bats or indirectly from bats through another species. Transmission of a virus from animals to humans is called a zoonosis.

A small but growing number of scientists have considered another hypothesis: that an ancestral bat coronavirus was collected in the wild, genetically manipulated in a laboratory to make it more infectious, training it to infect human cells, and ultimately released, probably by accident, in Wuhan, China. For most of 2020 this hypothesis was considered a crackpot idea, but in the last few weeks, more media attention has been given to the possibility that the Wuhan Institute of Virology, located near the Wuhan city center and with a population of over 11 million inhabitants, may have been the source of the field specimen collection effort, laboratory genetic manipulation, and subsequent leak. On January 15, 2021, the U.S. Department of State issued a statement requesting the WHO investigation of the origin of COVID-19 include specific assertions related to a laboratory origin of the pandemic.[2]

Given the strong sentiment in the scientific community in favor of a zoonosis and the massive effort undertaken by China to find the natural animal source, one can assume that any evidence in favor of a natural origin, no matter how trivial, would become widely disseminated and known. This provides a potential evidence bias within the scientific community in favor of a natural origin which isn't quantifiable but should be kept in mind.

This becomes especially important background when evidence that could support a laboratory origin has been directly provided by leading Chinese scientists themselves, like Dr. Zhengli Shi, head of coronavirus research at the Wuhan Institute of Virology and Gao Fu (George Fu Gao), Director of Chinese CDC; by the Chinese government, as well as by powerful and vocal, pro-natural origin scientists, like Dr. Peter Daszak, of the NYC-based NGO, EcoHealth Alliance.

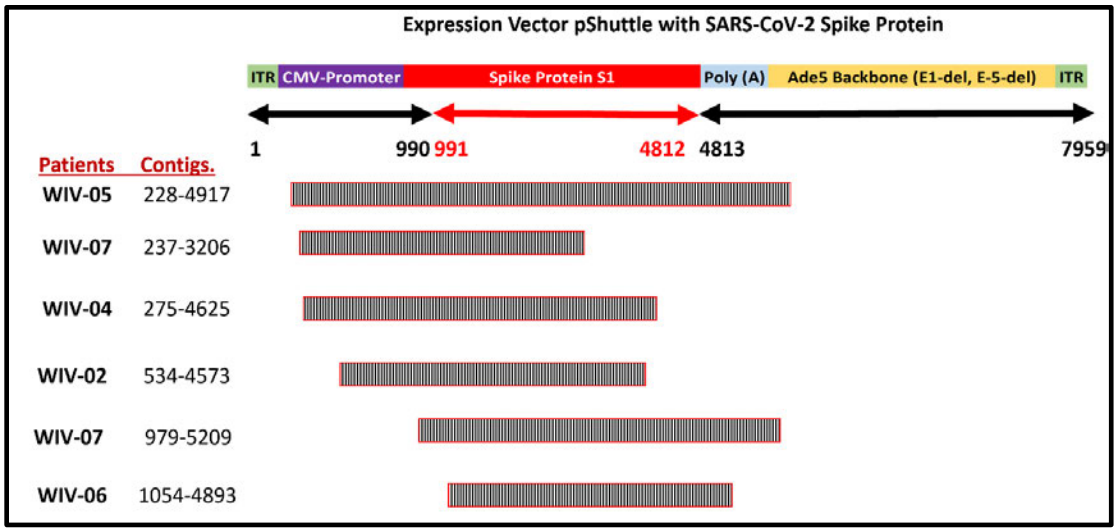
[1] https://www.worldometers.info/coronavirus/?
[2] https://www.state.gov/ensuring-a-transparent-thorough-investigation-of-covid-19s-origin/

This report uses Bayesian inference, a common statistical tool in which Bayes' theorem, a well-known statistical equation, is used to update the likelihood for a particular hypothesis as more evidence or information becomes available. It is widely used in the sciences and medicine and has begun to be used in the law.

The starting probability for origin of SARS-CoV-2 was set with the zoonotic or natural hypothesis at 98.8% likelihood with the laboratory origin hypothesis set at 1.2%. The initial state was biased as much as possible towards a zoonotic origin, with the starting point selected as the upper bounds of the 95% confidence interval for the mean and standard deviation of three independent estimates, including one by Daszak and colleagues. Each piece of new evidence for or against each hypothesis was then used to adjust the probabilities. If evidence favored a natural origin the math adjusts upward the probability of a natural origin, and so on.

The most significant evidence provided herein is the finding from RNA-Seq performed by the Wuhan Institute of Virology (WIV) of lavage patient samples collected on December 30, 2019.[3] These ICU patients were the subject of the seminal paper, entitled, "A pneumonia outbreak associated with a new coronavirus of probable bat origin," from Dr. Zhengli Shi and colleagues that first characterized SARS-CoV-2.[4] This author has confirmed that the RNA-Seq of all five patients contained SARS-CoV-2 sequences.

Surprisingly the specimens also contained the adenovirus "pShuttle" vector, developed by Chinese scientists in 2005 for SARS-CoV-1.[5] Two immunogens were identified, the Spike Protein gene of SARS-CoV-2 and the synthetic construct H7N9 HA gene.[6] Hundreds of perfectly homologous (150/150) raw reads suggest this is not an artefact. Reads that cross the vector-immunogen junction are identified. An example of the read contigs for CoV-2 is shown in this figure:



[3] The detailed evidence for the adenovirus vaccine sequences is given at the end of this document.
[4] https://www.nature.com/articles/s41586-020-2012-7
[5] https://www.ncbi.nlm.nih.gov/nuccore/AY862402.1
[6] https://www.ncbi.nlm.nih.gov/nuccore/KY199425.1/

While adenovirus is a common infection the wildtype viruses have low homology to the vaccine vector sequence, by design, to avoid rejection of the vaccine due to prior exposure to wildtype adenoviruses.

Two patients from the same hospital who had bronchial lavage on the same day but had their specimens sent to the Hubei CDC did not have adenovirus vaccine sequences.

Three explanations come to mind from this evidence:

1. **These represent sample preparation artifacts at the WIV, such as sample spillover on the sequencer.**
2. **These patients were admitted with an unknown infection, were not responding to the treatment protocols for a infection of unknown origin, and they were vaccinated with an experimental vaccine in a desperate but compassionate therapeutic "Hail Mary."**
3. **A clinical trial of a combination influenza/SARS-CoV-2 vaccine was being conducted and an accidental release into Wuhan occurred.**

Only WIV scientists and Chinese authorities can answer these questions. Until the evidence of the adenovirus sequences has been confirmed by other scientists, this author will not include this evidence in the Bayesian analysis.

Obviously if a vaccine containing the Spike Protein of SARS-CoV-2 was being administered to patients in Wuhan in December 2019 the question of laboratory origin is a settled matter.

The remaining analysis is being conducted without the adenovirus vaccine evidence unless and until it is corroborated. The outcome of this report is the conclusion that the probability of a laboratory origin for CoV-2 is 99.8% with a corresponding probability of a zoonotic origin of 0.2%. This exceeds most academic law school discussions of how to quantify 'beyond a reasonable doubt,' the threshold for finding guilt in a criminal case. The report contains the detailed analysis and quantitative basis for the statistics and conclusion. It should be noted that because of the commutative property of the collected adjustments to the probabilities, the order in which they are used in the overall calculation is immaterial and the same end likelihoods will be reached regardless of the order of input.

The following Text-Table summarizes the evidence examined and the changes in probabilities:

Evidence	Zoonotic Origin	Laboratory Origin
Initial State	98.8%	1.2%
International committees to determine CoV-2 origin may not be impartial	98.8%	1.2%
Three key zoonotic papers: pros and cons	98.8%	1.2%
SARS-like infections among employees of the Wuhan Institute of Virology in the fall of 2019 reported by US Government	98.8%	1.2%
Location of first cases near Wuhan Institute of Virology	95.1%	4.9%
Lack of evidence of seroconversion in Wuhan and Shanghai	80.9%	19.1%
Lack of posterior diversity	30.8%	69.2%
Opportunity: The Wuhan Institute of Virology has publicly disclosed that by 2017 it had developed the techniques to collect novel coronaviruses, systematically modify the receptor binding domain to improve binding or alter zoonotic tropism and transmission, insert a furin site to permit human cell infection, make chimera and synthetic viruses, perform experiments in humanized mice, and optimize the ORF8 gene to increase human cell death.	30.8%	69.2%
Lack of furin cleavage sites in any other sarbecovirus	4.7%	95.3%
Rare usage of -CGG- single codons & no CGG-CGG pairs	0.5%	99.5%
Routine use of CGG in laboratory codon optimization, including Daszak & Shi	0.2%	99.8%
Spike Protein receptor binding region (200 amino acids) optimized for humans	0.2%	99.8%
Whole genome analysis shows pre-adaption of CoV-2	0.2%	99.8%
The finding of CoV-2 in Barcelona wastewater in early 2019 was an artifact	0.2%	99.8%
Shi and the WHO comment early on that CoV-2 seemed to begin with a single patient	0.2%	99.8%
Mammalian biodiversity between Yunnan and Hubei is significantly different, limiting a potential common intermediate host	0.2%	99.8%
The ancestor of CoV-2 can only obtain a furin site from other subgenera viruses but recombination is limited/non-existent between subgenera	0.2%	99.8%
Canvas of 410 animals shows humans and primates are the best, bats are the worst, for ACE2-Spike Protein interaction	0.2%	99.8%
A government requested review of samples collected from a mineshaft may have caused the COVID-19 pandemic	0.2%	99.8%
The Hunan Seafood Market and farmed animals in Hubei province are not the source of CoV-2	0.2%	99.8%
Line 2 of the Wuhan Metro System is the likely conduit of the pandemic and is the closest subway line to the WIV	0.2%	99.8%
Feral and domestic cats are not the intermediate host	0.2%	99.8%
Extraodinary pre-adaption for the use of human tRNA is observed	0.2%	99.8%
Evidence of lax operations and disregard of laboratory safety protocols and regulations in China	0.2%	99.8%
Previous SARS-CoV-1 laboratory accidents	0.2%	99.8%
Shi and Daszak use Wuhan residents as negative control for zoonotic coronavirus exposure	0.2%	99.8%
RaTG13 could be CoV-2 precursor using the synthetic biology 'No See 'Em' technique	0.2%	99.8%
Location, location, location: Based on the distance between known SARS-CoV-1 laboratory-acquired infections and the hospital of admission of the infected personnel, the WIV is within the expected hospital catchment for a CoV-2 LAI	0.2%	99.8%

The summary which follows will simply be a review and discussion of the evidence in the context of the two hypotheses.

Zoonosis Hypothesis

A viral zoonosis has at least three elements, a host, a virus, and the human population. With some viruses there are often two hosts. One is a ‘reservoir host’ where the virus can live for years or even decades in a relatively stable relationship. The reservoir host is never decimated by the virus, and the virus is never burned out by the reservoir host, disappearing completely. For coronaviruses the reservoir host is always one or more bat species. If there is a reservoir host that some viruses that cannot jump directly into the human population, there is a need for an second host, an intermediate host. In this case the virus spends time jumping into the intermediate host, ‘practicing’ adaption through random mutation and Darwinian selection for fitness to reproduce, infect, and transmit in the intermediate host. This process is then repeated between the intermediate host and the human population. Alternatively, the virus can jump directly between the bat reservoir and humans, without the need for an intermediate host.

For two prior human coronavirus epidemics, an intermediate or proximate host was identified. For SARS-CoV-1 in 2003-4 it was the civet cat while for Middle Eastern Respiratory Syndrome (MERS) in 2012-4 it was the camel. In both of these human epidemics, the intermediate host was identified within four to ten months of the first clinically identified human infection. With CoV-2 we are at 12 months since the pandemic began and still waiting for evidence of, despite a much larger effort inside China to find an intermediate host. For both of these previous pandemics, a bat species reservoir host was also identified, but not in the case of SARS-CoV-2.[7]

Based on the genome sequence of CoV-2, Drs. Shi and Daszak have proposed that the reservoir host for CoV-2 is the intermediate horseshoe bat (*Rhinolophus affinis*), which is found in Yunnan Province. Yunnan Province is in southern, rural China and about 1900 km from the north central province of Hubei, where the 11 million people of Wuhan live. In the US this would be equivalent in distance, climate change, and human population density difference to going from the Everglades in Florida to Manhattan, in New York City. The intermediate horseshow bat isn't found at all in Hubei province, making a direct bat-to-human transmission improbable.[8] Experiments in three independent laboratories also demonstrate that CoV-2 has changed genetically so much that it can no longer infect any bat species cell culture tested. So, while the leading US coronavirus expert, Dr. Ralph Baric of The University of North Carolina suggested in early 2020 that CoV-2 may have jumped into the human population directly from bats without an intermediate host, this hypothesis seems to no longer be viable.

For the zoonosis hypothesis to be advanced, it is now necessary to find an intermediate host. In January 2020 a theory was proposed that CoV-2 arose in the Huanan Seafood Market, a traditional Chinese "wet market" where live animals are butchered and sold for food. The market theory was based on the observation that about 40% of early patients worked or shopped there. This was reminiscent of the wet market sources for civet cats infected with SARS-CoV-1 or the camel markets for the MERS coronavirus. The Chinese authorities closed the market on December 31, 2019 after performing extensive environmental sampling and sanitation.

But by May 2020 Dr. Gao Fu, Director of the Chinese CDC, announced that the market was not the source of CoV-2, as all of the animal specimens tested negative for CoV-2. And while SARS-CoV-1 was found in 100% of local farmed civets when tested, CoV-2 was different. In July 2020 Dr. Shi reported that extensive testing of farmed animals throughout Hubei Province failed to find CoV-2 in any animals.

For about six months, the pangolin, a scaly anteater, was suspected to be the intermediate host but finally Dr. Daszak reported that CoV-2 was not found in pangolins in the wild or from the (illegal) market trade.[9] Domestic and feral cats also were ruled out as a possible source. A

[7] I am distinguishing here the difference between SARS-CoV-2 being a descendent of a bat coronavirus (with 3.8% or 1100 nucleotide (nt) differences between them) and the finding of the immediate precursor of SARS-CoV-2 in a bat colony population somewhere in the wild, which usually is <100 nt differences.
[8] "We have done bat virus surveillance in Hubei Province for many years but have not found that bats in Wuhan or even the wider Hubei Province carry any coronaviruses that are closely related to SARS-CoV-2. I don't think the spillover from bats to humans occurred in Wuhan or in Hubei Province," said Dr. Shi. Science, July 2020
[9] https://link.springer.com/article/10.1007/s10393-020-01503-x

comprehensive computer-based screen of 410 different animals reported the remarkable finding that the best ACE2 receptor matches to CoV-2 were human and other primates (or primate cells in the laboratory), including the favorite laboratory coronavirus host, the VERO monkey cell culture, and that all bat species were the worst host. At the time of this writing, there is not even a working hypothesis for the species of an intermediate host.

A typical zoonosis has a number of characteristic properties that can allow identification of a zoonotic infection, even in the absence of identifying an intermediate host. None of these properties are found for CoV-2.

All zoonotic infections have in common the principle that when a virus in nature uses evolution to move from, for example, a bat host to a camel host and then to a human host, it is a hit and miss, slow process. After all, evolution is the result of random genetic changes, mutations, and then enrichment of the ones that are helpful by amplification during reproduction. With both SARS-CoV-1 and MERS, the coronavirus spent months and years jumping from the intermediate host into humans, not having all of the necessary mutations needed to be aggressive, grow, and then spread, but spending enough time in humans to cause an infection and leaving behind a corresponding immune response.

The hallmark evidence of this ‘practice’ in abortive host jumping is in stored, archived human blood specimens taken from before the epidemic, where one can find evidence of pre-epidemic, usually sub-clinical, community spread from the antibodies to the eventual epidemic virus. For SARS-CoV-1 and MERS, about 0.6% of people in the region where the epidemic began showed signs of an infection in archived blood. With CoV-2, this seroconversion, as it is called, has never been observed, including in 540 specimens collected from ‘fever clinics’ in Wuhan between October 2019 and January 2020, reported by the WHO. Because this is such a potent signal of a zoonosis, and because I believe that China has over 100,000 stored specimens from Wuhan taken in the fall of 2019, the lack of reports of seroconversion, the silence from China on this evidence, speaks volumes.

Another hallmark of a slow, natural zoonosis can be found in the virus. In SARS-CoV-1 and MERS, the coronavirus spent years in the intermediate host, passing back and forth among populations of hosts, the civets or camels, that were living in close proximity. During this time, they would accumulate a background of genetic mistakes, i.e., mutations- usually about one mistake every two weeks. When the final chip falls, and a mutation(s) happens allowing the jump into humans, the virus with that new mutation(s) also jumps around within the intermediate host population. The consequence of this latter behavior for a true zoonosis is that the genome sequences found in humans don’t all descend from a single jump into a single human but show jumps from viruses that are only cousins of each other, not direct lineal descendants.

In a true zoonosis, the family tree of virus genome sequences doesn’t pass back through the first patient but instead tracks all the way back to an ancestor months or years earlier. This is called posterior diversity, and it is an easy genetic test to perform. With CoV-2, every one of the more than 294,000 virus genomes sequenced can be traced back to the first genomic cluster and in the first patient in that cluster, a 39-year-old man who was seen at the People’s Liberation Army

(PLA) Hospital about one mile from the Wuhan Institute of Virology. The CoV-2 pandemic has the phylogenetic signature of one pure virus sequence infecting one human, with human-to-human spread thereafter; there is just the one and only jump into the human population ever seen. This lack of posterior diversity has been alluded to by Dr. Shi, by the WHO, and by other prominent virologists; they just never take that critical piece of the evidence to the next the proper inference.

The virus in a true zoonosis also contains the signature record of the gradual changes and adaptions it made in the protein key, the Spike Protein, it uses to unlock human cells and cause infection. With SARS-CoV-1 the Spike Protein had fewer than one-third of all the changes it would later develop by the time it became an epidemic. With CoV-2 the Spike Protein was almost perfectly adapted to the human lock, using 99.5% of the best amino acids possible.

Since with CoV-2 we have no evidence from stored blood that it was quietly practicing on humans in the community of Wuhan, it is surprising that when it finds its first patient, it has perfected to 99.5% the spike protein amino acid sequence, its ability to attack and infect humans. If this adaption couldn't have happened in the community, the only place it could have happened is in a laboratory, by what is called serial passage, a common laboratory process that repeatedly gives the virus a chance to practice on humanized mice or VERO monkey cells.[10] A related study showing human adaption right from the start of the pandemic looked at which of the dozens of protein manufacturing tools that CoV-2 uses (called tRNAs). It showed the same uncanny adaptation to the human tools with no evidence that the tools from other potential intermediate hosts would be suitable.

This evidence presented makes a strong case that CoV-2 did not come from nature. But is there affirmative evidence that it could have come from a laboratory? The answer is yes.

Laboratory Origin Hypothesis

The spike protein that gives the coronavirus its name, corona or crown, is the key to match with the lock found in host cells. But before it can inject its genetic material in the host cell, the spike protein needs to be cut, to loosen it in preparation for infection. The host cell has the scissors or enzymes that do the cutting. The singular, unique feature of CoV-2 is that it requires a host enzyme called furin to activate it at a spot called the S1/S2 junction. No other coronavirus in the same subgenera has a furin cleavage site, as it is called. The other coronaviruses are cleaved at a site downstream from the S1/S2 site, called the S' site.

This is of course a major problem for the zoonosis theory, but it gets worse.

Since 1992 the virology community has known that the one sure way to make a virus deadlier is to give it a furin cleavage site at the S1/S2 junction in the laboratory. At least eleven gain-of-function experiments, adding a furin site to make a virus more infective, are published in the open literature, including Dr. Zhengli Shi, head of coronavirus research at the WIV. This has

[10] It is noteworthy that the furin cleavage site is actually unstable in passage in VERO cells and is often deleted within a few passages. A laboratory origin theory needs to account for this observation. On the other hand, mutations in the furin site among the human CoV-2 genomes are exceedingly rare.

caused a flurry of Chinese papers since the pandemic began trying to show a natural furin site in a related virus (this one example was later shown to be an error in interpretation) or to show that furin sites from distant cousins of CoV-2 might be the source through a process called recombination, where two different viruses infect the same host and then make a mistake in copying their genetic material, and swap sequences.

These convoluted, hypothetical methods each fail, however. It turns out that it is Daszak himself who has shown that the subgenera of coronaviruses that have furin sites are found in different bat hosts, which live in different regions of China, than the sarbecovirus subgenera of which CoV-2 is a member. And even with these barriers, they apparently are too far apart to recombine. "For the three focal subgenera, *Sarbecoviruses, Merbecoviruses and Embevoviruses*…none of the three focal subgenera recombines with one another."[11] As noted previously[2] Dr. Shi also does not believe the bats of Hubei province are capable of being a host for CoV-2-related coronaviruses.

But it gets worse still for the zoonosis theory. The gene sequence for the amino acids in the furin site in CoV-2 uses a very rare set of two codons, three letter words so six letters in a row, that are rarely used individually and have never been seen together in tandem in any coronaviruses in nature. But these same 'rare in nature' codons turn out to be the very ones that are always used by scientists in the laboratory when researchers want to add the amino acid arginine, the ones that are found in the furin site. When scientists add a dimer of arginine codons to a coronavirus, they invariably use the word, CGG-CGG, but coronaviruses in nature rarely (<1%) use this codon pair. For example, in the 580,000 codons of 58 Sarbecoviruses the only CGG pair is CoV-2; none of the other 57 sarbecoviruses have such a pair.[12]

So, there is no natural example of a furin protein site in nature that could be introduced into CoV-2 by recombination, there is no natural example of the particular gene sequence for the furin protein site contained in CoV-2 being used to code for anything in nature, but this particular coding is exactly what Dr. Shi, Baric, and others have used previously in published experiments to insert or optimize arginine codons.

It is telling that when Dr. Shi introduced the world to CoV-2 for the first time in January 2020 she showed hundreds of gene sequences of this novel virus but stopped just short of showing the furin site, the one she is purported to have introduced, seemingly not wanting to call attention to her handywork. She apparently failed to realize that an accomplished <u>but innocent</u> virologist, finding the first furin site ever seen in this class of viruses apparently coming from nature, would have featured the presence of the furin site prominently, and also would have used its presence and her experience with furin sites in other viruses to predict what it would foretell for the world due to its aggressive nature.

She could have perhaps saved many lives just by telling the world that she saw a furin site in the virus sequence. It would be left to a French and Canadian team to later identify the furin site in a

[11] CoV-2 is in the subgenera Sarbecoviruses.
https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1009272
[12] https://virological.org/t/alignment-of-58-sarbecovirus-genomes-for-conservation-analysis-of-sars-cov-2/430

paper.[13] They would write: "This furin-like cleavage site…may provide a **gain-of-function** to the 2019-nCoV for efficient spreading in the human population compared to other lineage b betacoronaviruses." [Emphasis added.]

Dr. Shi has denied the virus came from her lab, but she has created such a record of multiple examples of obfuscation, half-truths, contrived specimens, genetic sequences taken from thin air but published in premier journals and US NIH databases, etc. that her veracity is deeply damaged. Perhaps her words and actions on December 30, 2019 show the truth. Her very first response when told there was an unknown outbreak in Wuhan and to return back quickly from a meeting she was attending in Shanghai was to say, "Could this have come from our lab?"[14]

"I wondered if [the municipal health authority] got it wrong," she says. "I had never expected this kind of thing to happen in Wuhan, in central China." Her studies had shown that the southern, subtropical provinces of Guangdong, Guangxi and Yunnan have the greatest risk of coronaviruses jumping to humans from animals—particularly bats, a known reservoir. After all, the US equivalent of the distance, climate change, and human population density change between Yunnan and Wuhan is comparing the Everglades National Park in Florida and New York City.

Her other action on December 30 was to alter WIV computer databases of novel coronaviruses used by the world's virologists for research to make it more difficult to search for which coronaviruses she had in her building. In short, the day she was asked to address the pandemic in Wuhan, she chose to spend time to make unavailable to her fellow scientists of the world her decades of coronavirus work.

The notion that CoV-2 was a laboratory creation, designed for maximum virulence, that escaped the laboratory accidentally has additional rings of evidence. From President Xi announcing in February new laws about laboratory security, to abundant evidence that the WIV was closed in October with few personnel inside, to the top military medical research doctor, General Chen Wei, being placed in charge of the WIV, to many more clues, it is clear an event occurred in Wuhan sometime in late 2019 that is most consistent with a laboratory escape.

The Asian region has a two-decade record of a little less than one laboratory-acquired infection per year. After the first SARS-CoV-1 epidemic was ended, SARS-CoV-1 jumped four more times into the human population, all from laboratories, with two in China. The last smallpox death in the entire world was a secretary who worked two floors above a research lab in England and contracted it through the ventilation system. The head of that laboratory committed suicide over his anguish for causing her death.

Over and over again. there is a long history and record of laboratory acquired infections that provides the background for considering what happened here.

[13] https://www.sciencedirect.com/science/article/pii/S0166354220300528?via%3Dihub
[14] https://www.scientificamerican.com/index.cfm/_api/render/file/?method=inline&fileID=E1FDF8DE-9E22-4CE5-AD8B2E4682F52A86

Lab-made Bio-Weapon Hypothesis

But was SARS-CoV-2 more than just a gain-of-function experiment that escaped a laboratory? Could it have been one part of a two-part novel virus-vaccine bioweapons program?

General Chen Wei has been involved in vaccine research since joining the People's Liberation Army after college. In a 2017 internal speech at the AMMS (Academy of Military Medical Sciences) she said: *"只要有矛, 才能研究盾."* which translates roughly as, "you need to have an arrow to study a shield." I believe a Rubicon has been crossed by the world with this pandemic and framing the proper understanding of how we got here, and the proper response will be the critical next steps.

Evidence of adenovirus vaccine sequences in early patients would suggest both that SARS-CoV-2 was created in a laboratory and that there was sufficient priority set on this project to create a specific vaccine for the chimera coronavirus.

When Oppenheimer saw the application of Einstein's physics in the embodiment of the atomic bomb, he is said to have quoted a line from the Hindu scripture, the Bhagavad Gita, which reads: 'Now I am become Death, the destroyer of worlds.' The contribution of physics' research to human killing would total less than 300,000 people in two ten-square mile zones in Japan, and the horrors of those events led the world to regulate the raw materials of such bombs and to sanction sovereign nations who attempted to violate the rules.

This had followed the contribution of chemistry to human killing in the form of chemical warfare during World War I, in which 100,000 were killed, and led the nations of the world to an historic agreement to never use chemical warfare again. It is now only 'rogue' operators who violate the norms civilized nations have agreed to.

It seems to be biology's turn to show its dark arts. If it is generally understood that biology/biotechnology has been harnessed to create a pandemic that has killed more people than physics and chemistry research combined, and to be a weapon where no place on earth is safe from its effects (SARS-CoV-2 has been detected in the deepest Amazon jungles and at research stations in Antarctica), there needs to be developed a new set of regulations, rules, etc. to both honor the 1.8 million innocent people who died from COVID-19 and to protect the world so this never happens again. It is also urgent to gather further data to support or refute if this was a Chinese bioweapons program, as the consequences of that would be significant.

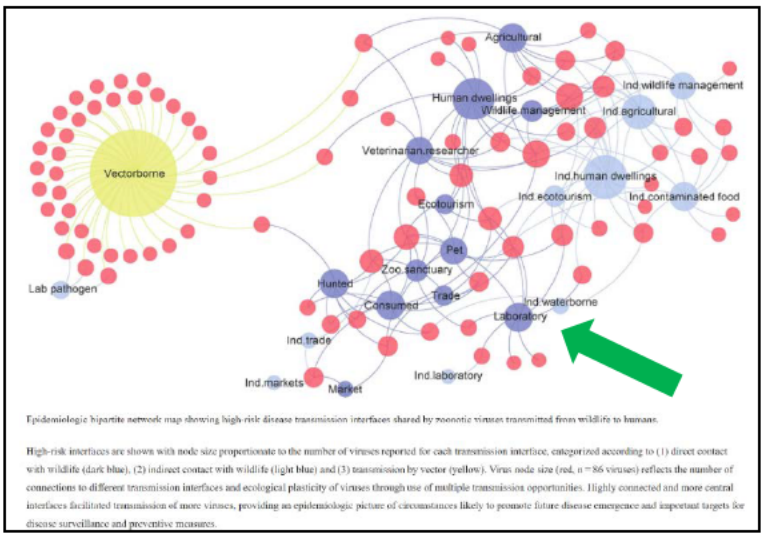
A Bayesian analysis concludes beyond a reasonable doubt that SARS-CoV-2 is not a natural zoonosis but instead is laboratory derived

Introduction. A two-hypothesis, Bayesian analysis was conducted to determine the origin of the SARS-CoV-2 pandemic. The conclusion was that it was created in a laboratory with synthetic biology tools from a bat beta coronavirus, subgenera sarbecovirus backbone (98.9% probability) and not from a natural, zoonotic transmission (1.1%).

There is no direct evidence of whether the release was accidental, or deliberate but circumstantial evidence makes it is highly likely it was accidental.

At the one-year anniversary of the first cases of COVID-19, the coronavirus pandemic caused by the SARS-CoV-2 virus, the origin of the virus remains unknown. While leading institutions and experts have been consistently adamant that it is a zoonotic disease which jumped from a bat reservoir host to humans directly or through an intermediate host the alternative possibility that it escaped from a laboratory conducting research remains a viable option.

In fact, in 2015 Peter Daszak, a leading zoonotic proponent of CoV-2 origin, wrote in, "Spillover and pandemic properties of zoonotic viruses with high host plasticity,"[15] that transmission from laboratories was a major source of zoonotic disease. The Figure below from the Daszak paper shows this important relationship (green arrow):



Epidemiologic bipartite network map showing high-risk disease transmission interfaces shared by zoonotic viruses transmitted from wildlife to humans.

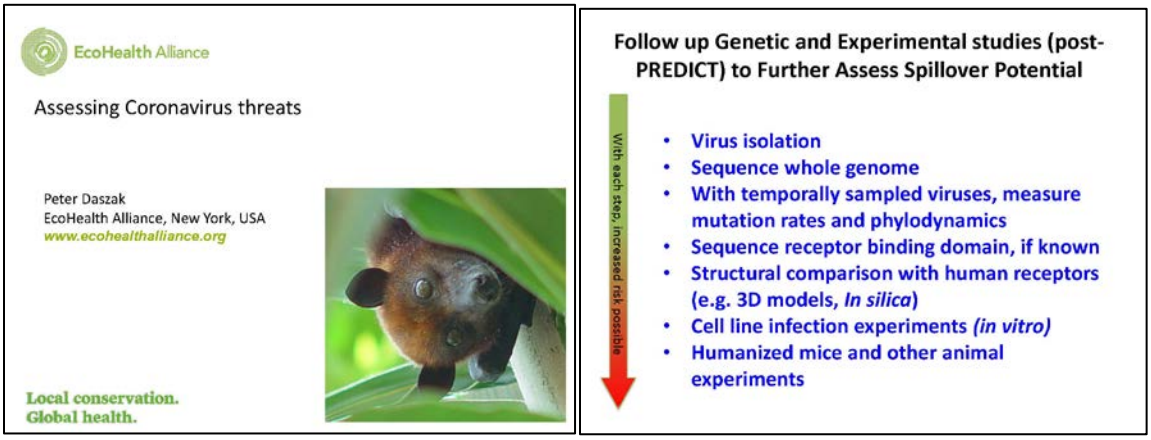
High-risk interfaces are shown with node size proportionate to the number of viruses reported for each transmission interface, categorized according to (1) direct contact with wildlife (dark blue), (2) indirect contact with wildlife (light blue) and (3) transmission by vector (yellow). Virus node size (red, n = 86 viruses) reflects the number of connections to different transmission interfaces and ecological plasticity of viruses through use of multiple transmission opportunities. Highly connected and more central interfaces facilitated transmission of more viruses, providing an epidemiologic picture of circumstances likely to promote future disease emergence and important targets for disease surveillance and preventive measures.

Daszak et al. also writes: "**Zoonotic virus spillover** from wildlife was most frequent in and around human dwellings and in agricultural fields, as well as **at interfaces with occupational exposure to animals** (hunters, **laboratory workers**, veterinarians, **researchers**, wildlife management, zoo and sanctuary staff). **Primate hosts were most frequently cited as the source of viruses transmitted by direct contact** during hunting (exact P = 0.051) and **in laboratories**

[15] https://www.nature.com/articles/srep14830

(exact P = 0.009)." [Emphasis added]. Primate "hosts" can presumably include monkey cell culture, such as the ubiquitous VERO cell used in all virology laboratories, including the WIV.

In 2015 Dr. Daszak spoke of the spillover danger of certain types of laboratory research:



He writes: "with each step, increased risk possible" with "Humanized mice and other animal experiments" the highest risk work.

In a prescient Twitter post in November 2019, he highlights the work he is doing using recombinant viruses with humanized mice and making viruses that "**don't respond to MAbs, vaccines…**" in response to criticism his work is of limited value:

Clearly, before the beginning of the pandemic, Daszak, now a member of both the WHO and Lancet teams being sent to China to explore the origin of CoV-2, could entertain the eal possibility of a laboratory created virus escaping into the human population/community.

The purpose of this analysis is to use a Bayesian Inference Network approach to the collected circumstantial evidence that is available to provide likelihoods of the alternative hypotheses as to the origin of SARS-CoV-2. The analysis also will include certain prior probabilistic conclusions to help set the initial state before the proprietary evidence is used.

Origin hypotheses: Initial States to establish the posterior probabilities.

Two published Bayesian analyses and two independent studies of zoonotic spillover from nature and laboratory-acquired infections in Asia will be used to establish the posterior probabilities for this analysis.

Zoonotic spillover frequency versus laboratory acquired infection frequency based on two published papers, one by Daszak et al.

In 2015 Daszak et al. published a paper entitled, "Spillover and pandemic properties of zoonotic viruses with high host plasticity,"[1] in which they identified 162 zoonotic viruses with naturally occurring animal-to-human transmission from 1990-2010. This is a frequency of 162/20 = 8.1 events per year.

They also note: "The majority (94%) of zoonotic viruses described to date (n = 162) are RNA viruses, which is 28 times higher (95% CI 13.9–62.5, exact $P < 0.001$) than the proportion of RNA viruses among all vertebrate viruses recognized, indicating that RNA viruses are far more likely to be zoonotic than DNA viruses." CoV-2 is an RNA virus.

Finally, they note that: "In general, wild animals were suggested as the source of zoonotic transmission for 91% (86/95) of zoonotic viruses compared to 34% (32/95) of viruses transmitted from domestic animals and 25% (24/95) with transmission described from both wild and domestic animals."

One of the caveats of the Daszak data is that it categorizes a laboratory-acquired infection (LAI) from an animal collected from the wild as a zoonotic spillover. There is no data in the paper to assess this issue and leaving it uncorrected is a conservative approach since it only inflates the natural zoonotic frequency.

In 2018 a paper by Siengsanan-Lamont entitled, "A Review of Laboratory-Acquired Infections in the Asia-Pacific: Understanding Risk and the Need for Improved Biosafety for Veterinary and Zoonotic Diseases," was published.[16] They reported 27 LAIs between 1982 and 2016, a frequency of 27/(2016 – 1982) = 0.8 events per year.

Using these historical frequencies of zoonotic spillover versus LAI to predict a future event can be calculated in the following manner:

Evidence	Zoonotic Origin	Laboratory Origin
Frequency per year from Daszak paper	8.1	NA
Frequency per year from Siengsanan-Lamont paper	NA	0.8
Total events per year	8.1 + 0.8 = 8.9	8.1 + 0.8 = 8.9
Likelihood of future event based on historical frequency	8.1/8.9 X 100 = 0.91	0.8/8.9 X 100 = 0.9

Daszak's initial state analysis. This evidence sets the likelihood that CoV-2 was a zoonotic origin event at 91% and a laboratory origin event at 9%.

[16] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6073996/

Independent prior analyses: Rootclaim.

The next data that will be used is a recent analysis published on the Rootclaim website.[17] Three hypotheses below were analyzed through a series of evidence statements and the probabilities that each was the origin of SARS-CoV-2 determined:

Hypothesis	Calculated Probability
Lab escape: The virus was the subject of genetic research, including gain-of-function, and was released by accident	81%
Zoonotic: The virus evolved in nature and was transmitted to humans from a non-human vertebrate animal	16%
Bioweapon: The virus was genetically engineered as a bioweapon and was deliberately released	3%

As can be seen, the highest likelihood probability is an accidental lab escape, the lowest a bioweapon. The details of the evidence used to arrive at this conclusion is contained in Appendix 1. A summary of the changes in probability at each level of evidence analysis is shown in this table:

Evidence	Laboratory	Zoonosis	Bioweapon
Starting point	1.2%	82%	16%
Contagion and mortality	1.4%	97%	1.9%
Outbreak location: Wuhan	42%	56%	2.8%
Virus sources near Wuhan	16%	83%	1.0%
Chimera	37%	60%	2.5%
Furin cleavage	72%	23%	4.8%
WIV lab procedures	80%	17%	3.5%
WIV disassociation	89%	9%	2.0%
Chinese response	90%	8%	1.7%
No reported infections at WIV	86%	11%	2.4%
No whistleblowers	81%	16%	2.8%

As can be seen, the starting point assumed an 82% probability of a zoonotic origin. This starting point is a reasonable value and will be used here. Since some of the evidence in the above analysis will be used here, only the starting point will be used and not the probability changes from there.

For purposes of this analysis only the Rootclaim initial state will be used since much of their evidence is also covered in the analysis here.

[17] https://www.rootclaim.com/analysis/what-is-the-source-of-covid-19-sars-cov-2

In a paper by Daszak and colleagues it states: "In general, wild animals were suggested as the source of zoonotic transmission for 91% (86/95) of zoonotic viruses compared to 34% (32/95) of viruses transmitted from domestic animals and 25% (24/95) with transmission described from both wild and domestic animals."[1]

On the other hand, domestic animals seem to have been ruled out for SARS-CoV-2. In an interview for *Science* in July 2020, Dr. Zhengli Shi, head of coronavirus research at the Wuhan Institute of Virology, stated: "Under the deployment of the Hubei Provincial Government, our team and researchers from Huazhong Agricultural University collected samples of farmed animals and livestock from farms around Wuhan and in other places in Hubei Province. We did not detect any SARS-CoV-2 nucleic acids in these samples."[18]

Reanalysis of Rootclaim initial state to remove Bioweapons option.

The US government uses the following definitions:

"Gain-of-function (GOF) studies, or research that improves the ability of a pathogen to cause disease, help define the fundamental nature of human-pathogen interactions, thereby enabling assessment of the pandemic potential of emerging infectious agents, informing public health and preparedness efforts, and furthering medical countermeasure development.

Gain-of-function studies may entail biosafety and biosecurity risks; therefore, the risks and benefits of gain-of function research must be evaluated, both in the context of recent U.S. biosafety incidents and to keep pace with new technological developments, in order to determine which types of studies should go forward and under what conditions."[19]

"Dual use research of concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops, and other plants, animals, the environment, materiel, or national security. "[20]

For this analysis, the assumption is made that GOF and DURC are largely the same processes and techniques in the laboratory and thus can only be distinguished by direct, documentary evidence of the intent of the research from administers in the facilities conducting the work.

In the absence of any such documentary evidence that bioweapon research was being conducted or that SARS-CoV-2 is a bioweapon and to take the least inflammatory posture, the initial state for the above prior analysis will be recalculated by eliminating the hypothesis, and its accompanying probability, that SARS-CoV-2 was created as a bioweapon. The revised initial state calculation is shown in this table:[21]

[18] https://www.sciencemag.org/sites/default/files/Shi%20Zhengli%20Q%26A.pdf
[19] https://www.phe.gov/s3/dualuse/Pages/GainOfFunction.aspx
[20] https://www.phe.gov/s3/dualuse/Pages/default.aspx
[21] For clarity, the 3% bioweapon probability was simply dropped and the remaining likelihoods, 81% and 16%, were normalized.

Evidence	Zoonotic Origin	Laboratory Origin	Bioweapons Origin
Rootclaim initial state	0.86	0.012	0.16
Remove bioweapons	NA	NA	0
Normalize remaining hypotheses	0.86/(0.86 + 0.012) = 0.986	0.012/(0.86 + 0.012) = 0.014	NA

Rootclaim Initial state analysis, adjusted. This evidence sets the likelihood that CoV-2 was a zoonotic origin event at 98.6% and a laboratory origin event at 1.4%.

Additional Prior Evidence by Demaneuf and De Maistre. A second prior Bayesian analysis was performed by professionally educated risk assessment personnel and Chinese-language speaking professionals[22] and is included herein in its entirety. For the sake of brevity, the zoonotic origin evidence was based primarily on population size, distribution, and geographic distribution of bat populations relative to Wuhan. With respect to a lab accident, they separately analyze probabilities of a virus escape during collection, transport, and direct lab accidents and then separately the probability of a community outbreak following a lab escape. They also use primary Mandarin-language sources for Chinese estimates of the same events, showing corroboration of the probabilities. Their conclusion is that the probability of a lab escape ranges from 6% to 55% with a zoonotic origin a zoonotic origin probability being 45% to 94%.

Second Bayesian analysis. Using the most conservative probabilities, this evidence sets the likelihood that CoV-2 was a zoonotic origin event at 94% and a laboratory origin event at 6%.

Selection of initial state for Bayesian analysis.

The Text-Table below summarizes the three approaches to an initial state as to the origin of CoV-2. While the Demaneuf and De Maistre analyses set a range for the zoonotic origin of 45% to 94%, I have used the top of the range of their probability of a zoonotic origin to be conservative.

Prior Analysis	Zoonotic Origin	Laboratory Origin
Daszak et al. paper	91%	9%
Rootclaim Bayesian analysis	98.6%	1.4%
Demaneuf and De Maistre Bayesian analysis	94%	6%

Using a simple online calculator[23] the mean of these three value sets is 94.5%, the standard deviation is $\pm$ 3.8%, and the 95% confidence interval is $\pm$ 4.3%. Using these data, the upper bound of the 95% confidence interval is 98.8% and, to be most conservative, this will be used as the starting probability of a zoonotic origin.

Initial state for this analysis. The likelihood that SARS-CoV-2 began as a zoonotic event is 98.8% and the likelihood it began as a laboratory event is 1.2%.

[22] https://zenodo.org/record/4067919#.X-qIm9gzbOj . For reference purposes, this paper comes with a spreadsheet listing 112 individual BSL-3 labs in China across 62 lab-complexes.
[23] https://www.calculator.net/standard-deviation-calculator.html?numberinputs=91%2C+94%2C+98.6&ctype=s&x=48&y=19

1. General approach of this analysis[24]

This analysis is intended to examine two competing and mutually exclusive theories of the origin of the coronavirus, SARS-CoV-2 (CoV-2), and the pandemic it has caused, COVID-19.

At the time of this writing there have been 83 million confirmed cases and 1.8 million deaths.[25] Some sources place the economic damage at $21 trillion USD.

Bayes Theorem

This brief description of the Bayes Theorem was taken from the work of Jon Seymour:[26]

"The eponymously named Bayes Theorem was discovered by the Reverend Thomas Bayes in the 1700's and saved for posteriority by an archivist of his papers who discovered the work posthumously. In common language, it provides a rational technique for revising a prior belief in light of new evidence. The equation for Bayes Theorem is given below:

$$P(H|E) = \frac{P(E|H).P(H)}{P(E)}$$

where:

- H is the statement of the hypothesis of interest
- P(H) is the prior probability that the hypothesis is true, independent of the evidence.
- E is the evidence being used to revise the belief in hypothesis
- P(E) is the marginal likelihood of the evidence, independent of the hypothesis
- P(E|H) is the likelihood the evidence, given that the hypothesis is true
- P(H|E) is the posterior probability of the hypothesis, given the evidence.

P(E) is sometimes difficult to estimate, but the following identity must hold:

$$P(E) = P(E|H).P(H) + P(E|\hat{H}).P(\hat{H})$$

Here P(E|^H) is the probability of the evidence, assuming the hypothesis is false and P(^H) is the probability the hypothesis is false which is the same as 1-P(H). Estimating the two conditional probabilities P(E|H) and P(E|^H) is generally easier than estimating the unconditional probability, P(E)."

---

[24] The statistical approach and many of the individual statistical analyses were performed by Dr. Martin Lee, PhD, Adjunct Professor of Biostatistics, UCLA. https://ph.ucla.edu/faculty/lee The likelihood adjustments to the Bayesian analysis, which you can see are routine math, were conducted by the author.
[25] https://www.worldometers.info/coronavirus/coronavirus-cases/
[26] https://jonseymour.medium.com/a-bayesian-analysis-of-one-aspect-of-the-sars-cov-2-origin-story-where-the-first-recorded-1fbdcbea0a2b

Theory One. The zoonotic theory is that a vertebrate animal was infected with CoV-2 or an ancestor (Index Host) and that a human was infected with contact to that Index Host in some manner. Human-to-human spread then followed.

Theory Two. The laboratory origin theory is that CoV-2 or an ancestor was being used in laboratory experiments and that it 'escaped' from the lab via an infected person, lab animal, experimental waste, etc.

I have found no evidence of a deliberate release and early firsthand accounts of local officials and scientists suggest surprise and consternation. If this was a deliberate release, such evidence would be extremely local, limited in distribution, and highly compartmentalized. It is beyond the scope of this analysis.

Weight of the evidence. For purposes of the calculation of posterior probabilities in the Bayesian analysis, evidence which has a statistical basis will be used directly to adjust the probabilities.

Statistically significant evidence. Since some of the probability calculations have astronomical values which would make a single such evidence statement, if inputted directly, swamp any further calculation and make their later contribution mute, a decision was made to simply treat quantitative probabilities as significant at the $p = 0.05$ level, no matter how much 'more significant' the calculation suggested.

So, for example, a probability of certain codon usage coming from nature may be one in 440 or $p = 0.002$, the contribution of this evidence to the input to the posterior probability adjustment would be set at a p-value of 0.05. In such cases the adjustment would be to change the 'winning' hypothesis by multiplying by 19, since a $p = 0.05$ is the same as a 19 out of 20 likelihood event. This is a conservative treatment of what would be highly significant data.

Other quantitative evidence. If a piece of evidence can be quantified but it does not reach a significance of $p = 0.05$ it will be used directly in the likelihood adjustment.

Non-quantitative evidence. For evidence that cannot be quantified, the decision was made to treat these as quantitative outcomes with a 51% to 49% likelihood value with respect to the 'winning' hypothesis. This has the effect of increasing the probability of that hypothesis for that step in the Bayesian analysis by 1.04. This 51%/49% concept is related to the legal standard of the 'preponderance of the evidence' used in civil litigation.

Independence. An important qualitative assessment that must be made is whether or not two pieces of evidence are independent of each other. If they are independent, they can each be used in determining a new likelihood calculation. If they are dependent on each other then they must be combined and only a single new likelihood analysis can be made. Where ever possible, evidence statements that could be considered as dependent are called out and this rule is followed on their contribution to the analysis.

Subjective Discount Factor. The impact of each piece of evidence was adjusted further by a subjective discount factor. This is a qualitative assessment of the overall veracity of a particular

piece of evidence when all factors, samples, methods, data sources, etc. are taken into context. It varies from 60% to 100% and is used as a fraction to reduce the impact of a single piece of evidence even further.

Hearsay. Just as in a court of law, evidence, usually attributed to a given person or persons, that is not directly available but instead relies on statements of others is usually not allowed in a court trial and will accordingly not be used here to adjust the Bayesian analysis. It may be recorded and preserved as a placeholder and reminder for further research. If new, direct evidence can be found than the bar of using it is lifted and it can be used for adjustment.

Significant figures. Because of the overall nature of the analyses here, all math calculations related to likelihoods are performed and carried forward at the 'one significant figure' level, with standard rounding rules applied. This has the effect, near the end of the cumulative evidence, of failing to change the relative probabilities as the small adjustments are reversed in the rounding process.

Evidence. International committees to investigate the origin of SARS-CoV-2 may not be impartial.

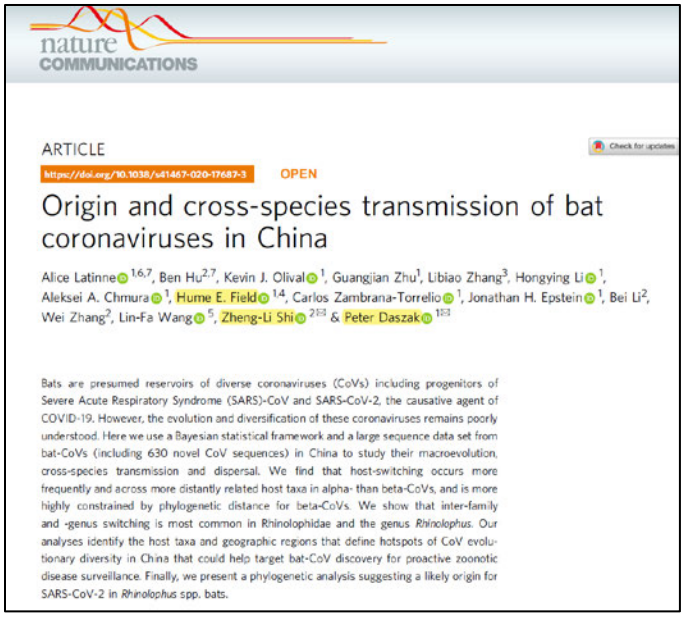
At the time of the writing of this manuscript there are two committees charged with examining the evidence and determining the origin of the SARS-CoV-2 virus. One committee is commissioned by the World Health Organization (WHO) and the other is an ad hoc committee established by the British medical journal, *The Lancet.*

The composition of the two committees is shown in the Text-Table below:

	Lancet Commission of CoV-2	WHO Commission on CoV-2 origin
	Dr. Peter Daszak, Chair	**Dr. Peter Daszak, Ph.D (EcoHealth Alliance, USA)**
	Dr. John Amuasi	Prof. John Watson (Public Health England, United Kingdom)
	Dr. Danielle Anderson	Prof. Dr. Marion Koopmans, DVM PhD (Erasmus MC, Netherlands)
	Dr. Isabella Eckerle	Prof. Dr. Dominic Dwyer, MD (Westmead Hospital, Australia)
Also co-author	Dr. Hume Field	Vladimir Dedkov, Ph.D (Institute Pasteur, Russia)
	Dr. Gerald Keusch	Dr. Hung Nguyen, PhD (International Livestock Research Institute (ILRI), Vietnam)
	Dr. Dato' Sai Kit (Ken) Lam	PD. Dr. med vet. Fabian Lendertz (Robert Koch-Institute, Germany)
	Dr. Carlos das Neves	Prof. Dr. Thea Fisher, MD, DMSc(PhD) (Nordsjællands Hospital, Denmark)
	Dr. Malik Peiris	Dr. Farag El Moubasher, Ph.D (Ministry of Public Health, Qatar)
	Dr. Stanley Perlman	Prof. Dr. Ken Maeda, PhD, DVM (National Institute of Infectious Diseases, Japan)
	Dr. Linda J. Saif	WHO Commission of CoV-2 origin
	Dr. Supaporn Wacharapluesadee	
	Lancet Commission on CoV-2	
	Signed Lancet letter	
	Co-author with Daszak	

There are a number of potential conflicts of interest:

Fully half of The Lancet's team had already suggested that any lab-leak hypothesis was a "conspiracy theory" in a January 2020 paper that has been shown elsewhere within to have been orchestrated behind the scenes to appear spontaneous.



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Origin and cross-species transmission of bat coronaviruses in China

Alice Latinne[1,6,7], Ben Hu[2,7], Kevin J. Olival[1], Guangjian Zhu[1], Libiao Zhang[3], Hongying Li[1], Aleksei A. Chmura[1], Hume E. Field[1,4], Carlos Zambrana-Torrelio[1], Jonathan H. Epstein[1], Bei Li[2], Wei Zhang[2], Lin-Fa Wang[5], Zheng-Li Shi[2,3] & Peter Daszak[1,3]

Bats are presumed reservoirs of diverse coronaviruses (CoVs) including progenitors of Severe Acute Respiratory Syndrome (SARS)-CoV and SARS-CoV-2, the causative agent of COVID-19. However, the evolution and diversification of these coronaviruses remains poorly understood. Here we use a Bayesian statistical framework and a large sequence data set from bat-CoVs (including 630 novel CoV sequences) in China to study their macroevolution, cross-species transmission and dispersal. We find that host-switching occurs more frequently and across more distantly related host taxa in alpha- than beta-CoVs, and is more highly constrained by phylogenetic distance for beta-CoVs. We show that inter-family and -genus switching is most common in Rhinolophidae and the genus *Rhinolophus*. Our analyses identify the host taxa and geographic regions that define hotspots of CoV evolutionary diversity in China that could help target bat-CoV discovery for proactive zoonotic disease surveillance. Finally, we present a phylogenetic analysis suggesting a likely origin for SARS-CoV-2 in *Rhinolophus* spp. bats.

The above paper published in August 2020 has as co-authors Drs. Hume, Daszak, and Shi. Having two of these scientists be asked to investigate a third co-author is a clear conflict of interest.

A newspaper piece about Peter Daszak entitled, "The doctor who denied COVID-19 was leaked from a lab had this major bias,"[27] questions his ability to be unbiased due to a deep, long history of work with Dr. Zhengli Shi of the WIV.

A lengthy piece in Wired was subtitled, "The two major investigations into the origins of the pandemic are compromised by potential conflicts of interest."[28]

Since the purpose of this manuscript is to evaluate the scientific evidence concerning the origin of SARS-CoV-2 no further effort will be put into these matters. If and when a report is prepared from either committee there will be time to analysis the work in the reports and compare it to prior publications and statements from the committee members to look for bias.

Likelihood from initial state is unchanged following this evidence analysis:

Zoonotic origin (98.8%) and laboratory origin (1.2%)

[27] https://nypost.com/2021/01/16/doctor-who-denied-covid-was-leaked-from-a-lab-had-this-major-bias/
[28] https://www.wired.com/story/if-covid-19-did-start-with-a-lab-leak-would-we-ever-know/?utm_source=twitter&utm_medium=social&utm_campaign=onsite-share&utm_brand=wired&utm_social-type=earned

Evidence. Three high visibility papers grounded the zoonotic origin hypothesis in the public conversation from February to May 2020: a pros and cons analysis.

Introduction. The two key data points from December 2019 concerning the origin of the SARS-CoV-2 coronavirus infection, the cause of COVID-19, are the observation that a large number of the earliest patients worked or had visited the Hunan Seafood Market in Wuhan, China and that the hospitals where the first patients were admitted were a short distance from the Wuhan Institute of Virology (WIV), the only high security, BSL-4 laboratory in all of China, and arguably the leading research institute in the world studying coronaviruses of the type causing COVID-19.

The first data point is reminiscent of the origin of SARS-CoV-1, a zoonosis with interspecies transmission from bats to civet cats and then to humans, identified in wet markets in southern China. The second data point is reminiscent of the four SARS-CoV-1 human spillovers that occurred after the 2003 epidemic ended and were each a laboratory-acquired infection (LAI) by a scientist working in a government research laboratory, much like the WIV, and then local human-to-human spread and nearby hospital admission.

To be clear in this paper, the term zoonosis will only be used to describe a interspecies transmission outside of a laboratory. This point seems important to clarify since Dr. Zhengli Shi, head of coronavirus research at the WIV, has previously reported: "An outbreak of hemorrhagic fever with renal syndrome occurred among students in a college (College A) in Kunming, Yunnan province, China in 2003. Subsequent investigations revealed the presence of hantavirus antibodies and antigens in laboratory rats at College A and two other institutions. Hantavirus antibodies were detected in 15 additional individuals other than the index case in these three locations. Epidemiologic data indicated that the human infections were a result of **zoonotic transmission** of the virus from laboratory rats."[29] [emphasis added.] The author has found no other support for the use of the term zoonotic transmission with respect to an LAI and its dual use could be confusing, and so will be avoided.

While the two initial data points would suggest that a balanced approach should be taken with respect to investigations of the origin of SARS-CoV-2, three high visibility publications that argued the laboratory origin idea was a "conspiracy theory" and strongly argued that it was of zoonotic origin foreclosed legitimate debate for much of 2019. The purpose of this evidence analysis is to examine these papers and weigh the strength of the evidence.

Paper 1: The February 3, 2020 paper by WIV scientist Dr. Shi et al. entitled: "A pneumonia outbreak associated with a new coronavirus of probable bat origin."

This seminal paper set the stage for the zoonotic origin of SARS-CoV-2 and has been accessed over one million times. According to *Nature*, this article is in the 99th percentile (ranked 24th) of the 326,159 tracked articles of a similar age in all journals and the 99th percentile (ranked 2nd) of the 783 tracked articles of a similar age in Nature.

[29] https://pubmed.ncbi.nlm.nih.gov/20380897/

However, a careful analysis of it shows serious issues which suggest it is unreliable. The following analysis is in the form of an independent manuscript:

The seminal paper from the Wuhan Institute of Virology claiming SARS-CoV-2 probably originated in bats appears to contain a contrived specimen, an incomplete and inaccurate genomic assembly, and the signature of laboratory-derived synthetic biology

The coronavirus RaTG13 was purportedly identified in a bat "fecal" specimen that is probably not feces, has significant unresolved method-dependent genome sequence errors and an incomplete assembly with significant gaps, and has an anomalous base substitution pattern that has never been seen in nature but is routinely used in codon-optimized synthetic genome constructions performed in the laboratory

Abstract. The species of origin for the SARS-CoV-2 coronavirus that has caused the COVID-19 pandemic remains unknown after over six months of intense research by investigators around the world. The current consensus theory among the scientific community is that it originated in bats and transferred to humans either directly or through an intermediate species; no credible intermediate species exists at this time. The suggested origin early on from a Wuhan "wet market" has been determined to be a red herring and the pangolin is no longer considered a likely intermediate by the virology community.

The basis for the hypothesis that SARS-CoV-2 probably evolved from bats initially came from a February 2020 paper[30] from Dr. Zheng-Li Shi's laboratory at the Wuhan Institute of Virology (WIV). In that paper the Wuhan laboratory made two claims: 1), "a bat fecal sample collected from Tongguan town, Mojiang county in Yunnan province in 2013" contained a coronavirus, originally designated "Rhinolophus bat coronavirus BtCoV/4991[31]" in 2016 but renamed in their paper, RaTG13; and 2), the genomes of RaTG13 and SARS-CoV-2 had an overall identity of 96.2%, making it the closest match to SARS-CoV-2 of any coronavirus identified at that time. RaTG13 remains the closest match to SARS-CoV-2 at the current time.

In this paper I document that:

1) The RaTG13 specimen was not a bat fecal specimen, based on a comparison of the relative bacterial and eukaryotic genetic material in the purported fecal specimen to nine authentic bat fecal specimens collected in the same field visits as RaTG13 was collected by the Wuhan laboratory, run on the same Illumina instrument (id ST-J00123), and published in a second paper in February 2020.[15] While the authentic bat fecal

[30] Zhou, P., Yang, X., Wang, X. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579,** 270–273 (2020). https://doi.org/10.1038/s41586-020-2012-7 .

[31] A Coronavirus BtCoV/4991 Genbank entry by Dr. Shi records: organism="Rhinolophus bat coronavirus BtCoV/4991." In July 2020 she wrote: "Ra4991 is the ID for a bat sample while RaTG13 is the ID for the coronavirus detected in the sample. We changed the name as we wanted it to reflect the time and location for the sample collection. 13 means it was collected in 2013, and TG is the abbreviation of Tongguan town, the location where the sample was collected."

samples were, as expected, largely bacterial (specifically, 65% bacteria and 12% eukaryotic genetic sequences), the purported RaTG13 specimen had a reversed composition, with mostly eukaryotic genes and almost no bacterial genetic material (0.7% bacteria and 68% eukaryotic). The RaTG13 specimen was also only 0.01% virus genes compared to an average of 1.4% for authentic bat fecal specimens. A Krona analysis identified 3% primate sequences consistent with VERO cell contamination, the standard monkey cell culture used for coronavirus research, including at the Wuhan laboratory. Based on using the mean and standard deviation of the nine authentic bat fecal specimens from the Wuhan laboratory, the probability that RaTG13 came from a true fecal sample but had the composition reported by the Wuhan laboratory is one in thirteen million;

2) According to multiple references, RaTG13 was identified via Sanger dideoxy sequencing before 2016, partially sequenced by amplicon sequencing in 2017 and 2018, and then complete sequencing and assembly by RNA-Seq in 2020, although some reports from WIV suggest the timing of the RNA-Seq experiments may have been performed earlier than 2020. In any case, a Blast analysis of sequences from the amplicon and RNA-Seq experiments indicates an approximate 5% nucleotide difference, 50-fold higher than the technical error rate for RNA-Seq of about 0.1%. At least two gaps of over 60 base-pairs, with no coverage in the RNA-Seq data, were easily identified. The incomplete assembly and anomalous, method-dependent sequence divergence for RaTG13 is troublesome;

3) The pattern of synonymous to non-synonymous (S/NS) sequence differences between RaTG13 and SARS-CoV-2 in a 2201 nucleotide region flanking the S1/S2 junction of the Spike Protein records 112 synonymous mutation differences with only three non-synonymous changes. Based on the S/NS mutational frequencies elsewhere in these two genomes and generally in other coronaviruses the probability that this mutation pattern arose naturally is approximately one in ten million. A similar pattern of unnatural S/SN substitutions was seen in a 10,818 nt region of the pp1ab gene. This pp1ab gene pattern has a probability of occurring naturally of less than one in 100 billion. A total of four regions of the RaTG13 genome, coding for 7,938 nt and about one-quarter of the entire genome, contain over 200 synonymous mutations without a single non-synonymous mutation. This has a probability of one in 10^{-17}. A possible explanation, the absolute criticality of the specific amino acid sequence in the regions which might make a non-synonymous change non-infective, is ruled out by the rapid appearance of an abundance of non-synonymous mutations in these very regions when examining the over 80,000 human SARS-CoV-2 specimens sequenced to date. An alternative hypothesis, that this arose by codon substitution is examined. It is demonstrated, by example from a published codon-optimized SARS-Cov-2 Spike Protein experiment, that the anomalous S/SN pattern is precisely the pattern which is produced, by design, when synthetic biology is used and represents a signature of laboratory construction.

Based on the findings concerning the RaTG13 data, including anomalies and inconsistent statements about RaTG13, its origin, renaming, and sequencing timing; the finding that the specimen it is purported to have come from is not bat feces and has a signature of cell culture contamination; the unexplained method-dependent 5% sequence difference for RaTG13; and the S/SN mutation pattern reported, which to my knowledge has never been seen in nature, it can be concluded that RaTG13 is not a pristine biological entity but shows evidence of genetic manipulation in the laboratory.

Until a satisfactory explanation of the findings in this paper have been offered by the Wuhan laboratory, all hypotheses of the proximal origin of the entry of SARS-CoV-2 into the human population should now include the likelihood that the seminal paper contains contrived data. For example, the hypothesis that SARS-CoV-2 was the subject of laboratory research and at some point escaped the laboratory should be included in the narrative of the origin of SARS-CoV-2 research.

Introduction. Since the first reported patient on December 1, 2019 with a SARS-CoV-2 infection, the virus has caused a pandemic that has led to twenty-five million cases worldwide and over 840,000 deaths as of August 30, 2020. To make progress on treating this disease and preventing the next viral outbreak, knowing the origin of the virus and how it entered the human population is critical.

On February 3, 2020 a paper was published from the Wuhan Institute of Virology that identified a bat coronavirus, RaTG13, as having a 96.2% identity to SARS-CoV-2, quickly providing support for a zoonotic origin, either from bats directly or from bats to humans through an unknown intermediary species. If true, this would replicate the model of SARS-CoV 2003 in which the transmission was from bats to civets to humans and for MERS in which the transmission was from bats to camels to humans. At the time of this paper and through August 30, 2020, no other virus has been identified with a closer sequence homology to SARS-CoV-2 than RaTG13. The publication containing the RaTG13 sequence has been cited over 1600 times in the six months since publication. None of these studies contain research on the isolated virus itself since the virus has never been isolated or cultured. It was apparently found in only one sample from 2013 and that sample has been exhausted.[32]

An examination of the raw data associated with RaTG13 immediately identified serious anomalies, bringing into question the existence of RaTG13 as a biological entity of completely nature origin.

[32] Dr. Shi Science interview July 2020

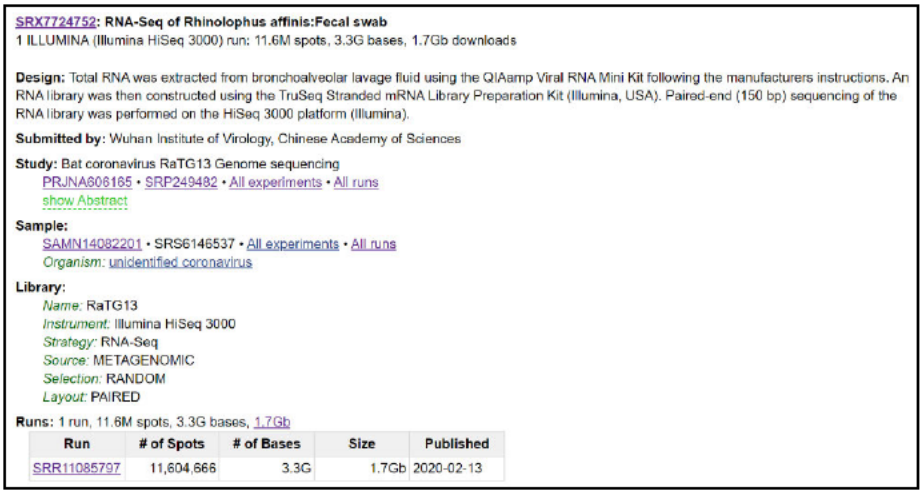
Materials and Methods.

GenBank accession URL table for sequences used in this paper.

The GenBank accession URLs for the specimens, raw reads, and sequences that are used in this paper are contained in the following Table, which can be used to reach the raw data.

Descriptor	URL Hyperlink
SARS-CoV-2 reference sequence in GenBank	SARS-CoV-2 complete genome
Bat coronavirus RaTG13, complete genome, Genbank	RaTG13 complete genome
RaTG13 purported bat fecal specimen	SRR11085797
Rhinolophus bat coronavirus BtCoV/4991 RNA-dependent RNA polymerase (RdRp) gene, partial cds	BtCoV/4991 RdRp gene
SRX8357956: amplicon_sequences of RaTG13	Specimen descriptor
RNA-Seq data for RaTG13	RNA-Seq data for RaTG13
Reference fecal bat specimens from WIV	SRR11085736
Reference fecal bat specimens from WIV	SRR11085734
Reference fecal bat specimens from WIV	SRR11085737
Reference fecal bat specimens from WIV	SRR11085733
Reference fecal bat specimens from WIV	SRR11085735
Reference fecal bat specimens from WIV	SRR11085738
Reference fecal bat specimens from WIV	SRR11085739
Reference fecal bat specimens from WIV	SRR11085740
Reference fecal bat specimens from WIV	SRR11085741

Below is a screen shot of the GenBank entry for the purported specimen from which RaTG13 was identified and upon which RNA-Seq was performed. While the title claims it is a "Rhinolophus affinis fecal swab" specimen it also records in the design of work entry that "(t)otal RNA was extracted from bronchoalveolar lavage fluid." These descriptions are clearly inconsistent.



SRX7724752: **RNA-Seq of Rhinolophus affinis:Fecal swab**
1 ILLUMINA (Illumina HiSeq 3000) run: 11.6M spots, 3.3G bases, 1.7Gb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit following the manufacturers instructions. An RNA library was then constructed using the TruSeq Stranded mRNA Library Preparation Kit (Illumina, USA). Paired-end (150 bp) sequencing of the RNA library was performed on the HiSeq 3000 platform (Illumina).

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Bat coronavirus RaTG13 Genome sequencing
PRJNA606165 • SRP249482 • All experiments • All runs
show Abstract

Sample:
SAMN14082201 • SRS6146537 • All experiments • All runs
Organism: unidentified coronavirus

Library:
Name: RaTG13
Instrument: Illumina HiSeq 3000
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

Runs: 1 run, 11.6M spots, 3.3G bases, 1.7Gb

Run	# of Spots	# of Bases	Size	Published
SRR11085797	11,604,666	3.3G	1.7Gb	2020-02-13

Apparent missing amplicon reads for RaTG13 in GenBank.

There are 33 amplicon reads in GenBank for RaTG13 from experiments recorded as having been performed in 2017 and 2018. A file naming pattern was noticed among the data sets which suggests there may be amplicon runs that were not deposited in GenBank. These files, if related to RaTG13, may contain useful sequence data and an effort should be made to retrieve them and, if appropriate, upload them to GenBank. A Table with the apparently missing data (yellow) is shown here.

Date	Amplicon file name endings							
3-Jun-17	A07	A08						
17-Jun-17	A05	A06						
20-Jun-17						F03	G03	H03
27-Sep-18	A06	B06	C06		E05	F05	G05/G06	H05/H06
29-Sep-18				D05	E05		G04	H04
30-Sep-18	A02	B11						
8-Oct-18			C11				G10	H11
11-Oct-18	A12	B12						
14-Oct-18	A02	B02	C02	D02				

Relationship of *Rhinolophus* bat coronavirus BtCoV/4991 and Bat coronavirus RaTG13.

The Wuhan laboratory has reported on the bat coronaviruses, BtCoV/4991 and RaTG13, in two peer-reviewed publications, one in 2016 and one in February 2020.[33] They have submitted three entries to GenBank for these two viruses, in 2016, February 2020, and May 2020.[34] The GenBank entries confirm sequencing experiments using Sanger dideoxy sequencing in 2016, PCR-generated amplicon sequencing performed on an AB 310 Genetic Analyzer in 2017 and 2018, and RNA-seq performed on an Illumina HiSeq 3000 (instrument id ST-J00123) in 2020. A single GISAID entry records that the RNA-seq data was obtained from an original specimen without passage.[35] This is an important detail since evidence of primate sequences, consistent with VERO cell contamination, is found in this specimen, as reported below, which would suggest laboratory passage.

None of these disclosures report that BtCoV/4991 and RaTG13 are the same coronavirus, simply renamed. This information was only disclosed in a written Question and Answer publication from *Science* magazine by Dr. Shi on July 31, 2020.[4, 36] Given this disclosure months after the original publication concerning RaTG13 in *Nature* it is possible that the omission of the original publication and sequence data concerning BtCoV/4991 violated the "Reporting

[33] 2016 Virologica Sinica paper and February 2020 Nature paper
[34] RaTG13 complete genome Feb 2020, Raw sequence reads for RaTG13 published Feb 2020, Amplicon reads for RaTG13 from 2017 and 2018 published in May 2020.
[35] The GISAID entry is EPI_ISL_402131.
[36] Dr. Shi wrote: "Ra4991 is the ID for a bat sample while RaTG13 is the ID for the coronavirus detected in the sample. We changed the name as we wanted it to reflect the time and location for the sample collection. 13 means it was collected in 2013, and TG is the abbreviation of Tongguan town, the location where the sample was collected."

standards and availability of data, materials, code and protocols" required for *Nature* publications.[37]

The February 2020 papers uses the RNA-Seq data for RaTG13 genome determination but fails to disclose the previous data obtained by Sanger dideoxy sequencing in 2016 and by amplicon sequencing in 2017 and 2018. Since these unrecorded data establish method-dependent sequencing differences of up to 4% the failure to disclose this data or to reconcile these differences is troubling.

In addition, the raw assembly accession data for RaTG13 are not described or linked to the Genbank entry, MN669532, and also no assembly method is specified in the raw data SRX7724752 12 and the Illumina run. And the amplicon sequencing data has sequence gaps of approximately 20% of the genome. Therefore, no primary assembly data has been made available by the WIV for the RaTG13 genome. This is contrary to the *Nature* Reporting Standards[9] as they state: "When publishing reference genomes, the assembly must be made available in addition to the sequence reads."

Relationship of RaTG13 and SARS-CoV-2.

There have been two descriptions of the process by which the RaTG13 genome was identified as closely homologous to SARS-CoV-2. These seem to be inconsistent with each other.

In the February 2020 *Nature* paper[5] it states:

"We then found that a short region of RNA-dependent RNA polymerase (RdRp) from a bat coronavirus (BatCoV RaTG13)—which was previously detected in Rhinolophus affinis from Yunnan province—showed high sequence identity to 2019-nCoV. We carried out full-length sequencing on this RNA sample (GISAID accession number EPI_ISL_402131). Simplot analysis showed that 2019-nCoV was highly similar throughout the genome to RaTG13, with an overall genome sequence identity of 96.2%."

In a July 2020 interview the process was described:

"We detected the virus by pan-coronavirus RT-PCR in a bat fecal sample collected from Tongguan town, Mojiang county in Yunnan province in 2013, and obtained its partial RdRp sequence. Because the low similarity of this virus to SARS-CoV, we did not pay special attention to this sequence. In 2018, as the NGS sequencing technology and capability in our lab was improved, we did further sequencing of the virus using our remaining samples, and obtained the full-length genome sequence of RaTG13 except the 15 nucleotides at the 5' end. As the sample was used many times for the purpose of viral nucleic acid extraction, there was no more sample after we finished genome sequencing, and we did not do virus isolation and other studies on it. Among all the bat samples we collected, the RaTG13 virus was detected in only one single sample. In 2020, we compared the sequence of SARS-CoV-2 and our unpublished bat

[37] Nature research reporting standards for availability of data

coronavirus sequences and found it shared a 96.2% identity with RaTG13. RaTG13 has never been isolated or cultured."

If the full-length genome of RaTG13 was available by 2018 it is unclear why a database search within the WIV for coronaviruses that resembled SARS-CoV-2 would lead to identifying the 370-nt segment representing the RdRp gene (as stated in the February paper) but not the full length RaTG13 genome (which was stated to have been sequenced by 2018). In addition, an assembly of all available amplicon data for RaTG13 from 2017 and 2018 contains gaps of approximately 20% of the genome. If the sample was completely consumed during the 2017-8 sequencing it is unclear how RNA-Seq was conducted in 2020 to permit the full-length genome to be determined.

Analytical methods. Taxonomy of specimens was determined in the NCBI Sequence Read Archive and KRONA.[38] Blast was used for sequence alignment and comparisons.[39]

To evaluate the data from the bat species relative to the RaTG13 fecal sample analysis, the latter was treated as a fixed result with the comparison to the taxonomy results of the nine bat feces specimens. It also was noted that the data were clearly right skewed (and descriptively both mean/median and standard deviation/interquartile range were used). Therefore, a non-parametric procedure, the Wilcoxon signed-rank test was used with the p-value calculated by an exact procedure because of the small sample size. Considering the synonymous to non-synonymous mutation frequency and how to evaluate that for the various protein coding regions of the virus, it was noted that for all of the genes pooled, the ratio of the synonymous to non-synonymous regions was approximately 0.83. To analyze the corresponding distribution for each gene, we assumed that each mutation was an independent observation from a Bernoulli random variable and, therefore the number of synonymous mutations in the gene would have a binomial distribution (with probability 0.83). A probability was then computed for the actual number of synonymous mutations on this basis (the probability was determined on a one-sided basis, i.e. excess mutations, and was calculated as a strict inequality).

Results.

Original characterization of RaBtCoV/4991 (RaTG13) and related bat fecal specimen.

In 2016 Dr. Shi and colleagues published a paper entitled, "Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft[40]" in which a number of novel bat coronaviruses were isolated from bat fecal specimens collected during 2012 and 2013. The viruses were named, according to the paper, in the following fashion:

[38] NCBI Sequence Archive
[39] Blast alignment
[40] Xing-Yi Ge, et. al., Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft, Virologica Sinica, 2016, 31 (1): 31–40. DOI: 10.1007/s12250-016-3713-9

> "The positive samples detected in this study were named using the abbreviated bat species name plus the bat sample number abbreviation. For example, a virus detected from *Rhinolophus sinicus* in sample number 4017 was named RsBtCoV/4017. If the bat was co-infected by two different coronaviruses, numbers were appended to the sample names, such as RsBtCoV/4017-1 and RsBtCoV/4017-2."

In the July 2020 interview Dr. Shi wrote:

> "Ra4991 is the ID for a bat sample while RaTG13 is the ID for the coronavirus detected in the sample. We changed the name as we wanted it to reflect the time and location for the sample collection. 13 means it was collected in 2013, and TG is the abbreviation of Tongguan town, the location where the sample was collected."

The 2016 and 2020 statements about the naming of virus RsBtCoV/4991 appear inconsistent with each other.

Of the 152 coronaviruses identified, 150 were classified as alphacoronaviruses while only two were classified as betacoronaviruses, HiBtCoV/3740-2 and RaBtCoV/4991. The naming convention from the paper means this latter coronavirus was identified in a fecal specimen from a *Rhinolophus affinis* bat and was sample number 4991.

The latter virus was described in the paper as follows:

> "Virus RaBtCoV/4991 was detected in a R. affinis sample and was related to SL-CoV. The conserved 440-bp RdRp fragment of RaBtCoV/4991 had 89% nt identity and 95% aa identity with SL-CoV Rs672. In the phylogenetic tree, RaBtCoV/4991 showed more divergence from human SARS-CoV than other bat SL-CoVs and could be considered as a new strain of this virus lineage."

The Genbank accession number for RaBtCoV/4991 is MN KP876546.1 and in Genbank it is identified as having been collected in July 2013 as a "feces/swabs" specimen.

The RATG13 genome sequence was assembled from low coverage RNA-Seq data.

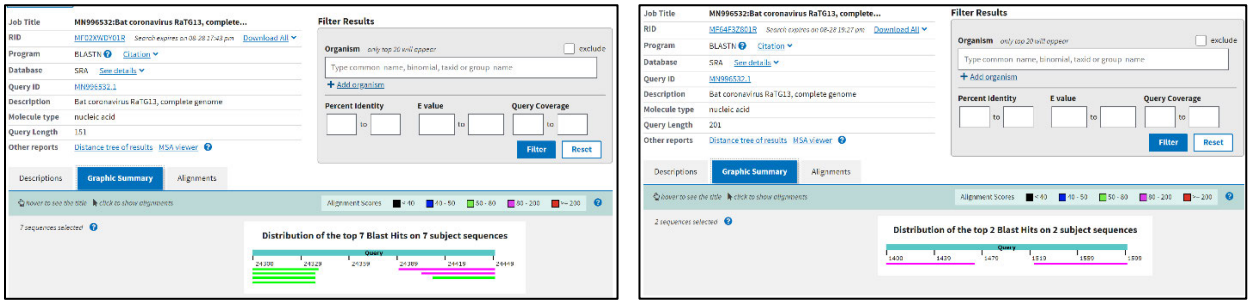
A Blast analysis of the RaTG13 genome against SRR11085797 retrieved about 1700 reads which covers only about 252,000 nt of the total reads of 3.3 Gb. Since the genome size of RaTG13 is known to be about 30,000 nt this represents an 8-fold coverage, typically insufficient for a definitive assembly. For example, some have suggested a 30-fold coverage is necessary to create high quality assemblies.[41]

[41] Sims, D. *et al.* Sequencing depth and coverage: key considerations in genomic analyses. Nature Reviews – Genetics. (2014) 15: 121-132. doi:10.1038/nrg3642.

At an eight-fold coverage and based on the typical practice of having four or more reads to call a SNP,[42] the 8-fold coverage of RaTG13 would have 4.2% bases or about 1260 calls of less than 4 reads and about 10 bases would be missed completely, with no calls at all.

A Blast of the RaTG13 published genome onto the RNA-Seq data documents at least two 60 base-pair gaps with no coverage, precluding a complete assembly.

Given the low coverage in the RNA-Seq data, an exploratory, non-exhaustive Blast search was conducted against the published RaTG13 sequence. Two gaps of over 60 nt, shown below, were easily found:

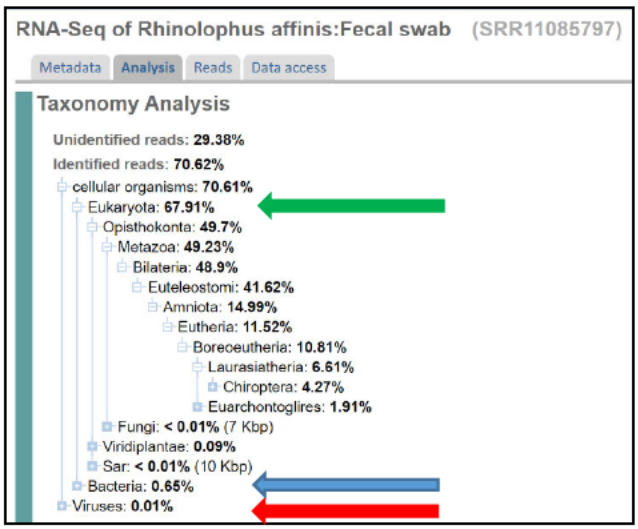


It is conceivable there are additional gaps but the above two are sufficient to document that the complete RaTG13 genome sequence could not have been assembled solely from the RNA-Seq data, as stated.[2]

Taxonomy analysis of the RaTG13 specimen is inconsistent with being from bat feces and shows evidence of laboratory cell culture contamination.

According to the Wuhan laboratory, the RaTG13 coronavirus was a fecal swab specimen collected from a *Rhinolophus affinis* bat in 2013. Unexpectedly, (Text-Figure below) the taxonomy analysis is primarily eukaryotic (green arrow; 67.91%) with only traces of bacteria (blue arrow; 0.65%). The viral genomes also make only a trace contribution (red arrow; 0.01%):

[42] Illumina Technical Bulletin Call Coverage



Taxonomy analysis for RaTG13 data SRR11085797

To compare this specimen composition to bat fecal specimens collected by Dr. Shi and her WIV colleagues and analyzed in other studies, a paper from Dr. Shi's laboratory, also published in February 2020, was identified. In this paper, entitled, "Discovery of Bat Coronaviruses through Surveillance and Probe Capture-Based Next-Generation Sequencing,"[43] a total of nine specimens "collected during previous bat CoV surveillance projects, (were) extracted from bat rectal swabs." According to the Methods section in this paper, the "previous bat CoV surveillance projects" include the field work in 2013 when the RaTG13 was said to have been collected. The comparison below is thus the same specimens collected on the same field surveillance projects by the same investigators from the Wuhan laboratory and sequenced on the same Illumina instrument. These nine specimens will be referred to as "reference fecal specimens" henceforth.

The following Text-Table compares the taxonomical analysis of the RaTG13 and reference fecal specimens. The reference fecal specimens have an average eukaryotic genome content of about 12% while RaTG13's eukaryotic content was 68%. On the other hand, the most abundant genes in the reference fecal specimens were bacterial, with an average of 65%; RaTG13 had less than 1% bacterial genes. And finally, the reference fecal specimens had 1.57% virus genes compared to the 0.01% virus genes of RaTG13.

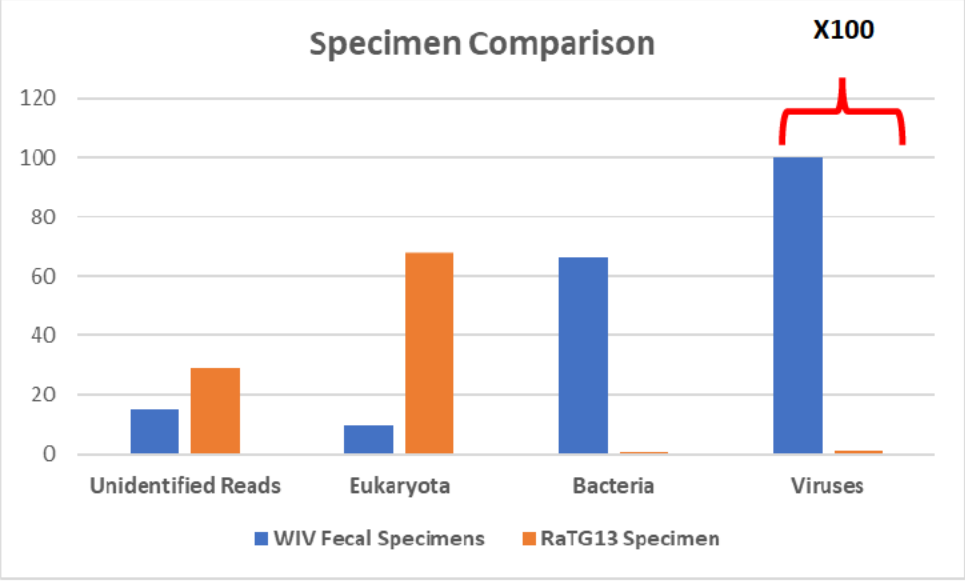
[43] Discovery of bat coronaviruses through surveillance and probe capture-based next-generation sequencing

Specimen ID	Specimen Type	Unidentified Reads	Eukaryota	Bacteria	Viruses	Sum
SRR11085736	*Rhinolophus affinis*	0.86	4.36	91.07	0.03	96.32
SRR11085734	*Miniopterus schreibersii*	3.81	16.03	76.15	0.11	96.1
SRR11085737	*Scotophilus kuhlii*	17.98	8.59	67.81	2.19	96.6
SRR11085733	*Hipposideros larvatus*	13.27	27.99	42.96	4.1	88.32
SRR11085735	*Hipposideros pomona*	34.33	7.96	54.78	0.71	97.78
SRR11085738	*Pipistrellus abramus*	20.33	21.44	47.3	6.45	95.52
SRR11085739	*Tylonycteris pachypus*	61.75	14.34	20.06	0.06	96.21
SRR11085740	*Miniopterus pusillus*	0.78	1.46	99.22	0.05	101.51
SRR11085741	*Rousettus aegyptiacus*	6.44	2.59	88.36	0.45	97.84
Mean +/- SD	Nine bat feces specimens	17.73+/-19.79	11.64+/-9.02	65.30+/-26.10	1.57+/-2.28	96.24+/-3.45
Median +/- IQR	Nine bat feces specimens	13.27+/-24.995	8.59+/-15.26	67.81+/-41.58	0.45+/-3.09	96.32+/-2.00
SRR11085797	**RaTG13 fecal specimen**	**29.38**	**67.91**	**0.65**	**0.01**	**97.95**
	P-value (exact Wilcoxon signed-rank test)	0.16	**0.0039**	**0.0048**	**0.0039**	0.098

As shown in the Text-Table above the RaTG13 specimen is significantly different from the reference fecal specimens in composition. The probabilities for each category, eukaryote, bacteria, and virus, are individually highly statistically significant. They are also independent of each other and therefore the overall probability that RaTG13 has the composition of eukaryote, bacteria, and virus genes that was reported by the Wuhan laboratory but is actually from an authentic bat fecal specimen is less than one in 13 million.

The alternative conclusion is that this sample was not a fecal specimen but was contrived. The data cannot, however, distinguish between a non-fecal specimen that came from true field work on the one hand and a specimen created *de novo* in the laboratory on the other hand.

A graphical comparison of the above data is shown below and visually shows the significant differences between the WIV fecal specimens and the RaTG13 specimen, despite the claim they were collected in the same field surveillance trips:



Another comparison can be made between the reference fecal specimens and the RaTG13 specimen by looking at the taxonomy of the nine to twelve "strong signals" identified on the NCBI Sequence Read Archive. The following Text-Table is a summary of these findings.

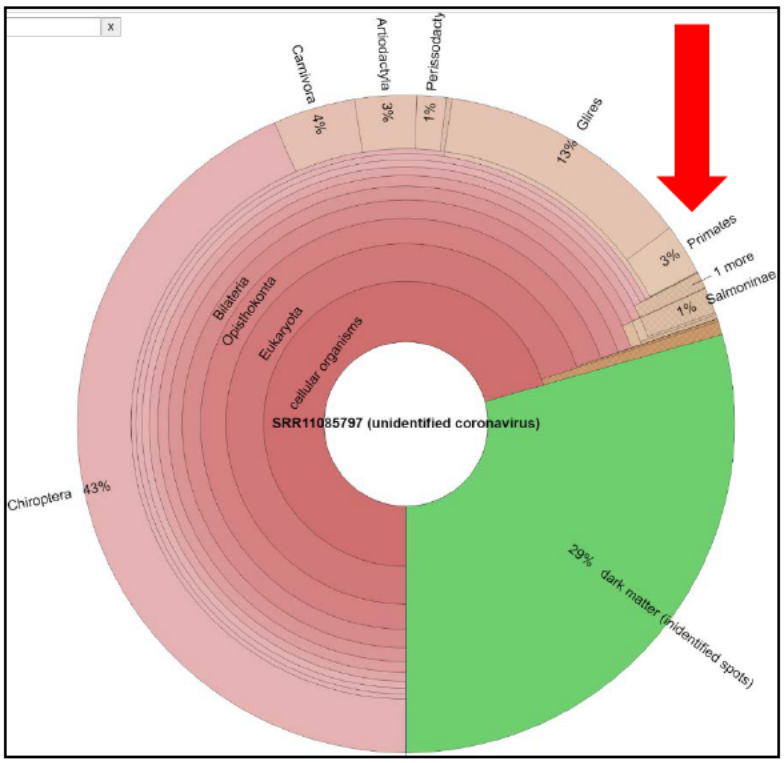
Specimen	The identity of the Strong Signals in the Specimens		
	Bacteria	Eukaryotes	Viruses
Rhinolophus affinis anal swab (SRR11085736)	92%	One magnaorder of placental mammals, includes bat	None
Miniopterus schreibersii anal swab (SRR11085734)	88%	**One bat**, the host bat, Miniopterus sp.	None
Scotophilus kuhlii anal swab (SRR11085737)	56%	**Two bats**, mouse-eared and big brown bats.	Two viruses, kobuvirus (host includes bats) and a Scotophilus kuhlii coronavirus
Hipposideros larvatus anal swab (SRR11085733)	56%	**One bat,** the host bat, Hipposideros sp. and one rodent.	Hipposideros pomona bat coronavirus
Hipposideros pomona: Anal swab (SRR11085735)	78%	**One bat**, the host bat, Hipposideros sp.	None
Pipistrellus abramus: Anal swab (SRR11085738)	73%	**Two bats**, the big brown bat and the mouse-eared bat.	Pipistrellus abramus bat coronavirus
Tylonycteris pachypus: Anal swab (SRR11085739)	67%	**Three bat**s, the microbat, the great roundleaf bat, and a superorder of mammals, which includes bats.	None
Miniopterus pusillus: Anal swab (SRR11085740)	89%	**One bat**, the Natal long-fingered bat.	None
Rousettus aegyptiacus: Anal swab (SRR11085741)	91%	One magnaorder of placental mammals, includes bats.	None
Average	77%		
RaTG13 Rhinolophus affinis:Fecal swab (SRR11085797)	None	All nine strong signals are eukaryotes. **Five bats**, the Great Roundleaf bat, resident of China, the Egyptian fruit bat, which is not found in China, a megabat, mouse-eared bat, and bent-winged bat. Two marmots, the Alpine marmot from Europe and the Yellow-bellied marmot of North America.The paraorder of whales. The red fox.	None

As can be seen, while the strong signals in the authentic specimens contain 56% to 92% (average 77%) bacterial signals, the RaTG13 specimen has no bacteria among the nine strong signals. Most specimens do not have virus strong signals but the three that do are host-related coronaviruses (four) or one host-related kobuvirus.

RaTG13 has no viral strong signals. Among the reference specimens with eukaryotic strong signals, they are either bat-related genes (eleven) or higher order taxonomy signals that include bats (three). There is one anomalous rodent-related signal among the reference specimens.

The RaTG13 specimen is again an outlier with all nine strong signals arising from eukaryotic genes. Five of the nine signals are bats, some resident to China and some with non-Chinese host ranges. Surprisingly, unlike three of the reference bat signals which are identified as host-related, the RaTG13 specimen did not contain *Rhinolophus* sp. host-related strong signals. The remaining four strong signals are marmot-related genes (two), whale-related gene (one), and red fox-related gene (one).

Finally, a Krona analysis (below) identifies 3% primate sequences (red arrow) in the RaTG13 sequence data. This is consistent with contamination by the standard laboratory coronavirus cell culture system, the VERO monkey kidney cell line.



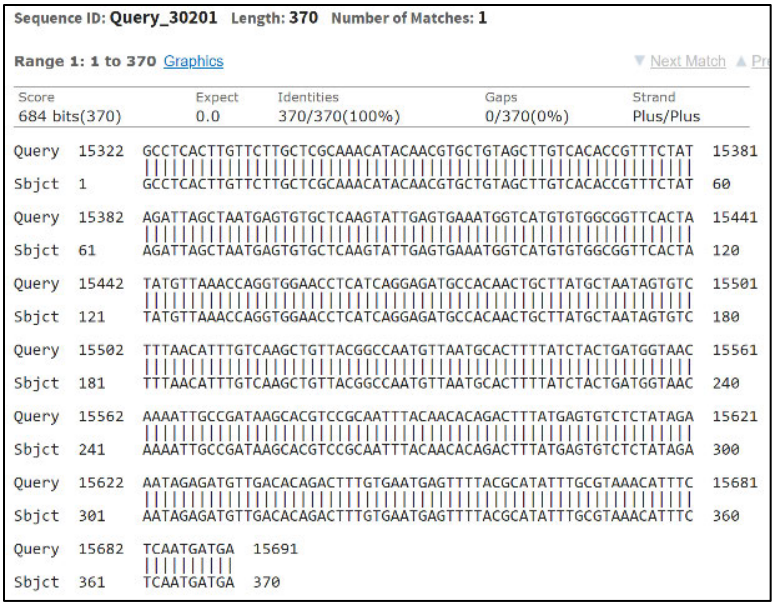
Source: [Krona analysis of RaTG13 specimen]

It is unclear why these obviously anomalous findings were not detected during the peer-review process prior to publication of this important work. At this point, an explanation is needed from the WIV to refute the conclusion that the specimen identified as the source of RaTG13 is **not** a bat fecal/anal specimen and that the primate genetic material is consistent with a VERO cell contaminated specimen.

Method-related nt base substitutions in RaTG13.

The original Sanger dideoxy RdRp sequence reported in 2016 is homologous to RNA-seq data from 2020 but is non-homologous to amplicon sequencing data from 2017 and 2018.

As expected, a comparison of the 2016 RdRp GenBank sequence for BtCoV/4991 obtained by Sanger dideoxy sequencing with the RNA-seq sequencing of RaTG13 reported in *Nature* shows 100% identity over the 370 nt segment.



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Range 1: 1 to 370 Graphics

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              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1      GCCTCACTTGTTCTTGCTCGCAAACATACAACGTGCTGTAGCTTGTCACACCGTTTCTAT  60

Query  15382  AGATTAGCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTTCACTA  15441
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61     AGATTAGCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTTCACTA  120

Query  15442  TATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTC  15501
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121    TATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTC  180

Query  15502  TTTAACATTTGTCAAGCTGTTACGGCCAATGTTAATGCACTTTTATCTACTGATGGTAAC  15561
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181    TTTAACATTTGTCAAGCTGTTACGGCCAATGTTAATGCACTTTTATCTACTGATGGTAAC  240

Query  15562  AAAATTGCCGATAAGCACGTCCGCAATTTACAACACAGACTTTATGAGTGTCTCTATAGA  15621
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Sbjct  241    AAAATTGCCGATAAGCACGTCCGCAATTTACAACACAGACTTTATGAGTGTCTCTATAGA  300

Query  15622  AATAGAGATGTTGACACAGACTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTC  15681
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301    AATAGAGATGTTGACACAGACTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTC  360

Query  15682  TCAATGATGA  15691
              ||||||||||
Sbjct  361    TCAATGATGA  370
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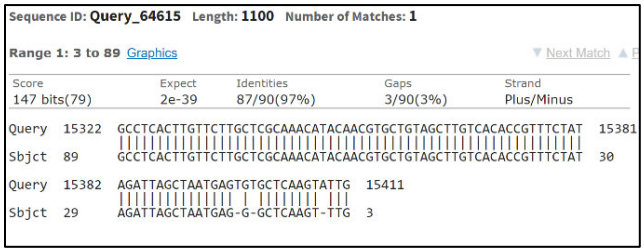
Surprisingly, the two amplicon sequences from 2017 that partially cover the 370 nt RdRp region have four base substitutions or gaps over a total segment of 219 nt (2% divergence).



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Range 1: 3 to 89 Graphics

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Sbjct  89     GCCTCACTTGTTCTTGCTCGCAAACATACAACGTGCTGTAGCTTGTCACACCGTTTCTAT  30

Query  15382  AGATTAGCTAATGAGTGTGCTCAAGTATTG  15411
              |||||||||||||| | |||||||| |||
Sbjct  29     AGATTAGCTAATGAG-G-GCTCAAGT-TTG  3
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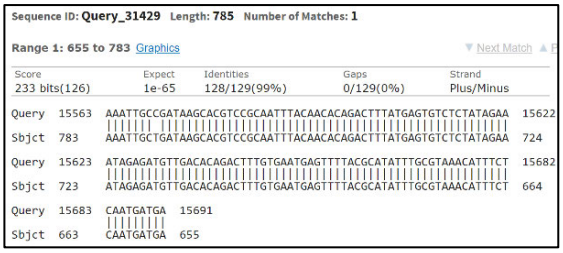

Sequence ID: **Query_31429** Length: **785** Number of Matches: **1**

Range 1: 655 to 783 Graphics

Score	Expect	Identities	Gaps	Strand
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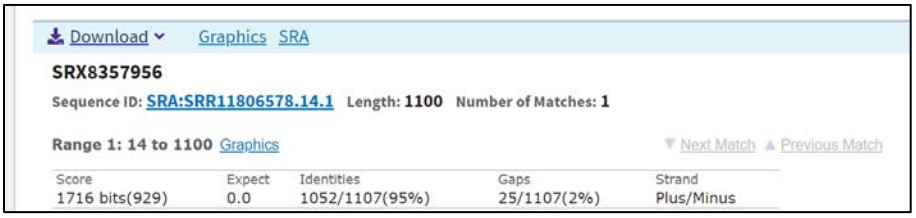
Query  15623  ATAGAGATGTTGACACAGACTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTCT  15682
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  723    ATAGAGATGTTGACACAGACTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTCT  664

Query  15683  CAATGATGA  15691
              |||||||||
Sbjct  663    CAATGATGA  655
```

RaTG13 Spike Protein gene has 5% substitutions when comparing 2020 RNA-Seq and 2017 amplicon sequencing data.

The segment of RaTG13 which shows the greatest sequence divergence between the RNA-seq and amplicon sequencing methods spans from A8886 to A9987 and is shown here below. It contains 80 base substitutions/indels in a 1107 nt sequence (5% substitution and 2% gaps).



Download Graphics SRA

SRX8357956

Sequence ID: SRA:SRR11806578.14.1 Length: **1100** Number of Matches: **1**

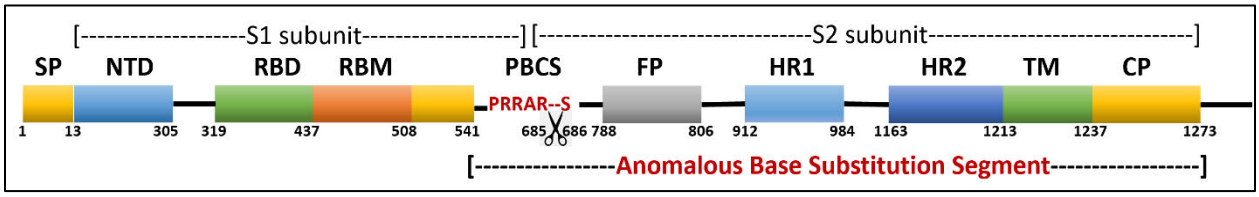
Range 1: 14 to 1100 Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1716 bits(929)	0.0	1052/1107(95%)	25/1107(2%)	Plus/Minus

No explanation has been offered in publications from the WIV for the method-dependent sequencing differences identified here, which are twenty- to 50-fold higher than the 0.1% technical error rate sometimes attributed to RNA-Seq data.

The Spike Protein gene sequence substitution divergence between RaTG13 and SARS-CoV-2 contains an improbable synonymous/non-synonymous pattern.

The functional structure of the SARS-CoV-2 Spike Protein is shown here:



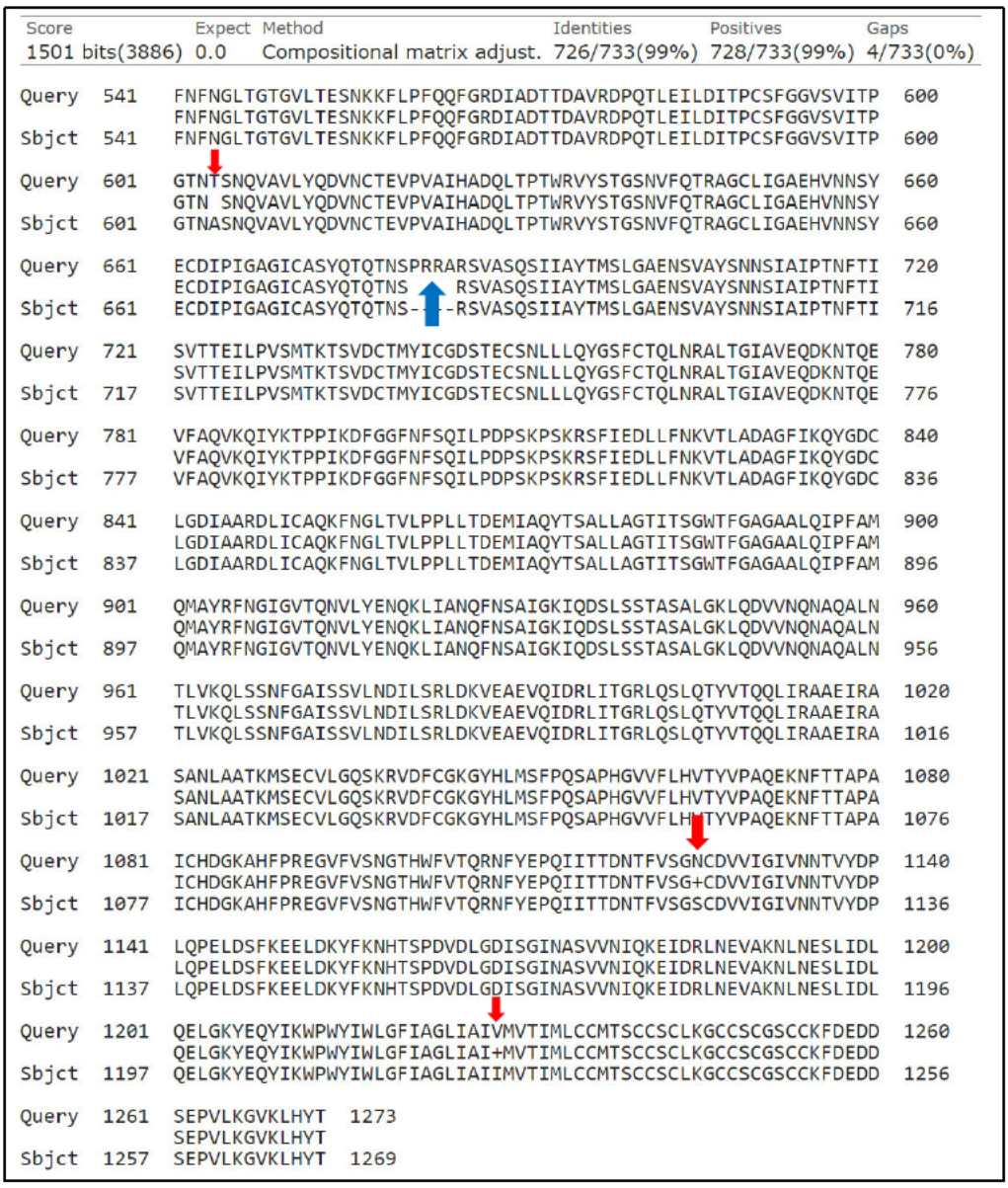
The SARS-CoV-2 Spike protein (above) contains an S1 subunit and S2 subunit with the Polybasic Cleavage Site (PBCS) between R685 and S686. This cleavage is performed by a host cell surface protease, furin, and is an important attribute in explaining the virulence of SARS-CoV-2 compared to other human coronaviruses, which do not have a furin cleavage site. The PBCS also contains the unusual PRRA insertion that has not been previously seen in Clade B coronaviruses and for which no natural mechanism for its appearance has been offered.[44]

The S1 subunit is located within the N-terminal 14–685 amino acids of S protein, containing N-terminal domain (NTD), receptor binding domain (RBD), and receptor binding motif (RBM). The S2 subunit contains a fusion peptide (FP), heptad repeat 1 (HR1), heptad repeat 2 (HR2), transmembrane domain (TM) and cytoplasmic domain (CP).

The base substitution pattern of synonymous and non-synonymous substitutions when comparing RaTG13 and the reference sequence of SARS-CoV-2 demonstrated an anomalous pattern for the coding region for aa 541 to 1273, a 733 aa protein segment representing over 60% of the SP gene.

As shown in the Text-Figure below, there are only three substitutions (red arrow) and the PBCS insertion (blue arrow) when comparing this segment of the RaTG13 and SARS-CoV-2 SP. Excluding the PBCS, the amino acid sequences are 99.6% identical.

[44] The proximal origin of SARS-CoV-2.



Score	Expect	Method	Identities	Positives	Gaps
1501 bits(3886)	0.0	Compositional matrix adjust.	726/733(99%)	728/733(99%)	4/733(0%)

```
Query  541   FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP  600
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Sbjct  541   FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP  600

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             GTN SNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY
Sbjct  601   GTNASNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY  660

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Sbjct  661   ECDIPIGAGICASYQTQTNS--  --RSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI  716

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             SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE
Sbjct  717   SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE  776

Query  781   VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC  840
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Sbjct  777   VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC  836

Query  841   LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM  900
             LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM
Sbjct  837   LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM  896

Query  901   QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN  960
             QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN
Sbjct  897   QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN  956

Query  961   TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA  1020
             TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA
Sbjct  957   TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA  1016

Query  1021  SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA  1080
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Sbjct  1017  SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA  1076

Query  1081  ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDP  1140
             ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSG+CDVVIGIVNNTVYDP
Sbjct  1077  ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGSCDVVIGIVNNTVYDP  1136

Query  1141  LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL  1200
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Sbjct  1137  LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL  1196

Query  1201  QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD  1260
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Sbjct  1197  QELGKYEQYIKWPWYIWLGFIAGLIAIIMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD  1256

Query  1261  SEPVLKGVKLHYT  1273
             SEPVLKGVKLHYT
Sbjct  1257  SEPVLKGVKLHYT  1269
```

Given the high amino acid identity of this 733 amino acid sequence (except for the PBCS insertion) and the typical coronavirus synonymous to non-synonymous mutation frequency of between three and five synonymous mutations for each non-synonymous mutation,[45] it was expected that a comparison of the nucleotide sequence for this region between SARS-CoV-2 and RaTG13 would show an almost identical sequence as well.

In fact, when the SARS-CoV-2 nt sequence 23,183-25,384 was compared to the RaTG13 nt sequence 23,165-25,354, the corresponding genome sequence to the 99.6% identical protein sequence above, the nucleotide identity was only 94.2% identical, with 122 synonymous substitutions and only the three non-synonymous substitutions.

[45] Comparative genomic analysis

To put this in context a comparison of thirteen other protein coding regions of SARS-CoV-2 and RaTG13 (Text-Table below) shows that the overall synonymous to non-synonymous mutation frequency is 549 synonymous to 109 non-synonymous or a ratio of about 5.0.

Gene	Region of Genome	Total Nucleotides	Synonymous mutations	Non-Synonymous mutations	S/NS	Probability of more than the number of synonymous mutations given the probability of a synonymous mutation is 0.83 (based on all genes pooled)
pp1ab	1-21,239	21,239	659	102	6.5	0.003
pp1ab ABSS	**7448-18266**	**10,818**	**283**	**13**	**21.8**	**5.73 x 10^-12**
Spike Protein RBD	1-1814	1814	131	27	4.9	0.48
Anomalous Base Substitution Segment	**23,183-25,384**	**2201**	**112**	**3**	**37.3**	**< 1.0 x 10^-7**
Entire Spike Protein	1-3810	3808	231	41	5.6	0.18
ORF1a polyprotein	1-13,215	13215	440	86	5.2	0.33
ORF3a protein	1-828	828	25	6	4.2	0.56
E Protein	1-228	228	1	0	Infinite	0.83
M Protein	1-669	669	27	3	9.0	0.1
ORF6 Protein	1-186	186	3	0	Infinite	0.17
ORF7a Protein	1-366	366	13	3	4.3	0.47
ORF7b Protein	1-132	132	0	1	0	0.83
ORF8 Protein	1-366	366	5	6	0.8	0.99
Nucleocapsid Phosphoprotein	1-1260	1260	35	4	8.75	0.083

With the exception of the anomalous base substitution segment (ABSS) in the Spike Protein gene and the pp1ab gene, the remainder of the S/SN substitution ratios are consistent with the literature values for coronaviruses. Only two genes or gene regions have a higher S/SN ratio than the ABSS because they have no non-synonymous mutations: the E protein gene with 228 nucleotides and the ORF6 protein gene with 186 nucleotides. Because of the short length of these two genes, the probabilities of the results for the E and ORF6 genes were not significant, with p-values of 0.86 and 0.17, respectively.

The p-value for the ABSS, on the other hand, was highly significant, with a p-value of <0.0000001. This strongly suggests a non-natural cause for this base substitution pattern, barring some unknown biological mechanism for such a result.

A second highly anomalous sequence was found in the pp1ab gene. This is about five-times larger than the Spike Protein region and is even more unlikely to have happened naturally, a chance of about one in 100 billion times.

Are there only synonymous mutations in these regions because non-synonymous mutations lead to non-replicative viruses?

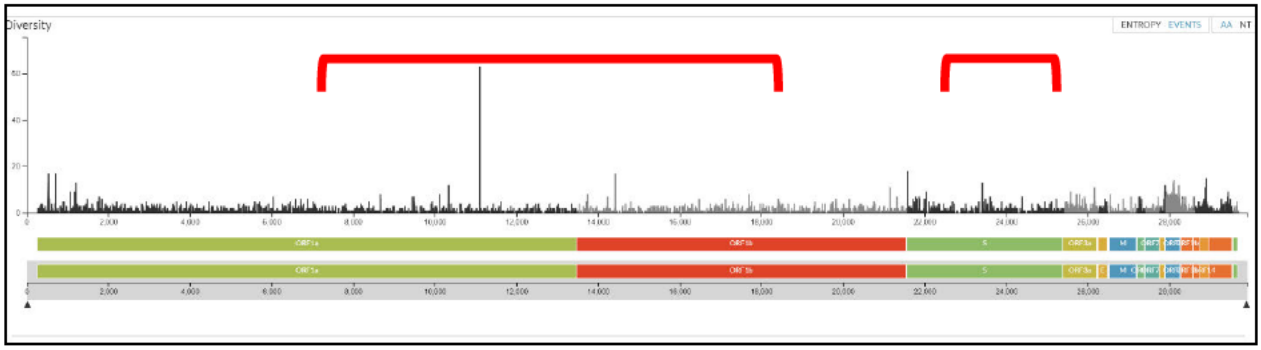
A simple explanation for these results would be an extreme criticality for the specific sequences of these regions with respect to infectivity. If a single amino acid change yielded a non-transmissible viral particle that strong negative purification process could explain the above results.

This hypothesis can be immediately rejected based on two observations.

In an examination of over 80,000 SARS-CoV-2 genome sequences, the most common Spike Protein non-synonymous mutation is within the ABSS (D614G) which was identified within weeks of the outbreak in January 2020 and which has become "the dominant virus…in every geographical region."[46] Specifically, as of August 28, 2020, GISAID reports that 65,738 full length SARS-CoV-2 genomes of a total of 83,387, or 79%, and comprising the G, GH, and GR clades, contain the D614G SNV. Under real world biological conditions, the ABSSN region has in fact, not a strong negative purification process in operation but in fact a strong positive selection process ongoing.

Secondly, in an analysis of mutations in 63,421 SARS-CoV-2 genomes the Spike Protein amino acid 605 to 1120 region had a total of 7,149 mutations. Fully 5,936 of these mutations (83%) are the above noted D614G non-synonymous change. Of the remaining 1213 mutations, 452 were non-synonymous while 755 were synonymous, a ratio of 1.7. There were also four indels and two stop codon mutations.

The following Text-Figure contains a map of the SARS-CoV-2 genome with the location of amino acid changes that have been found during the worldwide spread noted, with the frequency related to the height of the mark. The two ABSS in pp1ab and SP are marked with red brackets and clearly demonstrate an abundance of non-synonymous mutations in these regions during the human-to-human spread.



Nextstrain SARS-CoV-2 amino acid change events

Clearly, these regions can tolerate many non-synonymous mutations, rejecting the theory of a criticality for the amino acid sequence of this region. No other natural biological mechanism to explain these results has been identified.

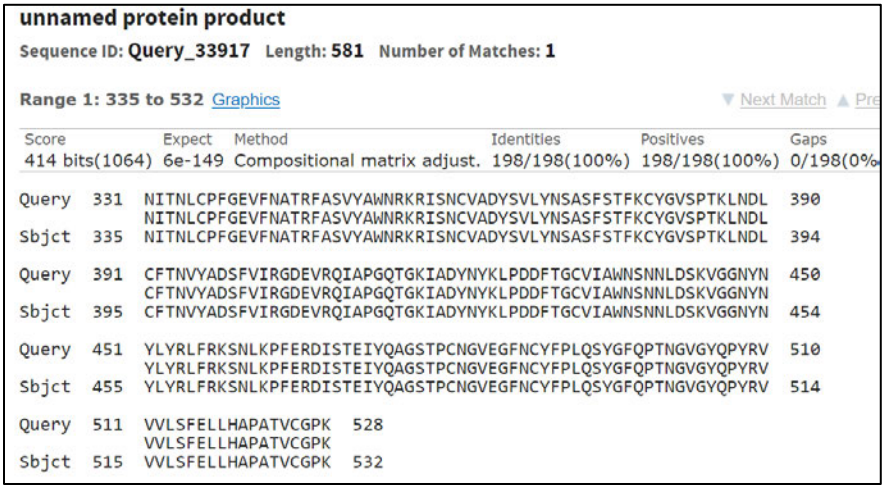
Codon modification, enhancement, or optimization is an example from synthetic biology in which the S/SN ratio is, by design, an anomaly when looked at through the lens of nature

[46] Biswas NK, Majumder PP. Analysis of RNA sequences of 3636 SARS-CoV-2 collected from 55 countries reveals selective sweep of one virus type. Indian J Med Res. 2020;151(5):450-458. doi:10.4103/ijmr.IJMR_1125_20.

Synonymous codon substitution is a decades old, well known method of enhancing gene expression when cloning exogenous genes in a laboratory experiment. In a paper on the immunogenicity of the SARS-CoV-2 Spike Protein[47] the following synthetic biology methods were used:

"We used the following structure coordinates of the coronavirus spike proteins from the PDB to define the boundaries for the design of RBD expression constructs: SARS-CoV-2 (6VSB), SARS-CoV-1 (6CRV), HKU-1 (5I08), OC43 (6NZK), 229E (6U7H) NL63 (6SZS). Accordingly, a codon-optimized gene encoding for S1-RBD [SARS-CoV-1 (318 – 514 aa, P59594), SARS-CoV-2 (331 – 528 aa, QIS60558.1), OC43 (329 – 613 aa, P36334.1), HKU-1 (310 – 611 aa, Q0ZME7.1), 229E (295 – 433 aa, P15423.1) and NL63 (480 – 617 aa, Q6Q1S2.1)] containing human serum albumin secretion signal sequence, three purification tags (6xHistidine tag, Halo tag, and TwinStrep tag) and two TEV protease cleavage sites was cloned into the mammalian expression vector pαH. S1 RBDs were expressed in Expi293 cells (ThermoFisher) and purified from the culture supernatant by nickel-nitrilotriacetic acid agarose (Qiagen)."

The Genbank alignment (below) confirms that the authentic SARS-CoV-2 Spike Protein sequence (https://www.ncbi.nlm.nih.gov/nuccore/1798174254) and the Synthetic construct SARS CoV-2 spike protein receptor binding domain gene, complete cds are 100% homologous at the protein level:



unnamed protein product

Sequence ID: **Query_33917** Length: **581** Number of Matches: **1**

Range 1: 335 to 532 Graphics ▼ Next Match ▲ Pre

Score	Expect	Method	Identities	Positives	Gaps
414 bits(1064)	6e-149	Compositional matrix adjust.	198/198(100%)	198/198(100%)	0/198(0%

```
Query  331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDL  390
            NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDL
Sbjct  335  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDL  394

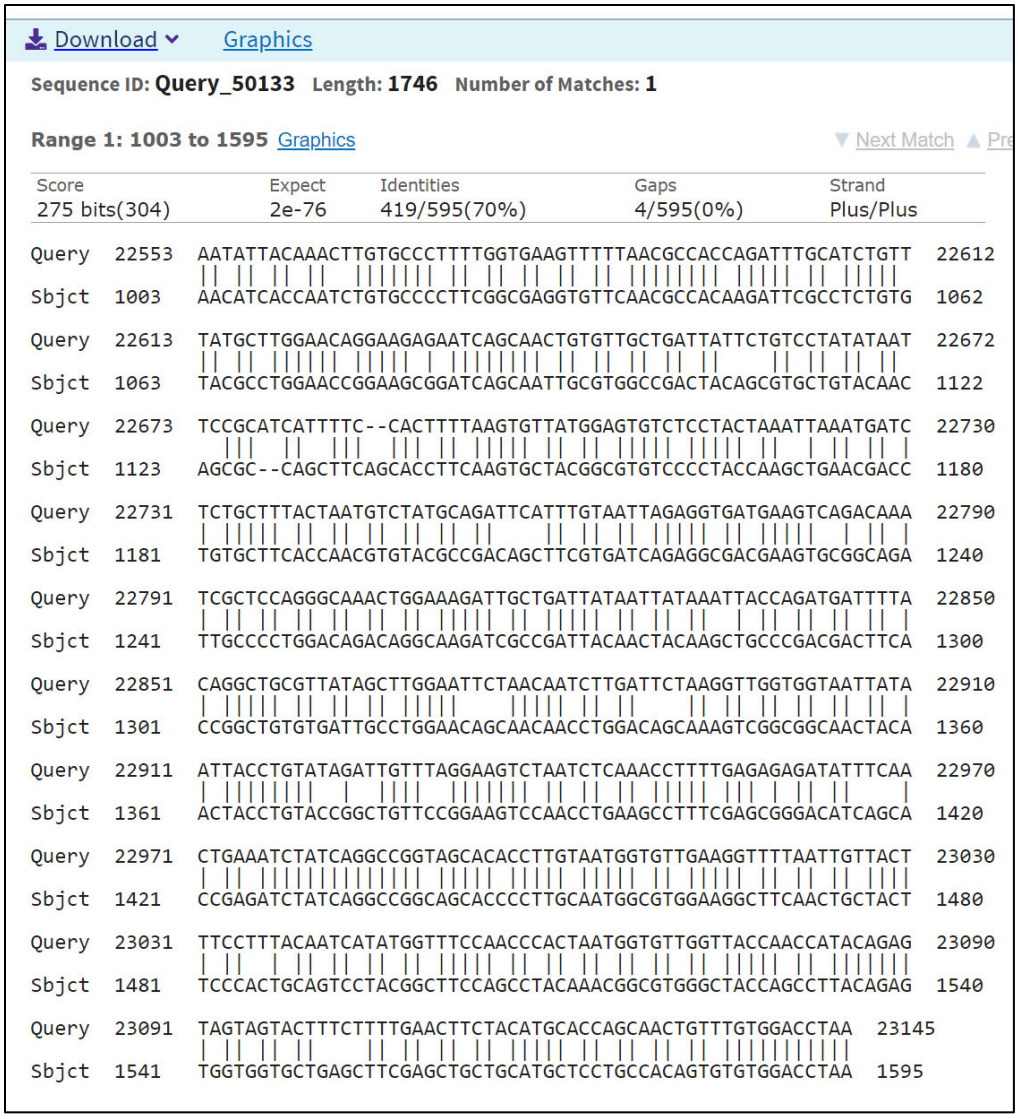
Query  391  CFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN  450
            CFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN
Sbjct  395  CFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN  454

Query  451  YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV  510
            YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV
Sbjct  455  YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV  514

Query  511  VVLSFELLHAPATVCGPK  528
            VVLSFELLHAPATVCGPK
Sbjct  515  VVLSFELLHAPATVCGPK  532
```

But a comparison of the authentic nucleotide sequence of SARS-CoV-2 to the codon-optimized synthetic construct shows no match using the "highly similar Megablast" algorithm setting. When the alignment algorithm is run in a more relaxed mode the impact of codon optimization in this case can be seen, a 70% homology:

[47] https://immunology.sciencemag.org/content/5/48/eabc8413/tab-pdf



Download Graphics

Sequence ID: Query_50133 **Length:** 1746 **Number of Matches:** 1

Range 1: 1003 to 1595 Graphics Next Match Pre

Score	Expect	Identities	Gaps	Strand
275 bits(304)	2e-76	419/595(70%)	4/595(0%)	Plus/Plus

```
Query  22553  AATATTACAAACTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACCAGATTTGCATCTGTT  22612
              || || || ||  ||||||| || || || || || |||||||| ||||| || |||||
Sbjct  1003   AACATCACCAATCTGTGCCCCTTCGGCGAGGTGTTCAACGCCACAAGATTCGCCTCTGTG  1062

Query  22613  TATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAAT  22672
              || || |||||| ||||| | |||||||| || || || || ||    || || || ||
Sbjct  1063   TACGCCTGGAACCGGAAGCGGATCAGCAATTGCGTGGCCGACTACAGCGTGCTGTACAAC  1122

Query  22673  TCCGCATCATTTTC--CACTTTTAAGTGTTATGGAGTGTCTCCTACTAAATTAAATGATC  22730
                 |||  ||  |||  ||| || ||||| || || ||||| ||||| ||  | || || |
Sbjct  1123   AGCGC--CAGCTTCAGCACCTTCAAGTGCTACGGCGTGTCCCCTACCAAGCTGAACGACC  1180

Query  22731  TCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCAGACAAA  22790
              | ||||| || || || || || ||    || || || ||||| || |||||  | || |
Sbjct  1181   TGTGCTTCACCAACGTGTACGCCGACAGCTTCGTGATCAGAGGCGACGAAGTGCGGCAGA  1240

Query  22791  TCGCTCCAGGGCAAACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTA  22850
              | || || || || || || ||||| || ||||| || || ||  | || || || || |
Sbjct  1241   TTGCCCCTGGACAGACAGGCAAGATCGCCGATTACAACTACAAGCTGCCCGACGACTTCA  1300

Query  22851  CAGGCTGCGTTATAGCTTGGAATTCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATA  22910
              | ||||| || || || |||||    ||||| || ||    || || || || || || |
Sbjct  1301   CCGGCTGTGTGATTGCCTGGAACAGCAACAACCTGGACAGCAAAGTCGGCGGCAACTACA  1360

Query  22911  ATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTTGAGAGAGATATTTCAA  22970
              | ||||||||  |  ||||  ||||||| || || || ||||| ||| | || ||    |
Sbjct  1361   ACTACCTGTACCGGCTGTTCCGGAAGTCCAACCTGAAGCCTTTCGAGCGGGACATCAGCA  1420

Query  22971  CTGAAATCTATCAGGCCGGTAGCACACCTTGTAATGGTGTTGAAGGTTTTAATTGTTACT  23030
              | || |||||||||||||| ||||| ||||| ||||| || ||||| || || || ||||
Sbjct  1421   CCGAGATCTATCAGGCCGGCAGCACCCCTTGCAATGGCGTGGAAGGCTTCAACTGCTACT  1480

Query  23031  TTCCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTTGGTTACCAACCATACAGAG  23090
              | ||  | || || || || ||||| || || || || || || ||||| || |||||||
Sbjct  1481   TCCCACTGCAGTCCTACGGCTTCCAGCCTACAAACGGCGTGGGCTACCAGCCTTACAGAG  1540

Query  23091  TAGTAGTACTTTCTTTTGAACTTCTACATGCACCAGCAACTGTTTGTGGACCTAA  23145
              | || || ||    || || || || ||||| || || || || ||||||||||
Sbjct  1541   TGGTGGTGCTGAGCTTCGAGCTGCTGCATGCTCCTGCCACAGTGTGTGGACCTAA  1595
```

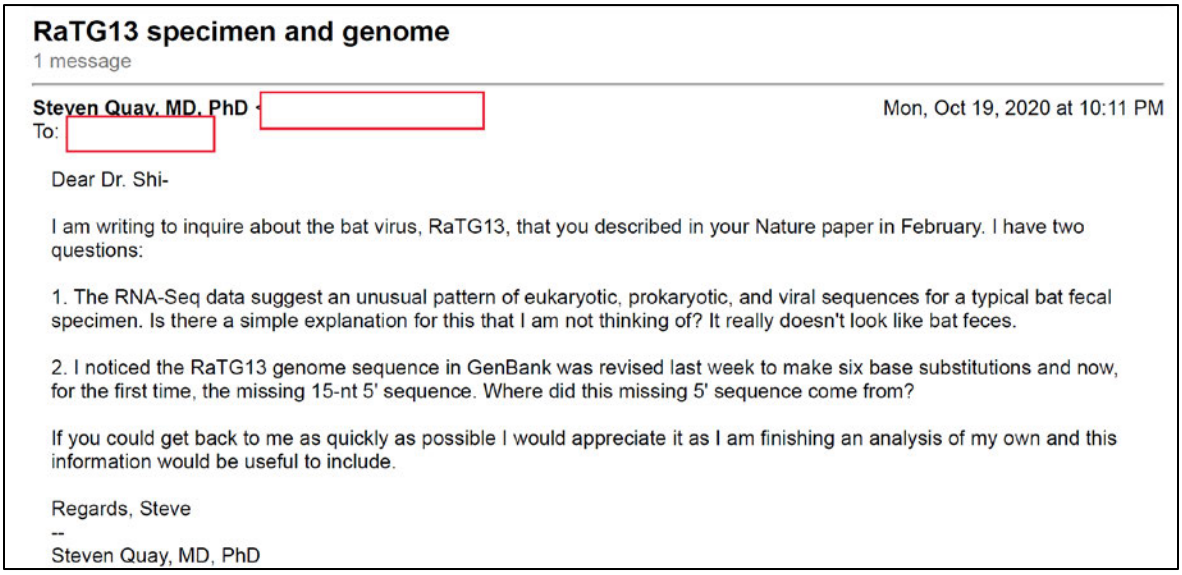
This is a situation in which there are 176 synonymous changes without a single non-synonymous change and is the genome signature of laboratory-derived synthetic biology. If these sequences were compared for phylogenetic divergence without the knowledge of their artificial construction, this synthetic laboratory experiment would create the impression that these two sequences had diverged in the wild from a common ancestor decades earlier.

The following Table identifies four regions of the RaTG13 and SARS-CoV-2 genomes in which there were a total of 220 synonymous mutations without a single non-synonymous change.

Protein/Gene	Protein Region	Total Nucleotides	Synonymous mutations	NS Mutations
S Protein	605-1124	1557	91	0
pp1ab	3607-4534	2781	66	0
pp1ab	4626-5111	1455	26	0
pp1ab	5113-5828	2145	37	0
	Total	7938	220	0

These regions represent over 26% of the entire genome and appear analogous to the outcome expected from the application of a synonymous codon modified, laboratory-derived synthetic biology project. They also represent about one-sixth of the 4% apparent phylogenetic divergence between RaTG13 and SARS-CoV-2.

October GenBank update. On October 13, 2020 the sequence for RaTG13 was updated. For the first time the first 15 nucleotides at the 5' end were present. However, these were not found in a blast of either the RNA-Seq raw reads or the Amplicons. The following email was sent to Dr. Shi asking for an explanation of the fecal specimen composition and the source for the 5' nt data.



RaTG13 specimen and genome
1 message

Steven Quay, MD, PhD [redacted] Mon, Oct 19, 2020 at 10:11 PM
To: [redacted]

Dear Dr. Shi-

I am writing to inquire about the bat virus, RaTG13, that you described in your Nature paper in February. I have two questions:

1. The RNA-Seq data suggest an unusual pattern of eukaryotic, prokaryotic, and viral sequences for a typical bat fecal specimen. Is there a simple explanation for this that I am not thinking of? It really doesn't look like bat feces.

2. I noticed the RaTG13 genome sequence in GenBank was revised last week to make six base substitutions and now, for the first time, the missing 15-nt 5' sequence. Where did this missing 5' sequence come from?

If you could get back to me as quickly as possible I would appreciate it as I am finishing an analysis of my own and this information would be useful to include.

Regards, Steve
--
Steven Quay, MD, PhD

At the time of this writing a response has not been received.

Discussion. The foundation of the working hypothesis that the COVID-19 pandemic arose via a natural zoonotic transfer from a non-human vertebrate host to man has been built on two publications: the February 3, 2020 *Nature* paper by Dr. Zheng-Li Shi and colleagues, in which the bat coronavirus RaTG13 is first identified as the closest sequence identity to SARS-CoV-2 at 96.2% and the March 17, 2020 *Nature Medicine* paper entitled, "The proximal origin of SARS-CoV-2," by Andersen *et al.,* in which the Shi *et al.* paper is cited as evidence for a bat origin for the pandemic. In the approximately six months since they were published, these two papers have been cited over 1600- and 200-times on PubMed, respectively.

However, research is beginning to question whether a bat species can be considered a natural reservoir for SARS-CoV-2. A recent paper performed an *in silico* simulation of the SARS-CoV-2 Spike Protein interaction with the cell surface receptor, ACE2, from 410 unique vertebrate species, including 252 mammals.[48] Among primates, 18/19 have an ACE2 receptor which is

[48] Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates Joana Damas, et al. Proc. of the Nat. Acad. of Sci. Aug 2020, 202010146; DOI: 10.1073/pnas.2010146117

100% homologous to the human protein in the 25 residues identified to be critical to infection, including the *Chlorocebus sabaeus* (the Old World African Green monkey) and the rhesus macaques.

It is noteworthy that the laboratory workhorse of coronavirus research is the VERO cell, isolated from a female African Green monkey in 1962, and containing an ACE2 receptor that is 100% homologous to the human ACE2 in the 25 critical amino acids for infectivity.

This *in silico* work was confirmed in the laboratory with respect to rhesus macaques. Within weeks of the identification of SARS-CoV-2, the Wuhan laboratory had demonstrated that the pandemic virus would infect and produce a pneumonia in rhesus macaques.[49]

A surprising finding from the ACE2 *in silico* surveillance work was the very poor predicted affinity of the ACE2 receptors in both bats and pangolins. Of 37 bat species studied, 8 scored low and 29 scored very low. As expected by these predictions, cell lines derived from big brown bat (*Eptesicus fuscus*),[50] Lander's horseshoe bat (*Rhinolophus landeri*), and Daubenton's bat (*Myotis daubentonii*) could not be infected with SARS-CoV-2.[51]

It is unfortunate that growth of the RaTG13 specimen could not have been attempted in the *Rhinolophus sinicus* primary or immortalized cells generated and maintained in the Wuhan laboratory: kidney primary cells (RsKi9409), lung primary cells (RsLu4323), lung immortalized cells (RsLuT), brain immortalized cells (RsBrT) and heart immortalized cells (RsHeT).[52] However it should be noted that a synthetically created RaTG13 was reported not to infect human cells expressing *Rhinolophus sinicus* ACE2, providing evidence that RaTG13 may not be a viable coronavirus in a wild bat population.[53]

The other proposed intermediate host, the pangolin, also had predicted ACE-2 affinity that was either low or very low.

A recent paper that examined the high synonymous mutation difference between RaTG13 and SARS-CoV-2 used an *in silico* methodology to suggest that the difference could be largely attributed to the RNA modification system of hosts.[54] However, the authors do not "(t)he

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[49] Infection with Novel Coronavirus (SARS-CoV-2) Causes Pneumonia in the *Rhesus Macaques.* C. Shan et al., Research Square, **DOI:** 10.21203/rs.2.25200/v1. Shan, C., Yao, Y., Yang, X. *et al.* Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in *Rhesus macaques*. *Cell Res* **30,** 670–677 (2020). https://doi.org/10.1038/s41422-020-0364-z

[50] J. Harcourt et al., Severe acute respiratory syndrome coronavirus 2 from patient with coronavirus disease, United States. Emerg. Infect. Dis. 26, 1266–1273 (2020).

[51] M. Hoffmann et al., SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181, 271–280.e8 (2020).

[52] Zhou, P., Fan, H., Lan, T. et al. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. Nature 556, 255–258 (2018). https://doi.org/10.1038/s41586-018-0010-9.

[53] Y. Li et al., Potential host range of multiple SARS-like coronaviruses and an improved ACE2-Fc variant that is potent against both SARS-CoV-2 and SARS-CoV-1. bioRxiv:10.1101/2020.04.10.032342 (18 May 2020).

[54] The divergence between SARS-CoV-2 and RaTG13 might be overestimated due to the extensive RNA modification

limitation of our study is that we were currently unable to provide experimental evidence for the modification on viral RNAs." The low S/SN ratio of 1.7 in the expansion of SARS-CoV-2 in the human population would argue against a robust host RNA modification mechanism.

In summary, the findings reported here are:

1. Inconsistences between published papers and interviews as to the source and sequencing history of the original specimen that was claimed to have been collected in 2013 (RaBtCoV/4991) and the specimen for the bat RaTG13 virus. For example, two explanations of the discovery of the close relationship between RaTG13 and SARS-Cov-2, a highly homologous match between the RdRp genes of the viruses noticed in 2020 followed by full genome sequencing, or identification in 2020 of a homologous match to full genome sequencing previously done in 2018. Current publicly available data for RaTG13 from 2017 and 2018 is a set of 33 amplicon sequencing runs but they cover only about 80% of the entire genome. In the *Science* interview Dr. Shi's says the specimen for RaTG was consumed during sequencing in 2018, but if this is true, the RNA-Seq referred to in the *Nature* paper could not have been performed in 2020. At this time, the Wuhan laboratory has not met the requirements of *Nature* with respect to the sharing of primary and sequence assembly data from their seminal paper[1] and this data should be provided immediately.
2. The specimen from which RaTG13 was reported to have been isolated and which has been repeatedly reported to have been a bat fecal specimen has a taxonomical composition of eukaryotes, bacteria, and viruses that is completely different from a set of nine bat fecal specimens collected in the same field visits by the same laboratory personnel from the Wuhan Institute of Virology. The probability that an authentic fecal specimen could have the composition reported is one in ten million, an impossibly low occurrence. Examination of the strong signals in the RaTG13 specimen identifies both a variety of bat genetic material, some that are not native to China, as well as unexpected species, such as marmots and a red fox. It also contains a telltale 3% primate sequence consistent with VERO cell contamination. I propose that this specimen is apparently either a mislabeled specimen (although I cannot conjure what the field source or specimen would be) or was artificially created in a laboratory.
3. The method-dependent sequence differences between the amplicon data and the RNA-Seq data are about 5% or about 50-times higher than expected as a technical error rate of 0.1%. This is an experimental quality issue that needs to be addressed; no explanation has been offered for this to date. In addition, no assembly methodology has been provided and at least two gaps, totaling over 60 nt, were easily identified.
4. The findings, reported here of a mutational drift of synonymous mutations only between SARS-CoV-2 and RaTG13 in the Spike Protein S1/S2 region and the pp1ab gene that has never been seen in nature before and which has a probability of having occurred by chance of less than one in ten million and one in one billion makes it more likely that, at least for these portions of the RaTG13 genome, comprising over one-

quarter of the entire genome, another process is underway. With the demonstration that codon-enhancement or optimization can produce this unnatural S/SN pattern, some form of laboratory-based synthetic biology was performed on RaTG13, SARS-CoV-2, or both.

Apparently, the entire specimen from which RaTG13 was purported to have been found has been consumed in previous sequencing experiments and the Principal Investigator has stated that no virus has ever been isolated or cultured from the specimen at any time in the past. Given the irregularities and anomalies identified in this paper it seems prudent to conclude that all data with respect to RaTG13 must be considered suspect. As such, reliance of the foundational papers of the origin of SARS-CoV-2 as having arisen from bats via a zoonotic mechanism must be reexamined and questioned.

Paper 2: The February 19, 2020 Lancet paper entitled: "Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19."

On February 19, 2020 *The Lancet* published a Correspondence entitled "Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19[55]" with 27 public health scientists from eight countries as authors. The statement seems to attempt to settle the question of the origin of SARS-CoV-2 and short circuit further debate, as the second sentence reads: "We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin." It goes on to state: "Conspiracy theories do nothing but create fear, rumors, and prejudice that jeopardize our global collaboration in the fight against this virus."

The letter provided an open solicitation for support and at this time has been signed by at over 20,300 people, as if to purport that science can be advanced through polling and the democratic process.[56] While it is a truism that conspiracy theories have no place in the academia, legitimate debate should not be foreclosed.

The statement itself provides a more nuanced discussion of the evidence for a zoonotic origin and contains 14 references, eight of which contain data about the COVID-19 pandemic and six of which are governmental policy statements without new data, background articles from 2003 and 2004 on zoonotic diseases, or a virus naming statement by the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses, which is responsible for developing the official classification of viruses and taxa naming (taxonomy) of the Coronaviridae family. The eight articles with data were written at the end of January or early February, when there were fewer than 10,000 patients.

[55] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30418-9/fulltext#back-bib1

[56] This is reminiscent of the story attributed to Albert Einstein by Stephen Hawkins in his *Brief History of Time.* According to Hawkins, a book was published in 1930 in pre-war Germany entitled, "One Hundred Authors Against Einstein." When he was asked about the book Einstein is reported to have retorted, "If I were wrong, then one would have been enough!"

An analysis of the evidence for a zoonotic source given in support of the above Statement is contained in Text-Table here. The analysis shows there was very little actual data available at the time to permit reaching such a definitive conclusion. There was also the absence of data or discussion that could support a laboratory origin.

Reference	Statements concerning origin of SARS-CoV-2	Response to statements
1.Gorbalenya AE Baker SC Baric RS et al. Severe acute respiratory syndrome-related coronavirus: the species and its viruses—a statement of the Coronavirus Study Group. bioRxiv. 2020; (published online Feb 11. DOI: 2020.02.07.937862 (preprint).)	A naming statement about SARS-CoV-2. The emergence of SARS-CoV-2 as a human pathogen in December 2019 may thus be perceived as completely independent from the SARS-CoV outbreak in 2002–2003. With respect to novelty, SARS-CoV-2 differs from the two other zoonotic coronaviruses, SARS-CoV and MERS-CoV, introduced to humans earlier in the twenty-first century.	Does not provide data on a potential zoonotic source.
2.Zhou P Yang X-L Wang X-G et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; (published online Feb 3.)	The sequences of 2019-nCoV BetaCoV/Wuhan/WIV04/2019 among patient specimens are almost identical and share 79.6% sequence identity to SARS-CoV. Furthermore, we show that 2019-nCoV is 96% identical at the whole-genome level to a bat coronavirus. Pairwise protein sequence analysis of seven conserved non-structural proteins domains show that this virus belongs to the species of SARSr-CoV. The close phylogenetic relationship to RaTG13 provides evidence that 2019-nCoV may have originated in bats.	The bat genome identity of 96% described here, coupled with the known mutation rate of SARS-CoV-2 of about 26/year, implies a **lowest common ancestor about 44 years ago.**

3.Lu R Zhao X Li J et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020; (published online Jan 30.)	Genome sequences of 2019-nCoV sampled from nine patients who were among the early cases of this severe infection are almost genetically identical, which suggests very recent emergence of this virus in humans and that the outbreak was detected relatively rapidly. 2019-nCoV is most closely related to other betacoronaviruses of bat origin, indicating that these animals are the likely reservoir hosts for this emerging viral pathogen.	Figure 1A shows 8 sequences and the concensus sequence. These 8 sequences show 3 with 0 mutations, 2 with 1 mutation, 3 with 2 mutations, and none with more than 2 mutations. Based on current estimates of 1 mutation per human passage, these are at most two human-to-human transfers apart. Importantly, there is no background diversity as would be seen in two or more resevoir-to-human events. Fig 2 states strain Bat-SL-CoVZC45 is 87.6% sequence identity to the human virus, which means a difference of about 3700 mutations or over 70 years from lowest common ancestor.
4.Zhu N Zhang D Wang W et al. A novel coronavirus from patients with pneumonia in China, 2019. NEJM. 2020; (published online Jan 24.)	"more than 85% identity with a bat SARS-like CoV (bat-SL-CoVZC45, MG772933.1) genome published previously. Since the sequence identity in conserved replicase domains (ORF 1ab) is less than 90% between 2019-nCoV and other members of betacoronavirus, the 2019-nCoV — the likely causative agent of the viral pneumonia in Wuhan — is a novel betacoronavirus belonging to the	A >85% identity with a bat coronavirus means the **human and bat virus have over 70 years to LCA.**

	sarbecovirus subgenus of Coronaviridae family."	
5.Ren L Wang Y-M Wu Z-Q et al. Identification of a novel coronavirus causing severe pneumonia in humans: a descriptive study. Chin Med J. 2020; (published online Feb 11.)	All five patients have sequence homology of 99.8% to 99.9%. These isolates showed 79.0% nucleotide identity with the sequence of SARS-CoV (GenBank NC_004718) and 51.8% identity with the sequence of MERS-CoV (GenBank NC_019843). The virus is closest to a bat SARS-like CoV (SL-ZC45, GenBank MG772933) with 87.7% identity, but is in a separate clade. **Surprisingly, RNA-dependent RNA polymerase (RdRp), which is the most highly conserved sequence among different CoVs, only showed 86.3% to 86.5% nt identities with bat SL-CoV ZC45.**	Similar to reference 3 comments. Lack of conserved sequencing of the most highly conserved sequence with bat coronavirus would **suggest a non-bat source.**
6.Paraskevis D Kostaki EG Magiorkinis G Panayiotakopoulos G Tsiodras S Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infect Genet Evol. 2020; (published online Jan 29.)	A BLAST search of 2019-nCoV middle fragment revealed no considerable similarity with any of the previously characterized corona viruses. Bat_SARS-like coronavirus sequences cluster in different positions in the tree, suggesting that they are recombinants, and thus that the 2019-nCoV and RaTG13 are not recombinants. Codon usage analyses can resolve	**The middle segment with no similarity to other corona viruses is about 40% of the entire genome. I agree SARS-CoV-2 is not a recombinant of RaTG13. I agree, codon usage analysis here supports the furin binding site insertion as having been invented de novo. A recent recombination event is not necessary for a**

	the origin of proteins with deep ancestry and insufficient phylogenetic signal or **invented de novo**. Our study rejects the hypothesis of emergence as a result of a recent recombination event. Notably, the new coronavirus provides a new lineage for almost half of its genome, with **no close genetic relationships to other viruses** within the subgenus of sarbecovirus. This genomic part comprises half of the spike region encoding a multifunctional protein responsible also for virus entry into host cells	**laboratory derived theory of origin. Statements do not advance a zoonotic origin.**
7.Benvenuto D Giovanetti M Ciccozzi A Spoto S Angeletti S Ciccozzi M The 2019-new coronavirus epidemic: evidence for virus evolution. J Med Virol. 2020; (published online Jan 29.)	The epidemic originated in Wuhan, China. A phylogenetic tree has been built using the 15 available whole genome sequences of 2019-nCoV, 12 whole genome sequences of 2019-nCoV, and 12 highly similar whole genome sequences available in gene bank (five from the severe acute respiratory syndrome, two from Middle East respiratory syndrome, and five from bat SARS-like coronavirus). >97% maximum likelihood match to Bat SARS-like virus 2015 (Fig 1) is noted. The SARS and MERS viruses are excluded as a source of SARS-CoV-2. These results do not	A 3% genome **distance from the noted bat virus to human is about 34 years** at 26 mutations per year, the in-human mutation rate. Predicted a future mutation like the D614G mutation which is more infective.

	exclude the fact that **further mutation due to positive selective pressure, led by the epidemic evolution, could favor an enhancement of pathogenicity and transmission of this novel virus.**	
8.Wan Y Shang J Graham R Baric RS Li F Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. J Virol. 2020; (published online Jan 29.)	Based on predicted RBD-host ACE2 receptor affinities, civet, mice, and rats are fuled out as source species. Pigs, ferrets, cats, and **nonhuman primates** contain largely favorable 2019-nCoV-contacting residues in their ACE2. SARS-CoV was isolated in wild palm civets near Wuhan in 2005, and its RBD had already been well adapted to civet ACE2.	The **potential nonhuman primate ACE2 usage is noted. Consistent with a laboratory origin from VERO cells**, a monkey kidney cell line. It expresses an ACE2 that permits SARS-CoV-2 infection, making it a possible source for the virus. A common tissue culture cell line forSARS virus research.
9.US Center for Disease Control and Prevention Coronavirus disease 2019 (COVID-19) situation summary. https://www.cdc.gov/coronavirus/2019-nCoV/summary.html Date: Feb 16, 2020 Date accessed: February 8, 2020	Rarely, animal coronaviruses can infect people and then spread between people such as with MERS-CoV, SARS-CoV, and now with this new virus, named SARS-CoV-2. The SARS-CoV-2 virus is a betacoronavirus, like MERS-CoV and SARS-CoV. All three of these viruses have their origins in bats. The sequences from U.S. patients are similar to the one that China initially posted, suggesting a likely single, recent emergence of this virus from an animal reservoir.	There are no data to support these statements about bats as the source for SARS-CoV-2.

10.Andersen KG Rambaut A Lipkin WI Holmes EC Garry RF The proximal origin of SARS-CoV-2. http://virological.org/t/the-proximal-origin-of-sars-cov-2/398 Date: Feb 16, 2020 Date accessed: February 17, 2020	See Table 2.	See Table 2.
11.Bengis R Leighton F Fischer J Artois M Morner T Tate C The role of wildlife in emerging and re-emerging zoonoses. Rev Sci Tech. 2004; 23: 497-512	In one pattern, actual transmission of the pathogen to humans is a rare event but, once it has occurred, human-to-human transmission maintains the infection for some period of time or permanently. Some examples of pathogens with this pattern of transmission are human immunodeficiency virus/acquired immune deficiency syndrome, influenza A, Ebola virus and severe acute respiratory syndrome.	This 2004 paper describes the pattern of rare animal-to-human transmission followed by human-to-human spread as an example of the SARS virus. It does not address the origin of SARS-CoV-2.
12.Woolhouse ME Gowtage-Sequeria S Host range and emerging and reemerging pathogens. Emerg Infect Dis. 2005; 11: 1842-1847	Emerging and reemerging pathogens are disproportionately viruses, with 37% being RNA viruses. Emerging and reemerging pathogens more often are those with broad host ranges that often encompass several mammalian orders and even nonmammals. For pathogens that are minimally transmissible within human populations (R0 close to 0), outbreak size is determined largely by the number of introductions from the reservoir. For pathogens that are highly transmissible within human populations	This 2005 article has good general information about looking broadly for the reservoir species(s), identifies RNA viruses as a major source of human epidemics, predicts a large outbreak size for a high Ro virus, but does address the origin of SARS-CoV-2 origin.

	(R0>>1), outbreak size is determined largely by the size of the susceptible population.	
13.NASEM The National Academies of Science Engineering and Medicine of the USA. NAS, NAE, and NAM presidents' letter to the White House Office of Science and Technology Policy. https://www.nationalacademies.org/includes/NASEM%20Response%20to%20OSTP%20re%20Coronavirus_February%206,%202020.pdf Date: Feb 6, 2020 Date accessed: February 7, 2020	The closest known relative of 2019-nCoV appears to be a coronavirus identified from bat-derived samples collected in China.4 The experts informed us that additional genomic sequence data from geographically- and temporally-diverse viral samples are needed to determine the origin and evolution of the virus. Samples collected as early as possible in the outbreak in Wuhan and samples from wildlife would be particularly valuable. Understanding the driving forces behind viral evolution would help facilitate the development of more effective strategies for managing the 2019-nCoV outbreak and for preventing future outbreaks.	Agree. If additional genomic sequence data is available from geographically- and temporally-diverse viral samples are needed to determine the origin and evolution of the virus this should be made publicly available.
14.WHO Director-General's remarks at the media briefing on 2019 novel coronavirus on 8 February 2020. https://www.who.int/dg/speeches/detail/director-general-s-remarks-at-the-media-briefing-on-2019-novel-coronavirus---8-february-2020 Date: Feb 8, 2020 Date accessed: February 18, 2020	A general statement about the emerging pandemic without reference to the origin of SARS-CoV-2	There is no data about the origin of the pandemic.

In November 2020 the Watchdog group, US Right-to-Know, reported the following with respect to the *Lancet* article:[57]

"Emails obtained by U.S. Right to Know show that a statement in *The Lancet* authored by 27 prominent public health scientists condemning "conspiracy theories suggesting that COVID-19 does not have a natural origin" was organized by employees of EcoHealth Alliance, a non-profit group that has received millions of dollars of U.S. taxpayer funding to genetically manipulate coronaviruses with scientists at the Wuhan Institute of Virology."

"The emails obtained via public records requests show that EcoHealth Alliance President Peter Daszak drafted the Lancet statement, and that he intended it to "not be identifiable as coming from any one organization or person" but rather to be seen as "simply a letter from leading scientists". Daszak wrote that he wanted "to avoid the appearance of a political statement."

A separate, worrisome article entitled, "Peter Daszak's EcoHealth Alliance Has Hidden Almost $40 Million In Pentagon Funding And Militarized Pandemic Science,[58]" seems to indicate a serious conflict of interest with respect to Dr. Daszak's participation in any investigations on the origin of SARS-CoV-2.

Paper 3: The March 17, 2020 article in *Nature Medicine* entitled "The proximal origin of SARS-CoV-2" by Andersen et al.[59, 60]

According to the journal, this article is in the 99th percentile (ranked 2nd) of the 312,683 tracked articles of a similar age in all journals and the 99th percentile (ranked 1st) of the 147 tracked articles of a similar age in *Nature Medicine.* The metrics also indicate it has been accessed over five million times. It is clearly the most cited paper and since its title and topic are the origin of the pandemic it clearly has an outsized influence on the topic.

The following statements form the evidence in the article of the natural origin of CoV-2:

- "While the analyses above suggest that SARS-CoV-2 may bind human ACE2 with high affinity, computational analyses **predict that the interaction is not ideal** and that the RBD sequence is different from those shown in SARS-CoV to be optimal for receptor binding. Thus, the high-affinity binding of the SARS-CoV-2 spike protein to human ACE2 is **most likely the result of natural selection on a human or human-like ACE2** that permits another optimal binding solution to arise. **This is strong evidence that SARS-CoV-2 is not the product of purposeful manipulation."** [emphasis added.]

[57] https://usrtk.org/biohazards-blog/ecohealth-alliance-orchestrated-key-scientists-statement-on-natural-origin-of-sars-cov-2/
[58] https://www.independentsciencenews.org/news/peter-daszaks-ecohealth-alliance-has-hidden-almost-40-million-in-pentagon-funding/
[59] https://www.nature.com/articles/s41591-020-0820-9
[60] Two non-peer reviewed analyses are included here because they provide a nearly line-by-line analysis. They unfortunately include occasional colorful language but the content is worth noting: https://harvardtothebighouse.com/2020/03/19/china-owns-nature-magazines-ass-debunking-the-proximal-origin-of-sars-cov-2-claiming-covid-19-wasnt-from-a-lab/ ; https://www.youtube.com/watch?v=HmSCMb8Nds4

- A later analysis of over 3800 possible substitutions of amino acids in a 200 amino acid receptor binding region, much larger than the small, selective region referred to in this paper, shows that CoV-2 is 99.5% optimized for binding to the ACE-2 receptor. This near perfect binding has never been seen before in a recent interspecies transmission jump.

- "Polybasic cleavage sites have not been observed in related 'lineage B' betacoronaviruses, although other human betacoronaviruses, including HKU1 (lineage A), have those sites and predicted O-linked glycans. Given the level of genetic variation in the spike, **it is likely that SARS-CoV-2-like viruses with partial or full polybasic cleavage sites will be discovered in other species.**" [emphasis added.]

    - As of the writing of this manuscript no other lineage B (sarbecovirus) has been found to have a furin site. In addition, the furin site of CoV-2 has the unusual -CGG-CGG- codon dimer, which has never been seen in an analysis of 58 other sarbecoviruses, that is, 580,000 codons. Since recombination between subgenera of beta coronaviruses is rare, or unknown, there is no source for the CGG-CGG dimer via a natural recombination event.

- "The acquisition of polybasic cleavage sites by HA has also been observed after repeated passage in cell culture or through animals."

    - It is curious why the above statement did not lead to a hypothesis somewhere in the article about a similar mechanism on CoV-2, a clear indication of a laboratory origin.

- "It is improbable that SARS-CoV-2 emerged through laboratory manipulation of a related SARS-CoV-like coronavirus."

    - This conclusory statement is unsupported my evidence.

- "Furthermore, if genetic manipulation had been performed, one of the several reverse-genetic systems available for betacoronaviruses would **probably have been used**. However, the genetic data irrefutably show that SARS-CoV-2 is not derived from any previously used virus backbone." [emphasis added.]

    - There is no explanation for why a prior backbone would necessarily be used. All synthetic biology chimera coronaviruses created in the past as published in prior papers have each used a unique backbone with no particular pattern in backbone selection. Each backbone was selected for the particular needs of those current experiments. This non-repeating prior pattern of reverse-genetic systems makes the above statement untenable. And with 16,000+ reported coronavirus specimens at the WIV it entirely reasonable a non-published virus could have been used.

- "Natural selection in an animal host before zoonotic transfer. For a precursor virus to acquire both the polybasic cleavage site and mutations in the spike protein suitable for binding to human ACE2, **an animal host would probably have to have a high population density (to allow natural selection to proceed efficiently**) and an ACE2-encoding gene that is similar to the human ortholog." [emphasis added.]

    - The paragraph discusses the pangolin as the possible intermediate host but at the time of this manuscript the coronavirus data from pangolins has been discredited. This author agrees with statement that selection of the two unique features of CoV-2 require a high population density of the animal host. Of course, in the laboratory the animal hosts for either *in vitro* cell culture experiments or in animal experiments are a single species at high density.

- Natural selection in humans following zoonotic transfer. "It is possible that a progenitor of SARS-CoV-2 jumped into humans, acquiring the genomic features described above through adaptation during **undetected human-to-human transmission**. Once acquired, these adaptations would enable the pandemic to take off and produce a sufficiently large cluster of cases to trigger the surveillance system that detected it." [emphasis added.]

- "Studies of banked human samples could provide information on whether such cryptic spread has occurred. Further serological studies should be conducted to determine the extent of prior human exposure to SARS-CoV-2."

    - As will be shown in later sections, this prior undetected human-to-human transmission would be evident in archived specimens from before the fall of 2019. In both SARS-CoV-1 and MERS, this prior seroconversion averaged about 0.6% with almost 5% among workers exposed to the intermediate hosts. At the time of the writing of this manuscript, in limited sampling of archived specimens there has been no seroconversion detected. The author believes there are thousands of archived specimens from Wuhan taken in the fall of 2019 and these should be immediately examined for evidence of seroconversion. Since finding seroconversion among these specimens would be strong evidence for a zoonotic origin and not a laboratory accident, the absence of any information from China on this important evidence is hard to understand.

- Selection during passage. "Basic research involving passage of bat SARS-CoV-like coronaviruses in cell culture and/or animal models has been ongoing for many years in biosafety level 2 laboratories across the world, and there are documented instances of laboratory escapes of SARS-CoV. We must therefore examine the possibility of an inadvertent laboratory release of SARS-CoV-2."

- "In theory, it is possible that SARS-CoV-2 acquired RBD mutations during adaptation to passage in cell culture, as has been observed in studies of SARS-CoV."

- "New polybasic cleavage sites have been observed only after prolonged passage of low-pathogenicity avian influenza virus in vitro or in vivo. Furthermore, a hypothetical generation of SARS-CoV-2 by cell culture or animal passage would have required prior isolation of a progenitor virus with very high genetic similarity, **which has not been described.** Subsequent generation of a polybasic cleavage site would have then required repeated passage in cell culture or animals with ACE2 receptors similar to those of humans, **but such work has also not previously been described**." [emphasis added.]

    - The authors correctly describe a method for CoV-2 to have been generated in the laboratory and then dismiss it because the work has not been published previously. As active scientists themselves, the authors must know how disingenuous this sounds. Almost by definition elite scientists, like Dr. Shi of the WIV, work in secret until the publication of any given line of research. As the say, the absence of evidence cannot be used as evidence of its absence.

    - A peer-reviewed paper[61] entitled, "Might SARS-CoV-2 Have Arisen via Serial Passage through an Animal Host or Cell Culture? A potential explanation for much of the novel coronavirus' distinctive genome," provides a compelling argument that serial passage in the laboratory might indeed have been the manner in which CoV-2 acquired many of its devastating traits.

- "Although the **evidence shows that SARS-CoV-2 is not a purposefully manipulated virus**, it is currently impossible to prove or disprove the other theories of its origin described here. However, **since we observed all notable SARS-CoV-2 features, including the optimized RBD and polybasic cleavage site, in related coronaviruses in nature, we do not believe that any type of laboratory-based scenario is plausible**." [emphasis added.]

    - This author could identify no prior evidence in the paper to warrant saying it is not a purposefully manipulated virus. There is also no evidence that would point to a purposely manipulated virus.

    - The evidence in the paper shows that no prior zoonotic interspecies transmission has ever had an RBD as optimized as the CoV-2 RBD for the human ACE2. The evidence also shows that there is no natural source for the polybasic cleavage site (PCS). No other member of the subgenera to which CoV-2 belongs has a PCS. Since these are the only coronaviruses from which recombination could supply a polybasic cleavage site, the data in this paper refutes the natural origin.

[61] https://onlinelibrary.wiley.com/doi/full/10.1002/bies.202000091

- The belief statement concerning a laboratory-based scenario would be closer to the evidence if it was professed with, “despite evidence which is consistent with a laboratory-based scenario.”

Based on the author’s analysis of the paper, the following email was sent to the lead author:

Gmail Steven Quay, MD, PhD

SARS-CoV-2 origin

Steven Quay, MD, PhD Mon, May 25, 2020 at 7:14 PM
To:

Dr. Andersen-

I read with interest your paper titled, The proximal origin of SARS-CoV-2, in which you conclude this is a natural, zoonotic-sourced infection. Three different approaches of analysis that I have done do not support this conclusion. Can you comment please:

1. The furin cleavage site insert has the unusual codon usage for the RR dimer of CGG-CGG. As you probably know, the frequency of this codon usage in the SARS-CoV-2 genome is 0.09. So having two next to each other is not likely as a random event. As support for the unlikeliness of these codons, using GISAID data, by March there is evidence of the third G being mutated out for either A or T at three-times the rate of the background mutation rate, 26/year from Nextstrain.org. Since codon usage in coronaviruses are not greatly influenced by the host it resides in, this means a jump to humans must have been in a host which did not have even a few months history with the virus, otherwise the terminal G would have been purified out. On the other hand, most laboratory use optimized codon primers and kits use CGG routinely; including in primers in published papers from the Wuhan Institute of Virology. So a laboratory source for a gain-of-function furin cleavage site would probably use these codons.

2. In the over 16,000 genomes in GISAID there is not one example of posterior diversity. With MERS, 93% of sequenced genomes did not pass through the index case but represented separate reservoir host to human jumps and this was apparent within 60 days of the index case. They collectively showed the most recent common ancestors among themselves was over 12 months before the index case. With SARS-CoV-2 it is acting like a 'pure culture' growth from the index case outward with no evidence of a reservoir host in the background. This would be the case for a laboratory acquired infection.

3. If you use a map of Wuhan and overlay the first four hospitals that saw cases with a map of the Metro system, you see that the hospitals straddle Line 2, which runs approximately east to west, carries 1,000,000 people a day, and is the Metro line with stops closest to both the Wuhan Institute of Virology and the original wet market that was considered an early source for the infection. There are 11 Metro lines in Wuhan, hundreds of stops on those lines, and over a dozen hospitals spread out over the city. I am working with a UCLA statistician to perform tests about the probability of this being simply an accident of statistics but the gestalt is, it does not look like a chance occurrence. But it is consistent with someone getting infected in the lab, riding Line 2 for a few days, and off you go.

It might be a truism to say that the six proven cases of laboratory derived SARS escapes occurred in big cities, Beijing, Singapore, or Taipei where the labs are located. But if you follow it with the fact that MERS and SARS, both proven as true zoonotic sources, on other hand began in rural settings in China and the Middle East, respectively. I am not sure why this obvious correlation was not at least pointed out in your paper and then addressed with a cogent argument.

I look forward to hearing your thoughts.

Regards, Steve

Soon after this email was written Dr. Andersen blocked the author from following his Twitter account. A reply to the above email was never received.

Conclusion. Three high visibility papers were published between January and May 202 which purported to settle the question of the origin of SARS-CoV-2 as a zoonotic transmission and not a laboratory accident. The analysis above concludes that these papers are not persuasive. The

author has elected to not use evidence within these papers to change the prior likelihood of a zoonotic versus laboratory origin. They are presented here as neutral evidence that supports neither theory.

Likelihood from initial state is unchanged following this evidence analysis:

Zoonotic origin (98.8%) and laboratory origin (1.2%)

Evidence. SARS-like infections among employees of the Wuhan Institute of Virology in the fall of 2019

The State Department of the United States issued the following statement on January 15, 2021[62]:

"1. Illnesses inside the Wuhan Institute of Virology (WIV):

- The U.S. government has reason to believe that several researchers inside the WIV became sick in autumn 2019, before the first identified case of the outbreak, with symptoms consistent with both COVID-19 and common seasonal illnesses. This raises questions about the credibility of WIV senior researcher Shi Zhengli's public claim that there was "zero infection" among the WIV's staff and students of SARS-CoV-2 or SARS-related viruses."

There is no additional evidence to support either parties position in the above statement. The U.S. Government statement would be considered hearsay in a court of law and probably not admissible. The veracity of Dr. Shi's statement above could be called into question due to other inconsistencies in some of her testimony, as reported elsewhere in this document.

At this time, the above evidence cannot be used to change the likelihood of either theory about the origin of SARS-CoV-2. The statement is kept within this analysis with the hope that in the future new information will come to light that could make this evidence a useful addition to the overall analysis.

Likelihood from initial state is unchanged following this evidence analysis:

Zoonotic origin (98.8%) and laboratory origin (1.2%)

[62] https://2017-2021.state.gov/fact-sheet-activity-at-the-wuhan-institute-of-virology//index.html

Evidence. A Bayesian Analysis of one aspect of the SARS-CoV-2 origin, where the first recorded outbreak occurred, increases the probability of a laboratory origin.

Introduction. The two competing hypotheses of the origin of SARS-CoV-2 as a natural, zoonotic spillover event versus a laboratory-acquired infection (LAI) or other laboratory accident each had supporting evidence from the very beginning of the pandemic.

On the one hand, about 40% of early patients with COVID-19 had an association with the Hunan Seafood Market in Wuhan. Since this mirrored SARS-CoV-1, where markets selling civet cats were determined to be the origin of that human epidemic, the natural origin hypothesis seemed logical. The Chinese CDC have now ruled out the market as a source for the outbreak.

On the other hand, the laboratory origin hypothesis also had an early beginning with the fact that the outbreak began adjacent to the only high security, BSL-4 laboratory in all of China, and one of the top coronavirus research centers in the world, was the Wuhan Institute of Virology (WIV). The hospitals of the first COVID patients were very close to the WIV.

This evidence statement is taken from an article applying a Bayesian analysis to the hypothesis that the proximal origin of SARS-CoV-2 was an uncontrolled[63] release from a laboratory using, as evidence, one aspect of the SARS-CoV-2 origin story — where the first recorded outbreak occurred.[64]

Hypothesis: The first recorded outbreak of SARS-CoV-2 in the human population occurred in a city that is also home to a virology laboratory that actively performs research on closely related viruses.

In this case, the city is Wuhan, and the virology laboratory is run by the Wuhan Institute of Virology.

Analysis. This analysis set the likelihood of a laboratory escape (the prior probability the hypothesis was true) at three values, 0.01%, 0.1%, and 1.0%. The second term was the conditional probability of the evidence, given that the hypothesis is actually false. This was set at 0.01. Finally, the third term was the conditional probability of the evidence, given the hypothesis is true. This was set, biasing to the natural origin, at 0.71.

Results. The paper provides the three-by-three cube of results for the three parameters of interest.

The ardent sceptic's probability begins at 0.01% and the revised estimate is no more than 0.05% or 5/10000. It applies to someone who was initially very skeptical about a lab origin (0.01% probability), who believes there is no more than 51% chance that an uncontrolled release of a highly contagious disease would lead to a local outbreak, and who thinks there was at least a

[63] By using the term uncontrolled release, the author was specifically excluding from consideration the possibility that the pathogen was deliberately released from the laboratory.
[64] https://jonseymour.medium.com/a-bayesian-analysis-of-one-aspect-of-the-sars-cov-2-origin-story-where-the-first-recorded-1fbdcbea0a2b

10% chance that a natural outbreak of a virus native to Yunnan would have occurred in Wuhan before any place else.

On the other extreme, is the ardent believer who started with at least a 1% belief in a laboratory outbreak, is 100% certain that an uncontrolled laboratory release would result in a local outbreak and believes that the probability that a natural outbreak of a virus native to Yunnan would occur in Wuhan before any place else is less than 0.1%. The ardent believer's revised belief is that the probability that the Wuhan outbreak was caused by an uncontrolled laboratory release changes from 1% to at least 91%.

In the center, is the so-called "central" observer who accepts that the central values for each of the parameter ranges are reasonable estimates of the true values of the probability being estimated. The central observer started with an initially skeptical belief in the hypothesis of 0.1%, believes that average citizen in Wuhan was a likely as any other citizen of China to be the initial vector of the virus into the human population and believes that there is no more or less than a 71% chance that an uncontrolled release from a laboratory of a highly contagious pathogen such as SARS-CoV-2 would result in a local outbreak as opposed to an outbreak in some other location. The central observer's revised belief in the hypothesis is 6.8%. If the central observer began with a 1% belief in a laboratory origin, this analysis would change that to 41.8%.

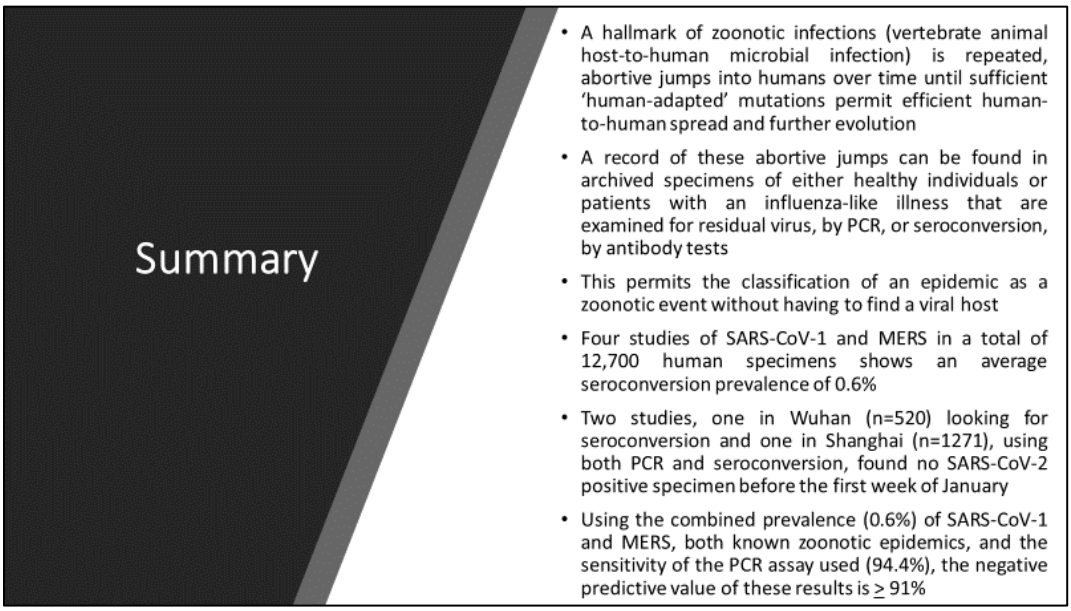
Conclusion. For purposes of this analysis and to be as conservative as possible, the assumptions will be that there is at least a 1% prior belief in a laboratory outbreak (because that was our starting probabilities), but there is no more than a 51% chance that an uncontrolled release of a highly contagious disease would lead to a local outbreak, and that there was at least a 10% chance that a natural outbreak of a virus native to Yunnan would have occurred in Wuhan before any place else. Using these assumptions, the initial likelihood of a 1% laboratory origin changes to 4.9%.

Starting likelihood from initial state: Zoonotic origin (98.8%) and laboratory origin (1.2%)

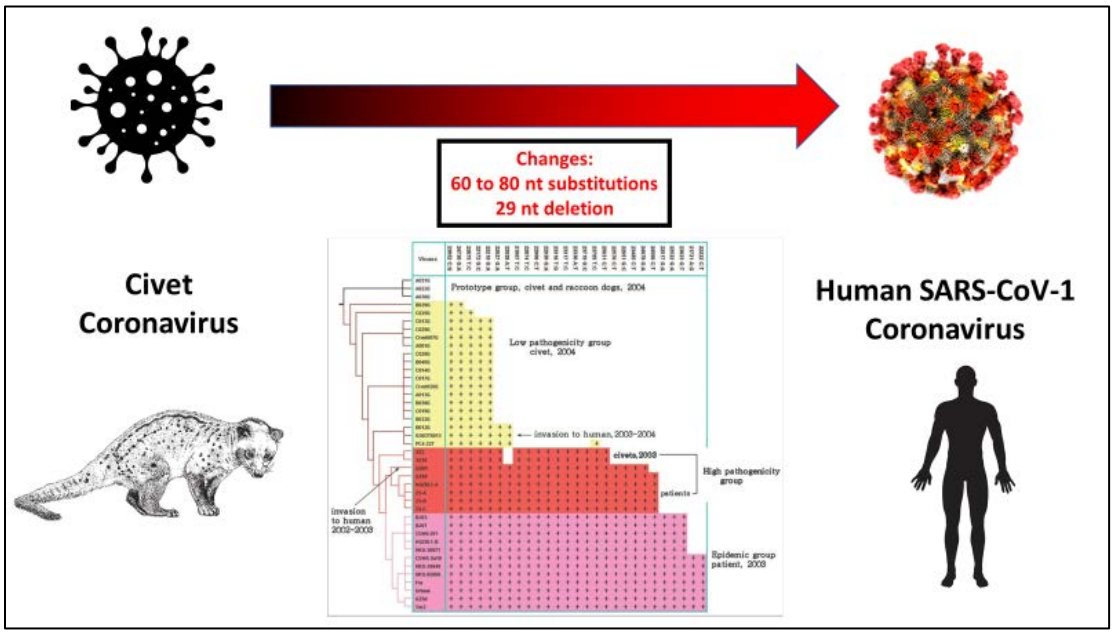
Adjusted likelihood: Zoonotic origin (95.1%) and laboratory origin (4.9%)

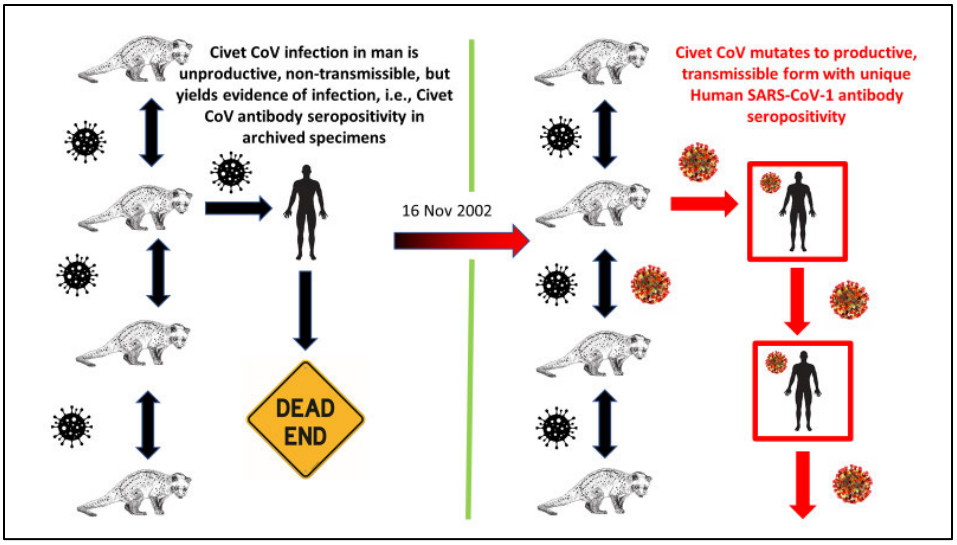
Evidence: Lack of seroconversion in Wuhan and Shanghai. Summary of evidence:

- A hallmark of zoonotic infections (vertebrate animal host-to-human microbial infection) is repeated, abortive jumps into humans over time until sufficient 'human-adapted' mutations permit efficient human-to-human spread and further evolution

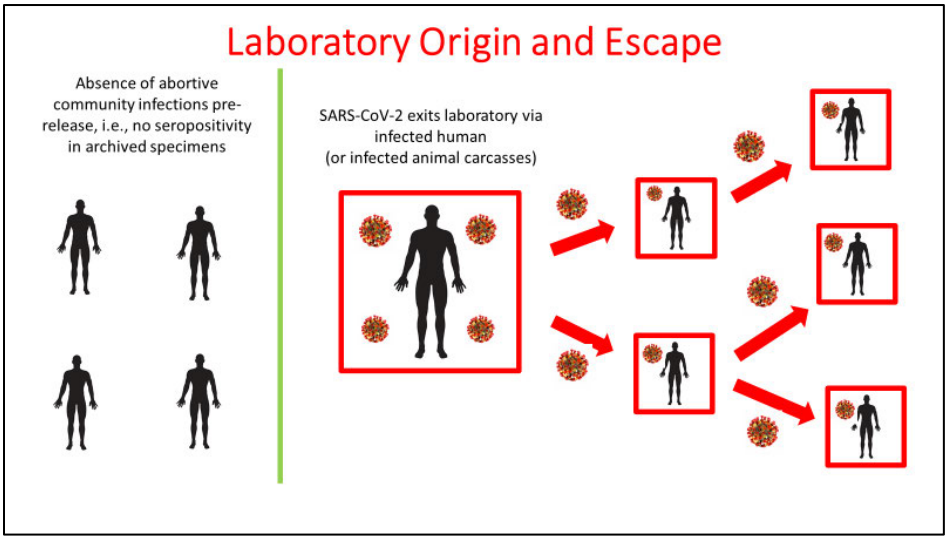


- A record of these abortive jumps can be found in archived specimens of either healthy individuals or patients with an influenza-like illness that are examined for residual virus, by PCR, or seroconversion, by antibody tests





- This permits the classification of an epidemic as a zoonotic event without having to find a viral host
- A laboratory accident is a situation in which there are no prior exposures within the human population as shown in the Figure below:



- Four studies of SARS-CoV-1 and MERS in a total of 12,700 human specimens shows an average seroconversion prevalence of 0.6%

SARS-related Virus Predating SARS Outbreak, Hong Kong

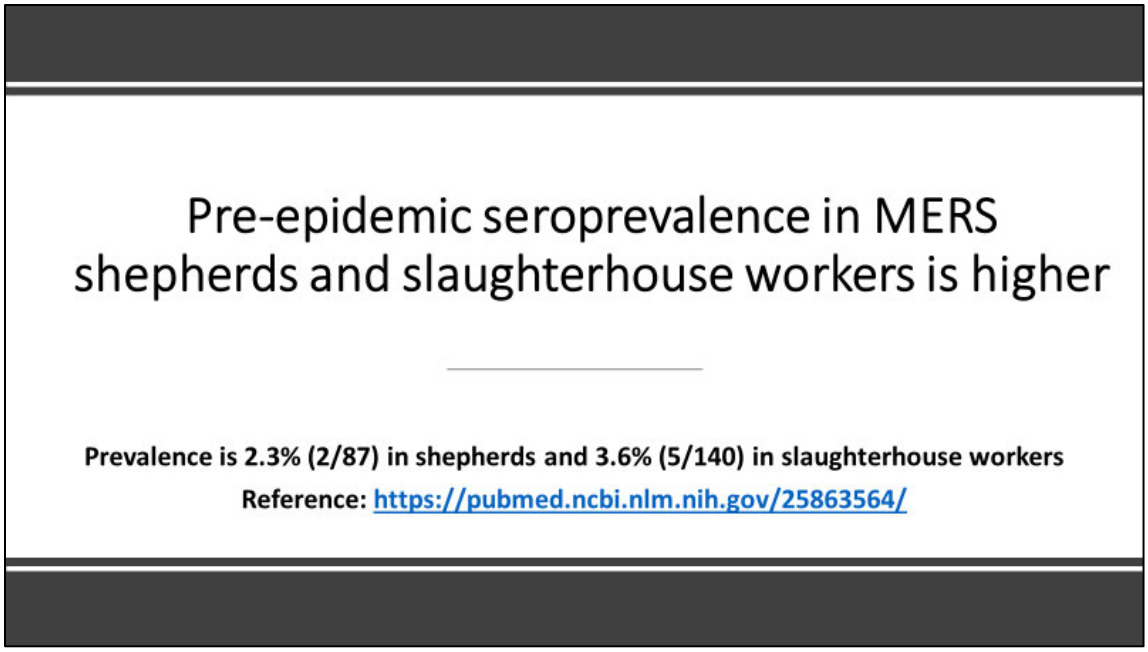
SARS-CoV-1 began in fall of 2002 in southern China

Patient Population	Serum samples collected in May 2001 from 938 healthy adults in Hong Kong	48 confirmed SARS patients diagnosed in February and March 2003 in Guangdong
Civet CoV > SARS-CoV-1 Seropositivity	13	0
SARS-CoV-1 > Civet CoV Seropositivity	4	48
Total	17 out of 938 = 1.8%	48 out of 48 = 100%

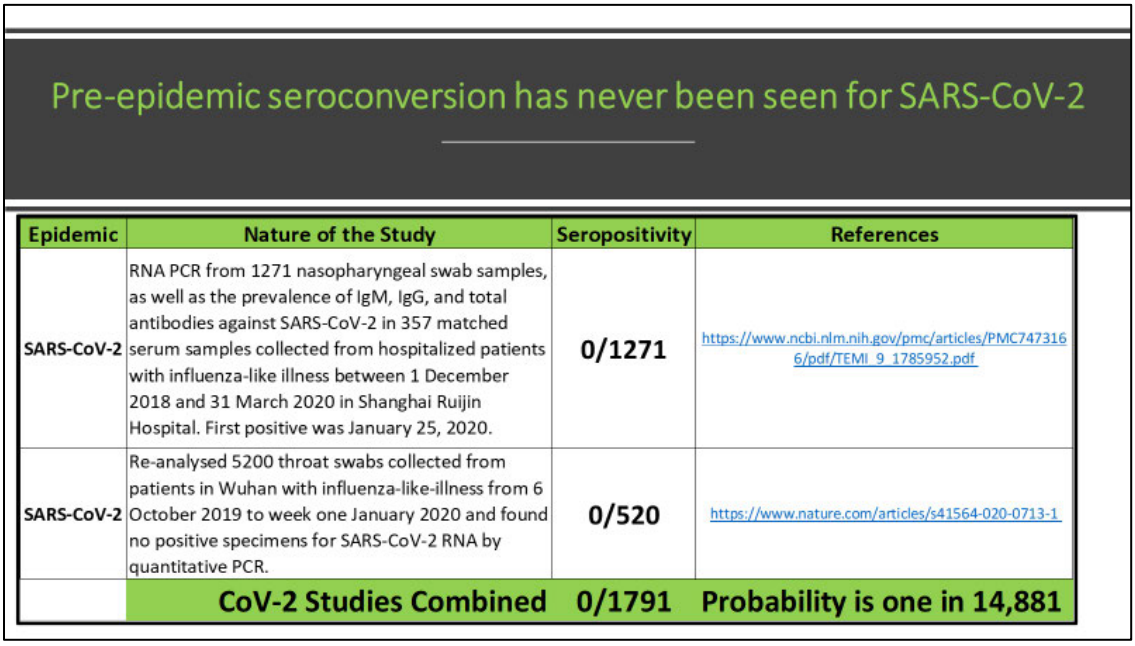
Pre-epidemic seroprevalence in the adult community

Prevalence is 0.6% for SARS-CoV-1 and MERS in 12,700 specimens

Epidemic	Nature of the Study	Seropositivity	Reference
SARS-CoV-1	Archived specimens from healthy adults in Hong Kong collected two years before CoV-1 were tested for Ab to civet or human CoV	17/938	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3322899/
MERS	Archived human sera collected in 2011 was tested for MERS-CoV S1-specific antibodies by ELISA	1/90	https://www.sciencedirect.com/science/article/pii/S1876034120300010#fig0010
SARS-CoV-1	Serum specimens collected from military recruits from the People's Republic of China in 2002 were tested for SARS-CoV-1 antibodies.	11/1621	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1074388/
MERS	Between Dec 1, 2012, and Dec 1, 2013, 10,009 individual serum samples were tested for anti-MERS-CoV antibodies in regions without cases.	15/10,009	https://pubmed.ncbi.nlm.nih.gov/25863564/
SARS-CoV-1	Serum samples that were collected from 42 individuals during 2001-2002, before the SARS outbreak, and tested for IgG antibody against SARS-CoV.	28/42	https://arxiv.org/ftp/arxiv/papers/1305/1305.2659.pdf

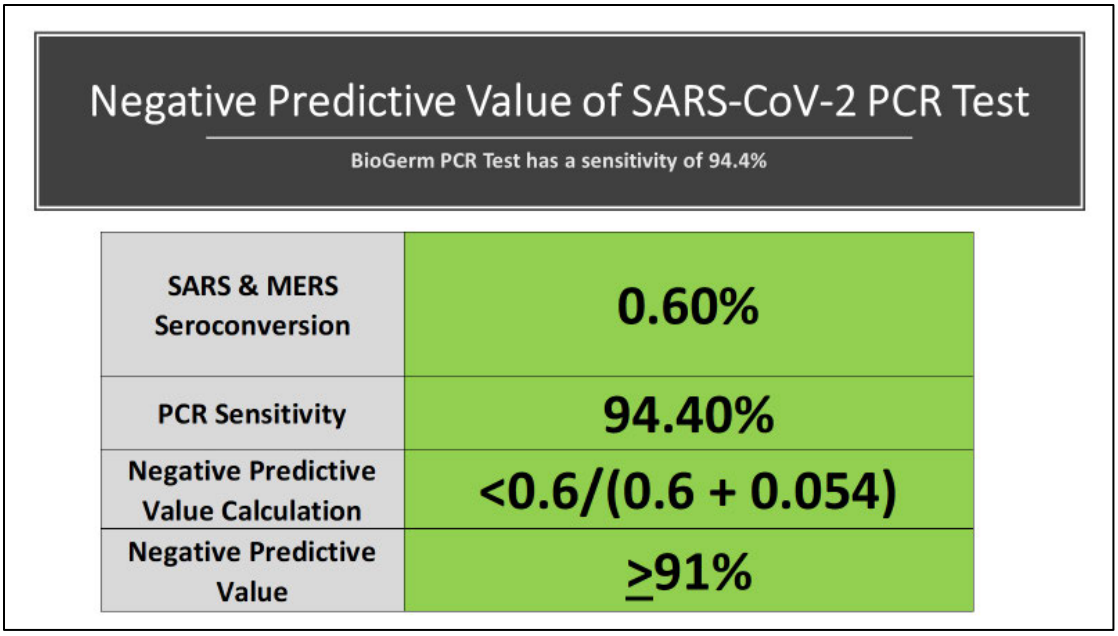


- Two studies, one in Wuhan (n=520) looking for seroconversion and one in Shanghai (n=1271), using both PCR and seroconversion, found no SARS-CoV-2 positive specimen before the first week of January



Epidemic	Nature of the Study	Seropositivity	References
SARS-CoV-2	RNA PCR from 1271 nasopharyngeal swab samples, as well as the prevalence of IgM, IgG, and total antibodies against SARS-CoV-2 in 357 matched serum samples collected from hospitalized patients with influenza-like illness between 1 December 2018 and 31 March 2020 in Shanghai Ruijin Hospital. First positive was January 25, 2020.	0/1271	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7473166/pdf/TEMI_9_1785952.pdf
SARS-CoV-2	Re-analysed 5200 throat swabs collected from patients in Wuhan with influenza-like-illness from 6 October 2019 to week one January 2020 and found no positive specimens for SARS-CoV-2 RNA by quantitative PCR.	0/520	https://www.nature.com/articles/s41564-020-0713-1
	CoV-2 Studies Combined	**0/1791**	**Probability is one in 14,881**

- Using the combined prevalence (0.6%) of SARS-CoV-1 and MERS, both known zoonotic epidemics, and the sensitivity of the PCR assay used (94.4%), the negative predictive value of these results is $\geq$ 91%



Here, the negative predictive value (NPV) represents the probability that a CoV-2 is not a zoonosis, given the negative seroconversion findings.

Subjective Discount Factor: 90% (a one in 10 chance this is wrong). This is a subjective value.

The change in origin likelihoods from this evidence and the calculations are shown in the Text-Table below.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin
Starting likelihood	0.951	0.049
Negative predictive value of lack of seroconversion	0.91	
Reduced by 90% Subjective Discount Factor	0.91 x 0.9 = 0.82	
Impact of this evidence	Reduces the likelihood of ZO by 82/18 or 4.6-fold. For every 100 tests, a true ZO would be seen 18 times and a non-ZO would be seen 82 times	
Impact of evidence calculation	0.951/4.6 = 0.207	
Normalize this step of analysis	0.207/(0.207 + 0.049) = 0.809	0.049/(0.207 + 0.049) = 0.191

Adjusted likelihood: Zoonotic origin (80.9%) and laboratory origin (19.1%)

Evidence: Lack of posterior diversity for SARS-CoV-2 compared to MERS and SARS-CoV-1

- The earliest stages of human CoV-1 and MERS infections were characterized by viral genome base diversity as expected for multiple, independent jumps from a large and diverse intermediate host population into humans.
- Combining MERS and CoV-1 studies, out of the earliest 255 human infections in which virus genome sequences are available, 137 could not be rooted in a prior human-to-human infection and so are attributed to an independent intermediate host-to-human infection.[65]
- That is about 54% non-human-to-human transmission.
- On the other hand, Ralph Baric has written[66] that CoV-2 is different: "SARS-CoV-2 probably emerged from bats, and early strains identified in Wuhan, China, showed limited genetic diversity, which suggests that the virus **may have been introduced from a single source**." [emphasis added.]
- With CoV-2, there are 249 viral genomes in GISAID from Hubei province, where Wuhan is located, collected between Dec 24, 2019 and Mar 29, 2020.
- From Dec 24, 2019 to November 2020, there are 1001 genomes sequenced from all of China and 198,862 worldwide.
- For CoV-2, every single genome sequence is rooted in the first sequence from the PLA Hospital in Wuhan.
- Not one case of posterior diversity.
- Using the frequency of non-rooted genome diversity seen with MERS and CoV-1, about 50:50 or a coin toss, the probability that CoV-2 is a zoonotic pandemic with 0/249 genomes is the chance of tossing a coin 249 times and getting heads every time!
- Mathematically that is nonexistent; specifically, one in 10 with 84 zeros.
- Since Wuhan had approximately 500,000 cases during the time interval of this sampling, the potential sampling error of testing only 249/500,000 or 0.05% is significant. This sampling error, while large, is unable to obliterate the overwhelming odds that this did not arise from an intermediate host in Wuhan.
- Therefore, to permit continued evidence analysis, this finding will be set at the boundary of customary statistical significance, a p-value of 0.05 or a 1 in 20 likelihood that this is zoonotic.

[65] https://elifesciences.org/articles/31257#abstract ; https://www.researchgate.net/publication/225726653_Molecular_phylogeny_of_coronaviruses_including_human_SARS-CoV ; https://science.sciencemag.org/content/300/5624/1394/tab-pdf ; https://pubmed.ncbi.nlm.nih.gov/14585636/ ; https://www.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.016378-0?crawler=true ; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7118731/

[66] https://www.nejm.org/doi/10.1056/NEJMcibr2032888

Detailed explanation

A fundamental difference between a laboratory and a non-laboratory acquired zoonotic disease, the imprint of phylogenetic diversity through pre-human spread within the source population, can be examined by the posterior diversity of human cases with no *a priori* knowledge of an intermediate host.

MERS. The MERS epidemic has been documented to have arisen from the initial jump from bats to camels, a three-to-five-year expansion within the camel population in which mutational diversity arose by random mistakes, and then a jump into humans. This model of spread predicts that there would, at some point, be additional jumps from other camels into other patients, and a pattern of "posterior diversity," would be found in the human specimens. If the COVID-19 pandemic arose by a similar mechanism the same pattern would be seen. The following Text-Table contains such data.

Phylogenetic Feature	MERS	SARS-CoV-2
Posteriority Diversity	28/30 (93%)	0
No Posteriority Diversity	2/30 (7%)	7666
Time from first patient to first example of posterior diversity	About 60 days	None at >120 days
Depth of posterior diversity to first patient	>365 days	None

The study of MERS noted above was published in 2013 in Lancet[67] in an article entitled, "Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study." Thirty specimens were used in the analysis. The features of a camel-to-human zoonotic epidemic are easily identified. Specimens taken within sixty days of the first patient, "Patient Zero," began to show a background diversity that could not be traced back through Patient Zero. The analysis of all thirty, in fact, documented that 93% were transmitted directly from the camel intermediate reservoir. And looking only at the "background" diversity permitted a calculation of the last common ancestor for the spread within the camel population of over 365 days.

A study of SARS-CoV-2[68] available May 5, 2020 and entitled, "Emergence of genomic diversity and recurrent mutations in SARS-CoV-2," looked at 7666 patient specimens from around the world for phylogenetic diversity. The authors state: "There is a robust temporal signal in the data, captured by a statistically significant correlation between sampling dates and 'root-to-tip' distances for the 7666 SARS-CoV-2 ($R^2 = 0.20$, $p < .001$). Such positive association between sampling time and evolution is expected to arise in the presence of measurable evolution over the timeframe over which the genetic data was collected." This conclusion also argues against a

[67] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3898949/
[68] https://www.sciencedirect.com/science/article/pii/S1567134820301829

MERS-like pattern of posterior diversity. In fact, the 95% upper bound for the probability of no posterior diversity being seen in SARS-CoV-2, given the data in MERS, is 3.9×10^{-4}.

The finding of posterior diversity in MERS was seen quickly, that is, within 60 days of the first patient and in only 30 specimens. In this study of COVID-19 the cutoff date of the 7666 specimens was April 19, 2020 or approximately 140 days after the first documented case. The lack of posterior diversity in COVID-19 at a much later date than what was seen with MERS also argues against a non-laboratory source for this pandemic.

A useful avenue of future research for those working to find an animal source for COVID-19 would be new mathematical models or statistical methods that might find a "hidden" signal of posterior diversity in the current data set which shows none. And given access to the unprecedented quantity of human data for COVID-19 which can be mined via bioinformatics, efforts to find the "missing link" in the wild through search and sample should be a second priority to mining the human specimen data set.

SARS-CoV-1. A similar pattern of clinical cases that do not show a common ancestor in the human population but instead is evidence of posterior diversity is shown in the Text-Table on the left for SARS-CoV-1[69] compared to CoV-2 on the right[70]. SARS-CoV-1 shows clusters of cases in humans that are connected only by phylogenetic branches that reach back in time (all of the branches inside the purple box. This is because of the extensive mutational background created while being in the intermediate host, the civet. With CoV-2 on the right, every clinical case descends from the first clinical case, in the 19A clade. There are no background mutations to account for. I will show elsewhere that the first Clade A patient was at the PLA Hospital about 3 km from the WIV.

[69] https://pubmed.ncbi.nlm.nih.gov/14585636/
[70] https://nextstrain.org/

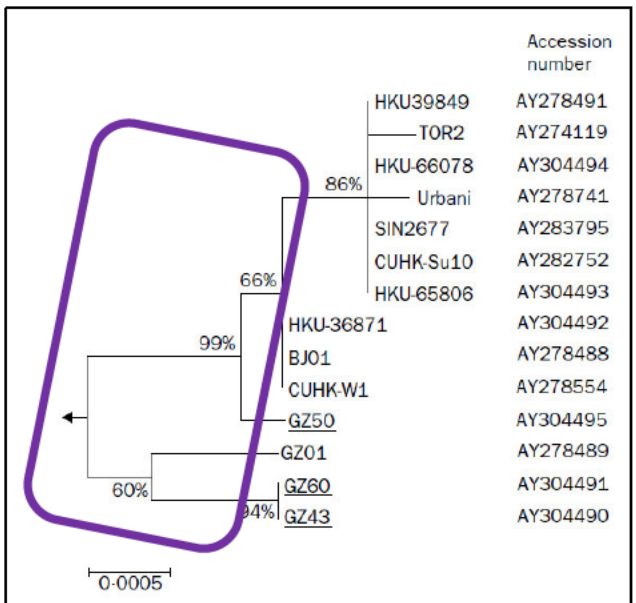
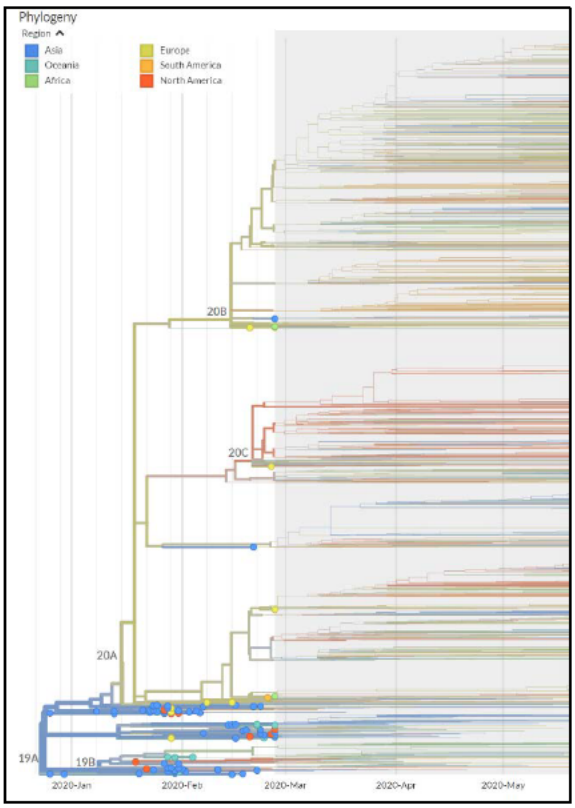
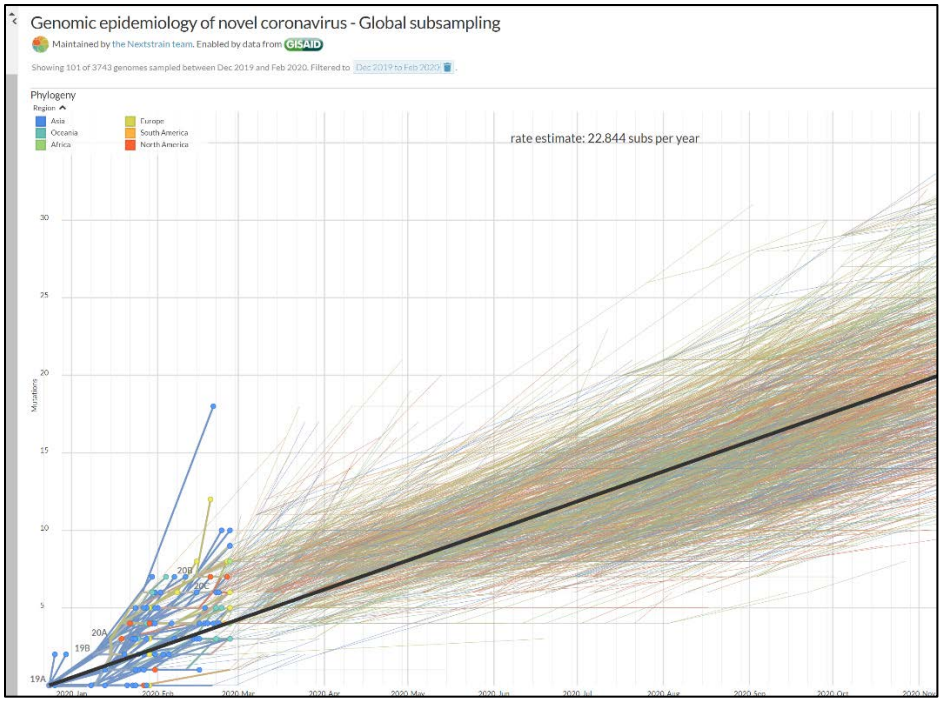


Figure 4: **Phylogenetic analysis of nucleotide acid sequence of spike gene of SARS CoV viruses**
Bootstrap values are shown as a percentage. The scale bar shows genetic distance estimated using Kimura's two parameter substitution model.[14] The nucleotide sequences of representative SARS CoV S genes (S gene coding region residue, 3765 bp) were analysed. Viruses sequenced in this study are underlined, and the other sequences used in the analysis can be accessed in GenBank with accession numbers as shown.



Given the rate of mutations of 22.8 per year for CoV-2 as shown in the Nextstrain graph below and a sequencing accuracy of about two calls per genome, CoV-2 could not have spent more than a few weeks in an intermediate host before a pattern of background mutations would be identified as posterior diversity. In the laboratory a pure culture on a single genome is used and the CoV-2 pattern is most consistent with a single pure culture infection a first human.

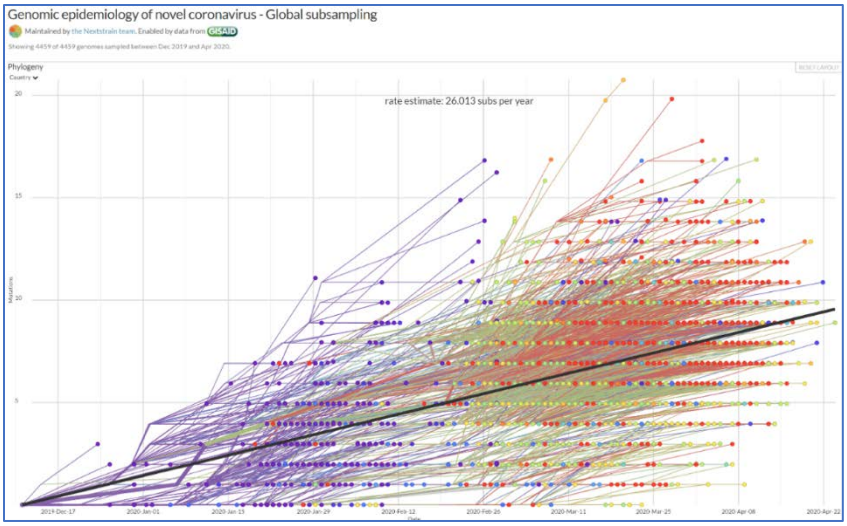


Non-zoonotic evolution. In a hypothetical in which there was a singular event in which one genetically pure virus infected one person and then the epidemic grow the development of the genetic diversity would have a clear, identifiable pattern: every new mutation would only appear on a background of the previous mutations.

The mutations in this virus are literally a personal tag. The general mutation rate leads to one mutation per patient. So, by definition, Patient Zero will have just one mutation. And then the 2-4 people that patient passes it to will have that mutation and then will add a new one, and so on. As time goes by two things happen: each patient gets a new mutation of their own and they pass on all the mutations of the past.

Since the virus has 29,900 nt and the mutation rate, as shown in this graph prepared by NextStrain is 26 mutations per year, there is very little chance a mutation will appear and then later get undone. By carefully going back in time, it is possible to literally name each person at each generation by the one (on average) new mutation they have and all of those that went before.

This graph of mutations on the Y-axis shows them gradually increasing and the color coding shows where they came from. In this infection, they only came from a previous patient and from the next previous patient and so on.



A NextStrain graphic.

How is that different from MERS, which was passed from camels to humans in a true zoonotic process?

In a true zoonotic spread to humans there is usually an initiating species (in MERS it is bats), and then an intermediate species (in MERS it is camels), and then it moves to humans, either because of a new "enabling mutation" or for a non-domestic species, a chance encounter, and Source Zero and Patient Zero meet, and a cross species event occurs. But "Source Zero" doesn't stop there with one infection in one human; the virus also transmits itself vertically into the intermediate species. Source Zero also creates a vertical infection in the camels. Whether it is mild or not doesn't matter. The new human jumping gene is moving into a very diverse population of viruses, who have themselves been evolving since the first bat to camel transmission.

What is the outcome in terms of a test to show this is happening?

The diversity of the virus in humans becomes great, and the spots where the mutations occur don't match up to MERS Patient Zero like they do in COVID-19. In MERS, the virus in Patient Zero and the virus in a later infection are not direct descendants but cousins and only descended from an earlier virus that spent time in another camel population, collecting random mutations until it got the one it needed to infect humans, and then it begins again.

The chart below, from Lancet. 2013 Dec 14; 382(9909): 1993–2002, shows just how this works. The patient at Bisha is the earliest case in this chart (Patient Zero in the red circle). But notice, no other case comes from that patient. The viruses have such a diverse genetic background they appear to only be related to the Bisha virus with a posterior timeline of about one year. Their background is in the green boxes and it skips Patient Zero.

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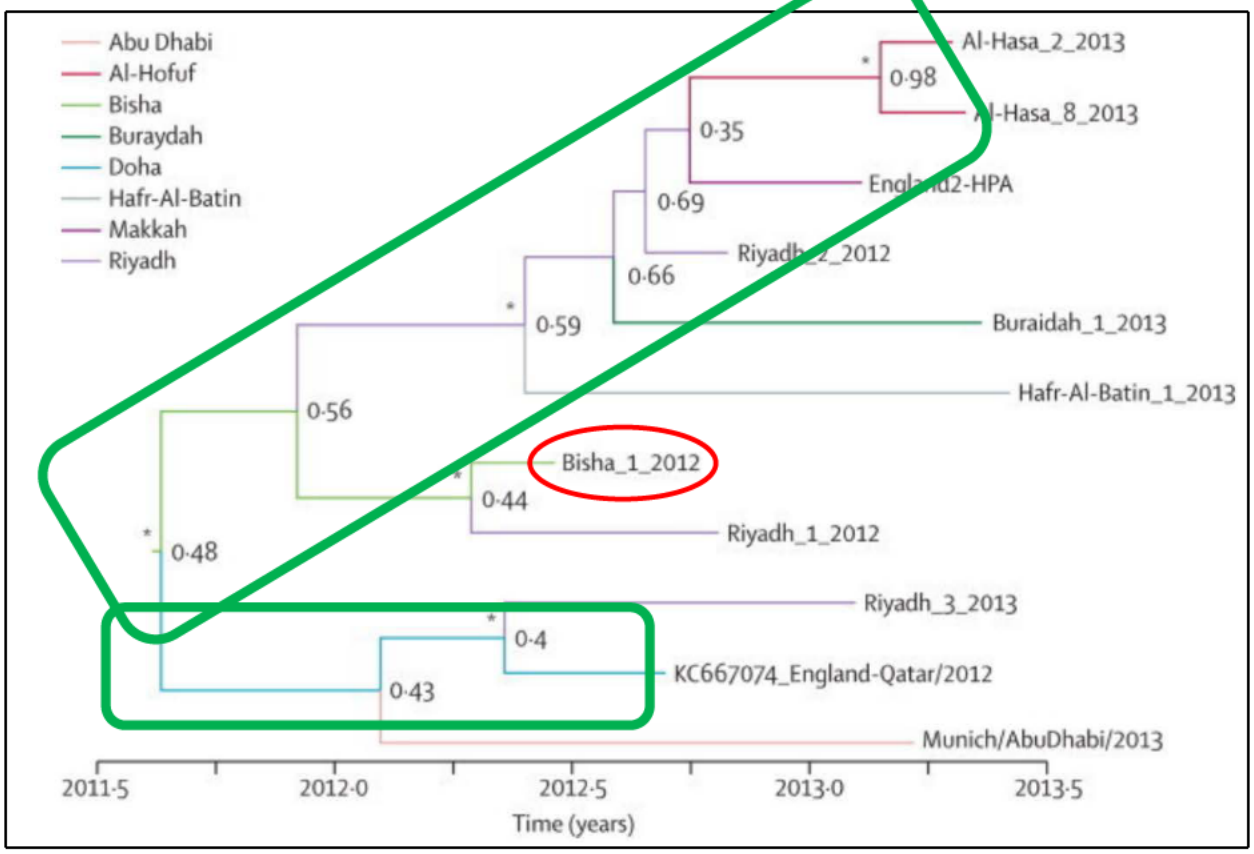

Even without knowing that camels are the zoonotic source for MERS, this data, from clinical sample only and without any field work in cave or camels, is all you need to know that this arose in the wild.

A paper just appeared with this analysis for a region of China and the posterior genomic diversity indicated a single starting point on December 1, 2019 for all cases. There was no posterior diversity. At this point with over 322,000 full genomes sequenced[71] and all showing an additive pattern of mutations and with none showing background diversity before the known appearance in Wuhan, the only conclusion is that there is no reservoir of genetic diversity.

On January 26, 2020 in an article in *Science* written by Jon Cohen, Kristian Andersen, an evolutionary biologist at the Scripps Research Institute who had analyzed sequences of CoV-2 to try to clarify its origin said: "The scenario of somebody being infected outside the market and then later bringing it to the market is one of the three scenarios we have considered that is still consistent with the data. It's entirely plausible given our current data and knowledge."

The negative predictive value of finding no posterior diversity in CoV-2 with 322,000 total infections sequenced, over 1000 in China, is 95%

Subjective Discount Factor: 95% (a one in 20 chance this is wrong)

[71] https://www.gisaid.org/

Below is the impact of the pack of posterior diversity on the likelihood of a zoonotic versus laboratory origin

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin
Starting likelihood	0.809	0.191
Negative predictive value of lack of posterior diversity	0.95	
Reduced by 95% Subjective Discount Factor	0.95 x 0.95 = 0.90	
Impact of this evidence	Reduces the likelihood of ZO by 90/10 or 9-fold. For every 100 tests, a true ZO would be seen 10 times and a non-ZO would be seen 90 times	
Impact of evidence calculation	0.809/9 = 0.085	
Normalize this step of analysis	0.085/(0.085 + 0.191) = 0.308	0.191/(0.085 + 0.191) = 0.692

Adjusted likelihood: Zoonotic origin (30.8%) and laboratory origin (69.2%)

Evidence: Opportunity.

The Wuhan Institute of Virology has publicly disclosed that by 2017 it had developed the techniques to collect novel coronaviruses, systematically modify the receptor binding domain to improve binding or alter zoonotic tropism and transmission, insert a furin site to permit human cell infection, make chimera and synthetic viruses, perform experiments in humanized mice, and optimize the ORF8 gene to increase human cell death (apoptosis).

Wuhan Institute of Virology scientists maps RBD and then takes a civet coronavirus that won't infect human cells, changes two amino acids in the receptor binding domain & it infects human cells.[72]

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Identification of Two Critical Amino Acid Residues of the Severe Acute Respiratory Syndrome Coronavirus Spike Protein for Its Variation in Zoonotic Tropism Transition via a Double Substitution Strategy*

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Baric & Shi at WIV take bat coronavirus that won't infect human cells, change S746R to add an ARG at S1/S2 site to make furin-like cleavage site, & the new coronavirus infects human cells.[73]

Baric & Shi of WIV create completely synthetic coronavirus from bat spike & mouse adapted backbone that no treatment, monoclonal antibody, or vaccine will touch.[74]

- "Using the SARS-CoV reverse genetics system2, we generated and characterized a chimeric virus expressing the spike of bat coronavirus SHC014 in a mouse-adapted SARS-CoV backbone.

- The results indicate that group 2b viruses encoding the SHC014 spike in a wild-type backbone can efficiently use multiple orthologs of the SARS receptor human angiotensin

[72] http://www.paper.edu.cn/scholar/showpdf/NUT2kN0INTT0gxeQh
[73] https://jvi.asm.org/content/jvi/89/17/9119.full.pdf
[74] https://pubmed.ncbi.nlm.nih.gov/26552008/

converting enzyme II (ACE2), replicate efficiently in primary human airway cells and achieve in vitro titers equivalent to epidemic strains of SARS-CoV.

- Additionally, in vivo experiments demonstrate replication of the chimeric virus in **mouse lung with notable pathogenesis.**

- Evaluation of available SARS-based immune-therapeutic and prophylactic modalities revealed poor efficacy; both monoclonal antibody and vaccine approaches failed to neutralize and protect from infection with CoVs using the novel spike protein.

- On the basis of these findings, we **synthetically re-derived an infectious full-length** SHC014 recombinant virus and demonstrate robust viral replication both in vitro and in vivo."

This study was conducted, with permission, during the gain of function moratorium put in place by NIH in 2014:

"These studies were initiated before the US Government Deliberative Process Research Funding Pause on Selected Gain-of-Function Research Involving Influenza, MERS and SARS Viruses (http://www.phe.gov/s3/dualuse/Documents/gain-of-function.pdf). This paper has been reviewed by the funding agency, the NIH. Continuation of these studies was requested, and this has been approved by the NIH."

Drs. Daszak and Shi becomes world's expert on ORF8 induced apoptosis by CoVs in human cells (HeLa) & maximizing lethality.[75]

> The full-length ORF8 protein of SARS-CoV is a luminal endoplasmic reticulum (ER) membrane-associated protein that induces the activation of ATF6, an ER stress-regulated transcription factor that activates the transcription of ER chaperones involved in protein folding [35]. We amplified the ORF8 genes of Rf1, Rf4092 and WIV1, which represent three different genotypes of bat SARSr-CoV ORF8 (S3C Fig), and constructed the expression plasmids. All of the three ORF8 proteins transiently expressed in HeLa cells can stimulate the ATF6-dependent transcription. Among them, the WIV1 ORF8, which is highly divergent from the SARS-CoV ORF8, exhibited the strongest activation. The results indicate that the variants of bat SARSr-CoV ORF8 proteins may play a role in modulating ER stress by activating the ATF6 pathway. In addition, the ORF8a protein of SARS-CoV from the later phase has been demonstrated to induce apoptosis [28]. In this study, we have found that the ORF8a protein of the newly identified SARSr-CoV Rs4084, which contained an 8-aa insertion compared with the SARS-CoV ORF8a, significantly triggered apoptosis in 293T cells as well.

This paper also demonstrates the collection of 64 novel bat coronaviruses from caves in southern China, including Yunnan where Dr. Shi has said is the location of the bat ancestor of CoV-2.

This evidence is necessary for a laboratory origin hypothesis in which genetic manipulation to create CoV-2 is a precursor to a laboratory accident. However, it does not per se, provide

[75] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5708621/

increased weight in favor of a laboratory origin. It is however provided here to be a guide for the kinds of investigations to be conducted if access to the WIV records is ever provided.

Likelihood from prior state is unchanged following this evidence analysis:

Zoonotic origin (30.8%) and laboratory origin (69.2%)

Evidence and Motive for laboratory furin site insertion:

A key to infectivity of coronaviruses is the addition, in nature or the laboratory, of a furin cleavage site (FCS) at the S1/S2 junction of the Spike Protein.

Furin cleavage sites (FCS) have been widely understood to be important for many viral infections, including HIV, influenza, and others. It has also been widely understood before now that lineage B coronaviruses do not have FCS.

It was therefore surprising when an examination of SARS-CoV-2 Spike Protein found an insertion of a 12-nt, 4-AA sequence near the junction of the S1/S2 subunits which creates a furin site that is essential to human infectivity and transmission. As expected from previous work, no lineage B (sarbecovirus) coronavirus has this feature. This is the most difficult "molecular fingerprint" of SARS-CoV-2 to explain having been acquired in the wild and for that reason there are no even passingly feasible theories.

One database of whole genome sequences of 386 coronaviruses was devoid of furin cleavage sites.[76] Another database of 2956 genomes of sarbecovirus strains sequences shows that none have a furin site.[77] This is a highly significant finding with a probability that sarbecovirus has a furin site in the wild of one in about 985.[78]

It has been known since 1994 that viral glycoproteins can be cleaved by secreted proteases, including furin.[79] Even before that, in 1992, it was known the peptide sequence R-X-K/R-R in surface glycoproteins was required for avian influenza viruses of Serotype H7 pathogenesis.[80] The first paper using furin inhibitors to define a role for an FCS in coronavirus-cell fusion was published in 2004.[81]

Since that time, it has become common practice to insert FCS during laboratory gain-of-function experiments to increase infectivity. The following Text-Table illustrates the scope of just a few of the experiments conducted, with the hyperlink to the paper in column one.

URL for Paper	Title of Paper
One	Characterization of a panel of insertion mutants in human cytomegalovirus glycoprotein B.
Two	Insertion of the two cleavage sites of the respiratory syncytial virus fusion protein in Sendai virus fusion protein leads to enhanced cell-cell fusion and a decreased dependency on the HN attachment protein for activity.

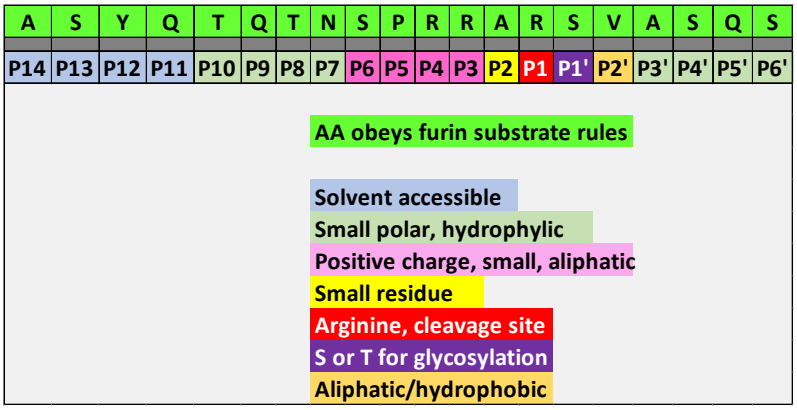
[76] https://academic.oup.com/bioinformatics/article/36/11/3552/5766118
[77] https://academic.oup.com/database/advance-article/doi/10.1093/database/baaa070/5909701
[78] When a series of samples are taken and none produce the result expected, the probability that this is a false negative finding can be estimated by taking the number of samples and dividing by three. Here, 2956 sarbecoviruses without a single furin site is a probability of one in 2956/3 or 985.
[79] https://www.ncbi.nlm.nih.gov/pubmed/8162439
[80] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7172898/pdf/main.pdf
[81] https://www.ncbi.nlm.nih.gov/pubmed/15141003

Three	Recombinant Sendai viruses expressing fusion proteins with two furin cleavage sites mimic the syncytial and receptor-independent infection properties of respiratory syncytial virus.
Four	Amino acid substitutions and an insertion in the spike glycoprotein extend the host range of the murine coronavirus MHV-A59
Five	Induction of IL-8 release in lung cells via activator protein-1 by recombinant baculovirus displaying severe acute respiratory syndrome-coronavirus spike proteins: identification of two functional regions.
Six	Coronaviruses as vectors: stability of foreign gene expression.
Seven	Experimental infection of a US spike-insertion deletion porcine epidemic diarrhea virus in conventional nursing piglets and cross-protection to the original US PEDV infection.
Eight	Minimum Determinants of Transmissible Gastroenteritis Virus Enteric Tropism Are Located in the N-Terminus of Spike Protein.
Nine	Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus.
Ten	Construction of a non-infectious SARS coronavirus replicon for application in drug screening and analysis of viral protein function
Eleven	A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo.

The creation in the wild of a coronavirus FCS that is used as an example of what might have happened in SARS-CoV-2 is uninformative. In this case, a strain of influenza, in which a new polybasic site appears spontaneously leads to increased infectivity and lethality,[82] was reported by Tse *et al*. 2014. The mechanism of the FCS acquisition in this paper is an RNA polymerase dependent stuttering at a small, constrained loop in which one or more A nt were inserted, removing the strain in the loop and inserting an AAA codon which represents the basic amino acid lysine. No such method exists for the insertion of arginine, the amino acid in the CoV-2 furin site that needs to be created.

The insert generates a canonical 20 AA furin site sequence. In 2011 Tian et al.[83] published an analysis of 126 furin cleavage sites from three species: mammals, bacteria and viruses. The analysis showed that when the furin sites are recorded as a 20-residue motif, a canonical structure emerges. It includes one core cationic region (eight amino acids, P6–P2′) and two flanking solvent accessible regions (eight amino acids, P7–P14, and four amino acids, P3′–P6′).

[82] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911587/
[83] https://www.nature.com/articles/srep00261



This figure above shows the 20-AA of the furin motif in SARS-CoV-2 (in green) with the P14 to P6' AA positions marked with the cleavage site being the amide bond between P1-R and the P1' residue. The motif is color coded with the requirements (in most cases, except for the positively charged AA requirements, most position requirements can be relaxed).

With the insertion, all 20 residues obey the rules as established by Tian. Since there are 20^4 different 4-AA peptides or 160,000 choices, it is remarkable that the 4 AA insert created a sequence that contained a small or cationic AA (8 AA/20 qualify), a cationic AA (3/20), another cationic AA (3/20), and a small AA (5/20) in that order. In fact, there are only 360 or the total or about 0.2% of all four amino acid inserts that would be expected to follow the exact rules for furin substrates. Of course, given the increase in infectivity SARS-CoV-2 has over other coronaviruses that do not have a well-designed furin cleavage site, selection pressure would drive this rare mutational event once it happened randomly. It would also be a likely choice for a laboratory designed furin cleavage site created *de novo*.

Based on the evidence that there are no furin cleavage sites in 2956 sarbecovirus (beta coronavirus) genome sequences[84], the likelihood that CoV-2 acquired the furin site from a wild sarbecovirus is one in 985 or 0.001. Because this is highly significant, we will use the conservative rule established in the beginning and use a likelihood of 0.05 for this evidence.

Subjective Discount Factor. 95% confidence (only a one in 20 chance this is wrong). Below is the calculation of the Bayesian adjustment.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin
Starting likelihood	0 308	0.692
Negative predictive value of a lack of furin sites in sarbecovirus genomes	0.95	
Reduced by 95% Subjective Discount Factor	0.95 x 0.95 = 0.90	
Impact of this evidence	Reduces the likelihood of ZO by 90/10 or 9-fold. For every 100 tests, a true ZO would be seen 10 times and a non-ZO would be seen 90 times	
Impact of evidence calculation	0 308/9 = 0 034	
Normalize this step of analysis	0.034/(0 034 + 0.692) = 0.047	0.692/(0.692 + 0.034) = 0.953

Adjusted likelihood. Zoonotic origin (4.7%), laboratory origin (95.3%).

[84] https://academic.oup.com/database/advance-article/doi/10.1093/database/baaa070/5909701

Evidence: Codon usage can distinguish insertion events in the wild from those created in the laboratory.

Not only is the insertion of an FCS peptide unique among lineage B coronaviruses, the nt sequence used for the process is more broadly unique among coronaviruses in general, regardless of lineage:

-CCT-**CGG-CGG**-GCA-

I will now use synonymous codon bias methods to try to inform the question of the origin of SARS-CoV-2.

Because of the redundancy of the genetic code, more than one 3-nt sequence specifies any given amino acid. For example, there are six codons that specify arginine, R. The frequencies with which such synonymous codons are used are unequal and have coevolved with the cell's translation machinery to avoid excessive use of suboptimal codons that often correspond to rare or otherwise disadvantaged tRNAs. This results in a phenomenon termed "synonymous codon bias," which varies greatly between evolutionarily distant species and possibly even between different tissues in the same species.

Decades of research has identified that all life forms, viruses, bacteria, and humans alike, use the codons in a signature pattern of frequency which can be used to identify a particular sequence of RNA or DNA as human or non-human; viral or non-viral.

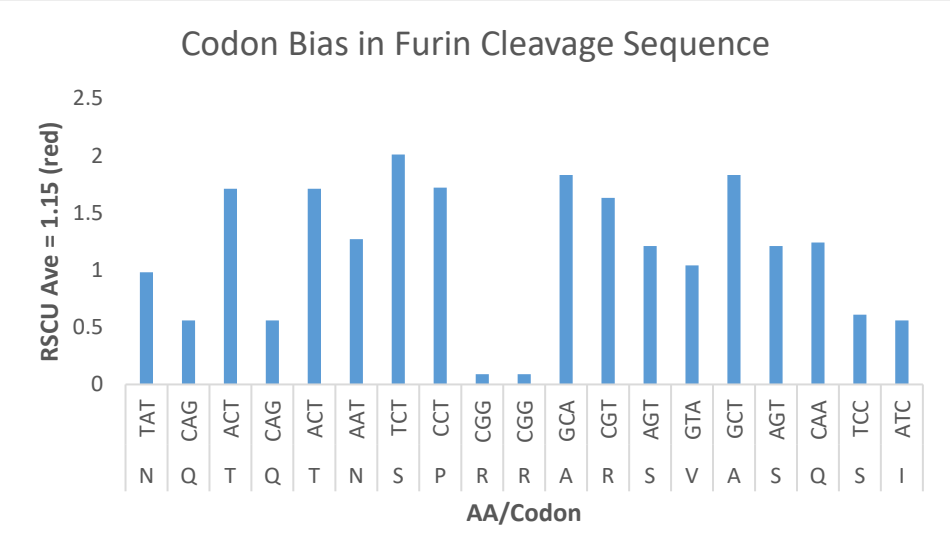
In this way, viruses in nature and scientists in the laboratory, with different goals and motivations, make distinguishing codon usage decisions which can sometimes provide a fingerprint of their source.

The Text-Table below contains the arginine codon usage for two populations, pooled data for SARS-CoV 2003 and related viruses and 13 Sars-CoV-2 human specimens from widely dispersed locations.

Codon	SARS-CoV 2003 and ten other evolutionary related viruses in the Nidovirales	SARS-CoV-2 from 13 Geo-locations
CGG	0.09	0.09
CGA	0.44	0.37
CGC	0.72	0.37
AGG	0.9	1.07
CGU	1.77	1.63
AGA	2.08	2.48

Since these values are of a type of multiplicative scale, they were fit using a log-normal distribution, which appears appropriate (although the sample size is small). Using the log mean and standard deviation and this distribution, the probability of finding a CGG codon is about 0.024. Assuming they are independent the probability of finding a CCG-CCG codon pair is effectively 0.024^2 or 0.00058. This is a likelihood of about one in 1700.

The following Figure shows the RSCU for the amino acids that comprise the new furin cleavage site in SARS-CoV-2. As one can see, the RSCU values are similar to each other with the exception of the RR dimer insert, which have a very low RSCU of 0.09.



The RSCU value for the CGG codon for R of 0.09 was taken from a 2004 paper of the RSCU for SARS-CoV 2003 and ten other evolutionary related viruses in the *Nidovirales* and is confirmed by 13 SARS-CoV-2 specimens obtained from diverse geographic locations. If one assumes that the RSCU observations are independent and that the probability distribution of these measurements is Gaussian (normal; a reasonable assumption), then one can calculate the probability of obtaining a result as small as 0.09. Removing the two 0.09 values, then the mean and standard deviation of the remaining values are 1.275 and 0.4992, respectively. Then the probability of a single 0.09 value is 0.0088. However, there are two 0.09 values. If we assume that these are independent findings, then the probability of both values being seen is 0.0088^2 or 7.7×10^{-5}. Using the RSCU of 0.2 from the Table above does not change the immense improbability of the usage of a CGGCGG codon pair in the wild.

Single Arginine CGG codon usage analysis suggests this will not be found in the wild.

The codon usage for SARS-CoV-2, like most coronaviruses studied, has a bias toward AT and away from GC nucleotides. The frequency of third position G use in CoV-2, for example, is 13%, 21%, 17%, and 16% for the spike protein, envelope, membrane, and nucleocapsid protein, respectively.

In that context, the scarcity of the CGG genome in SARS-CoV-2 and related coronaviruses, the relative synonymous codon usage, determined by the method of Behura and Severson,[85] was calculated and tabulated below. The color coding is blue for underutilized codons (RSCU < 1.0) and red for overutilized codons (RSCU > 1.0); light blue for RSCU values of 0.60 to 0.99 and

[85] https://www.ncbi.nlm.nih.gov/pubmed/22889422

light red for RSCU of 1.01 to 1.60. The highest RSCU usage of CGG is 1.21 in the membrane protein in the MERS virus but zero in SARS-CoV-2.

RSCU	SARS-CoV-2	Beta CoV Pangolin	SARS CoV	Bat SARS CoV	MERS CoV
Spike	0.29	0	0.19	0.08	0.25
Envelope	0	0	0	0	0
Membrane	0	0.35	0.74	0.24	1.21
Nucleocapsid	0.41	0.16	0.03	0.04	0.8

Looking at these five coronaviruses:

The largest structural protein of the coronaviruses is the spike protein, with 1273 amino acids. In SARS-CoV-2 there are 42 R residues, with only one RR dimer, the one in the insert that created SARS-CoV-2.

As a reminder none of these related coronaviruses have the 12-nucleotide insertion that forms the putative furin site in CoV-2. Interestingly, the pangolin coronavirus has no CGG residues in the spike protein. The significance of this is it makes the acquisition of this insert from pangolin by recombination impossible.

The smallest structural protein, the envelope protein, has 75 amino acids, including three R residues, but has no CGG codons in any of the related coronaviruses examined.

The SARS-CoV-2 membrane protein has 441 amino acids, 14 R residues and no CGG codons. Among related coronaviruses, this is the most unique finding of the four proteins for SARS-CoV-2 since the other four coronaviruses all utilize CGG to some extent in this protein. In the case of the MERS virus, this protein is the only occurrence in which this codon is overutilized.

The nucleocapsid protein has 418 amino acids and is responsible for packing the RNA genome. As expected for the role of R in protein-RNA interactions, it has 29 R residues and four RR dimers. None of the dimers use the CGGCGG sequence.

The nt usage of the 12-nt insert which forms the FCS cleavage site has a probability this sequence was selected for in the wild of one in 129,870.

A blast search was performed for the 12-nt inserted sequence and adjacent extensions and only the SARS-CoV-2 sequences were identified.

Shortening the search to just the two CGG-CGG codons was only slightly more fruitful. The Text-Table below shows the frequency of the middle half of the insert, CGGCGG, across the genomes of all seven known human coronaviruses, as well as a specimen bovine coronavirus and the bat and pangolin coronaviruses with greatest homology to SARS-CoV-2. Only a single example, outside of the Spike Protein gene, has been found.

Furin PBCS sequence	Beta Coronavirus	Total Arginine Dimers Anywhere	CGGCGG in Spike Protein *	CGGCGG Anywhere in genome *	CCGCCG Anywhere in genome
S**RR**K**RR**S	Human CoV-HKU1 GenBank: KF686346.1	12	0	0	0
K**RR**S**RR**A	Bovine CoV-Quebec GenBank: AF220295.1	12	0	0	0
PRRARSV	**SARS-CoV-2 Wuhan reference sequence GenBank: NC_045512.2**	**16**	**1; nt 23,606**	0	0
P**R**SV**R**S	MERS-CoV NCBI Reference Sequence: NC_019843.3	21	0	0	0
N**RR**S**R**GA	Human CoV-OC43 London/2011 GenBank: KU131570.1	16	0	0	0
None	Human CoV-229E GeneBank: KF514433.1	15	0	0	0
None	Human CoV NL63 NCBI Reference Sequence: NC_005831.2	9	0	0	0
None	SARS-CoV 2003 ZJ0301 from China GenBank: DQ182595.1	17	0	0	0
None	Bat coronavirus RaTG13 GeneBank: MN996532.1	11	0	1; nt 9394	0
None	Pangolin PCoV_GX-P4L GenBank: MT040333.1	10	0	0	0
	Total	139	1	0	0
* - Includes both in phase codons as well as out of phase, frameshift codons.					

To understand what this means for the search for the zoonotic source for SARS-CoV-2, a statistical approach was taken. Using the data from the nine viruses other than SARS-COV-2 there was a single incidence of the CGGCGG found in the bat coronavirus. Assuming 10,000 codons per genome, the frequency of CGGCGG in coronaviruses can be estimated at 2 per 45,000 codons or 4×10^{-5}. Therefore, the frequency of finding the center half of the SARS-CoV-2 insert is very small. This is consistent with the strong bias in all coronaviruses to place an A/U nt in the third codon position.

The last column above, the presence of -CCG-CCG- in these coronaviruses was included because it is the hybridization sequence partner for the negative strand sequence, which arises during genome replication. This eliminates the possibility of a strand jumping event to generate a CGGCGG codon dimer.

A similar analysis for the spike protein gene can be done. Since there are no instances of CGGCGG in the spike protein genome, and the gene is 3819 nucleotides long, there are 636 pairs of codons Thus, over the 9 other viruses, there are 5724 pairs of codons and no cases of the CGGCGG pair. To calculate the upper bound on the probability of such a pair from these data, one can use the Poisson “Rule of Three”, which yields a value of 3/5724 or 0.00052 with 95% confidence. Now examining the SARS-COV-2 genome, there was one instance of the pair in question out of 636 pairs. The probability of this happening if the true rate of this occurrence for a beta coronavirus is 0.00052 is 0.044. Obviously for smaller assumed rates of this occurrence, this would result in probabilities less than 0.044.

Since the 12-nt insert has been found nowhere in the coronavirus genomic universe, examining over 300,000 sequences and using the Poisson “Rule of Three” again, the upper bound on the frequency that it exists in nature is less than one in 100,000 with 95% confidence.

This observation in conjunction with the lack of finding the 12-nt sequence in any candidate zoonotic species makes unlikely a natural source for the virus. One line of investigation to establish a wild source for this infection would be to find a coronavirus strain with the 12-nt sequence somewhere in nature. The fact that 10 of the 12 nts are either G or C coupled, the documented bias against GC suggests this search would be futile.

Based on these analyses that demonstrate that the finding of a -CGG-CGG- codon pair in the furin site of CoV-2 is a highly improbable event, and using the conservative value of a one in 20 chance (the value for a p-value of 0.05), one can recalculate the likelihood of the choice between a zoonotic origin and a laboratory origin.

Subjective Discount Factor. 95% confidence (only a one in 20 chance this is wrong). Below is the calculation of the Bayesian adjustment.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin
Starting likelihood	0.047	0.953
Negative predictive value of the absence of the -CGG-CGG- pair in any coronavirus in nature	0.95	
Reduced by 95% Subjective Discount Factor	0.95 x 0.95 = 0.90	
Impact of this evidence	Reduces the likelihood of ZO by 90/10 or 9-fold. For every 100 tests, a true ZO would be seen 10 times and a non-ZO would be seen 90 times	
Impact of evidence calculation	0.047/9 = 0.005	
Normalize this step of analysis	0.005/(0.005 + 0.953) = 0.005	0.953/(0.953 + 0.005) = 0.995

Adjusted likelihood. Zoonotic origin (0.5%), laboratory origin (99.5%).

Evidence. Laboratory codon optimization uses CGG for laboratory insertions of arginine residues 50% of the time.

Codon optimization by recombinant methods (that is, to bring a gene's synonymous codon use into correspondence with the host cell's codon bias) has been widely used to improve cross-species expression of protein.

Though the opposite objective of reducing expression by intentional introduction of suboptimal synonymous codons has not been extensively investigated, isolated reports indicate that replacement of natural codons by rare codons can reduce the level of gene expression in different organisms. For example, one approach to vaccine development is to create an attenuated virus which comprises a modified viral genome containing nucleotide substitutions engineered in multiple locations in the genome, wherein the substitutions introduce synonymous de-optimized codons.

In US Patent 9,476,032[86] titled, "Attenuated viruses useful for vaccines," they state: "In one high-priority redesigned virus, most or all Arg codons are changed to CGC or **CGG** (the top two frequent human codons). This does not negatively affect translation." The patent contains numerous codon usages optimized for vaccine production, including the SARS-CoV virus, and in fact they use the CGG-CGG codon pair 45 times.

Beginning with a paper in 2004,[87] one motivation for codon-optimized SARS genomes is stated here: "The gene encoding the S protein of SARS-CoV contains many codons used infrequently in mammalian genes for efficiently expressed proteins. We therefore generated a codon-optimized form of the S-protein gene and compared its expression with the S-protein gene of the native viral sequence. S protein was readily detected in HEK293T cells transfected with a plasmid encoding the codon-optimized S protein."

Since that time, human optimized codons have been frequently used for coronavirus research, mostly in gain-of-function experiments. In that context the "molecular fingerprint" of CGG for R is one of those common laboratory reagent gene manipulators.

Other examples:

Examples of the use of CGG codon for arginine in coronavirus research	**Reference**
SARS was genetically modified to improve ACE2 binding using "human optimized" codons, like CGG for arginine, to grow better in the laboratory. The strains were more infective.Preparation of SARS-CoV S protein pseudotyped virus. "The full-length cDNA of	Wu, K. et al. Mechanisms of Host Receptor Adaptation by Severe Acute Respiratory Syndrome

[86] http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=9476032.PN.&OS=PN/9476032&RS=PN/9476032
[87] https://www.ncbi.nlm.nih.gov/pubmed/15367630

the SARS-CoV S gene was optimized according to human codon usage and cloned into the pCDNA3.1(+) vector (Invitrogen). The resulting "humanized" S sequence was identical with that of strain BJ01 at the amino acid level."	Coronavirus. J Biol Chem. 2012 Mar 16; 287(12): 8904–8911.
Predictions of future evolution of a virus are a difficult, if not completely impossible, task. However, our detailed structural analysis of the host receptor adaptation mutations in SARS-CoV RBD has allowed us to predict, design, and test optimized SARS-CoV RBDs that may resemble future evolved forms of the virus. "RBD might evolve into the human-optimized form by acquiring two mutations at the 442 and 472 position." SARS-CoV-2 acquired the mutation at position 472.	Fang Li. Receptor recognition and cross-species infections of SARS coronavirus. Antiviral Res. 2013 Oct; 100(1): 246–254.
Plasmid encoding a codon-optimized form of the SARS-CoV S protein of the TOR2 i	Wenhui Li, Chengsheng Z, et al., Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. EMBO J. 2005 Apr 20; 24(8): 1634–1643.
The gene encoding the S protein of SARS-CoV contains many codons used infrequently in mammalian genes for efficiently expressed proteins. We therefore generated a codon-optimized form of the S-protein gene and compared its expression with the S-protein gene of the native viral sequence. S protein was readily detected in HEK293T cells transfected with a plasmid encoding the codon-optimized S protein (Fig. (Fig.1).1). No S protein was detected in cells transfected with a plasmid encoding the native S-protein gene.	Moore, MJ, Dorfman, T. Retroviruses Pseudotyped with the Severe Acute Respiratory Syndrome Coronavirus Spike Protein Efficiently Infect Cells Expressing Angiotensin-Converting Enzyme 2. J Virol. 2004 Oct; 78(19): 10628–10635.
Published in 2019 by **Dr. Zhengl-Li Shi,** entitled "Origin and evolution of pathogenic coronaviruses," reviews genetic optimized SARS viruses using human codons.	Cui, J, Fang, L. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019; 17(3): 181–192.
In 2006, Montana scientists put a synthetic furin cleavage site into a SARS coronavirus by adding an R residue at position R667. They write: "We show that furin cleavage at the modified R667 position generates discrete S1 and S2 subunits and potentiates membrane fusion activity." Mutations were introduced by using	Follis, KE, York, J, Nunberg, JH. Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell–cell fusion but does not affect virion entry. Virology 350 (2006) 358–369

QuikChange mutagenesis (Stratagene)[88]	
Identification of murine CD8 T cell epitopes in codon-optimized SARS-associated coronavirus spike protein is the title of a paper that shows that the expression of spike protein in vitro was greatly increased by expression cassette optimization.	Zhia, Y, Kobinger, GP, Jordan, H, et al. Identification of murine CD8 T cell epitopes in codon-optimized SARS-associated coronavirus spike protein
As for the human clec4C_1 and mouse clec14A, they showed very similar profiles with spike genes, especially with bat SARS-CoV, in the arginine coding groups, showing the high RSCU values over 2.50 in AGA.	Ahn,I, Jeong, B-J, Son, HS. Comparative study of synonymous codon usage variations between the nucleocapsid and spike genes of coronavirus, and C-type lectin domain genes of human and mouse. Experimental & Molecular Medicine volume 41, pages746–756, 2009.

One relevant paper,[89] in which arginine residues were being inserted into bovine herpesvirus-1, used primers to create RR dimers with nine separate -CGG-CGG- codon pairs. as testament to their broad use in the Wuhan Institute of Virology laboratory.

Scientists from the Wuhan Institute of Virology provided the scientific community with a technical bulletin on how to make genetic inserts in coronaviruses and proposed using the very tool that would insert this CGGCGG codon.

A Technical Appendix[90] entitled, "Detailed methods and primer sequences used in a study of genetically diverse filoviruses in Rousettus and Eonycteris spp. bats, China, 2009 and 2015, by Yang, Xinglou & Zhang, Yunzhi & Jiang, Ren-Di & Guo, Hua & Zhang, Wei & Li, Bei & Wang, Ning & Wang, Li & Rumberia, Cecilia & Zhou, Ji-Hua & Li, Shi-Yue & **Daszak, Peter** & Wang, Lin-Fa & **Shi, Zheng-Li.** (2017), from the Wuhan Institute of Virology identifies primer sequences for doing genetic experiments in coronaviruses and identifies CGG containing primers when a R amino acid is being inserted.

[88] Since the codon usage here was not reported I contacted Professor Nunberg to inquire which arginine codons were used. He replied: "Unfortunately, those files have all been archived and access to the nt sequences would involve considerable digging. If it is useful to you, I typically choose codons that are more frequent in highly expressed human proteins."
[89] From the Wuhan Institute of Virology; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7125963/
[90] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5382765/

Given that there are two codons of six possibilities that are used in codon optimization, CGG and CGC, the finding of a CGG pair would have a likelihood of happening by chance of (2/6) times (2/6) or one in nine.

Subjective Discount Factor: 80% (this has a probability of being wrong one in five times). This is arbitrary. The calculation to make this adjustment in likelihood is shown here:

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.005	0.995
This is the outcome expected 8 of 9 times if this is codon optimization		0.88
Reduced by 80% confidence		0.88 x 0.8 = 0.704
Impact of this evidence		Increases the likelihood of LO by 70.4 divided by 29.6 or 2.378.
Impact of evidence calculation		0.995 x 2.378 = 2.37
Normalize this step of analysis	0.005/(2.37 + 0.005) = 0.002	2.37/(0.005 + 2.37) = 0.998

Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

Evidence: SARS-CoV-2 Spike Protein is Highly Optimized for ACE2 Binding and Human Cell Infectivity, a Finding that is Inconsistent with Natural Selection but is Consistent with Laboratory Creation

Summary:

- Andersen et al.[91] hypothesized that if the CoV-2 interaction with the human ACE2 was apparently "not ideal," it was evidence that CoV-2 arose by natural selection.

- The alternative hypothesis would be that a finding that CoV-2 was optimized for ACE2 binding and human infection from the initial infection would be evidence of laboratory creation.

- Andersen relied on a paper for the "not ideal" interaction that relied on a computer algorithm rather than laboratory data, was qualitative in nature, sampled only five amino acids or 0.45% of the interaction region, and was over-interpreted.

- The analysis of the Baric et al. paper cited by Andersen as evidence the interaction was not ideal was reexamined, and it was concluded that Andersen had over-interpreted the paper. The paper was a computer simulation study of only 5 of 201 amino acids in the CoV-2-ACE2 interaction region. Only one of the five amino acids discussed was said to be inferior to the equivalent amino acid in SARS-CoV-1; the remainder were either positive or neutral with respect to binding.

- More recently, Baric has clarified his thoughts concerning the CoV-2 ACE2 receptor binding interaction. In a December 31, 2020 *New England Journal of Medicine* paper[57] he wrote: "Early zoonotic variants in the novel coronavirus SARS-CoV that emerged in 2003 affected the receptor-binding domain (RBD) of the spike protein and thereby enhanced virus docking and entry through the human angiotensin-converting–enzyme 2 (hACE2) receptor. **In contrast, the spike-protein RBD of early SARS-CoV-2 strains was shown to interact efficiently with hACE2 receptors early on**." [emphasis added.]

- A comprehensive, laboratory-based, and quantitative paper by Starr et al. of all 201 amino acids in the receptor binding region, not just five amino acids, was examined. Fully 99.6% of all of the possible 3819[92] amino acid substitutions were tested for their effect on CoV-2 binding to ACE2. Only 21 substitutions of the 3819 improved ACE2 binding. Therefore, CoV-2 has been optimized for human ACE2 binding in 99.45% of the possible amino acids in its Spike Protein interaction region.

[91] https://www.nature.com/articles/s41591-020-0820-9
[92] There are 201 amino acids in the residue 331 to 531 interaction region and so 201 times the 19 possible alternative amino acids not found in CoV-2 equals 3819.

- To support this finding, Starr also made an examination of 31,570 CoV-2 sequences from human infections, looking for the 21 substitutions that had been shown to improve CoV-2 binding in the above in vitro laboratory experiments. Among the 31, 570 CoV-2 cases, they failed to find even a single case in which there was an amino acid substitution that improved binding at the time of writing this analysis.[93]

- Based on Andersen's hypothesis and its alternative, SARS-CoV-2 is fully optimized for interaction with the human ACE2 receptor and was at the time of the first patient. There is no evidence of an evolving SP binding region, as was seen with SARS-CoV-1. This is consistent with a laboratory optimized coronavirus which entered the human population fully evolved.

Analysis

Quote from Andersen: "While the analyses above suggest that SARS-CoV-2 may bind human ACE2 with high affinity, computational analyses predict that the interaction is not ideal (reference 7) and that the RBD sequence is different from those shown in SARS-CoV to be optimal for receptor binding (references 7,11).

Thus, the high-affinity binding of the SARS-CoV-2 spike protein to human ACE2 is most likely the result of natural selection on a human or human-like ACE2 that permits another optimal binding solution to arise. This is strong evidence that SARS-CoV-2 is not the product of purposeful manipulation."

The apparent **hypothesis** for the above conclusion is:

"If the SARS-CoV-2 (CoV-2) Spike Protein interaction with the ACE2 receptor is not maximized, then it is evidence that the interaction is the product of natural selection and not purposeful (laboratory) manipulation."

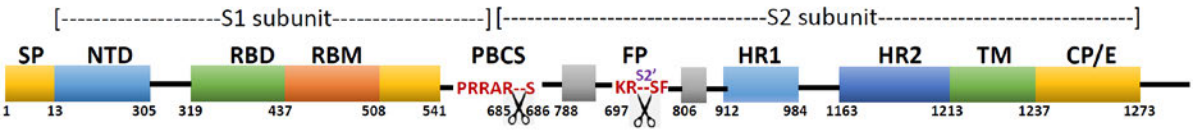
This would lead to an **alternative hypothesis**:

"If the CoV-2 Spike Protein interaction with the ACE2 receptor is maximized, then it is evidence that the interaction *was* the product of purposeful (laboratory) manipulation."

Background.

The Spike Protein (SP) structure and its functional domains are shown in this Figure. The S1 subunit is the initial host interaction portion while the S2 is the post-binding portion responsible for initiating host cell entry, with HR1, HR2, and TM being responsible for breaching the host cell membrane. Allowing viral RNA to enter the cell.

[93] The recent finding of the N501Y variant, first in the UK, and now spreading globally, is evidence of the power of this analysis. N501Y is one of only five potential substitutions in the Starr analysis that had a major effect in improving ACE2 binding.



The interaction of the SP portions which interact with the ACE2 of the host cell, which begins the internalization, infectious process, are contained in the Receptor Binding Domain (RBD) and to a lesser extent the Receptor Binding Motif (RBM), specifically residues 331 to 531. Herein, residues 331 to 531 are called the "interaction region."

Evidence given by Andersen:

Reference 7 in the Andersen paper above is a Ralph Baric paper[94] from early in the pandemic (submitted January 22, 2020) and examines five key residues in the receptor binding domain of the Spike Protein (SP) and whether they are "ideal" for interacting with the ACE2 of human cells. The entire paper is based on computer calculations or prior laboratory work but importantly does not do any new "wet" lab work with CoV-2.

Baric et al. had previously identified five amino acid residues that are important for SP-ACE2 interaction. Using the amino acid numbers of CoV-2, these amino acids are: 455, 486, 493, 494, and 501. Baric opines that the most critical residues are 493 and 501 and the next most important residues are 455, 486, and 494. The authors then discuss each amino acid in turn:

Residue 493: "Gln493 in 2019-nCoV RBD is compatible with hot spot 31, suggesting that 2019-nCoV is capable of recognizing human ACE2 and infecting human cells." In this analysis, 4 of the 20 amino acids are probed.

Residue 501: "This analysis suggests that 2019-nCoV recognizes human ACE2 less efficiently than human SARS-CoV (year 2002) but more efficiently than human SARS-CoV (year 2003). Hence, at least when considering the ACE2-RBD interactions, 2019-nCoV has gained some capability to transmit from human to human."

Direct binding evidence has shown that this statement is misleading, and CoV-2 binds the ACE2 receptor about ten-times better than SARS-CoV (year 2002).[95] In this analysis 3 of the 20 amino acids are probed.

Residues 455, 486, and 494: First, Baric et al. state: "Leu455 of 2019-nCoV RBD provides favorable interactions with hot spot 31, hence enhancing viral binding to human ACE2."

Next, they state: "Phe486 of 2019-nCoV RBD provides even more support for hot spot 31, hence also enhancing viral binding to human ACE2." Importantly, they also talk about their own laboratory work on an "optimized" receptor binding domain and state: "Leu472 of human and

[94] https://jvi.asm.org/content/94/7/e00127-20
[95] https://www.cell.com/action/showPdf?pii=S0092-8674%2820%2931003-5 ; https://www.nature.com/articles/s41586-020-2179-y ; https://www.sciencedirect.com/science/article/pii/S0092867420302622 ; https://science.sciencemag.org/content/367/6483/1260

civet SARS-CoV RBDs provides favorable support for hot spot 31 on human ACE2 through hydrophobic interactions with ACE2 residue Met82 and several other hydrophobic residues **(this residue has been mutated to Phe472 in the optimized RBD)**." [emphasis added.]

Finally, they state: Ser494 in 2019-nCoV RBD still provides positive support for hot spot 353, but the support is not as favorable as that provided by Asp480. Overall, Leu455, Phe486, and Ser494 of 2019-nCoV RBD support the idea that 2019-nCoV recognizes human ACE2 and infects human cells."

In this analysis they probe 3 of 20 amino acid residues for position 480, 4 of 20 for position 486, and 4 of 20 for position 442.

As shown in the Figure below from the Baric paper, the in vitro designed, optimized human SP (red arrow) had the amino acid residues F, F, N, D, and T at these five key residues. Since CoV-2 was identical in only one of these five it was not "optimal" and, according to Andersen, it therefore was not laboratory derived.

B

Virus	Year	442	472	479	480	487
SARS - human	2002	Y	L	N	D	T
SARS - civet	2002	Y	L	K	D	S
SARS - human/civet	2003	Y	P	N	G	S
SARS - civet	2005	Y	P	R	G	S
SARS - human	2008	F	F	N	D	S
Viral adaption to human ACE2		F > Y	F > L > P	N = R >>> K	D > G	T >>> S
Optimized - human	In vitro design	F	F	N	D	T
Viral adaptation to civet ACE2		Y > F	P = L > F	R > K = N	G > D	T > S
Optimized - civet	In vitro design	Y	P	R	G	T
SARS - bat	2013	S	F	N	D	N
2019-nCoV – human	2019	L (455)	F (486)	Q (493)	S (494)	N (501)

Conclusion from the above paper: by examining five amino acid residues of the 200 residues encompassing the interaction region, and calculating the expected interaction of a total of 18 of the 4000 possible residues or 0.45% of all possibilities, they conclude CoV-2 can infect human cells, but is not optimized to do so. This data was twisted by Andersen to show 'strong evidence' of natural selection.

An alternative and comprehensive analysis in another paper:[96]

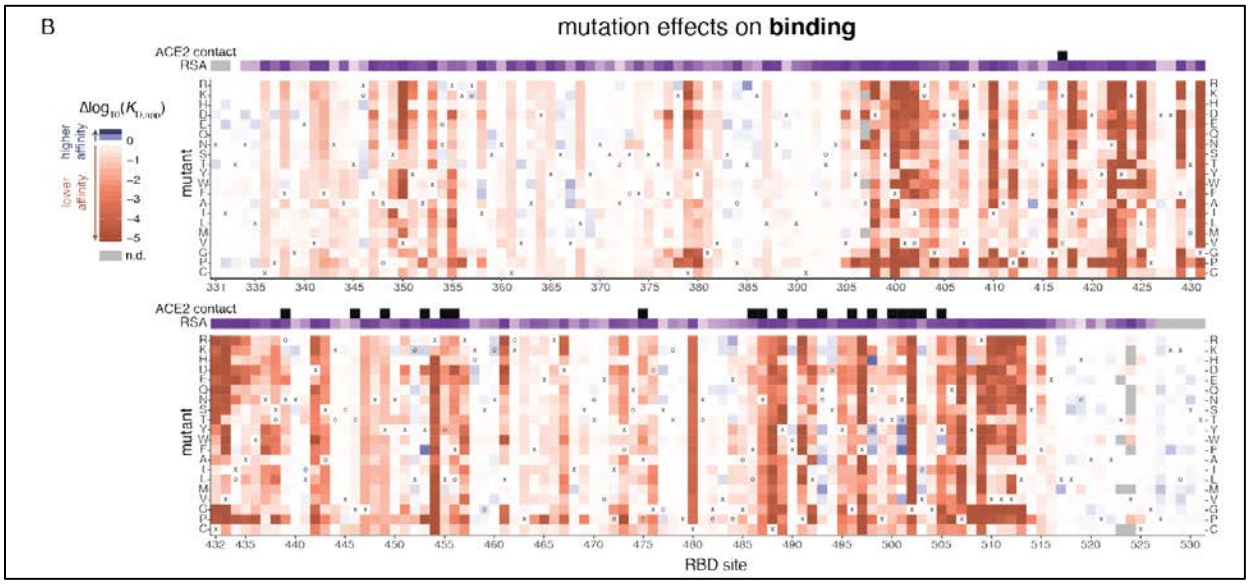
The receptor binding domain (RBD) of the CoV-2 SP is included in residues 331 to 531, a 201 amino acid sequence, of the SP. To examine the effect of each and every amino acid in each and every position, all 19 different amino acids were changed into all 201 positions of the RBD to the extent possible. Out of a total potential of 3819 different single amino acid variants, the scientists

[96] https://www.cell.com/action/showPdf?pii=S0092-8674%2820%2931003-5

were able to create 3804 of the potential variants or 99.6% of the possible variants. It is probable that the variants with the 0.4% amino acid substitutions could not be made for one reason or another. These 3804 were then tested for binding to the human ACE2. Finally, the RBD from SARS-CoV-1 also was tested.

The Figure below is the result of the experiment. Starting with amino acid 331 and ending with amino acid 531, the amino acids that were changed are in vertical columns and are color coded. Shades of brown are amino acid substitutions that reduce ACE2 binding affinity and blue are amino acid substitutions that improve binding, in all cases compared to the 'native' CoV-2 SP sequence. White is the color of a neutral substitution which neither enhances nor diminishes binding. Only the dark blue substitutions provide a strong improvement in ACE2 binding. There is a black square along the top row that denotes amino acids in the SP that interact with the ACE2 protein. Unlike in the Baric analysis above, in which only five amino acids were considered, this group of 19 amino acids provide a more complete interaction picture.

The first overarching observation is that most amino acid substitutions among the 201 amino acids are negative; while a large number are neutral. The fact that the vast majority of amino acid substitutions do not provide an improved ACE2 interaction is clear evidence that the CoV-2 SP interaction region is not newly evolved to the human ACE2 but arrived in the first patient having been "trained" to invade and kill human cells.

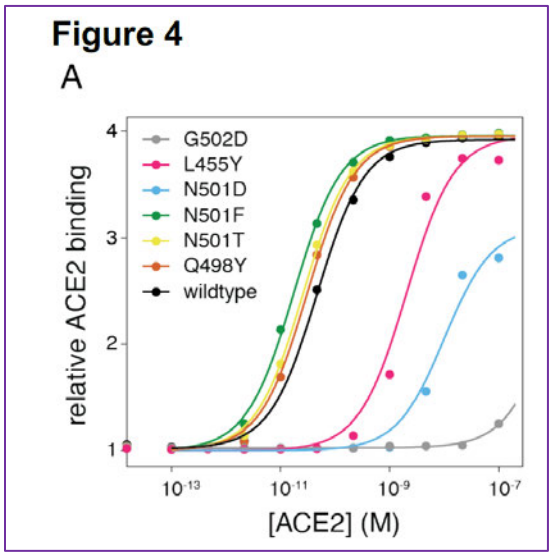


There are three levels of improved binding as designated by dark blue, medium blue, and pale blue. Out of the 3804 variants tested, there are 4 dark blue substitutions or 0.11% and 17 medium blue or 0.45%. According to the paper, the binding effect of the light blue could not be measured as different from the native sequence.

The conclusion of this comprehensive work is the demonstration that for 99.45% of the amino acids in the 201 amino acid interaction region, the CoV-2 choice is optimized, where any substitution is either detrimental or, at best, neutral with respect to the first step of CoV-2 entry to human cells, the binding step to the ACE2 receptor.

How much could CoV-2 binding be improved or made worse by substitutions during the human-to-human transmission of the pandemic?

The Figure 4 below, taken from the paper, shows that the three best amino acid substitutions have only a slight effect on the binding curve (Black is wildtype; curves to the left are better binding; curves to the right are worse binding). This is further evidence that CoV-2 is an optimized form of the original virus.



The authors also concluded that Anderson et al. was wrong: “An initially surprising feature of SARS-CoV-2 was that its RBD tightly binds ACE2 despite differing in sequence from SARS-CoV-1 at many residues that had been defined as important for ACE2 binding by that virus (Andersen et al., 2020; Wan et al., 2020).”

In fact, multiple studies have shown that CoV-2 binds ACE2 better than SARS-CoV-1, contradicting Andersen.

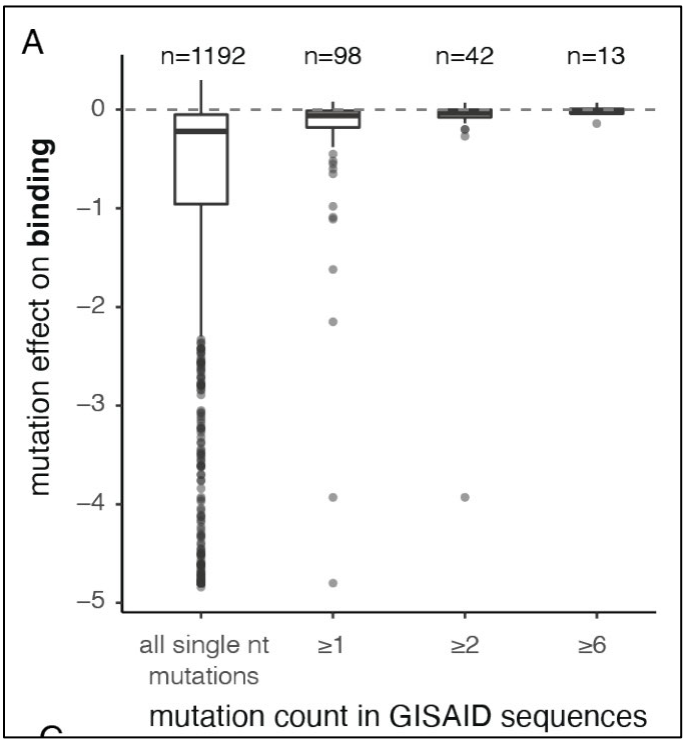
Is there evidence that CoV-2 in human circulation has mutations that enhance ACE2 binding?

Another measure of whether CoV-2 is optimized for human infection is to see if Spike Protein mutations have arisen during the pandemic that improve binding of the virus to the ACE2 receptor or if the SP amino acids are ideal from the very first human patient.

The Starr paper addressed this issue as well. A total of 31,570 human sequences were analyzed to see if any of the 21 amino acid substitutions from the binding experiments (or any other for that matter) were being selected for. That is, if there is any evidence of evolutionary pressure to improve SARS-CoV-2 infectivity.

Below is Figure 8 of the Starr paper. Of the 31,570 sequences, all mutations in the receptor interaction region were analyzed for their effect on ACE2 binding. The data below are for all examples of a single nt mutation (1192), two mutations (98), 3-5 mutations (42), and six or more (13) and the effect the mutation would have on ACE2 binding. The logarithmic scale has the

wildtype CoV-2 as 0 and each negative integer is a 10-fold reduction in affinity. Shockingly, there is not a single mutation that is above the 0 line, which would be an improved affinity for the ACE2 receptor. All of the mutations lower the receptor affinity.



Here are the results, in the words of Starr:

"Our discovery of multiple strong affinity-enhancing mutations to the SARS-CoV-2 RBD raises the question of whether positive selection will favor such mutations, since the relationship between receptor affinity and fitness can be complex for viruses that are well-adapted to their hosts (Callaway et al., 2018; Hensley et al., 2009; Lang et al., 2020). Strong affinity-enhancing mutations are accessible via single-nucleotide mutation from SARS-CoV-2 (Figure S8C), but **none are observed among circulating viral sequences in GISAID** (Figure 8A), and **there is no significant trend for actual observed mutations to enhance ACE2 affinity more than randomly drawn samples of all single nucleotide mutations** (see permutation tests in Figure S8D). **Taken together, we see no clear evidence of selection for stronger ACE2 binding, consistent with SARS-CoV-2 already possessing adequate ACE2 affinity at the beginning of the pandemic."** [emphasis added.]

It is striking that the authors, in observing the complete absence of any evidence for stronger ACE2 binding in over thirty thousand cases, would describe this as evidence of "adequate ACE2 affinity" and not as an exceptional finding of "optimized ACE2 affinity." Of course, calling the SP affinity exceptional from the beginning of the pandemic would beg the question of a laboratory derived virus.

Returning to the initial hypotheses, since the 3804 possible amino acids at the receptor interaction region of CoV-2 are 99.45% optimized for ACE2 binding, and there is not a single

example in 31,570 human CoV-2 genomes of a substitution that enhances ACE2 binding, the CoV-2 interaction with ACE-2 was maximized from the get-go.

Therefore, the hypothesis, "If the SARS-CoV-2 (CoV-2) Spike Protein interaction with the ACE2 receptor is not maximized, then it is evidence that the interaction is the product of natural selection and not purposeful (laboratory) manipulation," is **rejected**.

The alternative hypothesis, "If the CoV-2 Spike Protein interaction with the ACE2 receptor is maximized, then it is evidence that the interaction was the product of purposeful (laboratory) manipulation," is thus **accepted**.

At the time of this writing, a new RBD mutant N501Y has been observed. It is one of the five potential mutations that could be expected to increase RBD-ACE2 affinity.

This is the first example of evidence that will not be statistically quantified but treated as a 51%.49% preponderance of the evidence adjustment. The evidence is more consistent with having been optimized by various methods used in the laboratory than with the slow natural process as seen with SARS-CoV-1, and so the conservative rule that this is consistent with a laboratory origin (51%) versus zoonotic origin (49%) will be used. There will be no confidence adjustment.

The adjusted likelihoods are shown in the following table.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.002	0.998
This is the outcome favors LO over ZO at 51% versus 49%		0.51
Impact of this evidence		Increases the likelihood of LO by 51/49 = 1.041
Impact of evidence calculation		1.041 x 0.998 = 1.039
Normalize this step of analysis	0.002/(0.002 + 1.039) = 0.002	1.039/(0.002 + 1.039) = 0.998

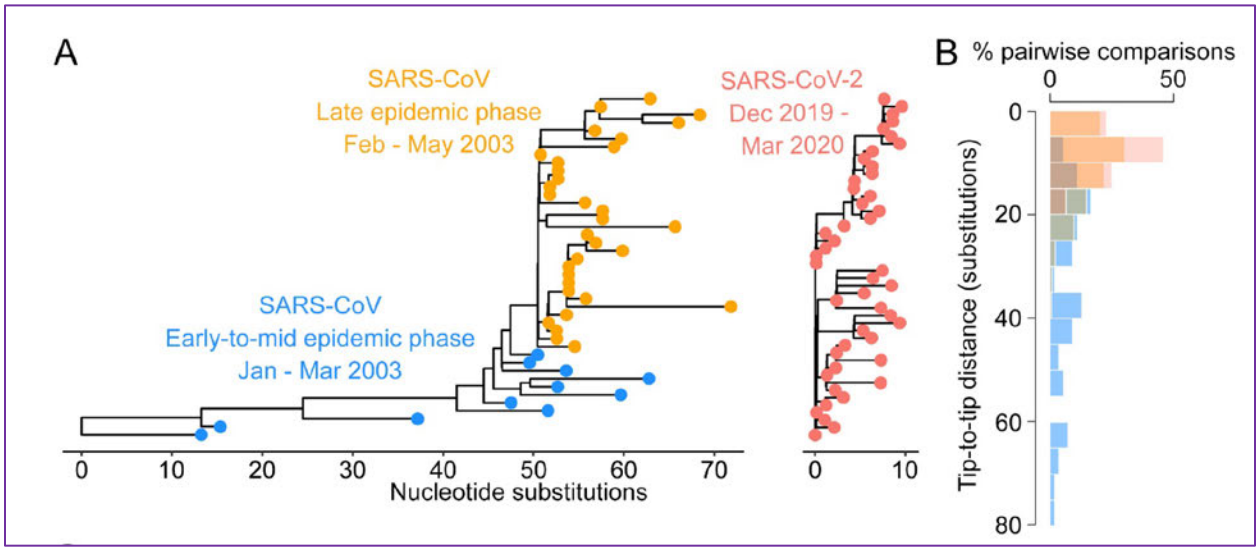
Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

Evidence. Whole genome comparison of human adaption of CoV-2 compared to SARS-CoV-1 is consistent with a "pre-adaption" of CoV-2 to the human host

A paper[97] entitled, "SARS-CoV-2 is well adapted for humans. What does this mean for re-emergence?" by Shing Hei Zhan, Benjamin E. Deverman, and Yujia Alina Chan states in the abstract:

"In a side-by-side comparison of evolutionary dynamics between the 2019/2020 SARS-CoV-2 and the 2003 SARS-CoV, we were surprised to find that SARS-CoV-2 resembles SARS-CoV in the late phase of the 2003 epidemic, after SARS-CoV had developed several advantageous adaptations for human transmission. Our observations suggest that **by the time SARS-CoV-2 was first detected in late 2019, it was already pre-adapted to human transmission to an extent similar to late epidemic SARS-CoV**. **However, no precursors or branches of evolution stemming from a less human-adapted SARS-CoV-2-like virus have been detected.** The sudden appearance of a highly infectious SARS-CoV-2 presents a major cause for concern that should motivate stronger international efforts to identify the source and prevent re-emergence in the near future. [Emphasis added.]

The following Figure from the paper best illustrates the relative SNV adaption for SARS-CoV-1 versus CoV-2.



The paper also makes a tangential comment about posterior diversity: "It would be curious if no precursors or branches of SARS-CoV-2 evolution are discovered in humans or animals."

This is another example of evidence that will not be statistically quantified. The evidence is more consistent with having been adapted by various known methods used in a laboratory than with the slow natural process as seen with SARS-CoV-1, and so the conservative rule that this is consistent with a laboratory origin (51%) versus zoonotic origin (49%) will be used. There will be no confidence adjustment.

[97] https://www.biorxiv.org/content/10.1101/2020.05.01.073262v1

The adjusted likelihoods are shown in the following table.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.002	0.998
This is the outcome favors LO over ZO at 51% versus 49%		0.51
Impact of this evidence		Increases the likelihood of LO by 51/49 = 1.041
Impact of evidence calculation		1.041 x 0.998 = 1.039
Normalize this step of analysis	0.002/(0.002 + 1.039) = 0.002	1.039/(0.002 + 1.039) = 0.998

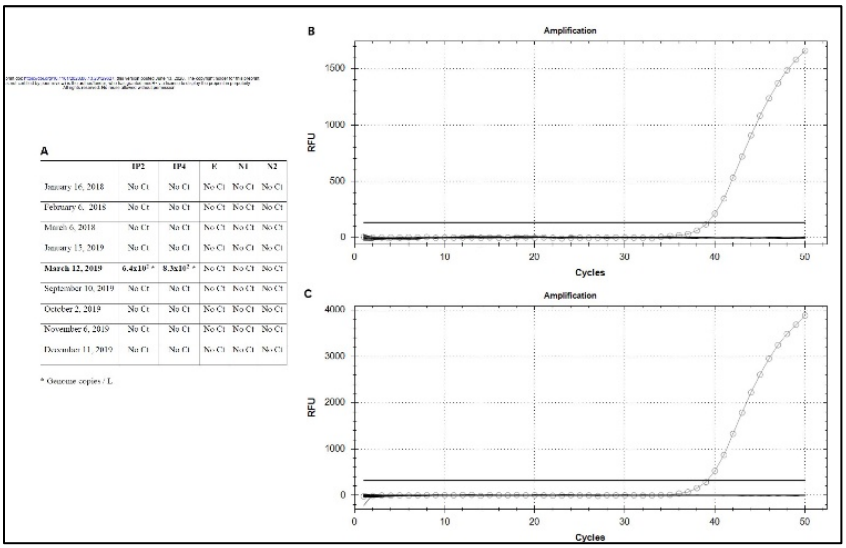
Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

Evidence: Evidence of CoV-2 during early 2019 in wastewater from Barcelona, Spain is a false positive artifact

A paper entitled "Sentinel surveillance of SARS-CoV-2 in wastewater anticipates the occurrence of COVID-19 cases"[98] claims CoV-2 was present in Barcelona, Spain in March 2019. Specifically, they state:

"This possibility prompted us to analyze some archival WWTP samples from January 2018 to December 2019 (Figure 2). All samples came out to be negative for the presence of SARS-CoV-2 genomes with the exception of March 12, 2019, in which both IP2 and IP4 target assays were positive. This striking finding indicates circulation of the virus in Barcelona long before the report of any COVID-19 case worldwide."

This is a false positive



As shown above from the paper, they found 43/45 runs with zero and two runs had only 600-800 CoV-2 copies/L

But the limit of detection (LoD) of their assay is 1,000,000 CoV-2/L.

According to the Promega PCR assay FDA clearance package, the Ct at the LoD is 33-34 for the N1 and N2, respectively (Table 17, page 51).[99] Here the LoD is listed as 1 RNA/µL.

In the paper the Ct is 40 or 6-7 above the LoD.

This evidence is neutral as to origin and will not be used to adjust the likelihoods. It does reduce the credibility of some of the new origin theories coming out of China.

[98] https://www.medrxiv.org/content/10.1101/2020.06.13.20129627v1.full.pdf
[99] https://twitter.com/quay_dr/status/1340572543548227585/photo/1

Evidence: WHO and Dr. Shi have spoken of the singular nature of the beginning of COVID-19

On January 23, 2020 Dr. Shi wrote in the draft of her paper: "The almost identical sequences of this virus in different patients imply a probably recent introduction in humans…"[100] By February 3, 2020, when the final version of this paper was published, this sentence had been **deleted**.[101]

On April 23, 2020 the WHO stated: "All the published genetic sequences of SARS-CoV-2 isolated from human cases are very similar. This suggests that the start of the outbreak resulted from a single point introduction in the human population around the time that the virus was first reported in humans in Wuhan, China in December 2019."[102]

The evidence, like the lack of posterior diversity and seroconversion reported earlier, is more consistent with a single introduction in a laboratory accident. This evidence will not be used to adjust probabilities but is included because it could be a form of party admissions of unfavorable facts.

[100] RaTG13 paper as a preprint
[101] RaTG13 final Nature paper
[102] WHO document page 2 of 12

Evidence. As documented by Drs. Daszak, Humes, and Shi, mammalian biodiversity and bat species differences between Yunnan and Hubei Provence are significant and do not support a zoonotic origin

Summary. SARS-CoV-2 is most closely related to bat coronaviruses from Yunnan, a rural province in South West China. Wuhan, where the pandemic began, is a large urban city of 11 million inhabitants in north central China. These two areas are approximately 1900 km apart.

This is the US equivalent of the difference between New York City (population 8.4 million) and the Everglades in Florida, 2000 km away. The incongruent image of a bat or intermediate host in the Everglades somehow finding its way to New York City is a clear demonstration of the difficulty in this hypothetical transmission process. Nonetheless, a strict literature-based analysis will be conducted.

If COVID-19 is a zoonotic disease it must have travelled from bats to humans or from bats to an intermediate species to humans. Therefore, an examination of mammalian biodiversity differences and commonalities between Yunnan and Wuhan might provide useful information about the intermediate host or the particular bat species.

Peter Daszak, Zhengli-li Shi and colleagues published an August 2020 paper entitled, "Origin and cross-species transmission of bat coronaviruses in China,"[103] in which they make a number of observations that are relevant to this analysis. It should be remembered that both lead authors have made multiple, strong, public statements over many months where they assert that SARS-CoV-2 is a natural virus of zoonotic origin.

Yunnan and Hubei Provinces have very dissimilar mammalian diversity

Quoting from the Methods section of the Daszak, Shi paper:

"Defining zoogeographic regions in China:

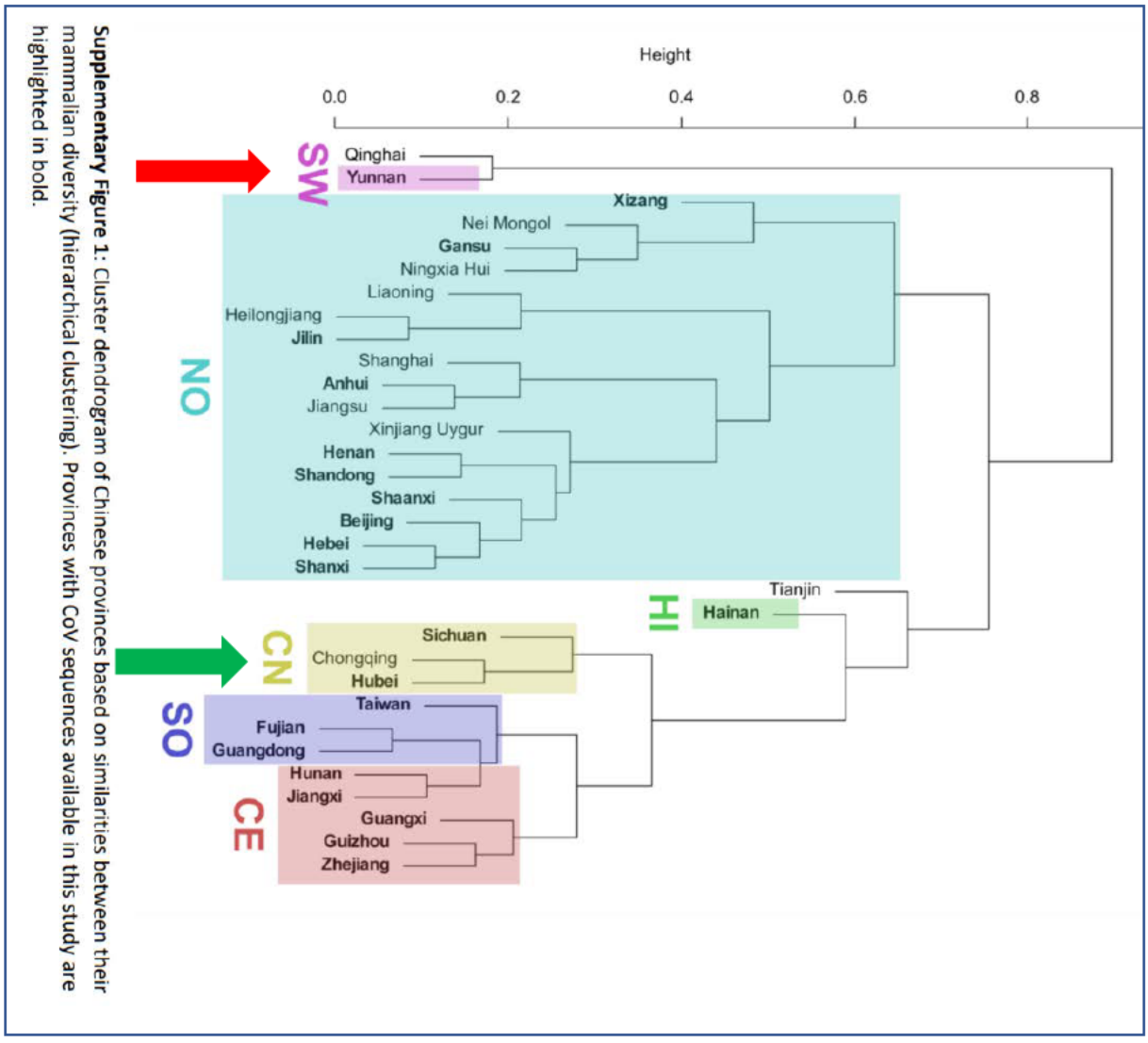
Hierarchical clustering was used to define zoogeographic regions within China by clustering provinces with similar mammalian diversity. Hierarchical cluster analysis classifies several objects into small groups based on similarities between them. To do this, we created a presence/absence matrix of all extant terrestrial mammals present in China using data from the IUCN spatial database and generated a cluster dendrogram using the function *hclust* with average method of the R package stats. Hong Kong and Macau were included within the neighboring Guangdong province. We then visually identified geographically contiguous clusters of provinces for which CoV sequences are available (Fig. 1 and Supplementary Fig. 1).

We identified six zoogeographic regions within China based on the similarity of the mammal community in these provinces: **SW (Yunnan province)**, NO (Xizang, Gansu, Jilin, Anhui, Henan, Shandong, Shaanxi, Hebei, and Shanxi provinces and Beijing municipality), **CN (Sichuan and Hubei provinces)**, CE (Guangxi, Guizhou, Hunan, Jiangxi, and Zhejiang provinces), SO (Guangdong and Fujian provinces, Hong Kong, Macau, and Taiwan), and HI.

[103] https://www.nature.com/articles/s41467-020-17687-3#Sec19

Hunan and Jiangxi, clustering with the SO provinces in our dendrogram, were included within the central region to create a geographically contiguous Central cluster (Supplementary Fig. 1). These six zoogeographic regions are very similar to the biogeographic regions traditionally recognized in China. The three β-CoV sequences from HI were included in the SO region to avoid creating a cluster with a very small number of sequences."

Below is a cluster dendrogram of Chinese provinces based on similarities between their mammalian diversity (hierarchical clustering). Provinces with CoV sequences available in this study are highlighted in bold.



Supplementary Figure 1: Cluster dendrogram of Chinese provinces based on similarities between their mammalian diversity (hierarchical clustering). Provinces with CoV sequences available in this study are highlighted in bold.

The y-axis height is a measure of the biodiversity with 1.0 being complete similarity and 0.0 being no similarity. As expected for the geography and location of the two provinces, Yunnan (red arrow above) and Hubei (green arrow above) have a height score of about 0.1, with seven branches and six nodes separating them. This is close to the biggest different in mammalian biodiversity of any two locations in all of China.

In conclusion, Daszak and Shi et al. demonstrate that the mammalian biodiversity between Yunnan and Hubei is very significant, reducing the options for a common intermediate host to be the natural conduit between bats and humans.

Shi, Humes, and Daszak statement: "SARS-CoV-2 is likely derived from a clade of viruses originating in horseshoe bats (*Rhinolophus* spp.). The geographic location of this origin appears to be Yunnan province."

This evidence will not be statistically quantified. The evidence reduces the biodiversity overlap needed to create a common intermediate species between the two provinces, and so the conservative rule that this is consistent with a laboratory origin (51%) versus zoonotic origin (49%) will be used. There will be no subjective discount factor adjustment.

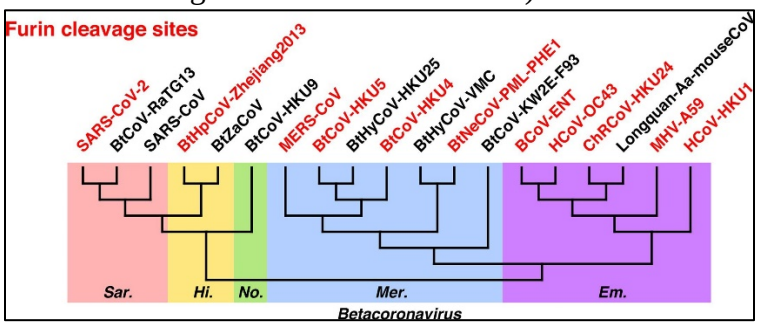
Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.002	0.998
This is the outcome favors LO over ZO at 51% versus 49%		0.51
Impact of this evidence		Increases the likelihood of LO by 51/49 = 1.041
Impact of evidence calculation		1.041 x 0.998 = 1.039
Normalize this step of analysis	0.002/(0.002 + 1.039) = 0.002	1.039/(0.002 + 1.039) = 0.998

Because of the rule on the use of significant figures, the likelihood does not change.

Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

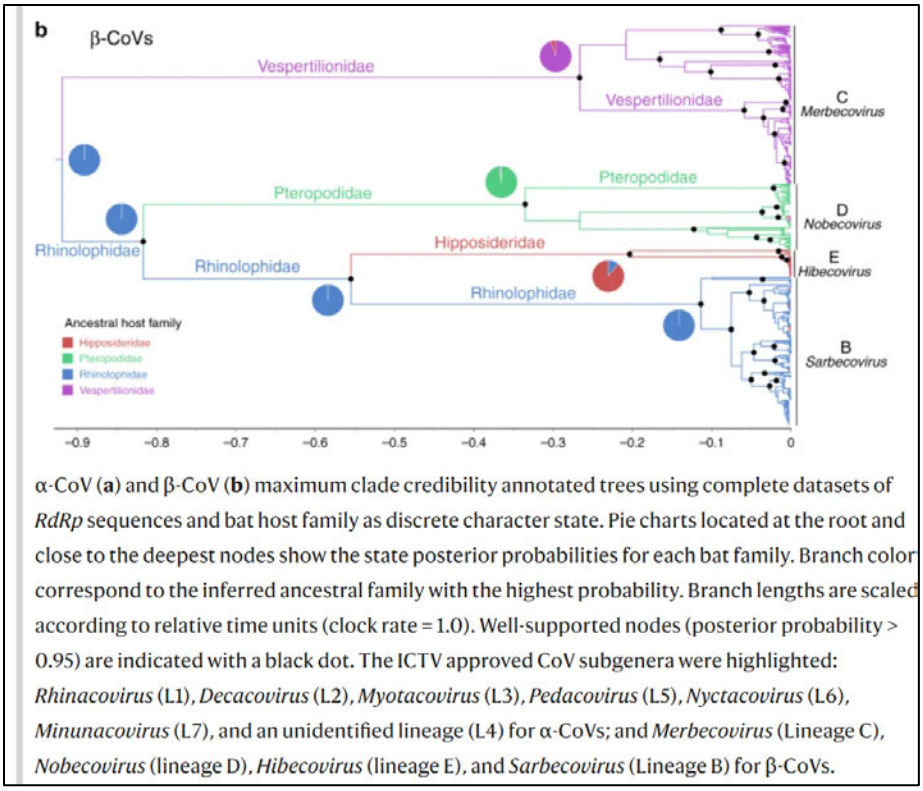
Evidence: The ancestor of SARS-CoV-2 can hypothetically only obtain a furin site by recombination outside of the sarbecovirus subgenera but there is strong evidence that coronavirus recombination is largely limited to the clade level, with limited evidence of sub-genera or genera recombination

- SARS-CoV-2 is a beta coronavirus, subgenera sarbecovirus and is the only sarbecovirus with a furin site.[104]
- Furin sites can be found in either alpha or gamma coronaviruses or the other beta coronavirus subgenera. The following Figure from reference 66 shows examples of such coronaviruses (furin containing viruses are shown in red):



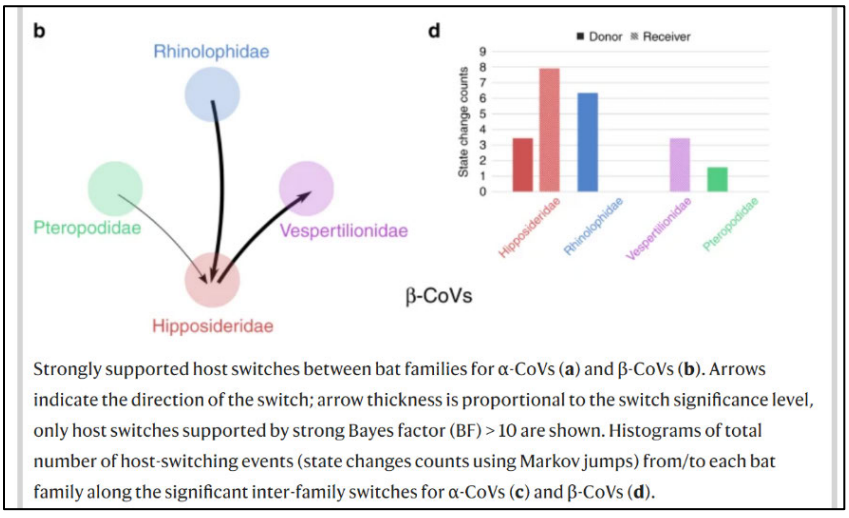
- To acquire a furin site in nature would require a co-infection between the CoV-2 sarbecovirus ancestor and a furin-containing non-sarbecovirus as shown above.
- However, there is no evidence of recombination in coronaviruses at either the genus level or the subgenus level; only at the clade level.[105][106]
- There is also evidence from Daszak and Shi that within the subgenera of the beta coronaviruses, there is bat host specificity. So, each subgenera of coronaviruses has a preferred bat host species. This reduces the opportunities for a co-host event to permit recombination.[107] The phylogeny below shows the problem of host incompatibility for beta coronaviruses:

[104] https://www.sciencedirect.com/science/article/pii/S1873506120304165#f0015
[105] file:///C:/Users/Steven%20Quay/Desktop/journal.pgen.1009272.pdf
[106] https://academic.oup.com/mbe/advance-article/doi/10.1093/molbev/msaa281/5955840
[107] https://www.nature.com/articles/s41467-020-17687-3#Sec2



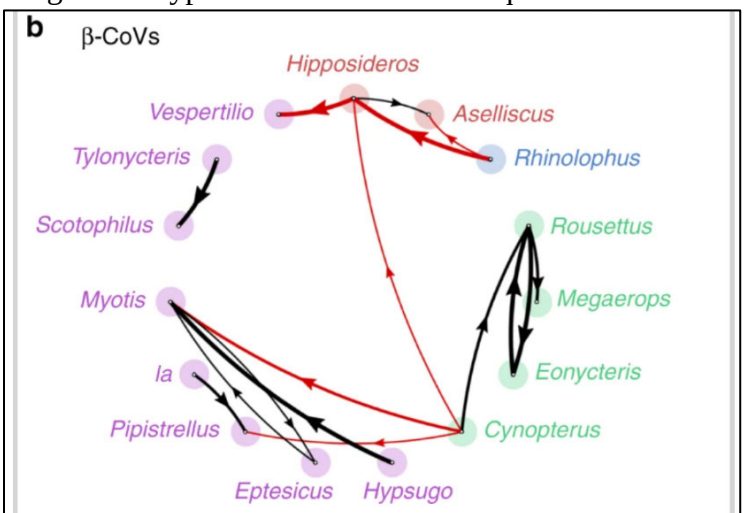
α-CoV (**a**) and β-CoV (**b**) maximum clade credibility annotated trees using complete datasets of *RdRp* sequences and bat host family as discrete character state. Pie charts located at the root and close to the deepest nodes show the state posterior probabilities for each bat family. Branch color correspond to the inferred ancestral family with the highest probability. Branch lengths are scaled according to relative time units (clock rate = 1.0). Well-supported nodes (posterior probability > 0.95) are indicated with a black dot. The ICTV approved CoV subgenera were highlighted: *Rhinacovirus* (L1), *Decacovirus* (L2), *Myotacovirus* (L3), *Pedacovirus* (L5), *Nyctacovirus* (L6), *Minunacovirus* (L7), and an unidentified lineage (L4) for α-CoVs; and *Merbecovirus* (Lineage C), *Nobecovirus* (lineage D), *Hibecovirus* (lineage E), and *Sarbecovirus* (Lineage B) for β-CoVs.

- Daszak and Shi also identified preferred directions of host switching. Since RaTG13, the closest coronavirus to SARS-CoV-2, is most closely related to viruses with bat hosts from the family, Rhinolophidae, it would be reasonable to expect furin-containing viruses from other bat hosts to migrate into Rhinolophidae, recombine by methods which have not been identified, and then the furin-containing sarbecovirus could evolve into the ancestor of SARS-CoV-2. Unexpectedly, Daszak et al. found host migration for the Rhinolophidae bats <u>only outward</u> and not inward, as required by the above, admittedly, convoluted process. The data Figure is shown here:



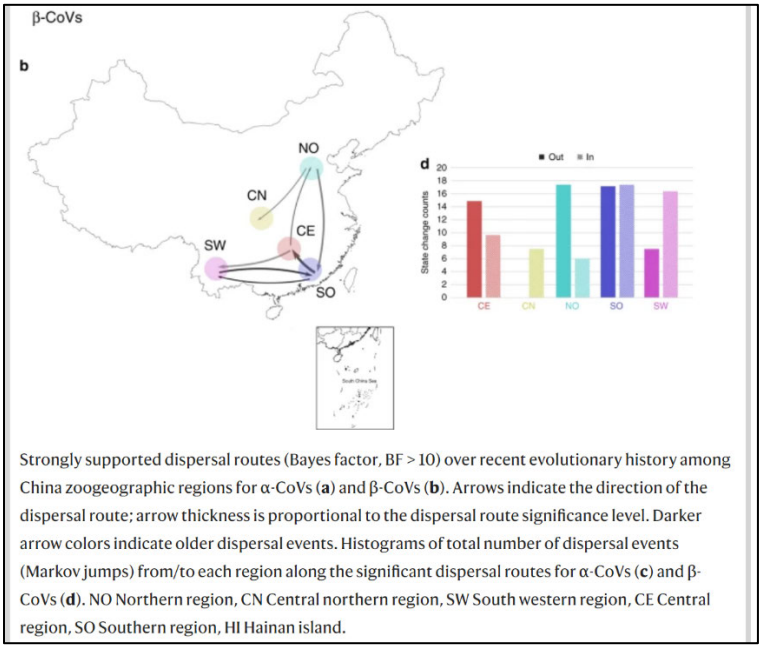
Strongly supported host switches between bat families for α-CoVs (**a**) and β-CoVs (**b**). Arrows indicate the direction of the switch; arrow thickness is proportional to the switch significance level, only host switches supported by strong Bayes factor (BF) > 10 are shown. Histograms of total number of host-switching events (state changes counts using Markov jumps) from/to each bat family along the significant inter-family switches for α-CoVs (**c**) and β-CoVs (**d**).

- Daszak and Shi also observed outward host switches from *Rhinolophus* at the genera level as well, also against a hypothesis for furin-site acquisition:



Strongly supported host switches between bat genera for α-CoVs (**a**) and β-CoVs (**b**) and their significance level (Bayes factor, BF). Only host switches supported by strong BF values >10 are shown. Line thickness is proportional to the switch significance level. Red lines correspond to host switches among bat genera belonging to different families, and black lines correspond to host switches among bat genera from the same family. Arrows indicate the direction of the switch. Genus names are colored according to the family they belong to using the same colors as in Figs. 2 and 3.

- Finally, this paper by Daszak and Shi states: "We used our Bayesian discrete phylogeographic model with zoogeographic regions as character states to reconstruct the spatiotemporal dynamics of CoV dispersal in China." If SARS-CoV-2 began in Yunnan and first crossed over into humans in Wuhan, this analysis should support a northernly spatiotemporal dispersal of beta coronaviruses. Unfortunately, Daszak and Shi cannot catch a break; their own data do not support the expected route of dispersion:



Strongly supported dispersal routes (Bayes factor, BF > 10) over recent evolutionary history among China zoogeographic regions for α-CoVs (**a**) and β-CoVs (**b**). Arrows indicate the direction of the dispersal route; arrow thickness is proportional to the dispersal route significance level. Darker arrow colors indicate older dispersal events. Histograms of total number of dispersal events (Markov jumps) from/to each region along the significant dispersal routes for α-CoVs (**c**) and β-CoVs (**d**). NO Northern region, CN Central northern region, SW South western region, CE Central region, SO Southern region, HI Hainan island.

As shown in the above Figure the only dispersal routes into Wuhan, which is in the CN region, are from the northern region. And the northern region has no inward dispersals from the SW, southwest region, where Yunnan and the origin of the ancestor of SARS-CoV-2, is located.

- Independent evidence documents that Hubei province does not have the bat species needed for SARS-CoV-2 reservoir host[108]

While statistical models of this data could be interesting and informative for general research about future spillovers, this is evidence will not be statistically quantified for this analysis. The evidence reduces the opportunities for subgenera co-infection and furin-site recombination into the CoV-2 ancestor and so the conservative rule that this is less consistent with a zoonotic origin (49%) versus laboratory origin (51%) will be used. There will be no subjective discount factor adjustment.

The results from the calculations are shown below.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.002	0.998
This is the outcome favors LO over ZO at 51% versus 49%		0.51
Impact of this evidence		Increases the likelihood of LO by 51/49 = 1.041
Impact of evidence calculation		1.041 x 0.998 = 1.039
Normalize this step of analysis	0.002/(0.002 + 1.039) = 0.002	1.039/(0.002 + 1.039) = 0.998

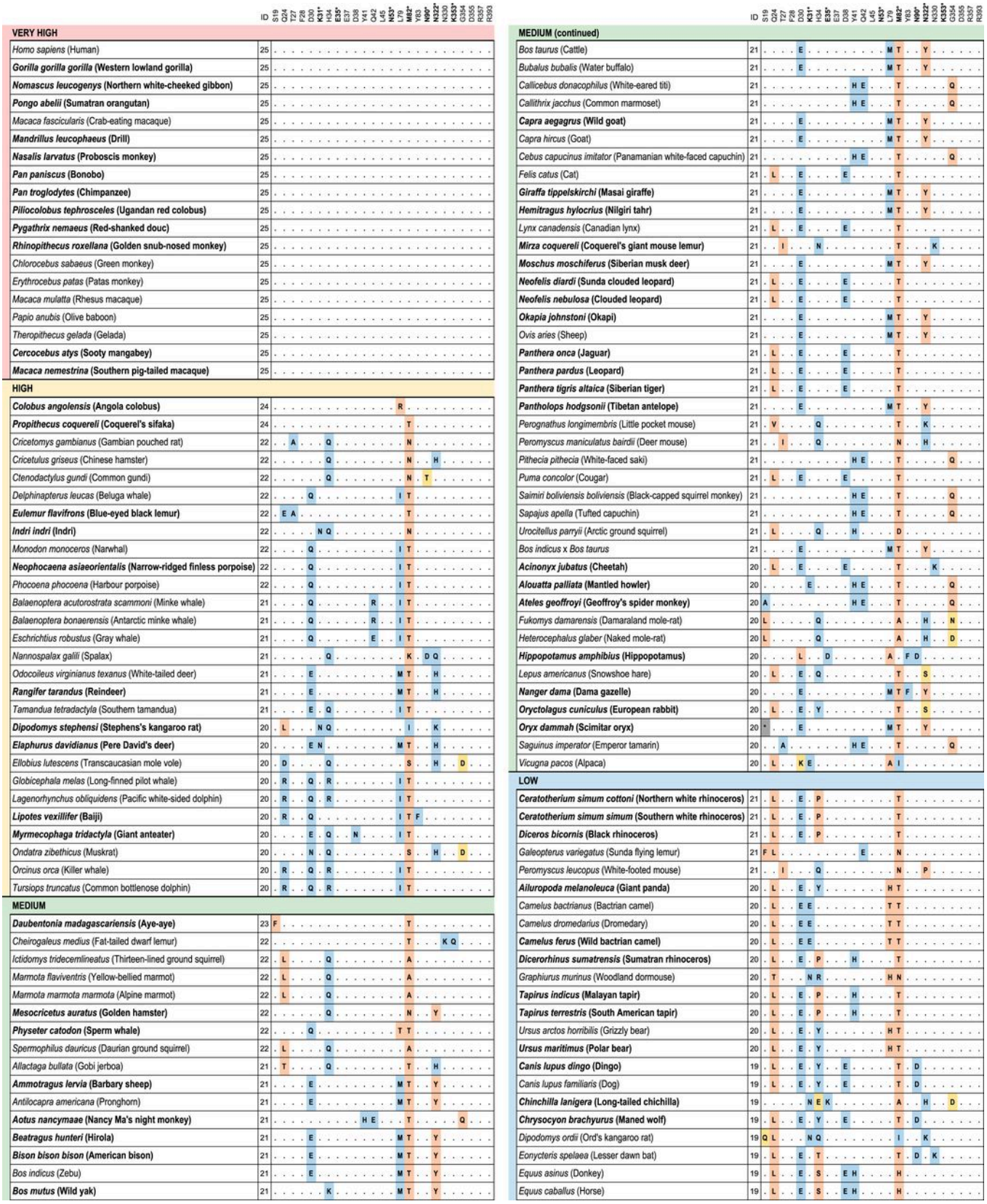
Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

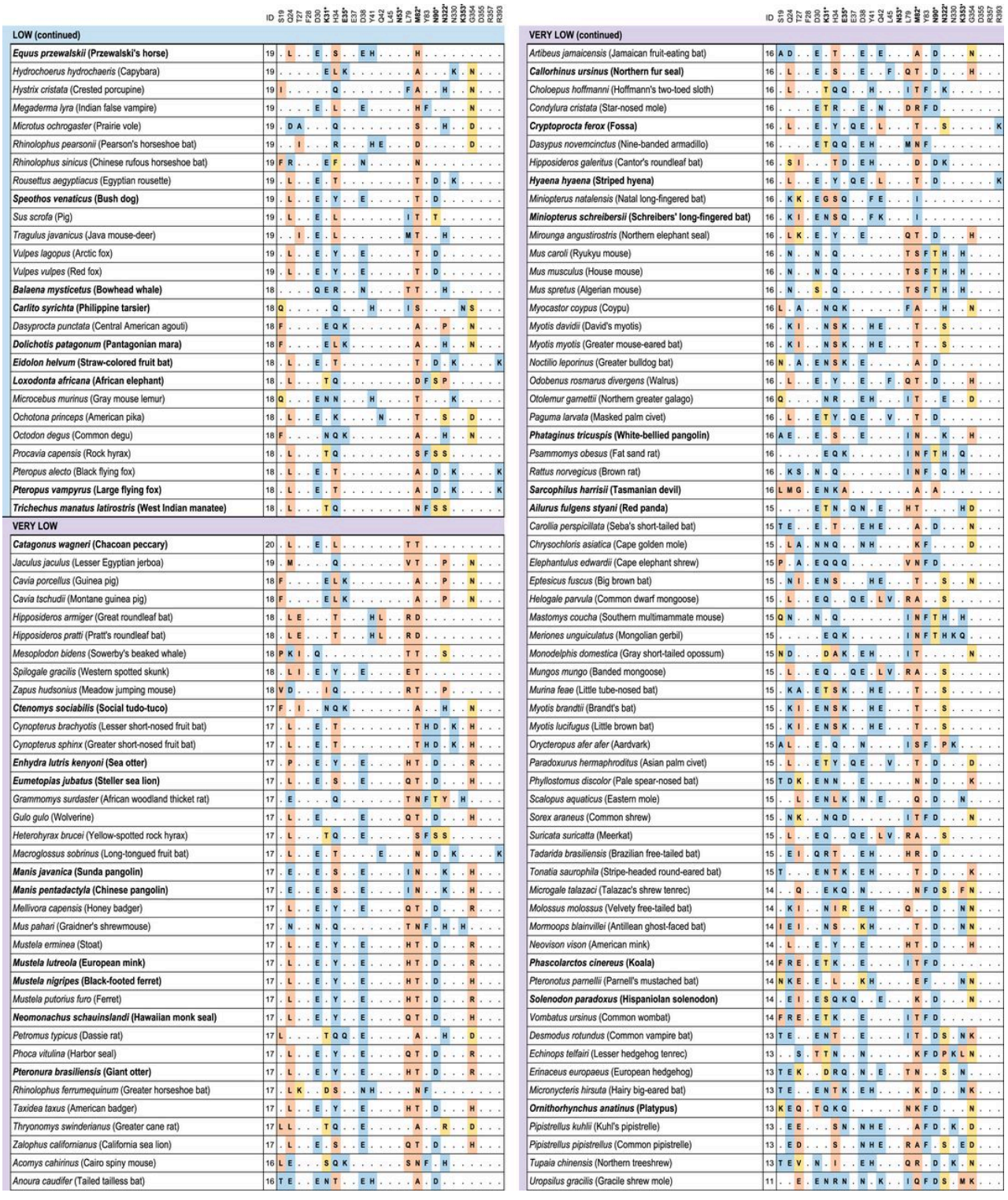
[108] file:///C:/Users/Steven%20Quay/Desktop/Zhangetal2009.pdf

Evidence: Of 410 vertebrate species tested for affinity to CoV-2 Spike Protein binding domain, primate ACE2 receptor, including human and VERO monkey cells, are the best at binding and bat species ACE2 are the worse, making direct bat-to-human host jumping extremely unlikely

- An examination of the ACE2 receptor binding domain amino acid sequences and their suitability for interacting with SARS-CoV-2 was performed in 410 vertebrates, including 252 mammals.[109]
- A five-category binding score was developed based on the conservation properties of 25 amino acids important for the binding between ACE2 and the SARS-CoV-2 spike protein.
- Only mammals fell into the medium to very high categories and only primates scored 25/25 for binding.
- This implies that SARS-CoV-2 is optimized for human ACE2-bearing cells from the first introduction into the human population, an observation that contradicts a zoonotic origin.
- It also suggests that other primates may be the proximate species from which SARS-CoV-2 entered the human population.
- Both VERO monkey kidney cells and ACE2 humanized mice would quality as an intermediate species by this criterion.
- Surprisingly, "all chiropterans (bats) scored low (n = 8) or very low (n = 29), including the Chinese rufous horseshoe bat, from which a coronavirus (SARSr-CoV ZC45) related to SARS-CoV-2 was identified."
- This is evidence that bats are probably not a reservoir host for SARS-CoV-2.
- A separate study observed: "Severe acute respiratory syndrome coronavirus 2 did not replicate efficiently in 13 bat cell lines."[110]
- The following two Tables are taken from the paper and are organized according to ACE2 SARS-CoV-2 affinity, from highest to lowest:

[109] https://www.pnas.org/content/117/36/22311
[110] https://wwwnc.cdc.gov/eid/article/26/12/20-2308_article


ID
S19 Q24 T27 F28 D30 K31* H34 E35* E37 D38 Y41 Q42 L45 N53* L79 M82* Y83 N90* N322* N330 K353* G354 D355 R357 R393
VERY HIGH
Homo sapiens (Human) 25
Gorilla gorilla gorilla (Western lowland gorilla) 25
Nomascus leucogenys (Northern white-cheeked gibbon) 25
Pongo abelii (Sumatran orangutan) 25
Macaca fascicularis (Crab-eating macaque) 25
Mandrillus leucophaeus (Drill) 25
Nasalis larvatus (Proboscis monkey) 25
Pan paniscus (Bonobo) 25
Pan troglodytes (Chimpanzee) 25
Piliocolobus tephrosceles (Ugandan red colobus) 25
Pygathrix nemaeus (Red-shanked douc) 25
Rhinopithecus roxellana (Golden snub-nosed monkey) 25
Chlorocebus sabaeus (Green monkey) 25
Erythrocebus patas (Patas monkey) 25
Macaca mulatta (Rhesus macaque) 25
Papio anubis (Olive baboon) 25
Theropithecus gelada (Gelada) 25
Cercocebus atys (Sooty mangabey) 25
Macaca nemestrina (Southern pig-tailed macaque) 25
HIGH
Colobus angolensis (Angola colobus) 24
Propithecus coquereli (Coquerel's sifaka) 24
Cricetomys gambianus (Gambian pouched rat) 22
Cricetulus griseus (Chinese hamster) 22
Ctenodactylus gundi (Common gundi) 22
Delphinapterus leucas (Beluga whale) 22
Eulemur flavifrons (Blue-eyed black lemur) 22
Indri indri (Indri) 22
Monodon monoceros (Narwhal) 22
Neophocaena asiaeorientalis (Narrow-ridged finless porpoise) 22
Phocoena phocoena (Harbour porpoise) 22
Balaenoptera acutorostrata scammoni (Minke whale) 21
Balaenoptera bonaerensis (Antarctic minke whale) 21
Eschrichtius robustus (Gray whale) 21
Nannospalax galili (Spalax) 21
Odocoileus virginianus texanus (White-tailed deer) 21
Rangifer tarandus (Reindeer) 21
Tamandua tetradactyla (Southern tamandua) 21
Dipodomys stephensi (Stephens's kangaroo rat) 20
Elaphurus davidianus (Pere David's deer) 20
Ellobius lutescens (Transcaucasian mole vole) 20
Globicephala melas (Long-finned pilot whale) 20
Lagenorhynchus obliquidens (Pacific white-sided dolphin) 20
Lipotes vexillifer (Baiji) 20
Myrmecophaga tridactyla (Giant anteater) 20
Ondatra zibethicus (Muskrat) 20
Orcinus orca (Killer whale) 20
Tursiops truncatus (Common bottlenose dolphin) 20
MEDIUM
Daubentonia madagascariensis (Aye-aye) 23
Cheirogaleus medius (Fat-tailed dwarf lemur) 22
Ictidomys tridecemlineatus (Thirteen-lined ground squirrel) 22
Marmota flaviventris (Yellow-bellied marmot) 22
Marmota marmota marmota (Alpine marmot) 22
Mesocricetus auratus (Golden hamster) 22
Physeter catodon (Sperm whale) 22
Spermophilus dauricus (Daurian ground squirrel) 22
Allactaga bullata (Gobi jerboa) 21
Ammotragus lervia (Barbary sheep) 21
Antilocapra americana (Pronghorn) 21
Aotus nancymaae (Nancy Ma's night monkey) 21
Beatragus hunteri (Hirola) 21
Bison bison bison (American bison) 21
Bos indicus (Zebu) 21
Bos mutus (Wild yak) 21
MEDIUM (continued)
Bos taurus (Cattle) 21
Bubalus bubalis (Water buffalo) 21
Callicebus donacophilus (White-eared titi) 21
Callithrix jacchus (Common marmoset) 21
Capra aegagrus (Wild goat) 21
Capra hircus (Goat) 21
Cebus capucinus imitator (Panamanian white-faced capuchin) 21
Felis catus (Cat) 21
Giraffa tippelskirchi (Masai giraffe) 21
Hemitragus hylocrius (Nilgiri tahr) 21
Lynx canadensis (Canadian lynx) 21
Mirza coquereli (Coquerel's giant mouse lemur) 21
Moschus moschiferus (Siberian musk deer) 21
Neofelis diardi (Sunda clouded leopard) 21
Neofelis nebulosa (Clouded leopard) 21
Okapia johnstoni (Okapi) 21
Ovis aries (Sheep) 21
Panthera onca (Jaguar) 21
Panthera pardus (Leopard) 21
Panthera tigris altaica (Siberian tiger) 21
Pantholops hodgsonii (Tibetan antelope) 21
Perognathus longimembris (Little pocket mouse) 21
Peromyscus maniculatus bairdii (Deer mouse) 21
Pithecia pithecia (White-faced saki) 21
Puma concolor (Cougar) 21
Saimiri boliviensis boliviensis (Black-capped squirrel monkey) 21
Sapajus apella (Tufted capuchin) 21
Urocitellus parryii (Arctic ground squirrel) 21
Bos indicus x Bos taurus 21
Acinonyx jubatus (Cheetah) 20
Alouatta palliata (Mantled howler) 20
Ateles geoffroyi (Geoffroy's spider monkey) 20
Fukomys damarensis (Damaraland mole-rat) 20
Heterocephalus glaber (Naked mole-rat) 20
Hippopotamus amphibius (Hippopotamus) 20
Lepus americanus (Snowshoe hare) 20
Nanger dama (Dama gazelle) 20
Oryctolagus cuniculus (European rabbit) 20
Oryx dammah (Scimitar oryx) 20
Saguinus imperator (Emperor tamarin) 20
Vicugna pacos (Alpaca) 20
LOW
Ceratotherium simum cottoni (Northern white rhinoceros) 21
Ceratotherium simum simum (Southern white rhinoceros) 21
Diceros bicornis (Black rhinoceros) 21
Galeopterus variegatus (Sunda flying lemur) 21
Peromyscus leucopus (White-footed mouse) 21
Ailuropoda melanoleuca (Giant panda) 20
Camelus bactrianus (Bactrian camel) 20
Camelus dromedarius (Dromedary) 20
Camelus ferus (Wild bactrian camel) 20
Dicerorhinus sumatrensis (Sumatran rhinoceros) 20
Graphiurus murinus (Woodland dormouse) 20
Tapirus indicus (Malayan tapir) 20
Tapirus terrestris (South American tapir) 20
Ursus arctos horribilis (Grizzly bear) 20
Ursus maritimus (Polar bear) 20
Canis lupus dingo (Dingo) 19
Canis lupus familiaris (Dog) 19
Chinchilla lanigera (Long-tailed chichilla) 19
Chrysocyon brachyurus (Maned wolf) 19
Dipodomys ordii (Ord's kangaroo rat) 19
Eonycteris spelaea (Lesser dawn bat) 19
Equus asinus (Donkey) 19
Equus caballus (Horse) 19



While statistical models of this data could be interesting and informative, this is evidence will not be statistically quantified for this analysis. The evidence is another way of looking at the pre-adapted state of the CoV-2 for humans and suggests that primate animals, monkey cell cultures like the VERO cell, and humanized mice could be likely laboratory models that were used by the

WIV in GoF research. This will contribute a 51%/49% contribution in favor of laboratory compared to zoonotic origin. There will be no subjective discount factor adjustment.

The results from the calculations are shown below.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.002	0.998
This is the outcome favors LO over ZO at 51% versus 49%		0.51
Impact of this evidence		Increases the likelihood of LO by 51/49 = 1.041
Impact of evidence calculation		1.041 x 0.998 = 1.039
Normalize this step of analysis	0.002/(0.002 + 1.039) = 0.002	1.039/(0.002 + 1.039) = 0.998

Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

Evidence: Did a Review of Samples Collected from a Mineshaft Cause the COVID-19 Pandemic?[111]

Abstract. The origin of the COVID-19 pandemic caused by SARS-CoV-2 has been hotly debated. Proponents of the natural spillover theory allege that the virus jumped species, possibly via an intermediary host, to cross over to humans via the wildlife trade or by other means. Proponents of a rival theory claim that the virus escaped from a laboratory in Wuhan. This research presents circumstantial evidence of a transmission route via a late 2019 review of samples collected from a mineshaft in Mojiang, Yunnan Province, China. It examines the activity at the Wuhan Institute of Virology in late 2019, when samples from a mineshaft associated with a suspected SARS outbreak were being reviewed. It proposes that spillover occurred during this review of samples including of a virus (BtCoV/4991) only 1% different to SARS-CoV-2 in its RNA-dependent RNA polymerase (RdRp).

It is a meticulous sourced analysis. It purposely avoids the question of whether SARS-CoV-2 was being grown or manipulated in the laboratory, but only addresses the evidence that events in the fall of 2019 are consistent with a laboratory accident.

This will not be used to adjust the likelihoods.

Current likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

[111] https://zenodo.org/record/4029545#.X-x_f9gzbOg. Author anonymous. A meticulously documented analysis that concludes an accident occurred at the Wuhan Institute of Virology during the fall of 2019. Includes many primary documents from Mandarin. No direct evidence of 'what' was the nature of the accident or if it was SARS-CoV-2.

Evidence: The Hunan market was not the source of SARS-CoV-2

From the WHO Terms of Reference for the investigation of the origin of SARS-CoV-2:[112]

"The Huanan wholesale market is a large market (653 stalls and more than 1180 employees) mainly supplying seafood products but also fresh fruits and vegetables, meat, and live animals. In late December 2019, 10 stall operators were trading live wild animals including chipmunks, foxes, racoons, wild boar, giant salamanders, hedgehogs, sika deer, and many others. Farmed, wild and domestic animals were also traded at the market including snakes, frogs, quails, bamboo rats, rabbits, crocodiles, and badgers. The market was closed on 1 January 2020, and several investigations followed, including environmental sampling, as well as sampling of frozen animal carcasses at the market. **Of the 336 samples collected from animals, none were PCR positive for SARS-CoV-2**, whereas 69 out of 842 environmental samples were positive by PCR for SARS-CoV-2. Sixty- one of those (88%) were from the western wing of the market. Of these, 22 samples were from 8 different drains and sewage, and 3 viruses were isolated, sequenced and shared on GISAID. These were virtually identical to the patient samples collected at the same time (>99.9 % homology)."

For contrast, with SARS-CoV-1 91 civets & 15 raccoon dogs in wet markets were tested with 106/106, 100% positive.[113]

This will not be used to adjust the likelihoods.

Current likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

[112] https://drive.google.com/file/d/1rx0W2efbE0R1Aq-lALWTqD22VsWbTlO-/view
[113] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1212604/

Evidence: Analysis of the hospital of admission for COVID-19 patients during December 2019 places "ground zero" for the outbreak somewhere along Line 2 of the Wuhan Metro System.

Line 2 carries one million people per day and services the Wuhan Institute of Virology, the Hunan Seafood Market, the high-speed rail system, and the Wuhan International Airport

A preprint manuscript[114] reported that the earliest genomic cluster of SARS-CoV-2 patients is a group of four individuals associated with the General Hospital of Central Theater Command of People's Liberation Army (PLA) of China in Wuhan. This cluster contains the "Founder Patients" of both Clade A and Clade B, from which every SARS-CoV-2 coronavirus that has infected every patient with COVID-19 anywhere in the world has arisen.

The PLA Hospital is about one mile from the Wuhan Institute of Virology (WIV) and the closest hospital to WIV. Both the PLA Hospital and WIV are serviced by Line 2 of the Wuhan Metro System. The Hunan Seafood Market is also located adjacent to Line 2. All patients between December 1st, 2019 and early January 2020 were first seen at hospitals that also are serviced by Line 2 of the Metro system.

With 40 hospitals located near seven of the nine Metro Lines, the likelihood that all early patients were seen at hospitals only near Line 2 by chance is about 1 in 68,500 (p-value = 0.0000146). The inference then would be that the early spread of SARS-CoV-2 was through human-to human transmission on Line 2.

Line 2 carries one million passengers per day and assuming most are round trip business workers going to and from work in the morning and evening, represents 500,000 riders or about 5% of the Wuhan population. A very recent publication determined that, in fact, 500,000 residents of Wuhan contracted COVID-19, a ten-fold upper estimate.[115] The coincidence of my prediction that 500,000 riders on Line 2 were likely exposed to SARS-CoV-2 in late 2019 and the recent admission from Chinese CDC that Wuhan had 500,000 COVID-19 cases is duly noted!

Line 2 connects to all eight other lines of the Wuhan Metro System (1, 3, 4, 6, 7, 8, 11, and Yanglu) facilitating rapid spread in Wuhan and Hubei Province, and also services both the high-speed rail station (Hankou Railway Station), facilitating rapid spread throughout China, and the Wuhan International Airport (Tianhe International Airport), facilitating rapid spread throughout Asia, Europe, and to the United States. In fact, direct human-to-human spread from the Reference Sequence patient to patients around the world is suggested by an unexpectedly reduced genome base substitution rate seen in patient specimens in cities with direct flights from Wuhan.

[114] https://zenodo.org/record/4119263#.X-rszNgzbOg
[115] https://mp.weixin.qq.com/s/LXTfDmsQLf3qZnu_S_MxcA ; https://thehill.com/policy/international/china/531935-study-shows-wuhan-coronavirus-cases-may-have-been-10-times-higher

In a separate paper by Quay and Dr. Martin Lee, Adjunct Professor of Statistics, UCLA, from May 2020, now accepted for publication in *Epidemics,*[116] the authors provide evidence that COVID-19 was appearing in California as early as the first week of 2020. This is likely due to direct flights connecting Line 2 to the Wuhan airport and then to San Francisco.

In conclusion, Line 2 of the Wuhan Metro System services the PLA Hospital with the first genomic cluster of patients with COVID-19, the hospitals where patients first went in December 2019 and early January 2020 and is the likely conduit for human-to-human spread throughout Wuhan, China, and the world.

The following slide overview provides a visual analysis of this evidence:

Zoonotic Origin	Laboratory Origin
Hunan Seafood Market	Wuhan Institute of Virology (WIV); Wuhan Center for Disease Control and Prevention (CDC)

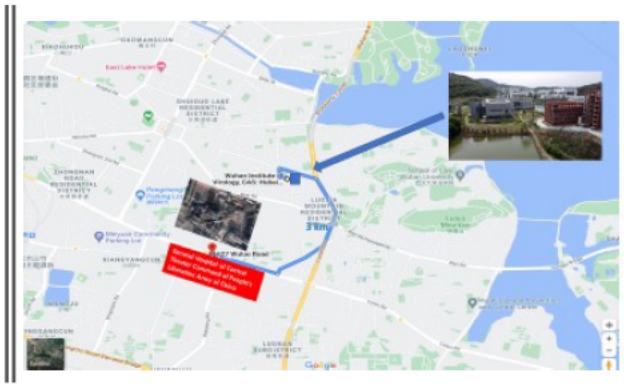
How did COVID start? • **After 10 months we still don't know!**

[116] https://www.researchgate.net/publication/341742303_COVID-19_May_Have_Have_Reached_United_States_in_January_2020_05272020

SARS 2003 and MERS 2015

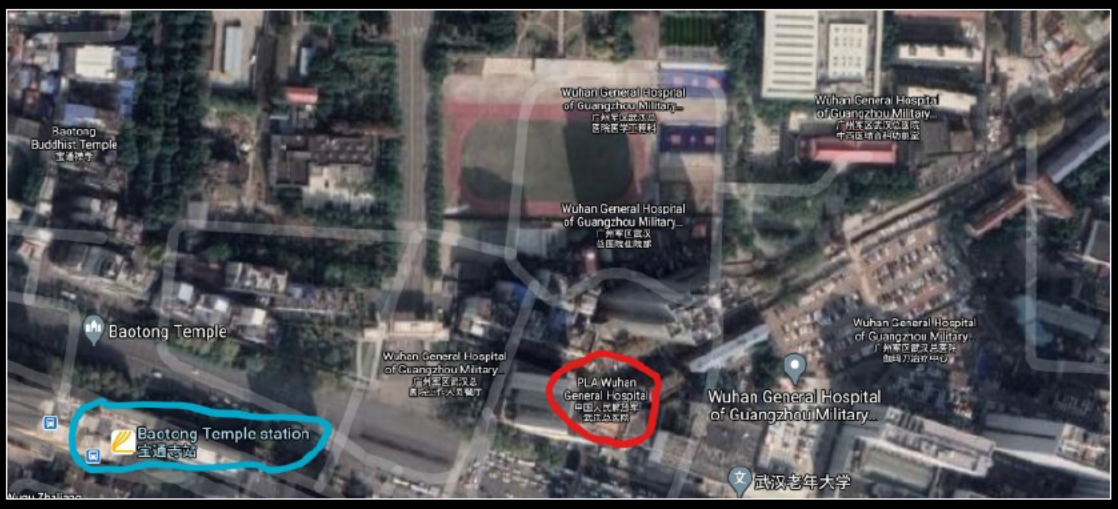
- Within four months (SARS 2003) and nine months (MERS 2015) we knew the zoonotic host

Virus detail
Virus name: hCoV-19/Wuhan/WH01/2019
Accession ID: EPI_ISL_406798
Type: Betacoronavirus
Lineage /GISAID Clade: B (2)
Passage details/history: Original
Sample information
Collection date: 2019-12-26
Location: Asia / China / Hubei / Wuhan
Host: Human
Additional location information:
Gender: male
Patient age: 44
Patient status: unknown
Specimen source: Bronchoalveolar Lavage NCIT:C51913
Additional host information:
Outbreak:
Last vaccinated:
Treatment:
Sequencing technology: DNBSEQ
Assembly method: SPAdes v.3.12.0
Coverage: 65x
Comment:
Institute information
Originating lab: General Hospital of Central Theater Command of People's Liberation Army of China
Address: NO.627 Wuhan Road, Wuchang District, Wuhan, China



GISAID Database

Earliest cases at the PLA Hospital



PLA Hospital is part of the Joint Logistic Support Force Complex

Position in RS	Bat-SL-CoVZC45	Bat-SL-CoVZXC21	RaTG13	PLA-4	PLA-3	PLA-2	Hu-1 Ref seq	PLA-1	GISAID #1
5' UTR	1-5 missing	1-5 missing	1-15 missing	1-16 missing	1-20 missing	1-36 missing	Intact	1-25 missing	Intact
3778	A	A	A	A	A	A	A	A	G
6968	T	T	C	C	C	C	C	A	C
8782	T	T	T	T	C	C	C	C	C
8987	T	T	T	T	T	T	T	T	A
11764	T	T	T	T	T	NA - Note 1	T	A	T
28144	C	C	C	C	T	T	T	T	T
3' UTR	last 4 poly-A missing	last 4 poly-A missing	last 13 poly-A missing	last 15 poly-A missing	last 15 poly-A missing	NA - Note 1	Intact	last 12 poly-A missing	last 4 poly-A missing
Genome length	29802	29732	29855	29872	29868	NA - Note 1	29903	29866	29899

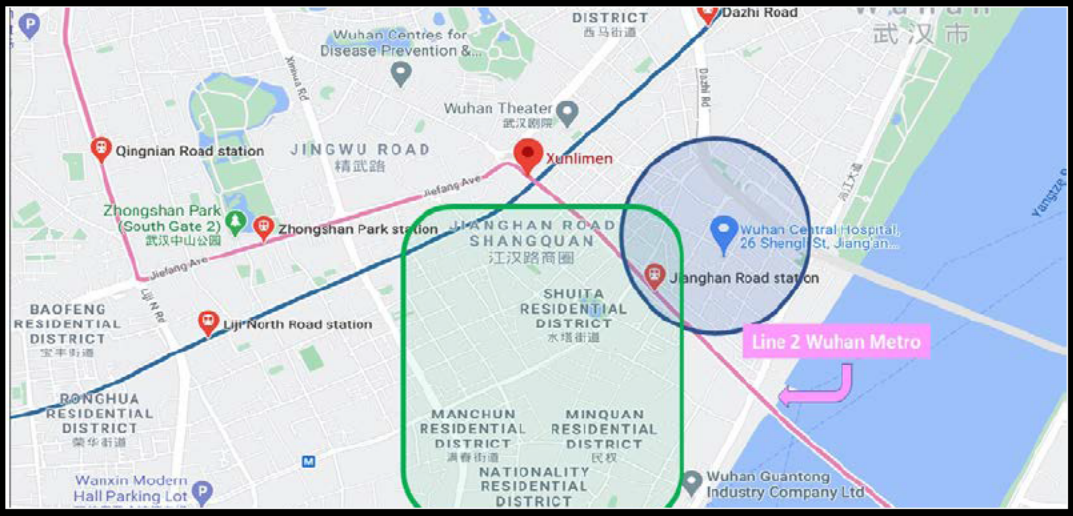
Clade A SNPs | Clade B SNPs | Non-RaTG13 DNPs

Note 1 - GISAID record: "Long stretches of NNNs (34.45% of overall sequence). Gap of 13 nucleotide(s) found at refpos 26171 (FRAMESHIFT). Gap of 13 nucleotides when compared to the reference sequence. 0.40% Unique Mutations."

The PLA patient cluster

- PLA-4 is genetically the closest human infection to the three closest bat viruses
- The four PLA patients have the close sequence pattern usually seen only in family transmissions

The Wuhan Metro System



Methodology: Metro station line for intersection of hospital catchment zone and residential living district zone was identified

All COVID patients from Dec 1 to early Jan were admitted to hospitals on Metro Line 2

This had the likelihood of happening by chance of one in 68,500

Feature	Relationship to Pandemic
Line 2 carried 1 MM passengers a day before COVID	Assuming 2 trips/d for commuters, about 5% of the Wuhan population uses this Line, making it an efficient transmission route for all of Wuhan as well as Hubei Provence. A single patient can leave a droplet/aerosol cloud for hours to infect others.
Line 2 shares stations with every other Metro Line	Permits human-to-human spread to every part of Wuhan at the stations shared with Line 2
Line 2, Hankou Railway Station	Connects Wuhan to all of China by high speed rail
Line 2, Tianhe International Airport	International destinations: New York City, San Francisco, London, Tokyo, Rome, Istanbul, Dubai, Paris, Sydney, Bali, Bangkok, Moscow, Osaka, Seoul, and Singapore.

The Line 2 COVID Conduit

The Line 2 COVID Conduit

- Line 2 Hankou High Speed Railway Station to all of China
- Line 2 Tianhe International Airport Station to the world

The Hunan Seafood Market, Wuhan Institute of Virology, and the Wuhan CDC, all locations suggested to be the possible source of SARS-CoV-2 in Wuhan, are also all serviced by Line 2 of the Metro system, suggesting this public transit line should become the focus for further investigations into the origin of this pandemic.

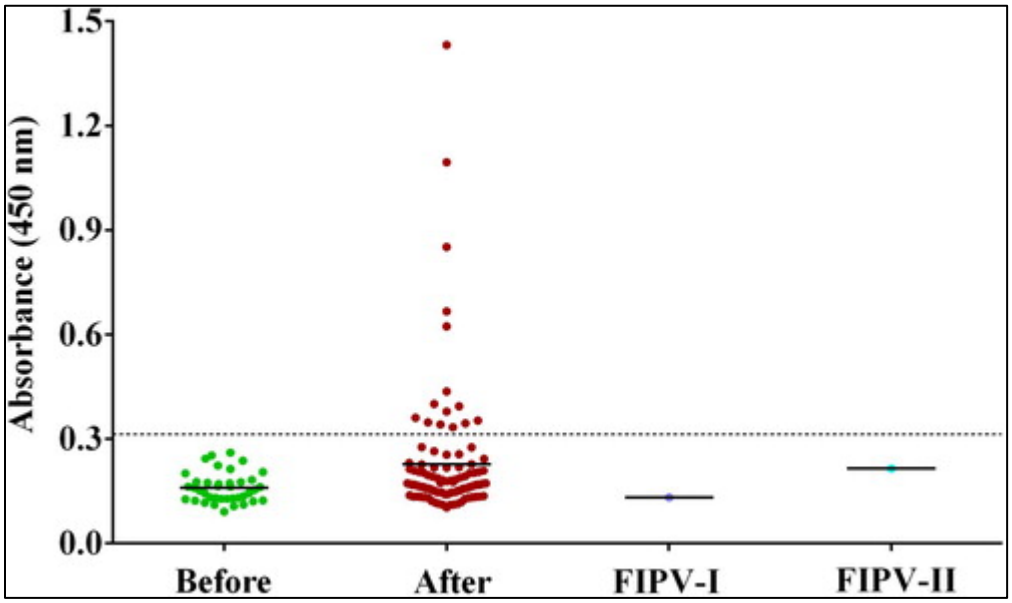
Given that the Hunan Seafood Market has been removed as a source for the origin of CoV-2, this evidence will contribute a 51%/49% contribution in favor of laboratory compared to zoonotic origin. There will be no Subjective Discount Factor adjustment.

The results from the calculations are shown below.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.002	0.998
This is the outcome favors LO over ZO at 51% versus 49%		0.51
Impact of this evidence		Increases the likelihood of LO by 51/49 = 1.041
Impact of evidence calculation		1.041 x 0.998 = 1.039
Normalize this step of analysis	0.002/(0.002 + 1.039) = 0.002	1.039/(0.002 + 1.039) = 0.998

Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

Evidence: SARS-CoV-2 infection, based on antibody seroconversion, was not found in 39 archived specimens taken from cats (1/3 feral) between March and May 2019[117]



Based on these results, the prevalence of SARS-CoV-2 in domestic and feral cats prior to January 2020 is less than 8% with a 90% confidence interval.

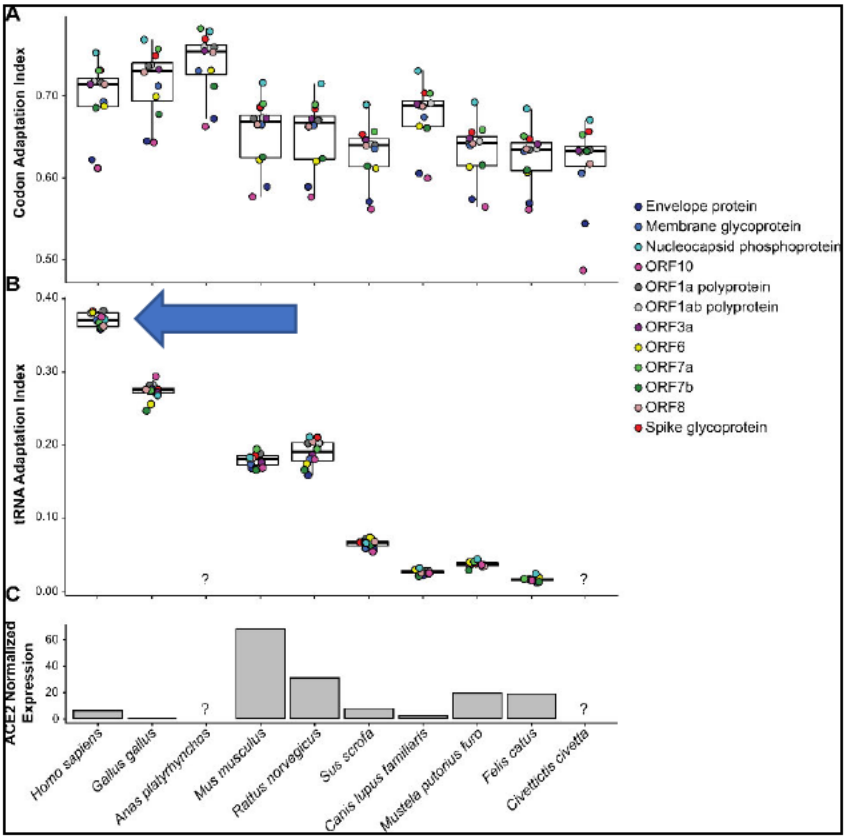
This will not be used to adjust the likelihoods.

Current likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

[117] https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1817796

Evidence: The extraordinary pre-adaption of SARS-CoV-2 for human cells is demonstrated by a paper looking at a tRNA adaption index.[118]

"The proteome of SARS-CoV-2 is mainly composed of the replicase polyprotein (ORF1ab) and of structural proteins: the spike glycoprotein, the membrane and envelope proteins, and the nucleoprotein [41]. Based on the genomic codon usage of each of the possible host species, we compute the codon adaptation index (CAI) and the tRNA adaptation index (tAI) to estimate the translational efficiency of SARS-CoV-2 proteins in each host (Fig 3A and 3B and S2 Table). Humans are among the top three species whose CAIs are mostly over 0.70, together with ducks and chickens. In terms of the tAI, humans show the highest translational adaptation among all others, followed by chickens, and, to some extent, mice and rats. On the other hand, cats, ferrets, pigs, and dogs are less translationally adapted than humans both by CAI and tAI."



As shown in panel B above, the tRNA Adaption Index is highest, by far, for humans (blue arrow) followed by the red junglefowl. This is additional evidence of the extraordinary adaption of SARS-CoV-2 to humans from the very beginning. This also is the first evidence of a reasonable intermediate host but based only on these *in silico* data.

This will not be used to adjust the likelihoods.

Current likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

[118] https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1008450#pcbi.1008450.s004

Evidence: **Evidence of Lax procedures and disregard of laboratory safety protocols and regulations in China, including the Wuhan Institute of Virology**

A collection[119] from the Chinese Q&A website, https://www.zhihu.com/, of first-hand documentation of laboratory safety breaches and incidents within a large number of laboratories with diverse research subjects and purposes in the People's Republic of China (PRC) is provided. The laboratories involved include Chemistry labs, Biolabs, Computer labs as well as Physics and Engineering labs.

From this first-hand documentation, we obtained evidence of relaxed safety regulations and frequent breaches of such regulations, with reasons ranging from poor training/education on lab safety and chronic ignorance of safety rules, to intentional breaches of protocols for purposes other than the research projects of the lab(s) of which the breach was documented in.

Such breaches often resulted in safety accidents ranging from physical injury, chemical burns, chemical leaks, and damage to property, to lab-acquired infection and escape of in-lab pathogens. With consequences ranging from personal-level to institution-wide impacts.

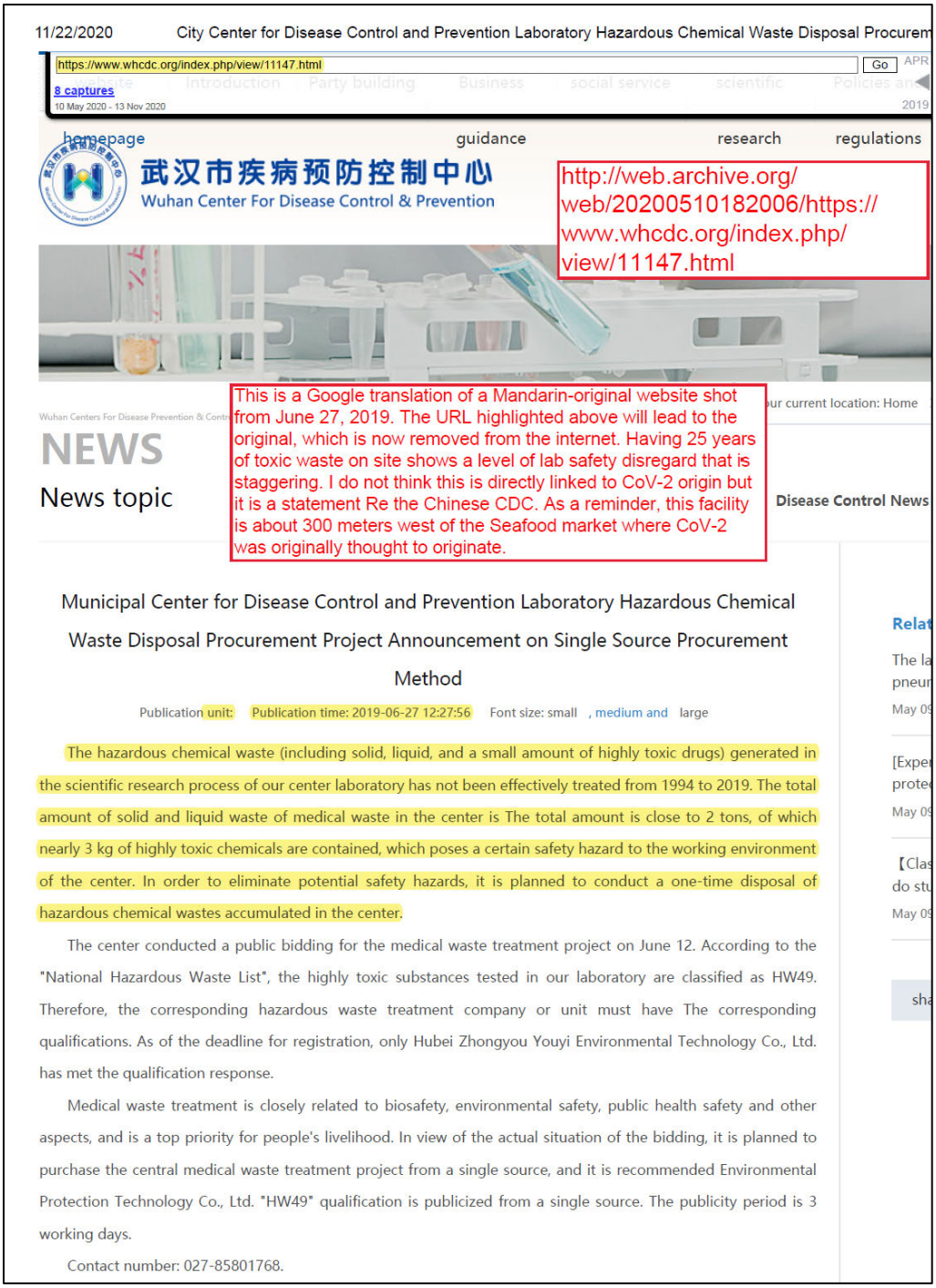
Here is the reference to the State Department cables concerning safety concerns at the WIV.[120]

The following document shows that in June 2019, the Chinese CDC was soliciting for the removal of 25-years-worth of solid and liquid medical waste. The total weight is close to two tons including three kg of highly toxic waste.

This is a Google translation of a Mandarin-original website shot from June 27, 2019. The URL highlighted above will lead to the original, which now has been removed from the internet. Having 25 years of toxic waste on site shows a staggering level of disregard for lab safety.

I do not think this is directly linked to CoV-2 origin, but it is a statement about the Chinese CDC. As a reminder, this facility is about 300 meters west of the Seafood market where CoV-2 was first thought to have originated.

[119] https://zenodo.org/record/4307879#.X-yUo9gzbOh
[120] https://foia.state.gov/Search/Results.aspx?caseNumber=F-2020-05255

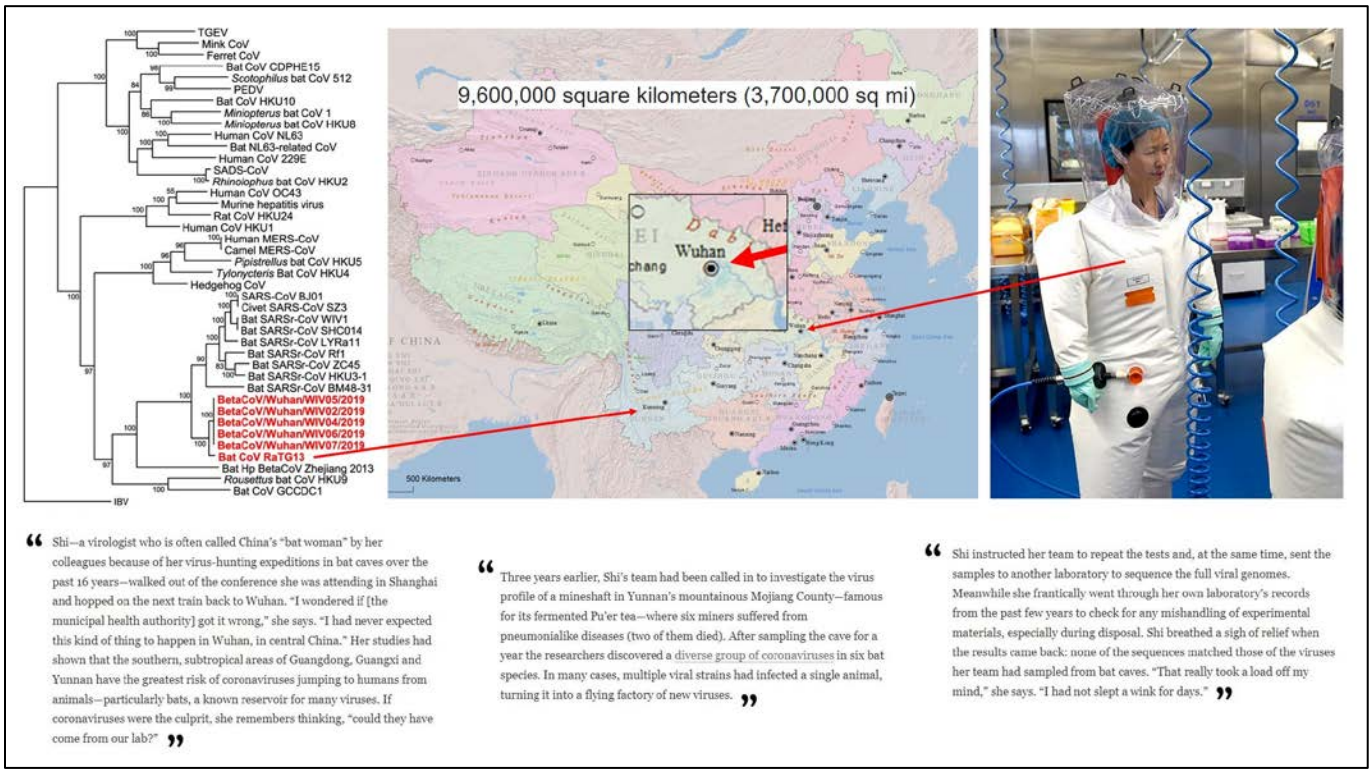


This will not be used to adjust the likelihoods.

Current likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

Evidence: The careful words of Dr. Shi do NOT say she did not have SARS-CoV-2 at the WIV.

This Figure contains quotes from an article about Dr. Shi and her reaction to the beginning of the COVID-19 pandemic.



Notice in the last frame Dr. Shi says two strange sentences:

Sentence 1: “…she frantically went through her own laboratory’s records from the past few years to check for any mishandling of experimental materials, **especially during disposal**.”

Why did she mention disposal? If you don’t know what you are looking for this, “especially during disposal,” is a bit of an odd qualifier. Other evidence from Wuhan suggests that, in fact, disposal may have been a likely source of the accidental lab release.

Sentence 2: “She breathed a sigh of relief when the results came back: none of the sequences matched those of the viruses her team had sampled from bat caves.”

If Dr. Shi had created SARS-CoV-2 as a chimera, perhaps starting with one of those cave viruses, of course you would no longer have a sequence match. This is a probably truthful statement that leaves open the question of lab creation.

This will not be used to adjust the likelihoods.

Current likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

Evidence: The Good, the Bad, and the Ugly: a review of SARS Lab Escapes[121]

In 2003–04, in the wake of the SARS epidemics, there were multiple cases of laboratory acquired infection (LAI) with SARS within just a few months: first in a P3 in Singapore, then in a military P4 in Taipei and last a protracted case in a P3 in Beijing. The 'WHO SARS Risk Assessment and Preparedness Framework' has a good summary of these lab accidents:

Since July 2003, there have been four occasions when SARS has reappeared. Three of these incidents [note: Singapore, Taipei and Beijing] were attributed to breaches in laboratory biosafety and resulted in one or more cases of SARS. The most recent laboratory incident [note: in Beijing] resulted in 9 cases, 7 of which were associated with one chain of transmission and with hospital spread. Two additional cases at the same laboratory with a history of illness compatible with SARS in February 2004 were detected as part of a survey of contacts at the facility.[i.1]

This article reviews some of these cases and discusses briefly some of the insights that were gained from these at the time.

Another article along the same lines is, "10 incidents discovered at the nation's biolabs"[122] This included Dr. Baric's laboratory in which "(b)etween April 2013 and September 2014, eight individual mouse escapes were reported at the University of North Carolina-Chapel Hill. Several of the mice were infected with either SARS or the H1N1 flu virus."

Dozens of holes in BSL-4 'spacesuits'

As a key protection against the world's most deadly pathogens, including the Ebola virus, scientists in the BSL-4 labs at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick in Maryland wear pressurized, full-body spacesuit-like gear and breathe purified air. Yet those suits ruptured or developed holes in at least 37 incidents during a 20-month period in 2013 and 2014, according to lab incident reports obtained by USA TODAY under the federal Freedom of Information Act.

This will contribute a 51%/49% contribution in favor of laboratory compared to zoonotic origin. There will be no confidence adjustment. The results from the calculations are shown below.

Evidence or process	Zoonotic Origin (ZO)	Laboratory Origin (LO)
Starting likelihood	0.011	0.989
The history of SARS laboratory accidents is consistent with the laboratory origin hypothesis		0.51
Impact of this evidence		Increases the likelihood of LO by 51/49 = 1.041
Impact of evidence calculation		1.041 x 0.989 = 1.030
Normalize this step of analysis	0.011/(0.011 + 1.030) = 0.011	1.030/(0.011 + 1.030) = 0.989

Adjusted likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

[121] https://gillesdemaneuf.medium.com/the-good-the-bad-and-the-ugly-a-review-of-sars-lab-escapes-898d203d175d
[122] https://www.usatoday.com/story/news/2015/05/29/some-recent-us-lab-incidents/25258237/

Evidence: Drs. Shi and Daszak use Wuhan residents as negative controls for zoonotic coronavirus seroconversion[123]

"As a control, we collected 240 serum samples from random blood donors in **Wuhan >1000 km away from Jinning & where inhabitants have a much lower likelihood of contact with bats due to its urban setting**" [emphasis added]. As expected, 0/240 samples from the patients from Wuhan had a positive serological evidence of prior coronavirus infection.

“The 2.7% seropositivity for the high-risk group of residents living in close proximity to bat colonies suggests that spillover is a **relatively rare event**, however this depends on how long antibodies persist in people, since other individuals may have been exposed and antibodies waned.”

In this paper from 2018, Drs. Shi and Daszak conclude that bat-to-human transfer is relatively rare for high-risk people living in close proximity to bat colonies and much less likely in Wuhan, a conclusion that does not support a hypothesis of bat-to-human transmission.

This will not be used to adjust the likelihoods.

Current likelihood: Zoonotic origin (0.2%), laboratory origin (99.8%).

[123] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6178078/

Evidence. The Bat Coronavirus RaTG13 has the Unique Genome Sequences Necessary to be the Precursor of SARS-CoV-2 Using the 'No See 'Em' Synthetic Biology Technology. ***The probability that RaTG13 acquired these 'No See 'Em' synthetic biology assembly sequences in nature is one in a billion.***

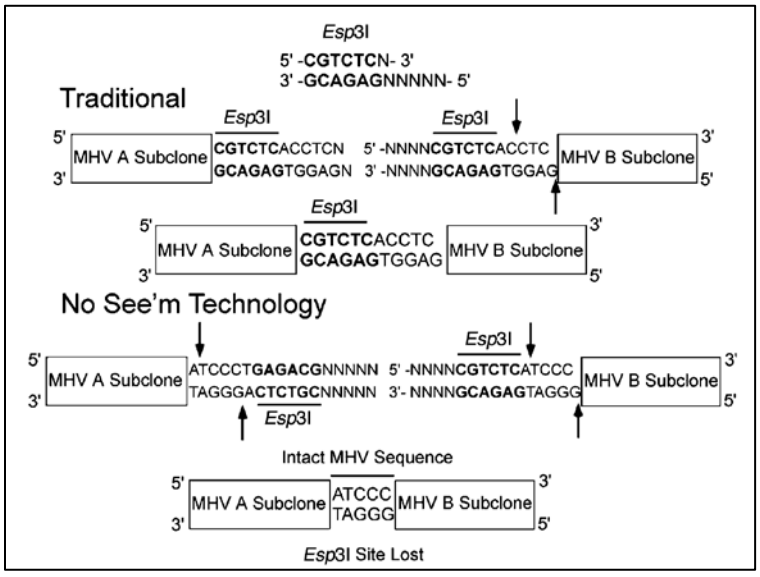
Summary.

- Synthetic biology techniques, like the engineered "No See 'Em'[124] restriction enzyme-enabled insertion method,[125] have been developed that, by design, extinguish the fingerprints of the insertion when only looking at the final genome.

- The use of these techniques is revealed however, if the precursor-product genome pair of such an insertion is available for inspection.

- **Hypothesis: the unique features of the SARS-CoV-2 Spike Protein, the receptor binding domain ACE2 contact amino acid residue region and the polybasic (furin) cleavage site, are the product of a genome insertion sequence into RaTG13 using engineered Esp3I restriction enzyme sites, the so-called, 'No See 'Em,' technology.**

- An example of the 'No See'm' Technology is shown below, taken from Baric and Sim.[1] By placing the restriction sites symmetrically on both strands of the cDNA, the resulting insertion no longer contains the identifying restriction site nts.



- According to Baric and Sims[1] "the type IIS restriction enzyme, Esp3I, recognizes an asymmetric sequence and makes a staggered cut 1 and 5 nucleotides downstream of the recognition sequence, leaving 256, mostly asymmetrical, 4-nucleotide overhangs

[124] Variably spelled 'No See 'Em,' 'No See 'um,' and 'No See'm.'
[125] https://www.researchgate.net/publication/8119695_Development_of_mouse_hepatitis_virus_and_SARS-CoV_infectious_cDNA_constructs

(GCTCTCN#NNNN). As identical Esp3I sites are generated every ~1,000,000 base pairs or so in a random DNA sequence, most restricted fragments usually do not self-assemble."

- Examination of RaTG13 identified two Esp3I cleavage sites in the Spike Protein gene, at nts 1366 and 2941 (positions 22,910 and 24,485 in the entire genome).

- As expected from the above rarity of such sites in an approximately 3800 nt gene, SARS-CoV-2 has no Esp3I sites in its SP gene. Neither do twelve other coronaviruses, including SARS-CoV-1, MERS, and other related human or bat coronaviruses.

- From all of the species other than bat RaTG13 gene source, the frequency of Esp3I sites **at any location** is 2 in 54,131 nucleotides or 0.000036947. If we assume the possibility of the occurrence of such a site at a given nucleotide is independent of any other nucleotide, then it is possible to use a binomial distribution calculation to determine the probability of 2 Esp3I sites in 3809 nucleotides for the bat RaTG13 gene. This calculation yields a probability of at least 2 sites anywhere in the Spike Protein gene of 0.009 or about one in a hundred. The probability of exactly 2 sites is 0.0086.[126]

- The 5' restriction site in RaTG13 begins at aa residue 455L, identified by Andersen et al, Nature, 2020. as the start of the "receptor-binding domain ACE2 contact residues." The downstream amino acids from this site are critical for why RaTG13 has such poor affinity for human ACE2 and the substitutions in CoV-2 are precisely why CoV-2 has such high affinity for human ACE2, why CoV-2 seems so 'preadapted' to human infections, etc. So this is the most important part of CoV-2 in explaining its ACE2 binding and infectivity. Further downstream is arguably the second most important site, the polybasic (furin) cleavage site.[127] Polybasic cleavage sites have not been observed in related 'lineage B' betacoronaviruses,' according to Andersen et al, Nature, 2020. and so there has been much speculation about how this site was acquired.

- The 3' restriction site in RaTG13 is at residue 980L. There is no protein-based rationale for this position.

- Comparing the nt sequences between RaTG13 and CoV-2, at the 5' restriction site, they are two codons in which only 2 of 6 nt bases are shared but, despite this low nt sequence homology, they are in fact synonymous base substitutions.

- Comparing the nt sequence between RaTG13 and CoV-2 at the 3' restriction site, this site has 5 of 6 identical nts with a single synonymous change in CoV-2 which destroys the restriction site. This is the only such five nt site in the RaTG13 spike protein gene and so

[126] Statistical analysis provided by Dr. Martin Lee, PhD, Adjunct Professor of Statistics, UCLA Fielding School of Public Health, UCLA, Los Angeles, CA.
[127] https://www.biorxiv.org/content/10.1101/2020.08.26.268854v1

is the easiest site in which a one nt substitution can create or destroy an Esp3I restriction site.

- The probability of having the restriction sites at **exactly these locations** can also be calculated.[2] Since there are 3809 nucleotides in the RaTG13 genome then, 3807 would not have a restriction site with probability (1-0.000036947), which was determined from the frequency of these restriction sites in other species. The other two sites would have this restriction site with probability 0.000036947. So the overall probability of this configuration has a probability of: $(1\text{-}0.00036947)^{3807} \times (0.000036947)^2 = 3.343 \times 10^{-10}$. This is a frequency of these site at their exact location being here from a natural process of approximately one in a billion.

- Dr. Zhengli-Li Shi, of the Wuhan Institute of Virology, collected the bat virus RaTG13 in 2013 and sequenced it between 2014 and 2018. In 2015, Dr. Shi and colleagues have also used the 'No See 'Em' technology' with a similar restriction enzyme, BgII, in the SARS-CoV reverse genetics system to generate chimeric coronaviruses. In that paper, they inserted a spike protein gene from a bat coronavirus into a mouse-adapted coronavirus, with a 'gain-of-function' phenotypic change.[128]

- **In conclusion:**

- **The bat coronavirus RaTG13 has two rare, Esp3I restriction sites strategically located to permit insertion of a genetic sequence that codes for the unique features of the SARS-CoV-2 Spike Protein, its receptor binding contact amino acids and its polybasic (furin) cleavage site, using the 'No See 'Em' synthetic biology techniques.**

- **This specific synthetic biology laboratory technique has been successfully performed previously by Wuhan Institute of Virology scientists to increase coronavirus infectivity.**

- **The probability these two sites are present and in their exact location in RaTG13 by an act of nature is one in a billion.**

[128] https://www.nature.com/articles/nm.3985

Text-Table. A record of the EspI restriction enzyme sites in the Spike Protein (SP) genes of fifteen coronaviruses, including RaTG13 and SARS-CoV-2. RaTG13 is unique in having two such sites, with SARS-CoV-2 and eleven other coronaviruses having no such site in the SP gene. The restriction sites were identified with the RestrictionMapper site algorithm: http://www.restrictionmapper.org/ .

Species	Spike Protein (SP) Gene Source	Nt Size of SP Gene	Esp3I Site Location in Spike Protein Gene	Reference
Bat	Bat Coronavirus RaTG13 from WIV	3809	1366, 2941 (22910, 24485 in genome)	
Human	SARS-CoV-2 Reference Sequence	3821	None	
Bat	Rhinolophus affinis coronavirus isolate LYRa11	3779	None	Daszak and Shi paper
Bat	Bat SARS coronavirus HKU3-1	3728	None	Daszak and Shi paper
Bat	SARS-like coronavirus isolate bat-SL-CoVZC45	3740	None	Third Military University publication
Bat	SARS-like coronavirus bat-SL-CoVZXC21	3737	None	Third Military University publication
Bat	hCoV-19/bat/Yunnan/RmYN02/2019	3873	None	Wild bat coronavirus with apparent furin-like insert
Bovine	Bovine coronavirus strain Quebec	4091	None	
Human	Human coronavirus HKU1 strain	4070	3208	
Human	MERS Reference Sequence	4061	None	
Human	Human coronavirus OC43 strain	4079	None	
Human	Human coronavirus 229E strain	3512	None	
Human	Human Coronavirus NL63 Reference Sequence	4070	None	
Human	SARS 2003 coronavirus ZJ0301	3767	None	
Pangolin	Pangolin coronavirus isolate PCoV_GX-P4L	3803	3351	
Human	SARS-CoV-1 Urbani	3767	None	

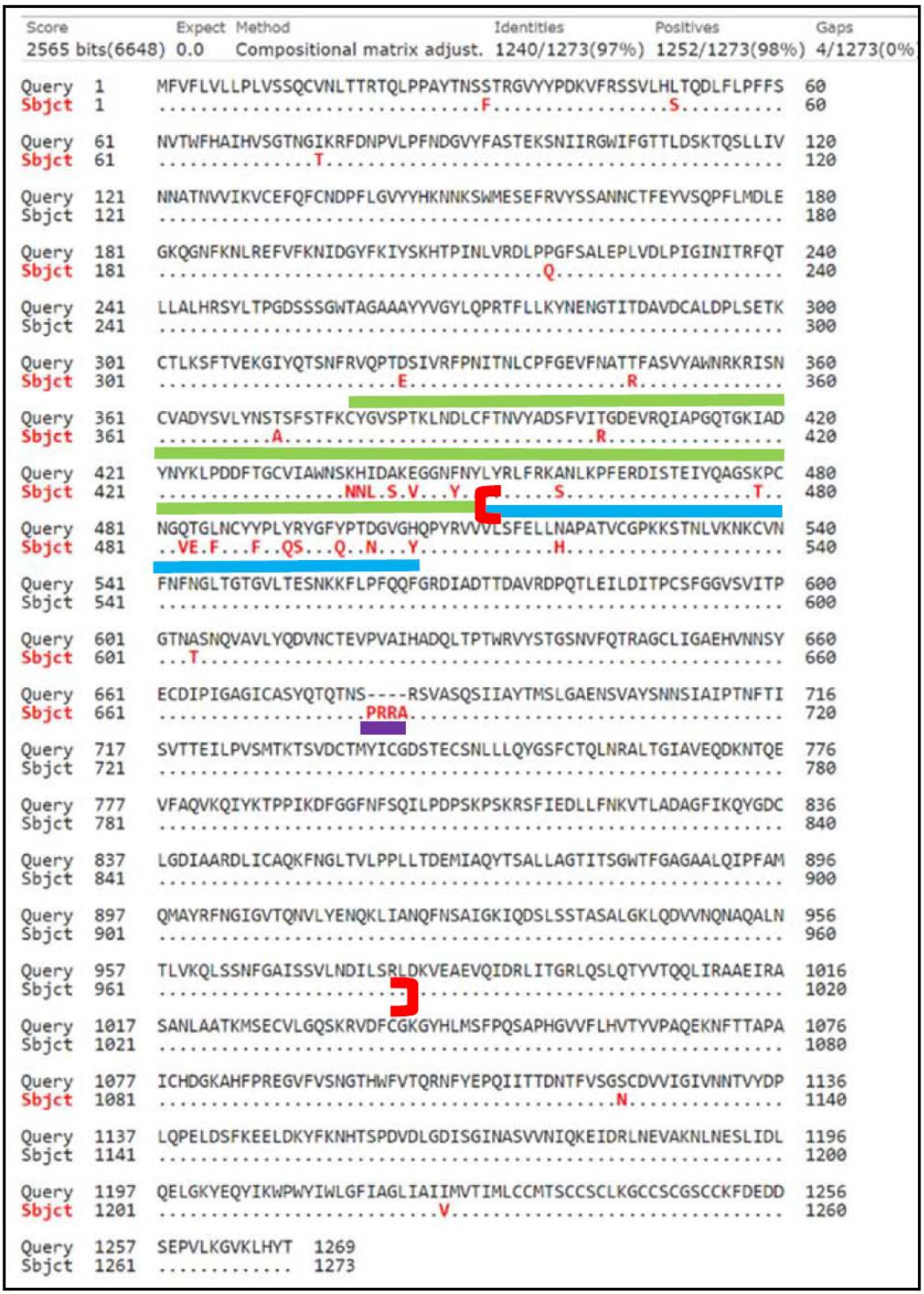
Figure. A comparison of the RaTG13 Spike Protein gene (Query) and the SARS-CoV-2 Reference Sequence (Sbjct) showing the only two Esp3I restriction enzyme cleavage site, both present in RaTG13 but absent in SARS-CoV-2. The restriction sites were identified with the RestrictionMapper site: http://www.restrictionmapper.org/ .The 5' cleavage site is strategically located at the beginning of the receptor binding domain ACE2 contact residues. Despite four of six nt are different these are synonymous changes.

```
Query  1321  ATTGATGCAAAAGAGGGCGGTAATTTTAACTATCTTTACCGTCTCTTTAGAAAAGCTAAT  1380
              |||||  |  ||  |  ||  |||||||  |||  ||  ||  ||   |   |||||  ||   |||||
Sbjct  1321  CTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAAT  1380
```

The 3' cleavage site is the only downstream -CGTCTN- sequence found in the CoV-2 Spike Protein, making it unique.

```
Query  2927  TCCTTTCACGTCTCGACAAAGTTGAGGCTGAAGTGCAGATTGACAGGTTGATCACAGGCA  2986
              ||||||||||||| ||||||||||||||||||||||| ||||| ||||||||||||||||
Sbjct  2939  TCCTTTCACGTCTTGACAAAGTTGAGGCTGAAGTGCAAATTGATAGGTTGATCACAGGCA  2998
```

Figure. Comparison of Spike Protein amino acid sequence between RaTG13 (Query) and SARS-CoV-2 (Sbjct). Amino acid substitutions in CoV-2 are shown in red, single letter abbreviation. Green band; receptor binding domain. Blue band; receptor binding domain ACE2 contact residues (Andersen et al, Nature, 2020.). Purple band; polybasic (furin) cleavage site. Red brackets; Esp3I cleavage sites in RaTG13.



```
 Score          Expect  Method                    Identities          Positives           Gaps
 2565 bits(6648)  0.0   Compositional matrix adjust.  1240/1273(97%)  1252/1273(98%)  4/1273(0%

Query  1     MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSSTRGVYYPDKVFRSSVLHLTQDLFLPFFS  60
Sbjct  1     ..............................F.................S.........  60

Query  61    NVTWFHAIHVSGTNGIKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV  120
Sbjct  61    ...............T.............................................  120

Query  121   NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE  180
Sbjct  121   ............................................................  180

Query  181   GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPPGFSALEPLVDLPIGINITRFQT  240
Sbjct  181   ......................................Q.....................  240

Query  241   LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK  300
Sbjct  241   ............................................................  300

Query  301   CTLKSFTVEKGIYQTSNFRVQPTDSIVRFPNITNLCPFGEVFNATTFASVYAWNRKRISN  360
Sbjct  301   ......................E.....................R..............  360

Query  361   CVADYSVLYNSTSFSTFKCYGVSPTKLNDLCFTNVYADSFVITGDEVRQIAPGQTGKIAD  420
Sbjct  361   ...........A................................R..............  420

Query  421   YNYKLPDDFTGCVIAWNSKHIDAKEGGNFNYLYRLFRKANLKPFERDISTEIYQAGSKPC  480
Sbjct  421   .................NNL.S.V...Y.....S..................T..  480

Query  481   NGQTGLNCYYPLYRYGFYPTDGVGHQPYRVVVLSFELLNAPATVCGPKKSTNLVKNKCVN  540
Sbjct  481   ..VE.F...F..QS...Q..N...Y.............H.....................  540

Query  541   FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP  600
Sbjct  541   ............................................................  600

Query  601   GTNASNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY  660
Sbjct  601   ...T........................................................  660

Query  661   ECDIPIGAGICASYQTQTNS----RSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI  716
Sbjct  661   ..................PRRA..................................  720

Query  717   SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE  776
Sbjct  721   ............................................................  780

Query  777   VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC  836
Sbjct  781   ............................................................  840

Query  837   LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM  896
Sbjct  841   ............................................................  900

Query  897   QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN  956
Sbjct  901   ............................................................  960

Query  957   TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA  1016
Sbjct  961   ................................................................  1020

Query  1017  SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA  1076
Sbjct  1021  ............................................................  1080

Query  1077  ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGSCDVVIGIVNNTVYDP  1136
Sbjct  1081  ..........................................N.................  1140

Query  1137  LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL  1196
Sbjct  1141  ............................................................  1200

Query  1197  QELGKYEQYIKWPWYIWLGFIAGLIAIIMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD  1256
Sbjct  1201  ............................V...............................  1260

Query  1257  SEPVLKGVKLHYT  1269
Sbjct  1261  .............  1273
```

Because it has not been established that RaTG13 was the precursor of CoV-2 this evidence statement will not be used at this time to adjust the likelihoods of the origin. If additional information is obtained at a later date this may be revisited.

Likelihood from prior state is unchanged following this evidence analysis:

Zoonotic origin (0.2%) and laboratory origin (99.8%)

Evidence. Location, location, location: Based on the distance between known SARS-CoV-1 laboratory-acquired infections and the hospital of admission of the infected personnel, the WIV is within the expected hospital catchment for a CoV-2 LAI

Hypothesis. Laboratory-acquired infections (LAI) have the property that the hospital of admission of the personnel from the laboratory with the acquired infection are close together, specifically they are within 24.64 km from the laboratory.

Prior data from SARS-CoV-1. There were four LAIs of SARS-CoV-1 that can be used to determine the distance between the laboratory where the infection occurred and the hospital of first admission. The data are here:

SARS-CoV-1 Laboratory Acquired Infection (LAI)	Hospital of admission	Distance (Google Maps)	
In September 2003, a 27-year-old student from the National University of Singapore (NUS) was infected with the SARS virus due to improper experimental procedures	Singapore General Hospital (SGH)	6.3 km	
Baiji Mountain, Sanxia, Taiwan	Taiwan Hoping Hospital, Taipei, Taiwan	27.8 km	
№100 Yingxin Street, Xicheng District, Bejing	Union Hospital, Beiijing, China	7.3 km	
№100 Yingxin Street, Xicheng District, Bejing	Friendship Hospital, Beijing, China	17.6 km	
		mean = 14.75	
		SD = 10.1	
		95% Confidence Interval	14.75 ±9.887

Based on these four cases, the 95% upper confidence limit for the distance from LAI patients to the hospitals of admission is 24.6 km of the laboratory where the infection was acquired.

SARS-CoV-2. Although it is not clear which hospital the first patient was admitted to the following Text-Table contains all likely candidates.

SARS-CoV-2 Potential LAI Source	Hospital of admission	Distance (Google Maps)	Probability of being closer than the average results for SARS-CoV-1	Probability of being farther than the average results for SARS-CoV-1
Wuhan Institute of Virology, Wuhan, China	PLA Hospital, NO. 627 Wuluo Road, Wuchang District, Wuhan, China	4.8 km	0.094	0.906
Wuhan Institute of Virology, Wuhan, China	Wuhan Central Hospital, Wuhan, China	9.1 km	0.338	0.662
Wuhan Institute of Virology, Wuhan, China	Zhongnan Hospital, Wuhan, China	2.8 km	0.019	0.981
Wuhan Institute of Virology, Wuhan, China	Tongji Hospital, Wuhan, China	5.1 km	0.109	0.891
Wuhan Institute of Virology, Wuhan, China	Hubei Maternity and Child Health Care Hospital, Wuhan, China	4.4 km	0.075	0.925
Hypothesis: Given the distance from the SARS-CoV-1 laboratory where an LAI occurred to the hospital of admission for the lab workers who became infected, what is the probability that CoV-2 is also an LAI, given the distance from the hospitals where the first patients were seen to the WIV, the hypothesized source.			Probability calculations based on the use of a log-normal distribution for distances	Probability calculations based on the use of a log-normal distribution for distances

Based on the data for actual LAI for SARS-CoV-1 the distance between the WIV and the hospitals of admission for CoV-2 is consistent with the WIV being the origin for the LAI. There is no evidence the putative LAI for CoV-2 is any different than the known LAIs for CoV-1.

This evidence is not independent of other evidence that is based on location and so it cannot be used independently in the Bayesian analysis. It is included here for completeness.

Likelihood from prior state is unchanged following this evidence analysis:

Zoonotic origin (0.2%) and laboratory origin (99.8%)

Evidence. Dr. Shi successfully identifies a laboratory-acquired infection outbreak from Hanta virus in laboratory rodents.

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Hantavirus outbreak associated with laboratory rats in Yunnan, China

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ABSTRACT

An outbreak of hemorrhagic fever with renal syndrome occurred among students in a college (College A) in Kunming, Yunnan province, China in 2003. Subsequent investigations revealed the presence of hantavirus antibodies and antigens in laboratory rats at College A and two other institutions. Hantavirus antibodies were detected in 15 additional individuals other than the index case in these three locations. Epidemiologic data indicated that the human infections were a result of zoonotic transmission of the virus from laboratory rats. A virus was isolated from rats in College A and the full-length genome sequence revealed that this was a new Hantaan virus isolate, designated strain KY. Sequence analysis of the three genome segments indicated that this new isolate is a reassortant derived from human and rat Hantaan viruses. Further sequence analysis of the medium (M) genome segment revealed that it originated from a recombination event between two rat Hantaan virus lineages.

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The significance of this evidence is that it demonstrates the methods used by Dr. Shi and the WIV to solve a laboratory-acquired infection outbreak. The methods described herein should be applied to the WIV in order to determine if CoV-2 was also a laboratory-acquired infection.

This will not be used to directly advance the Bayesian analysis.

Likelihood from prior state is unchanged following this evidence analysis:

Zoonotic origin (0.2%) and laboratory origin (99.8%)

Evidence. Bats hibernate when the temperature is below 10.5 C;[129] in Hubei province that begins in September and ends in May.

Average Hubei Temperature by Month

Month	Recommended Rate	Max Temp.	Min Temp.
Jan.	~	-17°C	-26°C
Feb.	√	-13°C	-23°C
Mar.	√√	-3°C	-14°C
Apr.	√√	7°C	-4°C
May.	√√	16°C	4°C
Jun.	√	23°C	11°C
Jul.	√	23°C	13°C
Aug.	√	21°C	11°C
Sep.	√√	15°C	4°C
Oct.	√√	6°C	-5°C
Nov.	√√	-5°C	-16°C
Dec.	√	-15°C	-23°C

Based on this evidence, they would have been hibernating at the time of the first human outbreak in the fall of 2019. Since this evidence is cumulative to the prior evidence from Dr. Shi that the bat host species for CoV-2 does not live in Hubei Province it will not be used to change the Bayesian analysis.

Likelihood from prior state is unchanged following this evidence analysis:

Zoonotic origin (0.2%) and laboratory origin (99.8%)

[129] https://zslpublications.onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-7998.1971.tb01323.x

Wuhan Institute of Virology analysis of lavage specimens from ICU patients at Wuhan Jinyintan Hospital in December 2019 contain both SARS-CoV-2 and adenovirus vaccine sequences consistent with a vaccine challenge trial

Summary. The most significant evidence provided herein is the finding from RNA-Seq performed by the Wuhan Institute of Virology (WIV) of lavage patient samples collected on December 30, 2019.[130] These ICU patients were the subject of the seminal paper, entitled, "A pneumonia outbreak associated with a new coronavirus of probable bat origin," from Dr. Zhengli Shi and colleagues that first characterized SARS-CoV-2.[131] This author has confirmed that the RNA-Seq of all five patients contained SARS-CoV-2 sequences.

Surprisingly the specimens also contained the adenovirus "pShuttle" vector, developed by Chinese scientists in 2005 for SARS-CoV-1.[132] Two immunogens were identified, the Spike Protein gene of SARS-CoV-2 and the synthetic construct H7N9 HA gene.[133] Hundreds of perfectly homologous (150/150) raw reads suggest this is not an artifact. Reads that cross the vector-immunogen junction are identified. While adenovirus is a common infection the wildtype viruses have low homology to the vaccine vector sequence, by design, to avoid rejection of the vaccine due to prior exposure to wildtype adenoviruses.

Two patients from the same hospital who had bronchial lavage on the same day but had their specimens sent to the Hubei CDC did not have adenovirus vaccine sequences.

Three explanations come to mind from this evidence:

1. These represent sample preparation artifacts at the WIV, such as sample spillover on the sequencer.
2. These patients were admitted with an unknown infection, were not responding to the treatment protocols for a infection of unknown origin, and they were vaccinated with an experimental vaccine in a desperate but compassionate therapeutic "Hail Mary."
3. A clinical trial of a combination[134] influenza/SARS-CoV-2 vaccine was being conducted and an accidental release into Wuhan occurred.

Only WIV scientists and Chinese authorities can answer these questions. Until the evidence of the adenovirus sequences has been confirmed by other scientists, this author will not include this evidence in the Bayesian analysis.

Obviously if a vaccine containing the Spike Protein of SARS-CoV-2 was being administered to patients in Wuhan in December 2019 the question of laboratory origin is a settled matter.

[130] The detailed evidence for the adenovirus vaccine sequences is given at the end of this document.
[131] https://www.nature.com/articles/s41586-020-2012-7
[132] https://www.ncbi.nlm.nih.gov/nuccore/AY862402.1
[133] https://www.ncbi.nlm.nih.gov/nuccore/KY199425.1/
[134] The proposal that this was, in fact, a combination vaccine was made by H. Lawrence Remmel, Department of Pathology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands.

Introduction. Following the 2003 SARS epidemic, Liu et al. developed an adenoviral expression vector of a truncated S1 subunit of SARS-CoV spike protein that resulted in specific humoral immune responses against SARS-CoV in rats.[135] This same vector was used to create the CoV-2 adenovirus vector vaccine.

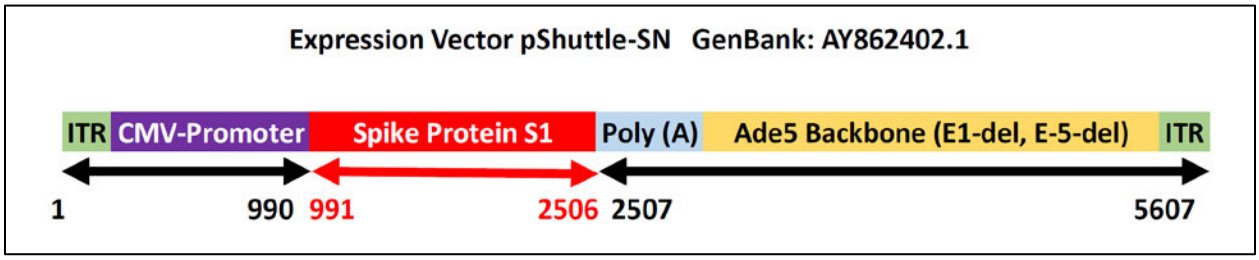
In order to test the hypothesis that CoV-2 began in the PLA Hospital as a vaccine challenge clinical trial that went awry, RNA-Seq raw reads from nasopharyngeal specimens of Wuhan COVID patients (Table below) were blasted against the published genome sequence of the SARS-CoV-1 vaccine (GenBank AY862402.1). I used the SARS-CoV-1 vaccine because the PLA CoV-2 vaccine has not been published at this time.

Adenovirus sequences detected	GenBank URL	GenBank Biosample URL	GISAID ID	CoV-2 Isolate	Sequencing Institution	Clinical Information from GISAID
>100	SRX7730879	SAMN14082200	EPI_ISL_402130	WIV07; Lineage B; mutations NSP3 D1761A, NSP4 T327I; passage original	Wuhan Institute of Virology, Chinese Academy of Sciences	56 y, male, hospitalized, ICU10G, 20 Dec 2019
>100	SRX7730880	SAMN14082196	EPI_ISL_402127	WIV02; Lineage B; mutations NSP16 D220N; passage original	Wuhan Institute of Virology, Chinese Academy of Sciences	32 y, male, hospitalized, ICU4G, outbreak 19 Dec 2019
>100	SRX7730881	SAMN14082197	EPI_ISL_402124	WIV04; Lineage B; no mutations; passage original	Wuhan Institute of Virology, Chinese Academy of Sciences	49 y, female, hopitalized, ICU-6, outbreak 27 Dec 2019, Retailer at Huanan Seafood Wholesale Market, patient alive
>100	SRX7730882	SAMN14082198	EPI_ISL_402128	WIV05; Lineage B; NSP3 G1433S, NSP16 K160R; passage original	Wuhan Institute of Virology, Chinese Academy of Sciences	52 y, female, hospitalized, ICU8G, outbreak 22 Dec 2019; recovered
>100	SRX7730883	SAMN14082199	EPI_ISL_402129	WIV06; Lineage B; no mutations; original passage	Wuhan Institute of Virology, Chinese Academy of Sciences	40 y, male, hospitalized, ICU9G, 25 Dec 2019
>100	SRX7730884	SAMN14082200	EPI_ISL_402130	WIV07; Lineage B; mutations NSP3 D1761A, NSP4 T327I; passage original	Wuhan Institute of Virology, Chinese Academy of Sciences	56 y, male, hospitalized, ICU10G, 20 Dec 2019
7 small	SRX7730885	SAMN14082196	EPI_ISL_402127	WIV02; Lineage B; mutations NSP16 D220N	Wuhan Institute of Virology, Chinese Academy of Sciences	32 y, male, hospitalized, ICU, outbreak 19 Dec 2019
1 small one	SRX7730886	SAMN14082197	EPI_ISL_402124	WIV04; Lineage B; no mutations; passage original	Wuhan Institute of Virology, Chinese Academy of Sciences	49 y, female, hopitalized, ICU-6, outbreak 27 Dec 2019, Retailer at Huanan Seafood Wholesale Market, patient alive
Very few	SRX7730887	SAMN14082199	EPI_ISL_402129	WIV06; Lineage B; no mutations; original passage	Wuhan Institute of Virology, Chinese Academy of Sciences	40 y, male, hospitalized, ICU9G, 25 Dec 2019
None	SRX8032202	SAMN14479127	EPI_ISL_412898	hCoV-19/Wuhan/HBCDC-HB-02/2019	Hubei Provincial Center for Disease Control and Prevention	male, "traveled from Wuhan"
None	SRX8032203	SAMN14479128	EPI_ISL_402132	Wuhan HBCDC-HB-01/2019; Lineage B; mutation Spike F32I; original passage	Hubei Provincial Center for Disease Control and Prevention	49 y, female, hospitalized

This is not related to the previous claim, now shown to be wrong, that SARS-CoV-2 itself contained adenovirus pShuttle sequences.[136]

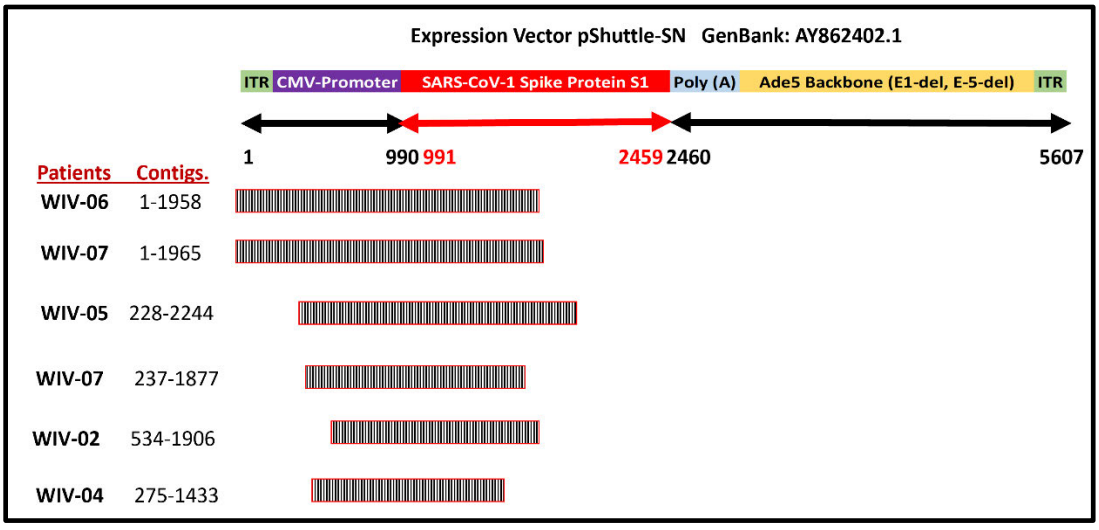
[135] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114075/
[136] https://sciencefeedback.co/claimreview/2019-novel-coronavirus-2019-ncov-does-not-contain-pshuttle-sn-sequence-no-evidence-that-virus-is-man-made/

According to Liu: "Adeno-X™ expression system (Clontech Laboratories, Inc.), comprising adenovirus type 5 genome with a deletion in the E1 and E3 regions (ΔE1, 343–3465 bp; ΔE3, 28,756–30,561 bp), was utilized to construct a recombinant adenovirus carrying nucleotides −45 to 1469 of Spike gene of SARS-CoV (Ad-SN) by *in vitro* ligation. This provides an immunogen which encoded a truncated S1 subunit of SARS-CoV S protein (490 N-terminal amino-acid residues)," as shown here:



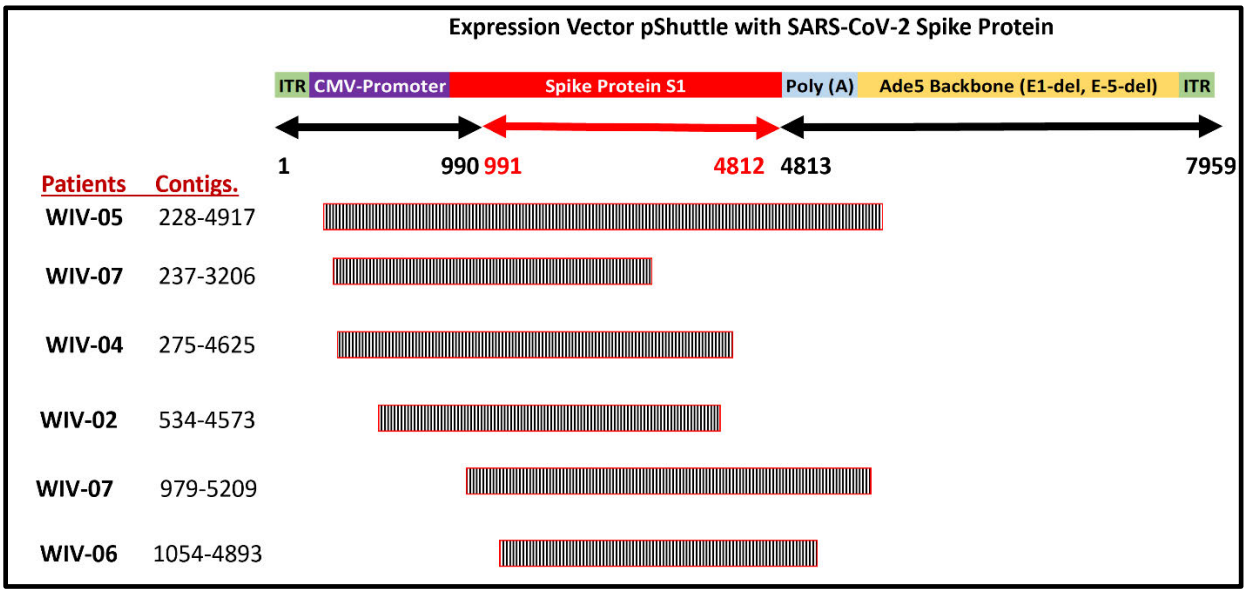
The expected result would be the finding of RNA-Seq sequence raw reads that were homologous to the two Adenovirus regions but only partially homologous (about 80%) to the SARS-CoV-1 regions.

The results are shown below. All five patients have adenovirus sequences that read through the 5' junction with the immunogen but do not read through the entire gene:

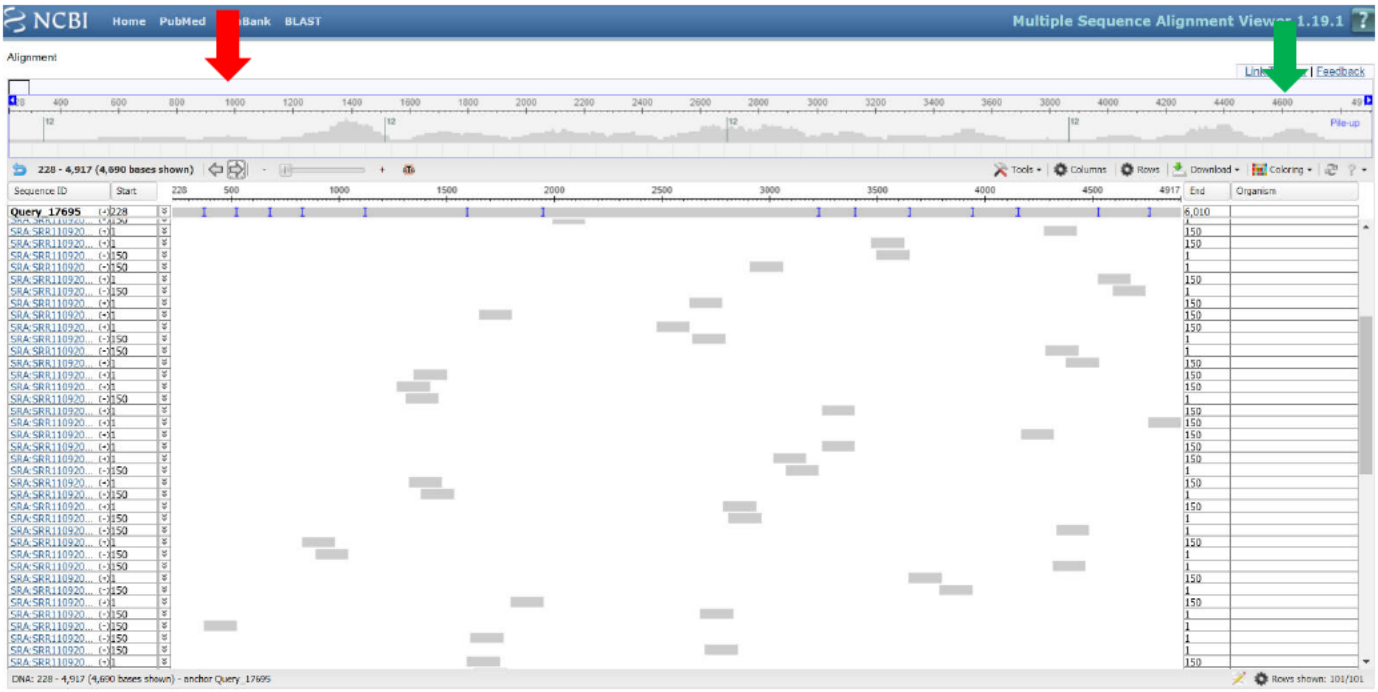


As can be seen above, all five patients have significant portions of the CMV-promoter as well as almost one-half of the truncated Spike Protein gene. This is the expected result if in fact the vaccine was not the previously described SARS-CoV-1, as in that case you would expect through reads covering the entire spike protein gene.

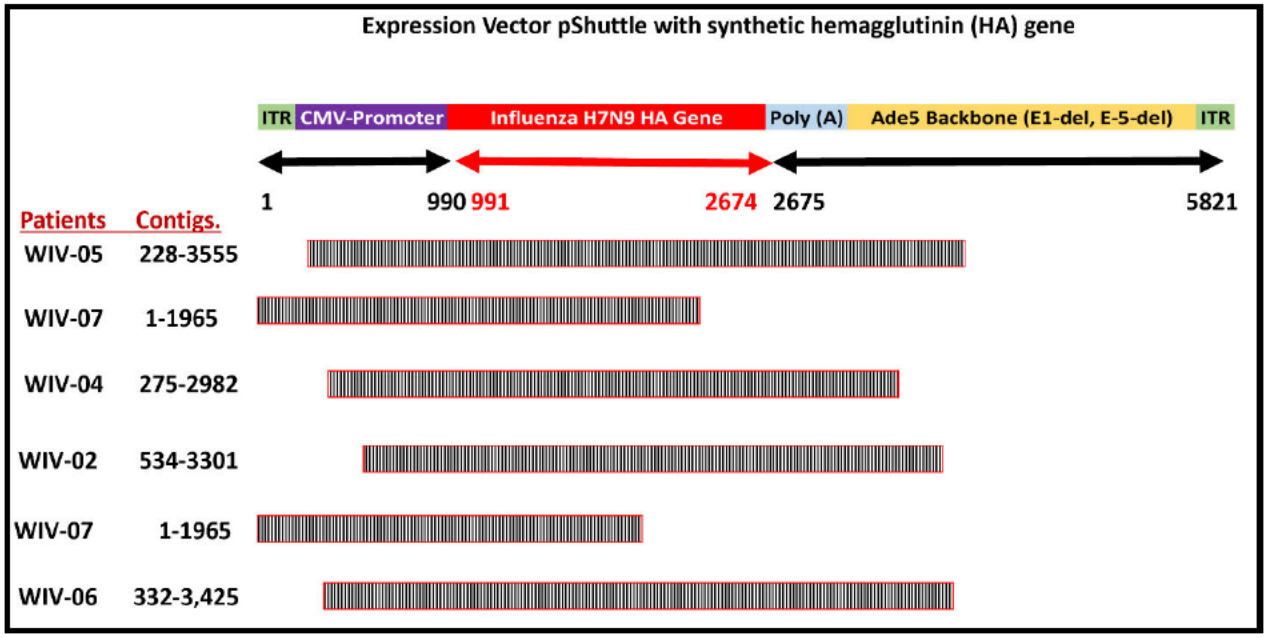
Next, an adenovirus vaccine vector sequence was created by substituting the full CoV-2 spike protein gene into the vector cassette. The results for this construct was much greater coverage within the specimens.



For example, the sequence alignment of patient WIV-05 is shown below. The red arrow and green arrow are at the 5’ and 3’ junctions of the adenovirus vector sequences and the CoV-2 Spike Protein gene sequence, showing cross junction contigs.



Another surprising finding was the presence of synthetic H7N9 gene sequences, again in all five WIV sequenced patients. The contigs are shown below.



1. **RNA-Seq** of Homo sapiens: hCov-19 infected patients Bronchoalveolar **lavage** uid
   1 ILLUMINA (Illumina MiSeq) run: 5.8M spots, 1.7G bases, 634.3Mb downloads
   Accession: SRX8032203

2. **RNA-Seq** of Homo sapiens: hCov-19 infected patients Bronchoalveolar **lavage** uid
   1 ILLUMINA (Illumina MiSeq) run: 5.2M spots, 1.6G bases, 583.4Mb downloads
   Accession: SRX8032202

3. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina MiSeq) run: 5.2M spots, 1.5G bases, 772.9Mb downloads
   Accession: SRX7730887

4. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina MiSeq) run: 5.2M spots, 1.5G bases, 768.3Mb downloads
   Accession: SRX7730886

5. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina MiSeq) run: 8.3M spots, 2.2G bases, 1.2Gb downloads
   Accession: SRX7730885

6. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 3000) run: 38.5M spots, 11.5G bases, 7.1Gb downloads
   Accession: SRX7730884

7. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 3000) run: 29.7M spots, 8.9G bases, 5.6Gb downloads
   Accession: SRX7730883

8. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 3000) run: 34.3M spots, 10.3G bases, 6.4Gb downloads
   Accession: SRX7730882

9. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 1000) run: 61.3M spots, 18.4G bases, 11.4Gb downloads
   Accession: SRX7730881

10. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
    1 ILLUMINA (Illumina HiSeq 3000) run: 67.1M spots, 20.1G bases, 12.6Gb downloads
    Accession: SRX7730880

11. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
    1 ILLUMINA (Illumina MiSeq) run: 3.6M spots, 1G bases, 548.1Mb downloads
    Accession: SRX7730879

The WIV entry with the greatest read depth, Number 10 above, is described below:

SRX7730880: RNA-Seq of Homo sapiens: bronchoalveolar lavage fluid
1 ILLUMINA (Illumina HiSeq 3000) run: 67.1M spots, 20.1G bases, 12.6Gb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit (50) following the manufacturers instructions. An RNA library was then constructed using the MGIEasy RNA Library Prep Set (96 RXN) (Cat. No.: 1000006384). Paired-end (150 bp) sequencing of the RNA library was performed on the MGISEQ-2000RS platform .

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Severe acute respiratory syndrome coronavirus 2 Raw sequence reads
PRJNA605983 • SRP249613 • All experiments • All runs
show Abstract

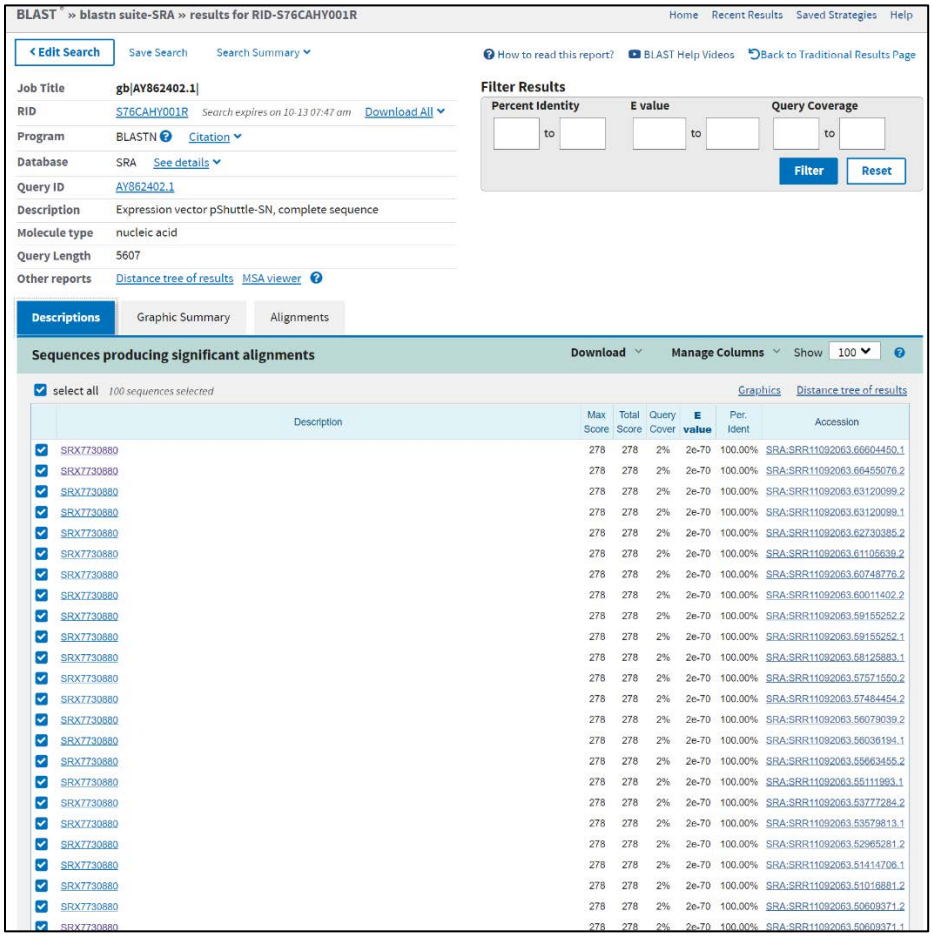
Sample:
SAMN14082196 • SRS6151291 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: WIV02-2
Instrument: Illumina HiSeq 3000
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

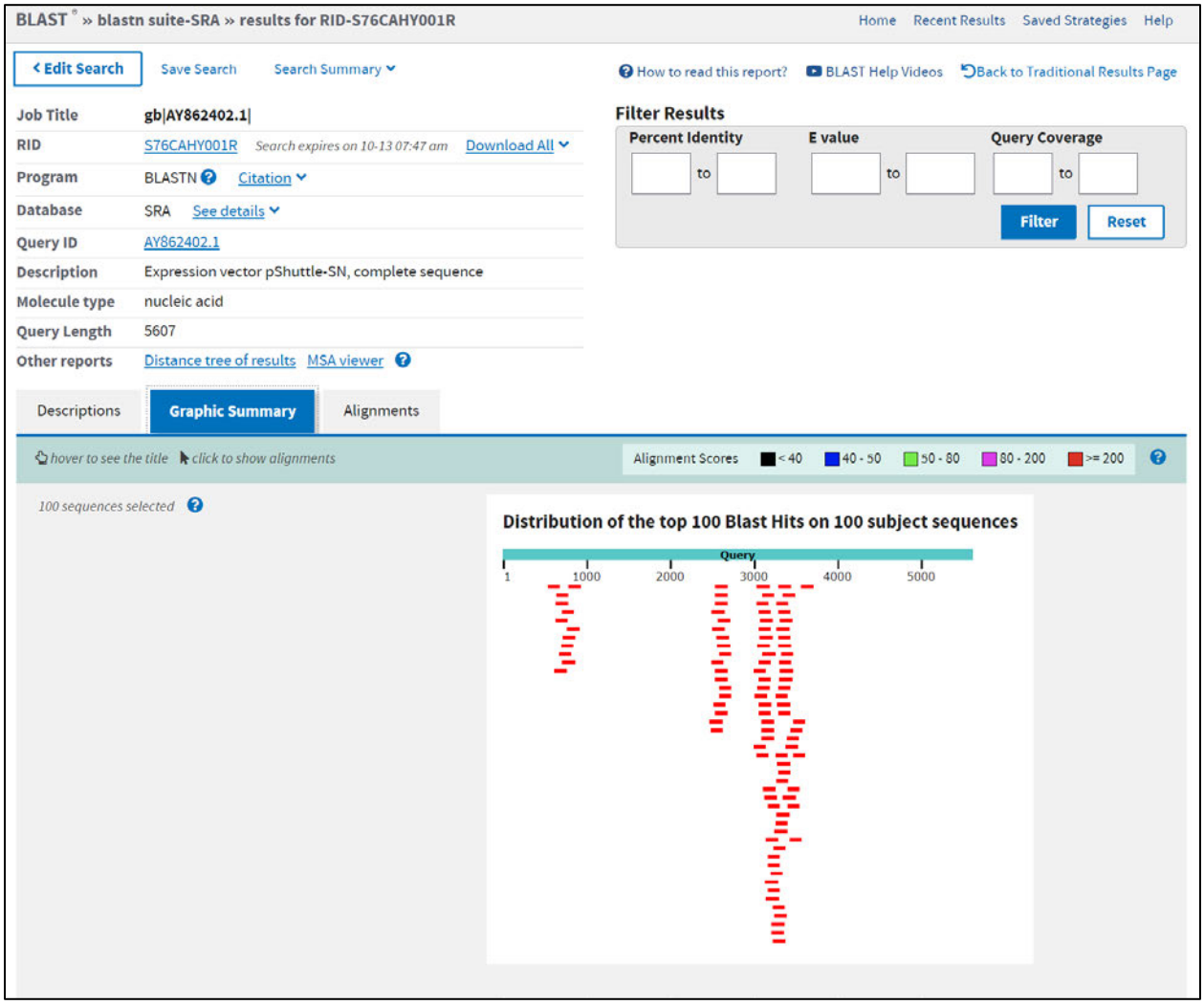
Runs: 1 run, 67.1M spots, 20.1G bases, 12.6Gb

Run	# of Spots	# of Bases	Size	Published
SRR11092063	67,083,195	20.1G	12.6Gb	2020-02-16

Unexpectedly, over 100 sequences producing significant alignment were identified:



A graphical display of the alignments shows they are not in the Spike Protein region (961 to 2507) of the adenovirus vector but outside of those regions.



An examination of individual reads show 100% homology over the entire 150 nt segments and outside of the Spike Protein region. The first set of reads are immediately downstream of the Spike Protein segment. The other read is from the region is from the 5' boundary of the Adenovirus vector with the Spike Protein region.

Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.66604450.1 **Length:** 150 **Number of Matches:** 1

Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Plus

```
Query  2536  CCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAG  2595
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     CCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAG  60

Query  2596  GAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTgggggtggggtggggCAG  2655
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    GAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG  120

Query  2656  GACAGCAAGGGGGAGGATTGGGAAGACAAT  2685
              |||||||||||||||||||||||||||||
Sbjct  121   GACAGCAAGGGGGAGGATTGGGAAGACAAT  150
```

Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.66455076.2 **Length:** 150 **Number of Matches:** 1

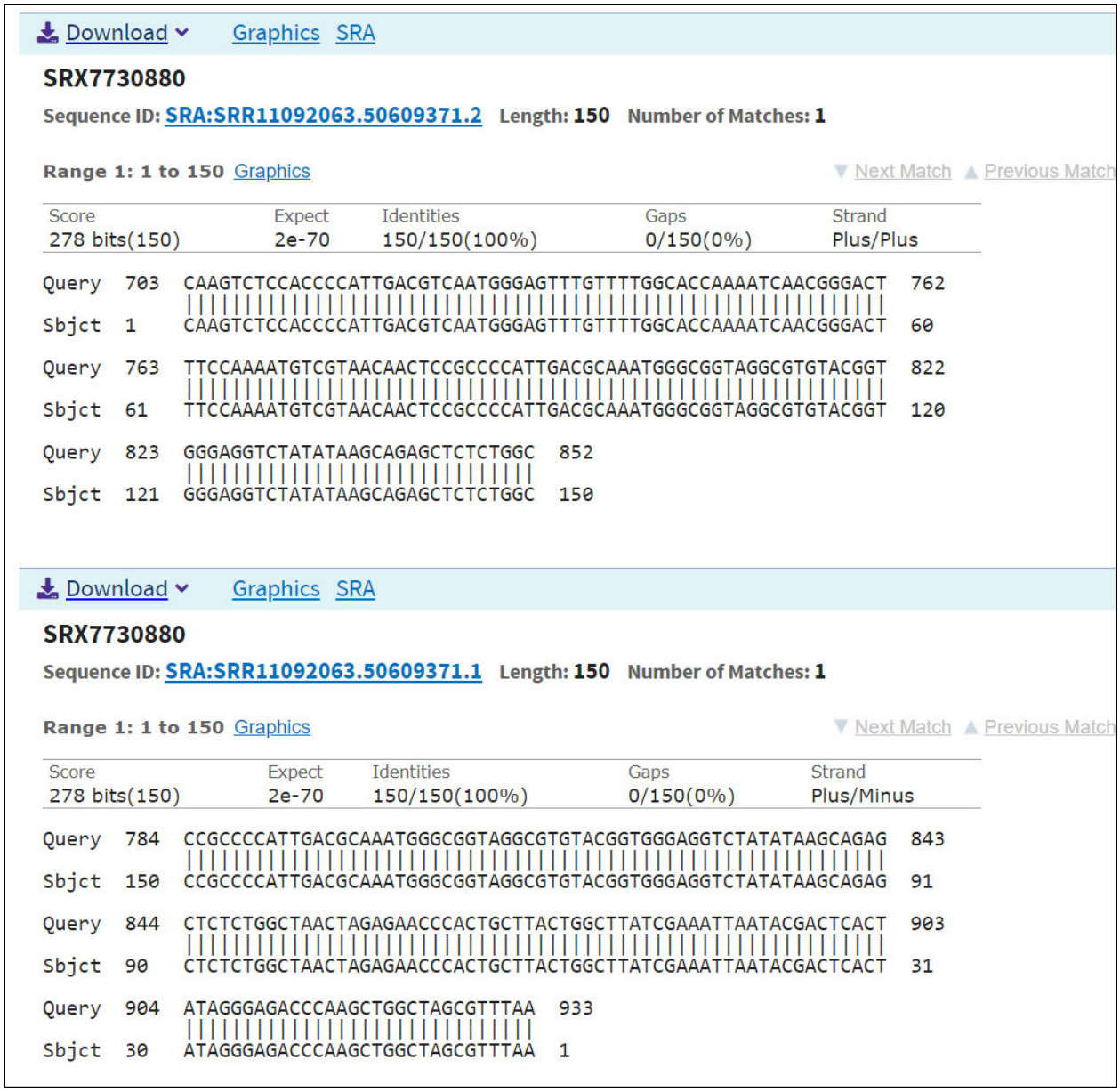
Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Minus

```
Query  3290  CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCC  3349
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150   CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCC  91

Query  3350  GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC  3409
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90    GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC  31

Query  3410  ACTGGTAACAGGATTAGCAGAGCGAGGTAT  3439
              |||||||||||||||||||||||||||||
Sbjct  30    ACTGGTAACAGGATTAGCAGAGCGAGGTAT  1
```



Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.50609371.2 Length: 150 Number of Matches: 1

Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Plus

```
Query  703  CAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT  762
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT  60

Query  763  TTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGT  822
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGT  120

Query  823  GGGAGGTCTATATAAGCAGAGCTCTCTGGC  852
            ||||||||||||||||||||||||||||||
Sbjct  121  GGGAGGTCTATATAAGCAGAGCTCTCTGGC  150
```

Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.50609371.1 Length: 150 Number of Matches: 1

Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Minus

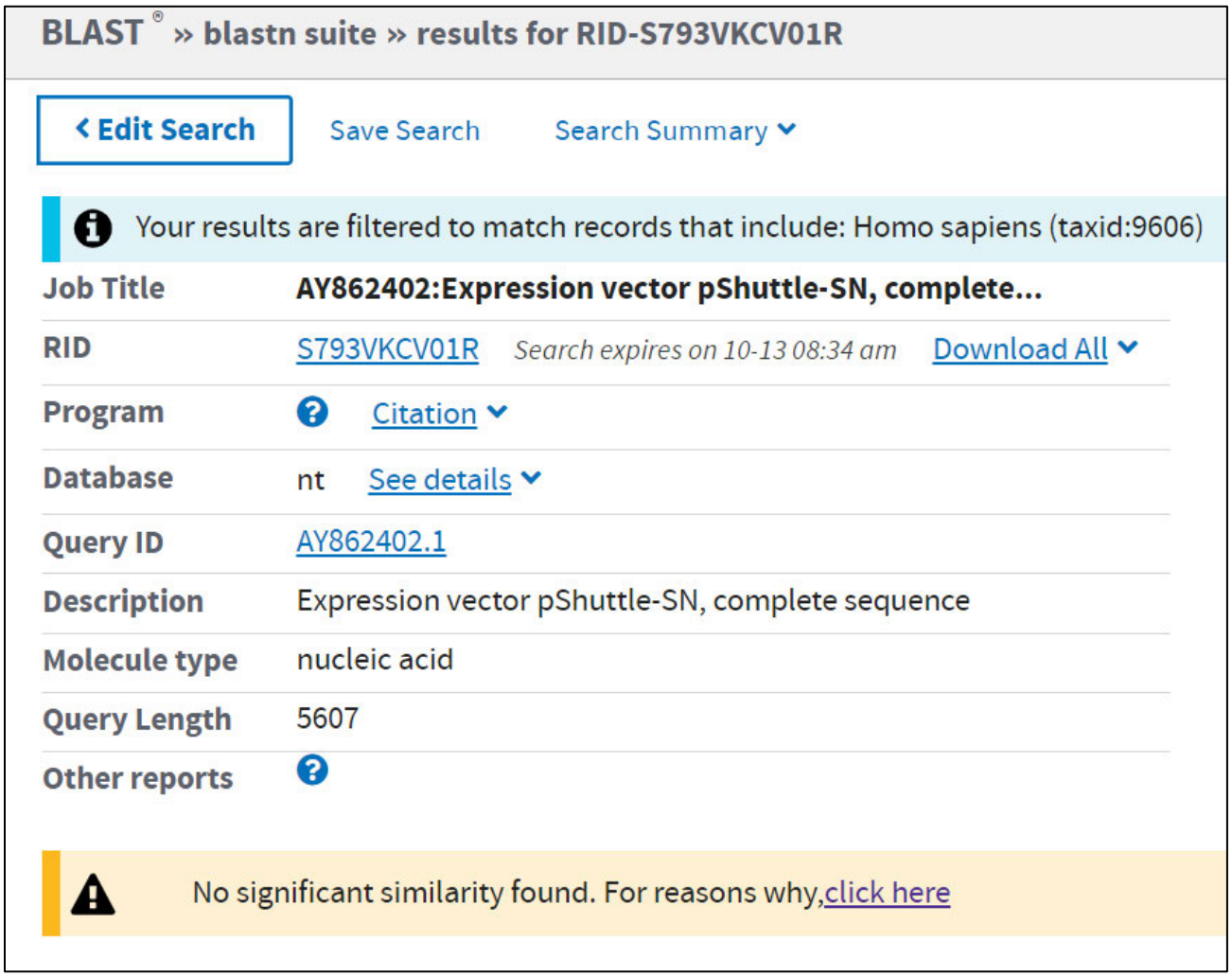
```
Query  784  CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAG  843
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150  CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAG  91

Query  844  CTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACT  903
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90   CTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACT  31

Query  904  ATAGGGAGACCCAAGCTGGCTAGCGTTTAA  933
            ||||||||||||||||||||||||||||||
Sbjct  30   ATAGGGAGACCCAAGCTGGCTAGCGTTTAA  1
```

To test if this was the actual SARS-CoV-1 vaccine vector and had been given to the patients as an desperate attempt to create immunity during an infection, the Spike Protein region of the vaccine was blasted against the above sample, looking for a near 100% homology. The only reads were a 38 nt segment of 1482-1518, with one gap, as expected. The absence of long reads for the SARS-CoV-1 Spike Protein suggests that this vaccine was not a CoV-1 vaccine.

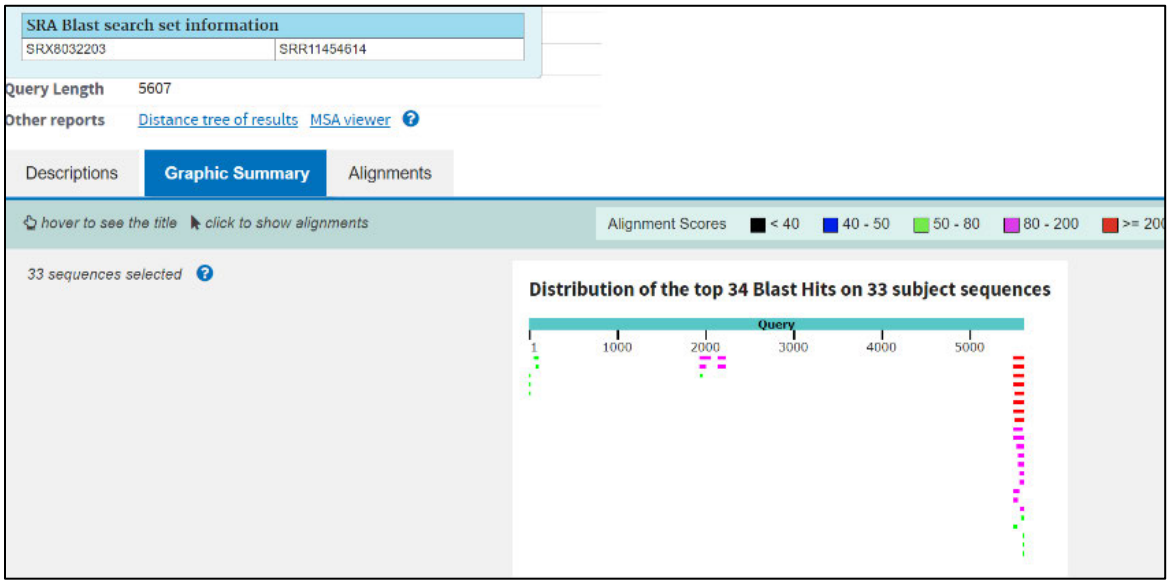
To test if the homology seen between lavage specimens of patients in Wuhan with the CoV-1 Adenovirus vaccine was due to homology with human sequencies the Expression vector was blasted against *Homo sapien* sequencies, but no matches were found, as shown below.



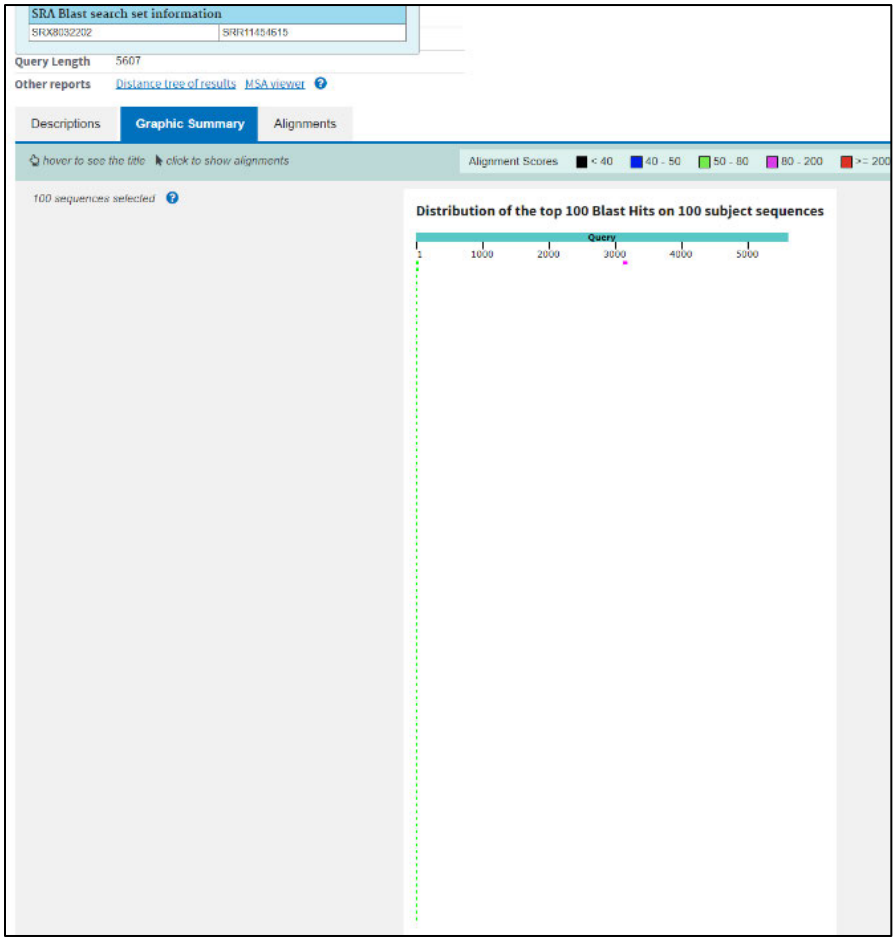
Background. Live attenuated adenovirus vectors for vaccine or gene therapy have been under development for decades.[137] Adenovirus vaccines against SARS-CoV-1[138] and MERS[139] have shown efficacy in animal models of disease. One of the earliest vaccines for CoV-2 is also an adenovirus vector vaccine, developed in collaboration with the PLA.[140]

[137] https://www.sciencedirect.com/science/article/pii/S1525001604013425
[138] https://www.sciencedirect.com/science/article/pii/S0140673603149628
[139] https://www.nih.gov/news-events/news-releases/investigational-chimp-adenovirus-mers-cov-vaccine-protects-monkeys
[140] https://www.nature.com/articles/d41586-020-02523-x ; https://www.nature.com/articles/s41467-020-18077-5

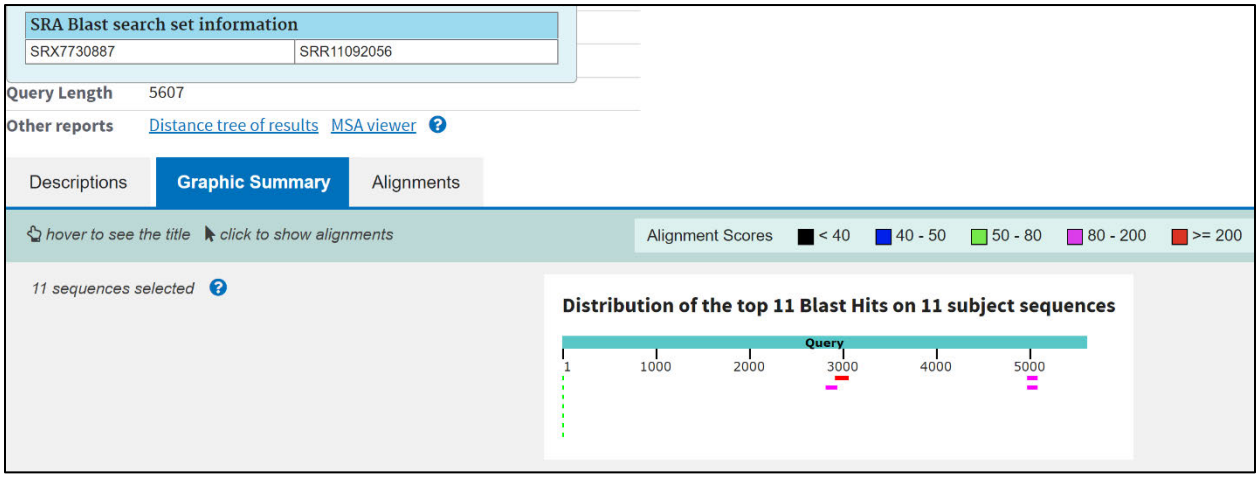
Below is a blast for sequences from the patients in the same hospital who had lavage on the same day but whose specimens went to the Hubei CDC. There are no adenovirus sequences below.

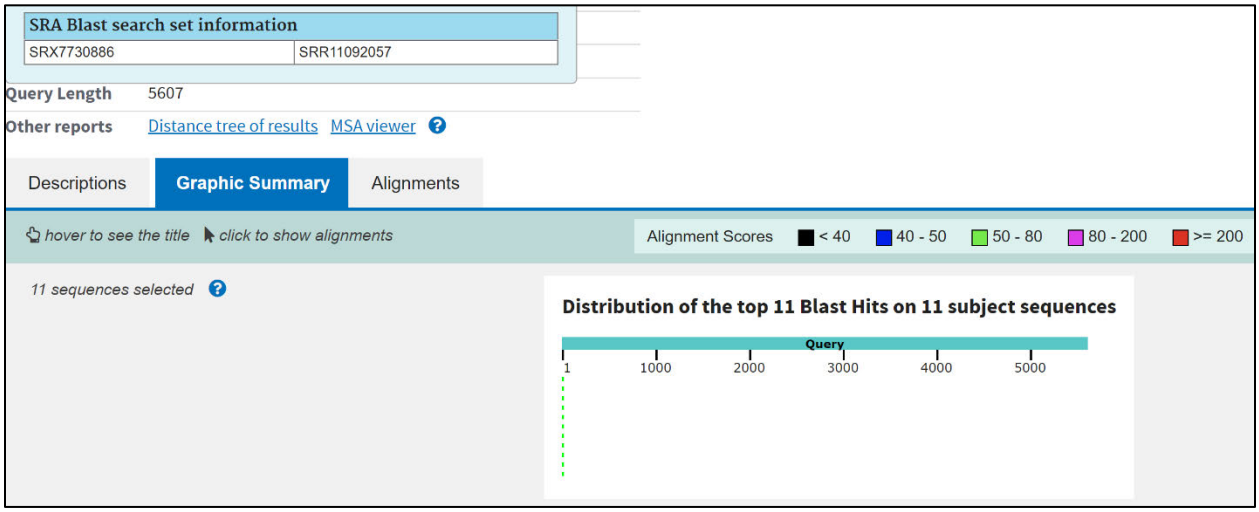


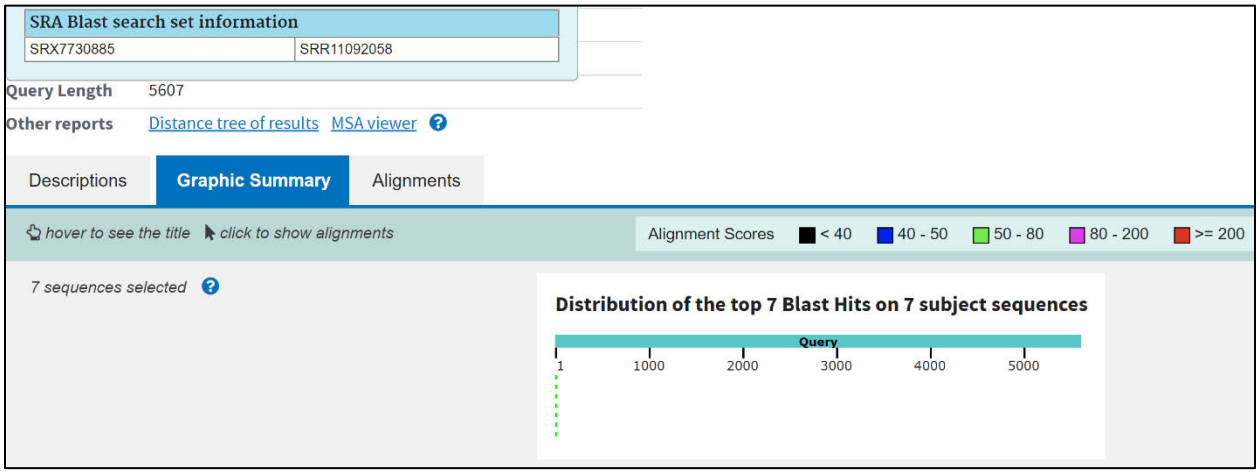
Or in this specimen.



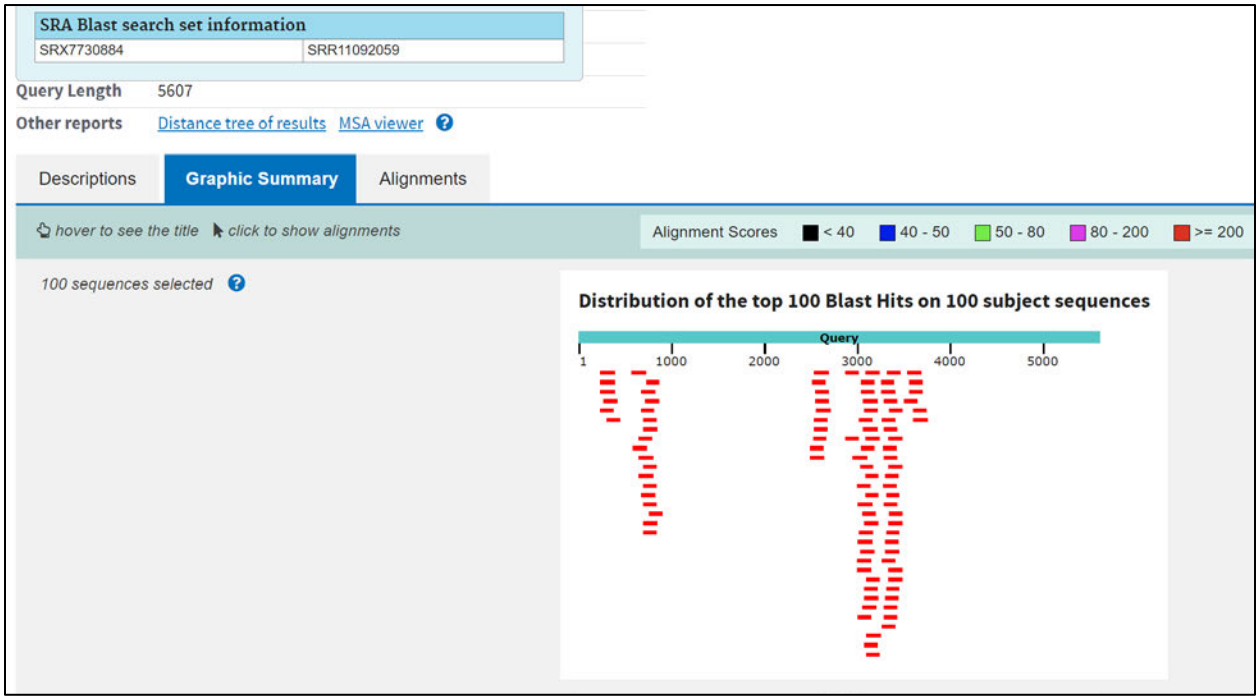
Or in these specimens.

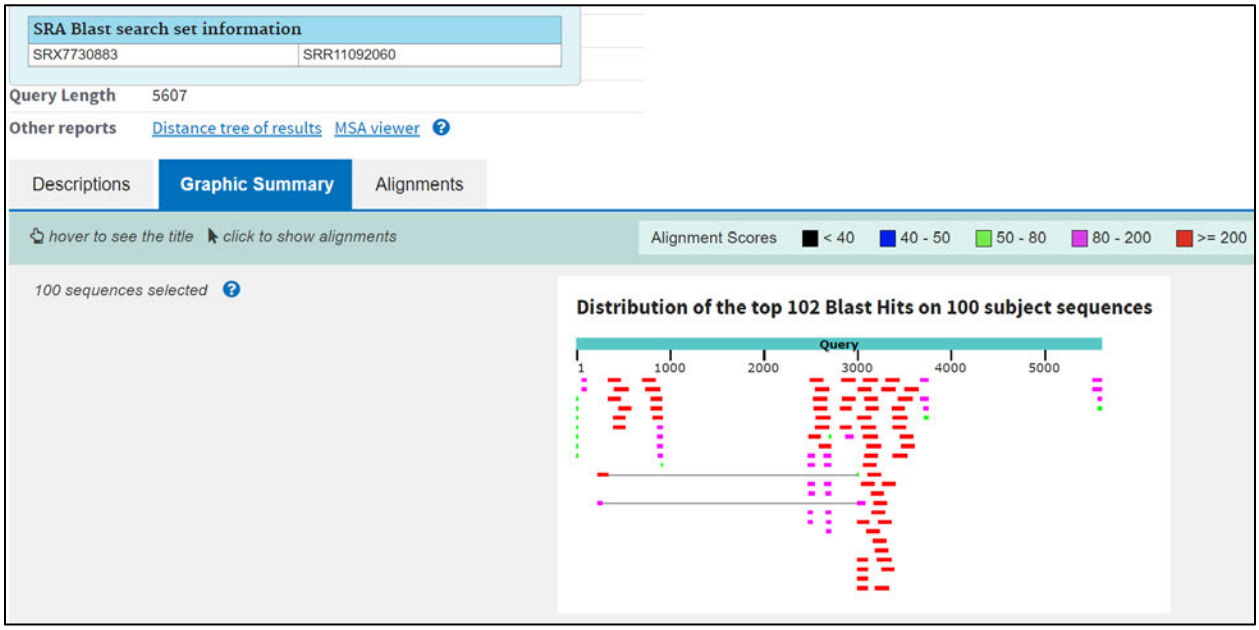


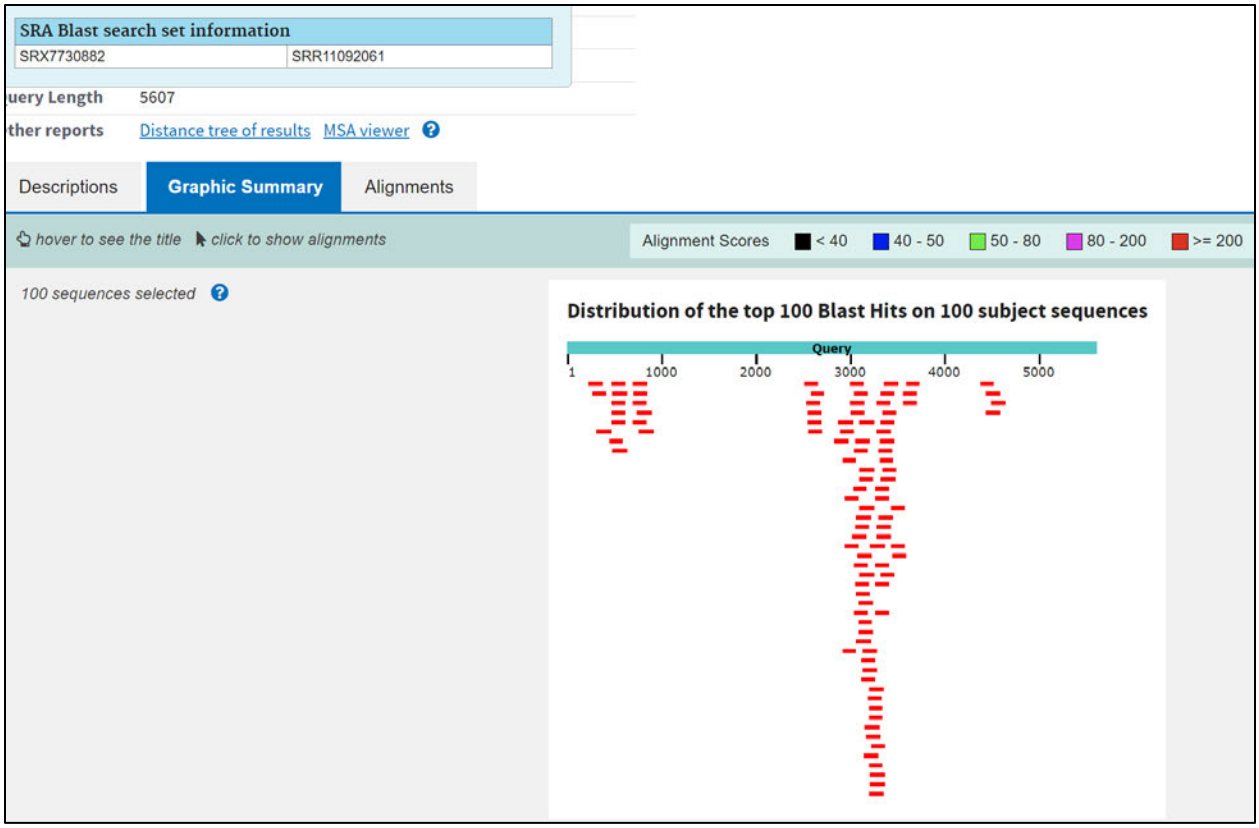


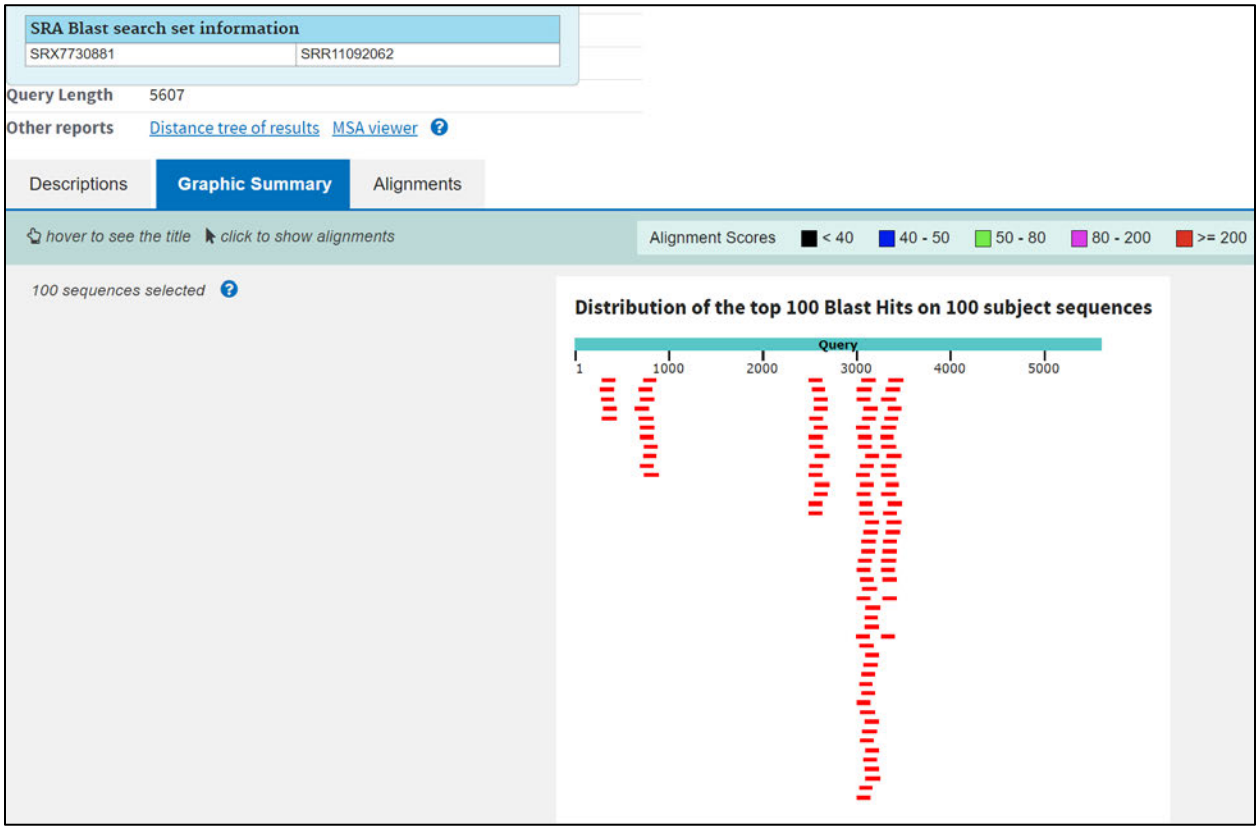


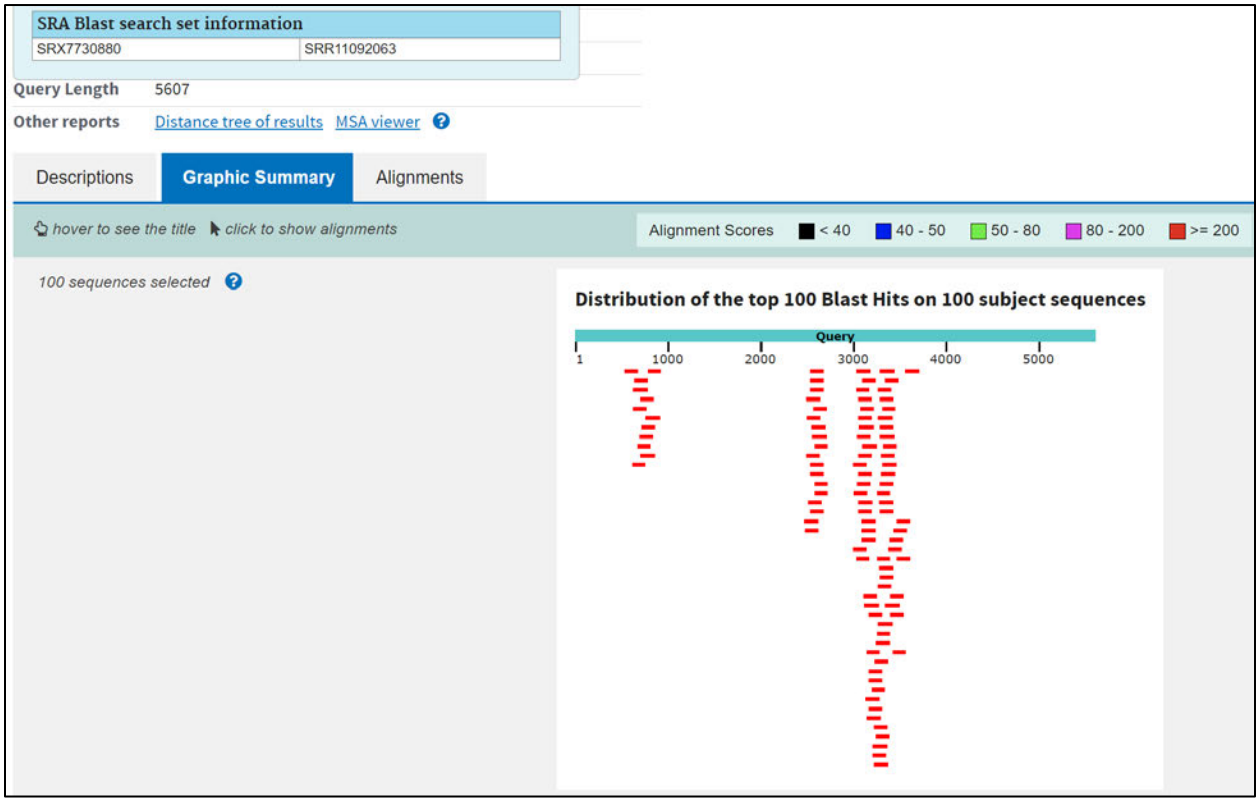
Below begins the specimens from the WIV.

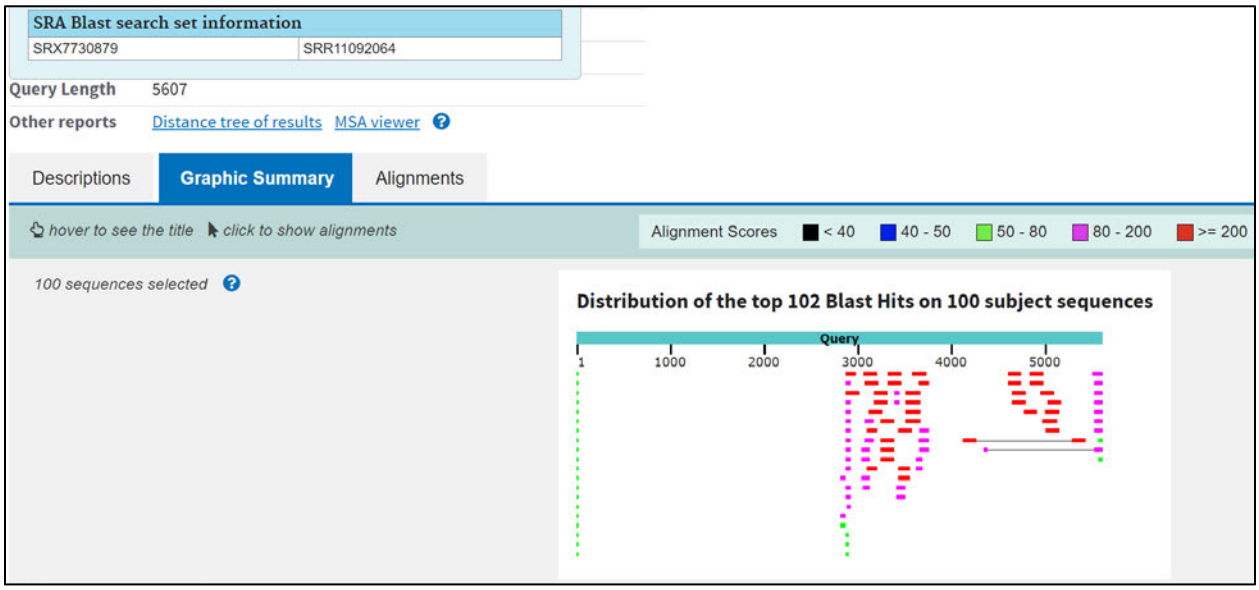





SRA Blast search set information
SRX7730882
SRR11092061
uery Length 5607
ther reports Distance tree of results MSA viewer
Descriptions
Graphic Summary
Alignments
hover to see the title
click to show alignments
Alignment Scores
< 40
40 - 50
50 - 80
80 - 200
>= 200
100 sequences selected
Distribution of the top 100 Blast Hits on 100 subject sequences
Query
1
1000
2000
3000
4000
5000


SRA Blast search set information
SRX7730881
SRR11092062
Query Length 5607
Other reports Distance tree of results MSA viewer
Descriptions
Graphic Summary
Alignments
hover to see the title
click to show alignments
Alignment Scores
< 40
40 - 50
50 - 80
80 - 200
>= 200
100 sequences selected
Distribution of the top 100 Blast Hits on 100 subject sequences
Query
1
1000
2000
3000
4000
5000


SRA Blast search set information
SRX7730880
SRR11092063
Query Length 5607
Other reports Distance tree of results MSA viewer
Descriptions Graphic Summary Alignments
hover to see the title click to show alignments
Alignment Scores < 40 40 - 50 50 - 80 80 - 200 >= 200
100 sequences selected
Distribution of the top 100 Blast Hits on 100 subject sequences
Query
1 1000 2000 3000 4000 5000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SRX7730879
SRR11092064
Query Length 5607
Other reports Distance tree of results MSA viewer
Descriptions Graphic Summary Alignments
hover to see the title click to show alignments
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Distribution of the top 102 Blast Hits on 100 subject sequences
Query
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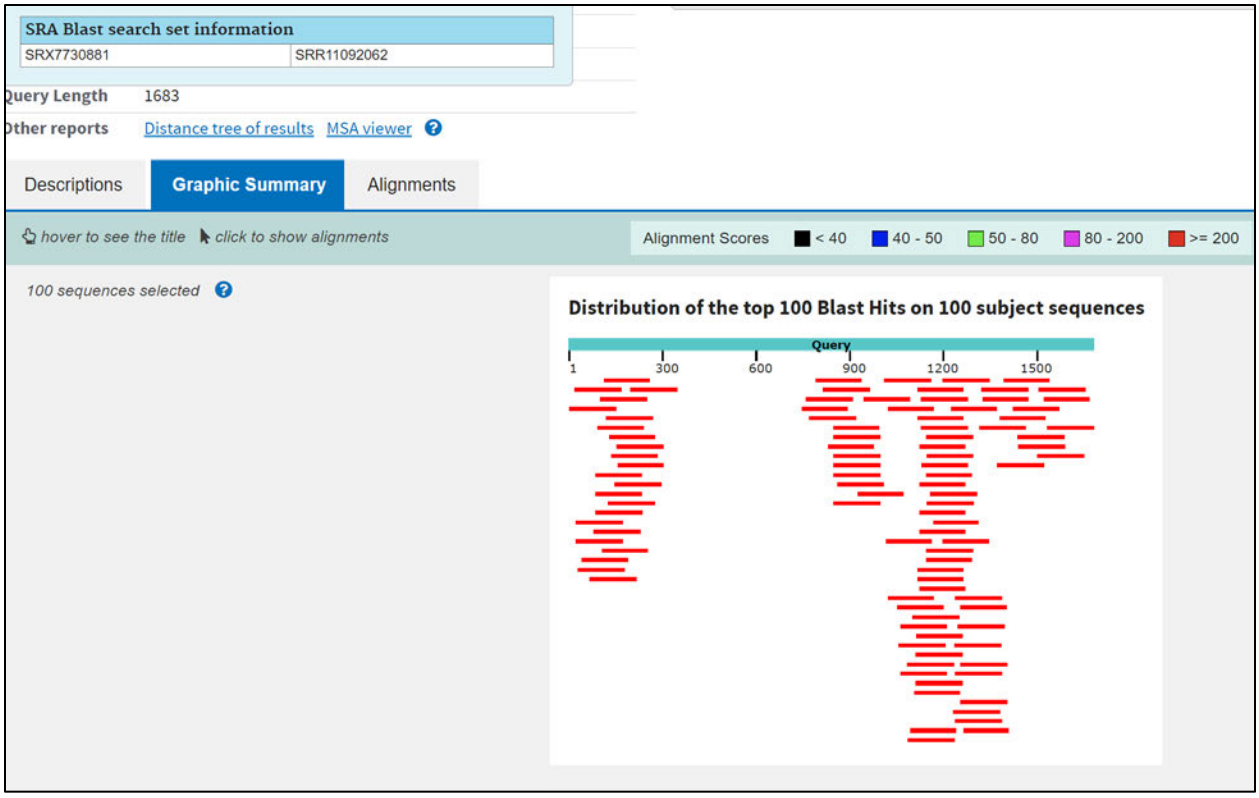

Above is a blast of Influenza A virus (A/swine/eastern China/HH24/2017(H7N9)) segment 4 hemagglutinin (HA) gene, complete cds in patient WIV-4-2 specimen https://www.ncbi.nlm.nih.gov/nucleotide/MG925503.1?report=genbank&log$=nuclalign&blast_rank=2&RID=WYG74MH9016

https://www.ncbi.nlm.nih.gov/nuccore/AY862402.1 Expression vector pShuttle-SN, complete sequence

AY862402.1

Specimen 1

https://www.ncbi.nlm.nih.gov/sra/SRX7730879[accn]

https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR11092064

SRX7730879: RNA-Seq of Homo sapiens: bronchoalveolar lavage fluid
1 ILLUMINA (Illumina MiSeq) run: 3.6M spots, 1G bases, 548.1Mb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit (50) following the manufacturers instructions. An RNA library was then constructed using the NEBNext Ultra II Directional RNA Kit (NEB, USA). Paired-end (150 bp) sequencing of the RNA library was performed on the Miseq platform (Illumina).

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Severe acute respiratory syndrome coronavirus 2 Raw sequence reads
PRJNA605983 • SRP249613 • All experiments • All runs
hide Abstract
Discovery and characterization of a novel human coronavirus from five patients at the early stage of the Wuhan seafood market pneumonia virus outbreak .

Sample:
SAMN14082200 • SRS6151290 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: WIV07
Instrument: Illumina MiSeq
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

Runs: 1 run, 3.6M spots, 1G bases, 548.1Mb

Run	# of Spots	# of Bases	Size	Published
SRR11092064	3,566,583	1G	548.1Mb	2020-02-15

ID: 10108892

BLAST » blastn suite-SRA » results for RID-SBB5ZMXM01R
Home Recent Results Saved Strategies Help

Edit Search Save Search Search Summary
How to read this report? BLAST Help Videos Back to Traditional Results Page

Job Title	AY862402:Expression vector pShuttle-SN, complete...
RID	SBB5ZMXM01R *Search expires on 10-14 21:33 pm* Download All
Program	BLASTN Citation
Database	SRA See details
Query ID	AY862402.1
Description	Expression vector pShuttle-SN, complete sequence
Molecule type	nucleic acid
Query Length	5607
Other reports	Distance tree of results MSA viewer

Filter Results
Percent Identity to
E value to
Query Coverage to
Filter Reset

Descriptions Graphic Summary Alignments

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
SRX7730879	279	279	2%	2e-72	100.00%	SRA:SRR11092064.3512575.2
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SRX7730879	279	279	2%	2e-72	100.00%	SRA:SRR11092064.2655789.2
SRX7730879	279	279	2%	2e-72	100.00%	SRA:SRR11092064.2415875.2
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SRX7730879	274	274	2%	1e-70	99.34%	SRA:SRR11092064.734472.1
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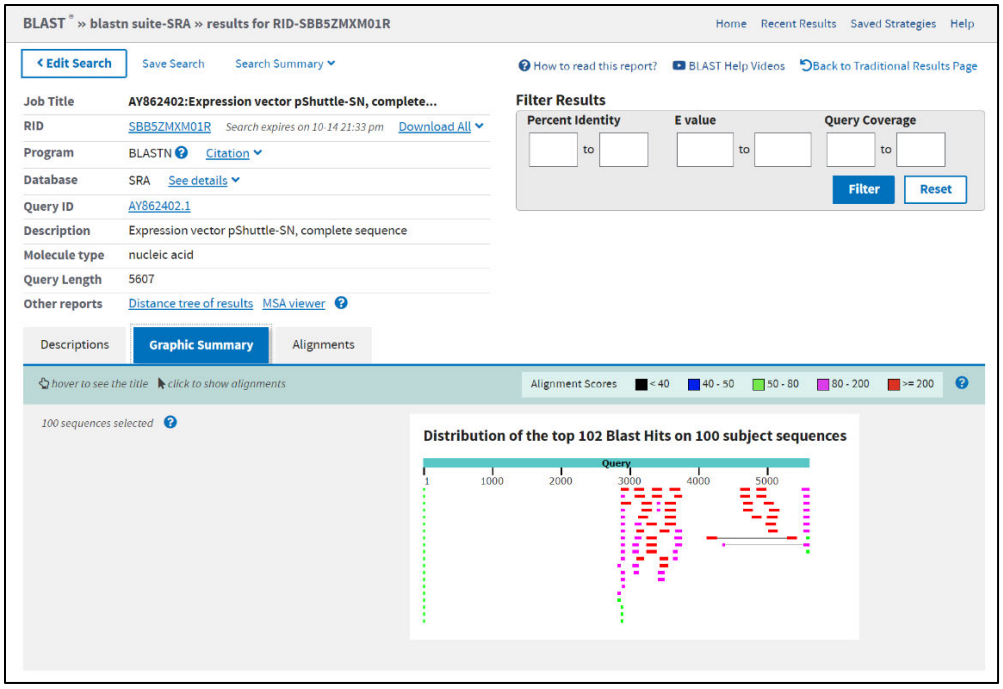
SRX7730879	274	274	2%	1e-70	99.34%	SRA:SRR11092064.612514.1
SRX7730879	272	272	2%	4e-70	99.33%	SRA:SRR11092064.674542.2
SRX7730879	268	268	2%	5e-69	98.68%	SRA:SRR11092064.2917500.2
SRX7730879	268	268	2%	5e-69	98.68%	SRA:SRR11092064.2655789.1
SRX7730879	267	267	2%	2e-68	98.67%	SRA:SRR11092064.612514.2
SRX7730879	259	259	2%	3e-66	99.30%	SRA:SRR11092064.3169732.2
SRX7730879	259	259	2%	3e-66	99.30%	SRA:SRR11092064.3169732.1


BLAST » blastn suite-SRA » results for RID-SBB5ZMXM01R
Home Recent Results Saved Strategies Help
Edit Search
Save Search
Search Summary
How to read this report?
BLAST Help Videos
Back to Traditional Results Page
Job Title AY862402:Expression vector pShuttle-SN, complete...
RID SBB5ZMXM01R Search expires on 10-14 21:33 pm Download All
Program BLASTN Citation
Database SRA See details
Query ID AY862402.1
Description Expression vector pShuttle-SN, complete sequence
Molecule type nucleic acid
Query Length 5607
Other reports Distance tree of results MSA viewer
Filter Results
Percent Identity
E value
Query Coverage
to
Filter
Reset
Descriptions
Graphic Summary
Alignments
hover to see the title
click to show alignments
Alignment Scores
< 40
40 - 50
50 - 80
80 - 200
>= 200
100 sequences selected
Distribution of the top 102 Blast Hits on 100 subject sequences
Query
1
1000
2000
3000
4000
5000

SRX7730879

Sequence ID: SRA:SRR11092064.3512575.2 **Length:** 151 **Number of Matches:** 1

Range 1: 1 to 151 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
279 bits(151)	2e-72	151/151(100%)	0/151(0%)	Plus/Minus

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Query  4830  ACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTA  4889
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  151   ACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTA  92

Query  4890  CGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACC  4949
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  91    CGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACC  32

Query  4950  ATCTCATCTGTAACATCATTGGCAACGCTAC  4980
             ||||||||||||||||||||||||||||||
Sbjct  31    ATCTCATCTGTAACATCATTGGCAACGCTAC  1
```

Download Graphics SRA

SRX7730879

Sequence ID: SRA:SRR11092064.2917500.1 **Length:** 151 **Number of Matches:** 1

Range 1: 1 to 151 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
279 bits(151)	2e-72	151/151(100%)	0/151(0%)	Plus/Minus

```
Query  3319  CCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGT  3378
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  151   CCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGT  92

Query  3379  AAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA  3438
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  91    AAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA  32

Query  3439  TGTAGGCGGTGCTACAGAGTTCTTGAAGTGG  3469
             ||||||||||||||||||||||||||||||
Sbjct  31    TGTAGGCGGTGCTACAGAGTTCTTGAAGTGG  1
```

Download Graphics SRA

SRX7730879

Sequence ID: SRA:SRR11092064.2878891.2 **Length:** 151 **Number of Matches:** 1

Range 1: 1 to 151 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
279 bits(151)	2e-72	151/151(100%)	0/151(0%)	Plus/Plus

```
Query  3059  CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA  3118
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA  60

Query  3119  AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT  3178
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT  120

Query  3179  CCTGTTCCGACCCTGCCGCTTACCGGATACC  3209
             ||||||||||||||||||||||||||||||
Sbjct  121   CCTGTTCCGACCCTGCCGCTTACCGGATACC  151
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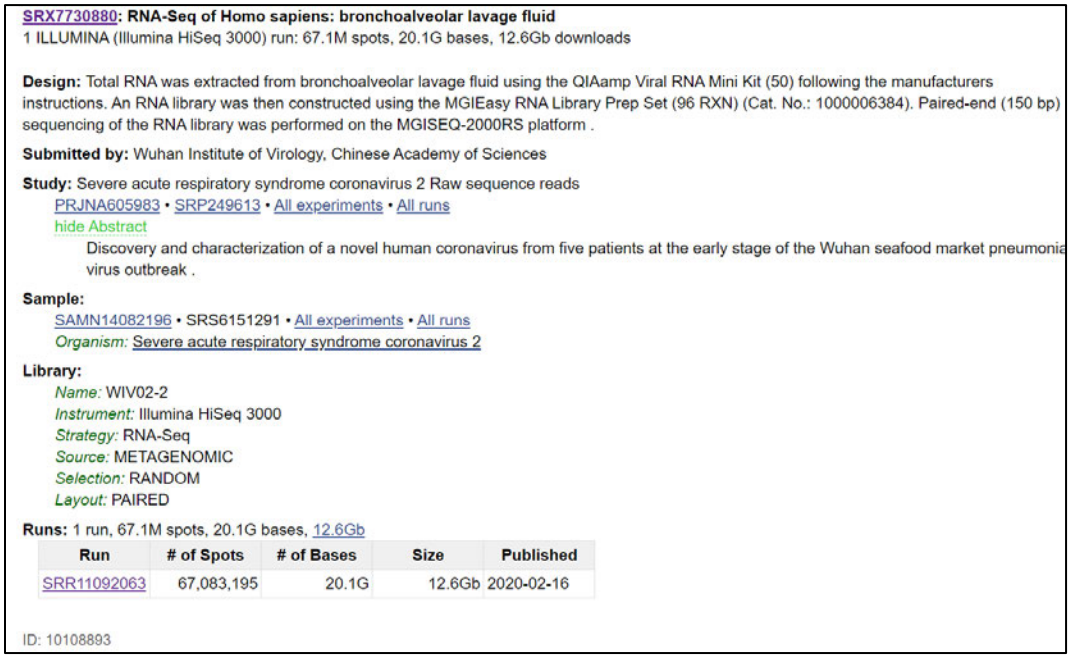
Specimen 2

https://www.ncbi.nlm.nih.gov/sra/SRX7730880[accn]



SRX7730880: **RNA-Seq of Homo sapiens: bronchoalveolar lavage fluid**
1 ILLUMINA (Illumina HiSeq 3000) run: 67.1M spots, 20.1G bases, 12.6Gb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit (50) following the manufacturers instructions. An RNA library was then constructed using the MGIEasy RNA Library Prep Set (96 RXN) (Cat. No.: 1000006384). Paired-end (150 bp) sequencing of the RNA library was performed on the MGISEQ-2000RS platform .

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Severe acute respiratory syndrome coronavirus 2 Raw sequence reads
PRJNA605983 • SRP249613 • All experiments • All runs
hide Abstract
Discovery and characterization of a novel human coronavirus from five patients at the early stage of the Wuhan seafood market pneumonia virus outbreak .

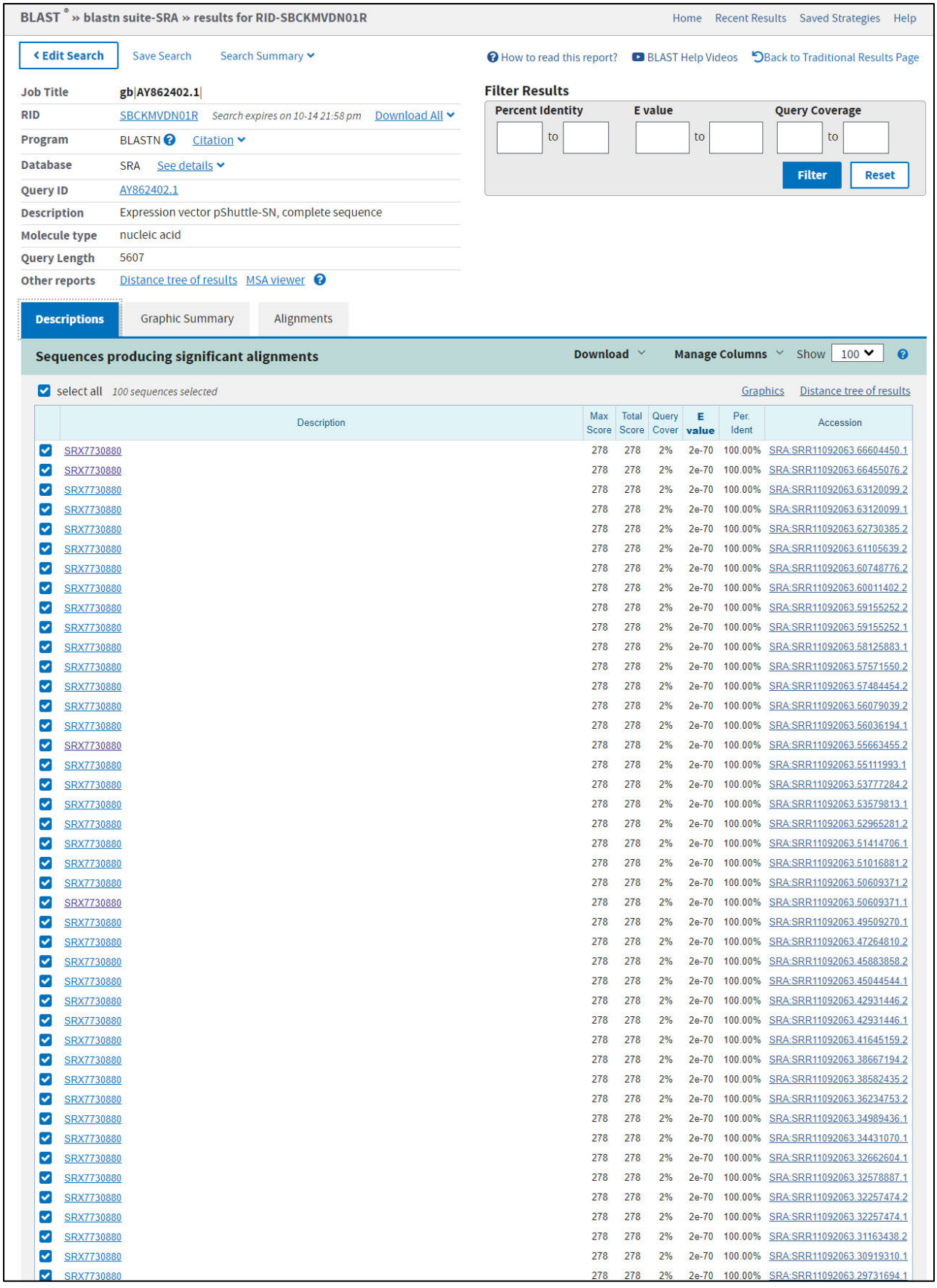
Sample:
SAMN14082196 • SRS6151291 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: WIV02-2
Instrument: Illumina HiSeq 3000
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

Runs: 1 run, 67.1M spots, 20.1G bases, 12.6Gb

Run	# of Spots	# of Bases	Size	Published
SRR11092063	67,083,195	20.1G	12.6Gb	2020-02-16

ID: 10108893



BLAST® » blastn suite-SRA » results for RID-SBCKMVDN01R

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Job Title	gb|AY862402.1
RID	SBCKMVDN01R *Search expires on 10-14 21:58 pm* Download All
Program	BLASTN Citation
Database	SRA See details
Query ID	AY862402.1
Description	Expression vector pShuttle-SN, complete sequence
Molecule type	nucleic acid
Query Length	5607
Other reports	Distance tree of results MSA viewer

Filter Results

Percent Identity to | E value to | Query Coverage to

Filter Reset

Descriptions | Graphic Summary | Alignments

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.66604450.1
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SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.63120099.2
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SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.60011402.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.59155252.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.59155252.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.58125883.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.57571550.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.57484454.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.56079039.2
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SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.55663455.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.55111993.1
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SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.51414706.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.51016881.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.50609371.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.50609371.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.49509270.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.47264810.2
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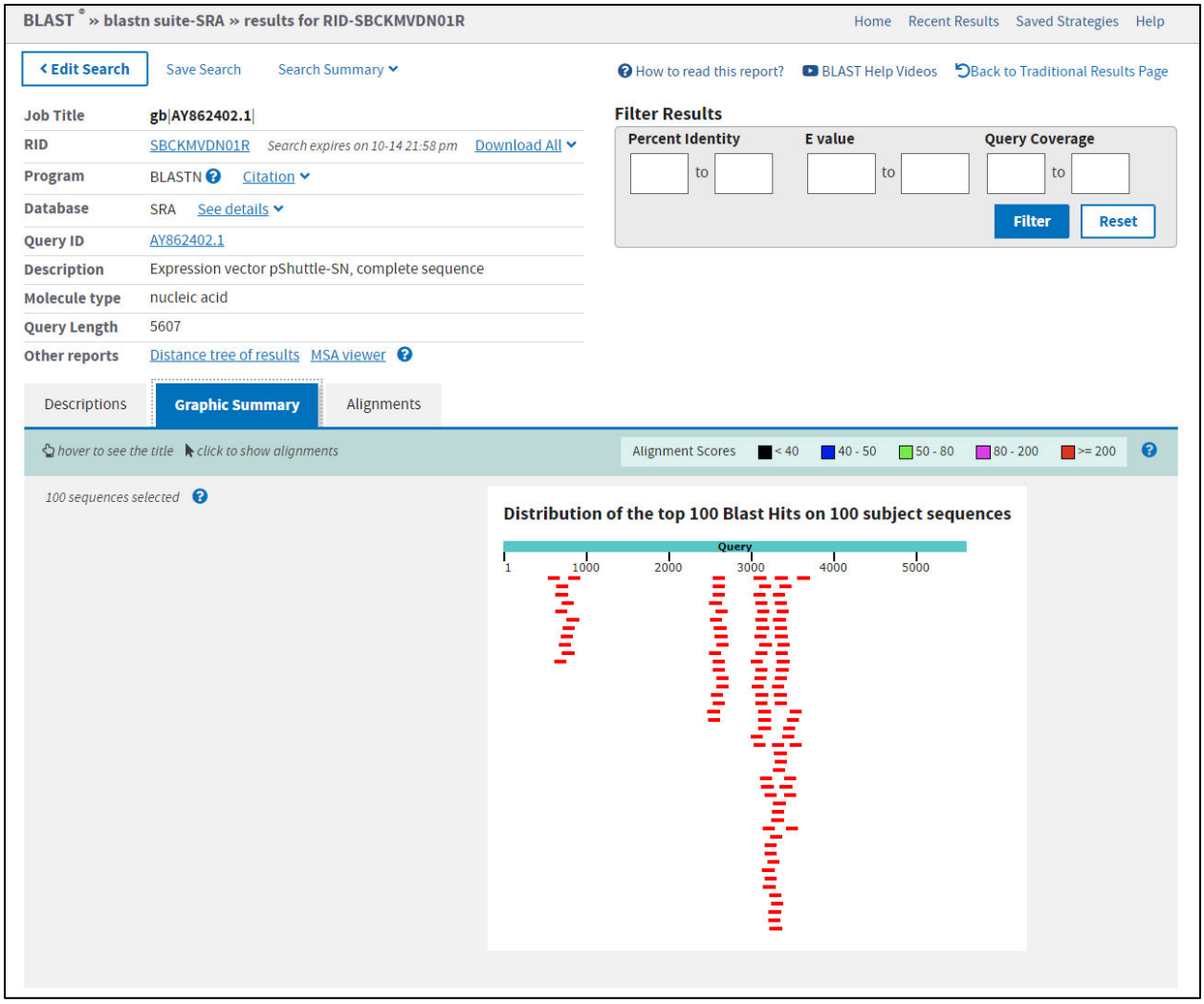
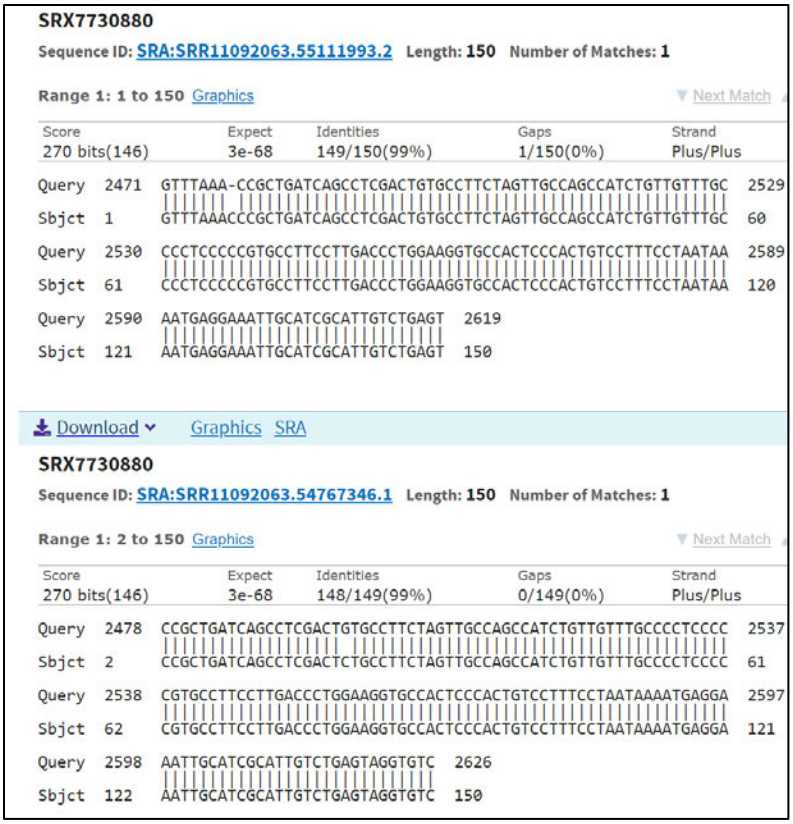
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.42931446.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.42931446.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.41645159.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.38667194.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.38582435.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.36234753.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.34989436.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.34431070.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.32662604.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.32578887.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.32257474.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.32257474.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.31163438.2
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.30919310.1
SRX7730880	278	278	2%	2e-70	100.00%	SRA:SRR11092063.29731694.1



The above distribution of hits appears to ‘invade’ the antigenic, Spike Protein region of the vaccine, residues 961 to 2507. To determine if this was the case, the hit that contained part of the antigen section was displayed (below).



SRX7730880

Sequence ID: SRA:SRR11092063.55111993.2 **Length:** 150 **Number of Matches:** 1

Range 1: 1 to 150 Graphics ▼ Next Match

Score	Expect	Identities	Gaps	Strand
270 bits(146)	3e-68	149/150(99%)	1/150(0%)	Plus/Plus

```
Query  2471  GTTTAAA-CCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGC  2529
             ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGC  60

Query  2530  CCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAA  2589
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    CCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAA  120

Query  2590  AATGAGGAAATTGCATCGCATTGTCTGAGT  2619
              |||||||||||||||||||||||||||||
Sbjct  121   AATGAGGAAATTGCATCGCATTGTCTGAGT  150
```

Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.54767346.1 **Length:** 150 **Number of Matches:** 1

Range 1: 2 to 150 Graphics ▼ Next Match

Score	Expect	Identities	Gaps	Strand
270 bits(146)	3e-68	148/149(99%)	0/149(0%)	Plus/Plus

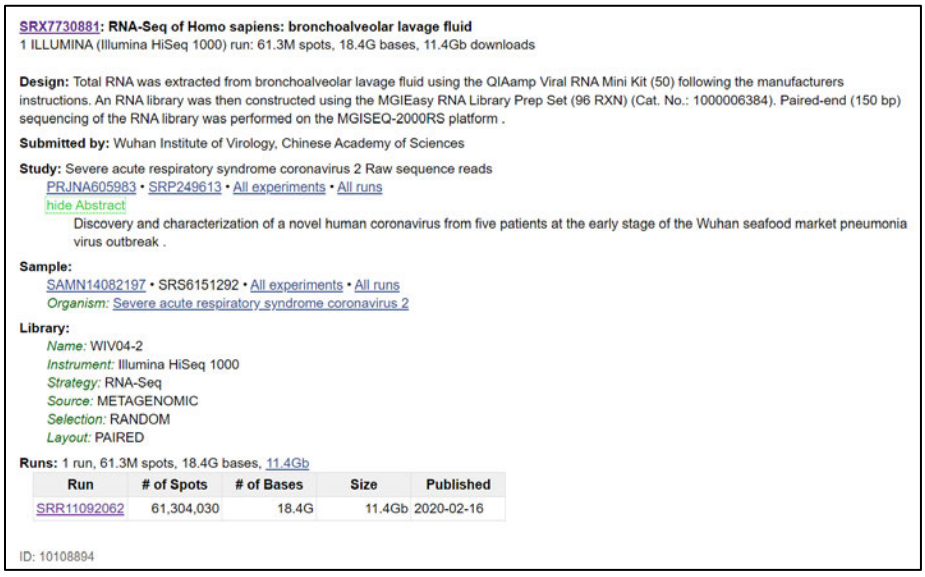
```
Query  2478  CCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCC  2537
             |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  2     CCGCTGATCAGCCTCGACTCTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCC  61

Query  2538  CGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGA  2597
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  62    CGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGA  121

Query  2598  AATTGCATCGCATTGTCTGAGTAGGTGTC  2626
              |||||||||||||||||||||||||||||
Sbjct  122   AATTGCATCGCATTGTCTGAGTAGGTGTC  150
```

As you can see, this 150 nt sequence starts at 2471 and within the antigen segment. However, there is no homology identified when this is blasted against the Reference Sequence of SARS-CoV-2.

Sample 3



SRX7730881: RNA-Seq of Homo sapiens: bronchoalveolar lavage fluid
1 ILLUMINA (Illumina HiSeq 1000) run: 61.3M spots, 18.4G bases, 11.4Gb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit (50) following the manufacturers instructions. An RNA library was then constructed using the MGIEasy RNA Library Prep Set (96 RXN) (Cat. No.: 1000006384). Paired-end (150 bp) sequencing of the RNA library was performed on the MGISEQ-2000RS platform .

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Severe acute respiratory syndrome coronavirus 2 Raw sequence reads
PRJNA605983 • SRP249613 • All experiments • All runs
hide Abstract
Discovery and characterization of a novel human coronavirus from five patients at the early stage of the Wuhan seafood market pneumonia virus outbreak .

Sample:
SAMN14082197 • SRS6151292 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: WIV04-2
Instrument: Illumina HiSeq 1000
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

Runs: 1 run, 61.3M spots, 18.4G bases, 11.4Gb

Run	# of Spots	# of Bases	Size	Published
SRR11092062	61,304,030	18.4G	11.4Gb	2020-02-16

ID: 10108894

Sample 4

SRX7730882: RNA-Seq of Homo sapiens: bronchoalveolar lavage fluid
1 ILLUMINA (Illumina HiSeq 3000) run: 34.3M spots, 10.3G bases, 6.4Gb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit (50) following the manufacturers instructions. An RNA library was then constructed using the MGIEasy RNA Library Prep Set (96 RXN) (Cat. No.: 1000006384). Paired-end (150 bp) sequencing of the RNA library was performed on the MGISEQ-2000RS platform .

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Severe acute respiratory syndrome coronavirus 2 Raw sequence reads
PRJNA605983 • SRP249613 • All experiments • All runs
hide Abstract
Discovery and characterization of a novel human coronavirus from five patients at the early stage of the Wuhan seafood market pneumonia virus outbreak .

Sample:
SAMN14082198 • SRS6151293 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: WIV05
Instrument: Illumina HiSeq 3000
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

Runs: 1 run, 34.3M spots, 10.3G bases, 6.4Gb

Run	# of Spots	# of Bases	Size	Published
SRR11092061	34,255,843	10.3G	6.4Gb	2020-02-16

ID: 10108895

https://www.ncbi.nlm.nih.gov/sra/SRX2913157[accn]

Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College

above has a few 125 nt hits between about 1950 to 3500 in adenovirus

Sequences used for the blast analyses

Adenovirus vaccine with CoV-1 Spike Protein

1 taactataac ggtcctaagg tagcgaaagc tcagatctgg atctcccgat cccctatggt
61 cgactctcag tacaatctgc tctgatgccg catagttaag ccagtatctg ctccctgctt
121 gtgtgttgga ggtcgctgag tagtgcgcga gcaaaattta agctacaaca aggcaaggct
181 tgaccgacaa ttgcatgaag aatctgctta gggttaggcg ttttgcgctg cttcgcgatg
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301 cggggtcatt agttcatagc ccatatatgg agttccgcgt tacataactt acggtaaatg
361 gcccgcctgg ctgaccgccc aacgacccccc gcccattgac gtcaataatg acgtatgttc
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481 ctgcccactt ggcagtacat caagtgtatc atatgccaag tacgcccccc attgacgtca
541 atgacggtaa atggcccgcc tggcattatg cccagtacat gaccttatgg gactttccta
601 cttggcagta catctacgta ttagtcatcg ctattaccat ggtgatgcgg ttttggcagt
661 acatcaatgg gcgtggatag cggtttgact cacggggatt tccaagtctc cacccccattg
721 acgtcaatgg gagtttgttt tggcaccaaa atcaacggga ctttccaaaa tgtcgtaaca
781 actccgcccc attgacgcaa atgggcggta ggcgtgtacg gtgggaggtc tatataagca
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961 gtgatattct tgttaataac taaacgaaca tgtttatttt cttattattt cttactctca
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1081 ctcaacatac ttcatctatg agggggggttt actatcctga tgaaattttt agatcagaca
1141 ctctttattt aactcaggat ttatttcttc cattttattc taatgttaca gggtttcata
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1261 ccacagagaa atcaaatgtt gtccgtggtt gggtttttgg ttctaccatg aacaacaagt
1321 cacagtcggt gattattatt aacaattcta ctaatgttgt tatacgagca tgtaactttg
1381 aattgtgtga caacccttttc tttgctgttt ctaaacccat gggtacacag acacatacta
1441 tgatattcga taatgcattt aattgcactt tcgagtacat atctgatgcc ttttcgcttg
1501 atgtttcaga aaagtcaggt aattttaaac acttacgaga gtttgtgttt aaaaataaag
1561 atgggtttct ctatgtttat aagggctatc aacctataga tgtagttcgt gatctacctt
1621 ctggttttaa cactttgaaa cctatttta agttgcctct tggtattaac attacaaatt
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2521 gttgtttgcc cctccccccgt gccttccttg accctggaag gtgccactcc cactgtcctt
2581 tcctaataaa atgaggaaat tgcatcgcat tgtctgagta ggtgtcattc tattctgggg
2641 ggtggggtgg ggcaggacag caaggggggag gattgggaag acaatagcag gcatgctggg
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SARS-CoV-2 Spike Protein gene

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25381 ataa

In silico construct with Adenovirus vector shuttle containing CoV-2 Spike Protein gene

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Artificial Spike Protein in Chinese patent (not found in any patient specimens)

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Adenovirus 5 vector shuttle with Synthetic construct H7N9 HA gene 7640-9302

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Expression vector pShuttle-SN, complete sequence

GenBank: AY862402.1

FASTA Graphics

Go to:

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  TITLE     Adenoviral expression of a truncated S1 subunit of SARS-CoV spike
            protein results in specific humoral immune responses against
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  TITLE     Constructing recombinant adenovirus carrying the spike gene
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  JOURNAL   Unpublished
REFERENCE   3  (bases 1 to 5607)
  AUTHORS   Liu,R.-Y., Huang,B.-J., Wu,L.-Z., Huang,J.-L., Zhang,R.-H.,
            Zeng,Y.-X. and Huang,W.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-DEC-2004) Cancer Center, Sun Yat-Sen University, 651
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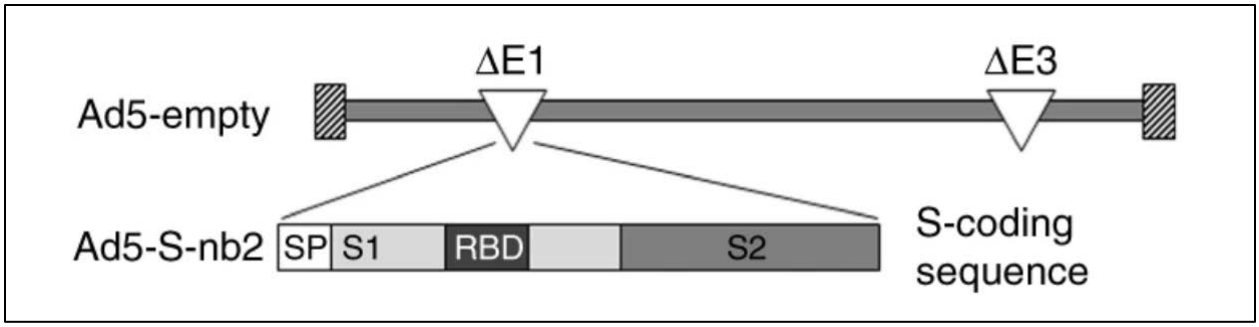

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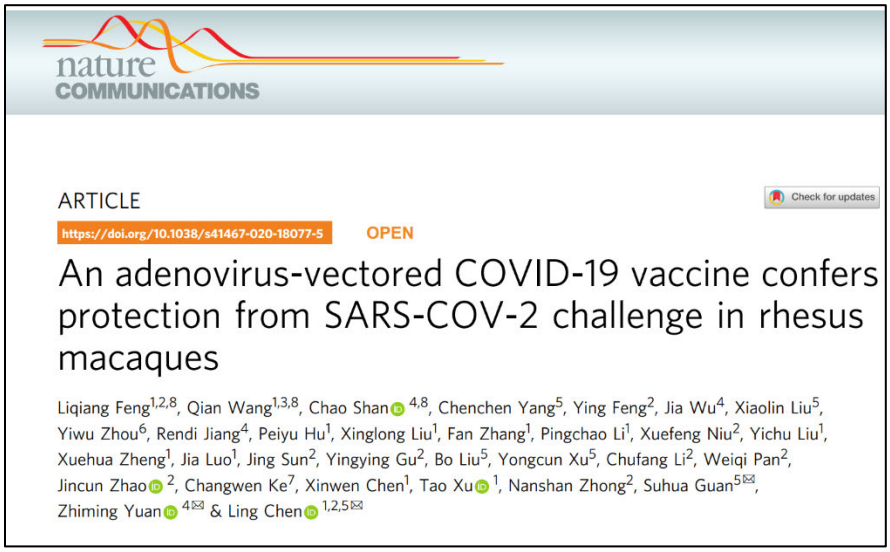

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ARTICLE

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https://doi.org/10.1038/s41467-020-18077-5 OPEN

An adenovirus-vectored COVID-19 vaccine confers protection from SARS-COV-2 challenge in rhesus macaques

Liqiang Feng[1,2,8], Qian Wang[1,3,8], Chao Shan[4,8], Chenchen Yang[5], Ying Feng[2], Jia Wu[4], Xiaolin Liu[5], Yiwu Zhou[6], Rendi Jiang[4], Peiyu Hu[1], Xinglong Liu[1], Fan Zhang[1], Pingchao Li[1], Xuefeng Niu[2], Yichu Liu[1], Xuehua Zheng[1], Jia Luo[1], Jing Sun[2], Yingying Gu[2], Bo Liu[5], Yongcun Xu[5], Chufang Li[2], Weiqi Pan[2], Jincun Zhao[2], Changwen Ke[7], Xinwen Chen[1], Tao Xu[1], Nanshan Zhong[2], Suhua Guan[5]✉, Zhiming Yuan[4]✉ & Ling Chen[1,2,5]✉

Adenovirus vaccine sequences in patient specimen WIV02 from patient who is 32 y, male, hospitalized, ICU4G, outbreak 19 Dec 2019.

SRX7730880: **RNA-Seq of Homo sapiens: bronchoalveolar lavage fluid**
1 ILLUMINA (Illumina HiSeq 3000) run: 67.1M spots, 20.1G bases, 12.6Gb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit (50) following the manufacturers instructions. An RNA library was then constructed using the MGIEasy RNA Library Prep Set (96 RXN) (Cat. No.: 1000006384). Paired-end (150 bp) sequencing of the RNA library was performed on the MGISEQ-2000RS platform .

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Severe acute respiratory syndrome coronavirus 2 Raw sequence reads
PRJNA605983 • SRP249613 • All experiments • All runs
hide Abstract
Discovery and characterization of a novel human coronavirus from five patients at the early stage of the Wuhan seafood market pneumonia virus outbreak .

Sample:
SAMN14082196 • SRS6151291 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: WIV02-2
Instrument: Illumina HiSeq 3000
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

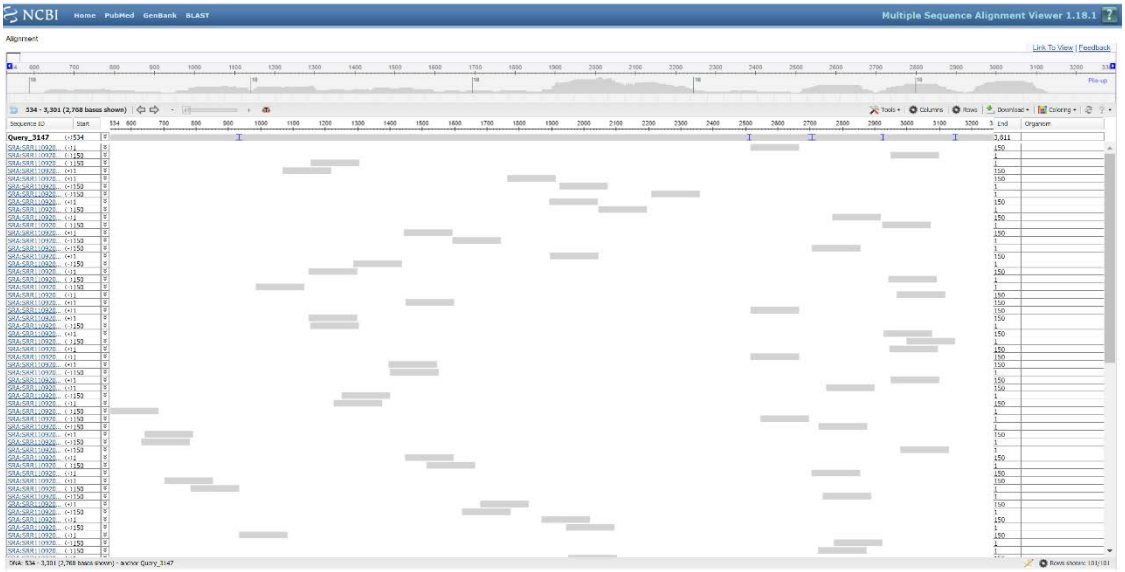
Runs: 1 run, 67.1M spots, 20.1G bases, 12.6Gb

Run	# of Spots	# of Bases	Size	Published
SRR11092063	67,083,195	20.1G	12.6Gb	2020-02-16

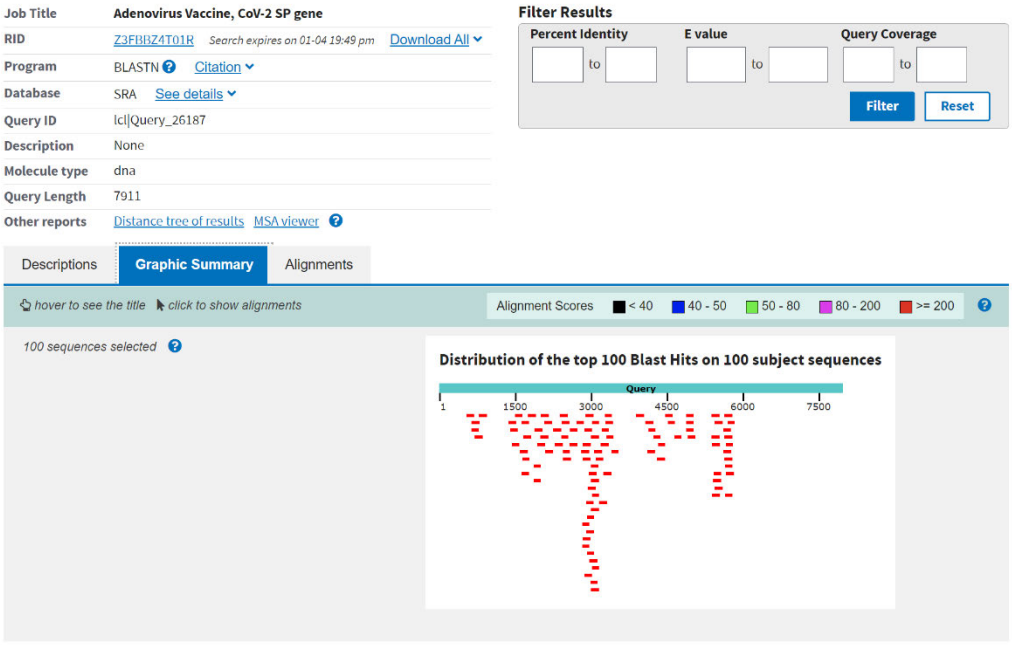
URL: https://www.ncbi.nlm.nih.gov/sra/SRX7730880%5baccn%5d

Adenovirus Expression vector pShuttle-SN, Synthetic construct H7N9 HA gene 7640-9302

534-3301 contiguous nt sequence (2768 nt) in H7N9 HA gene



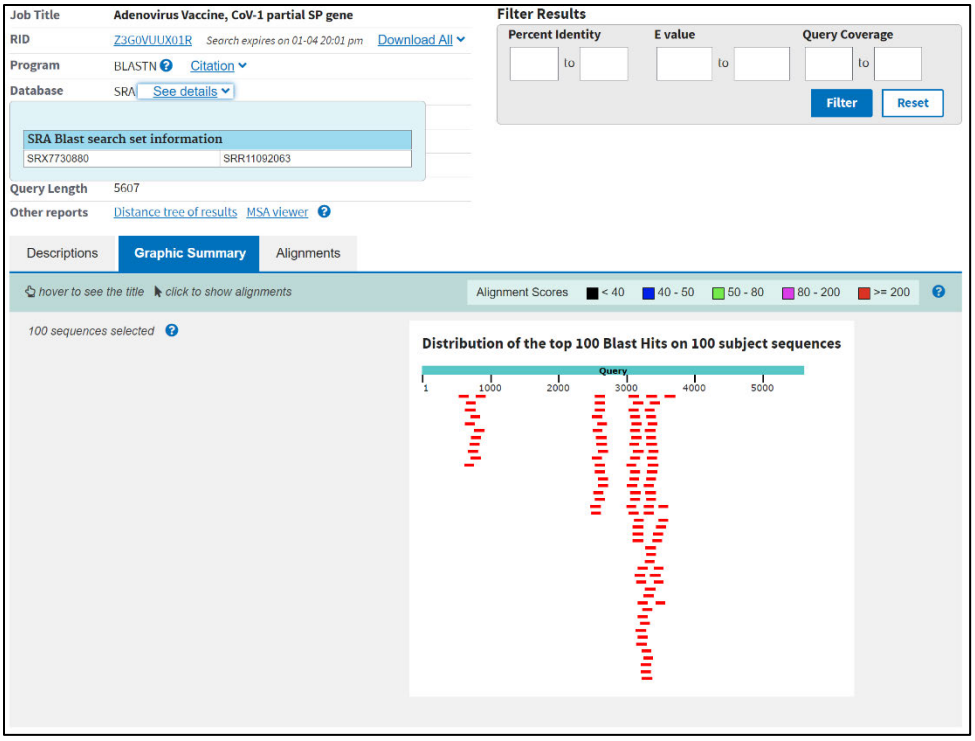
Adenovirus with CoV-2 Spike Protein, full sequence

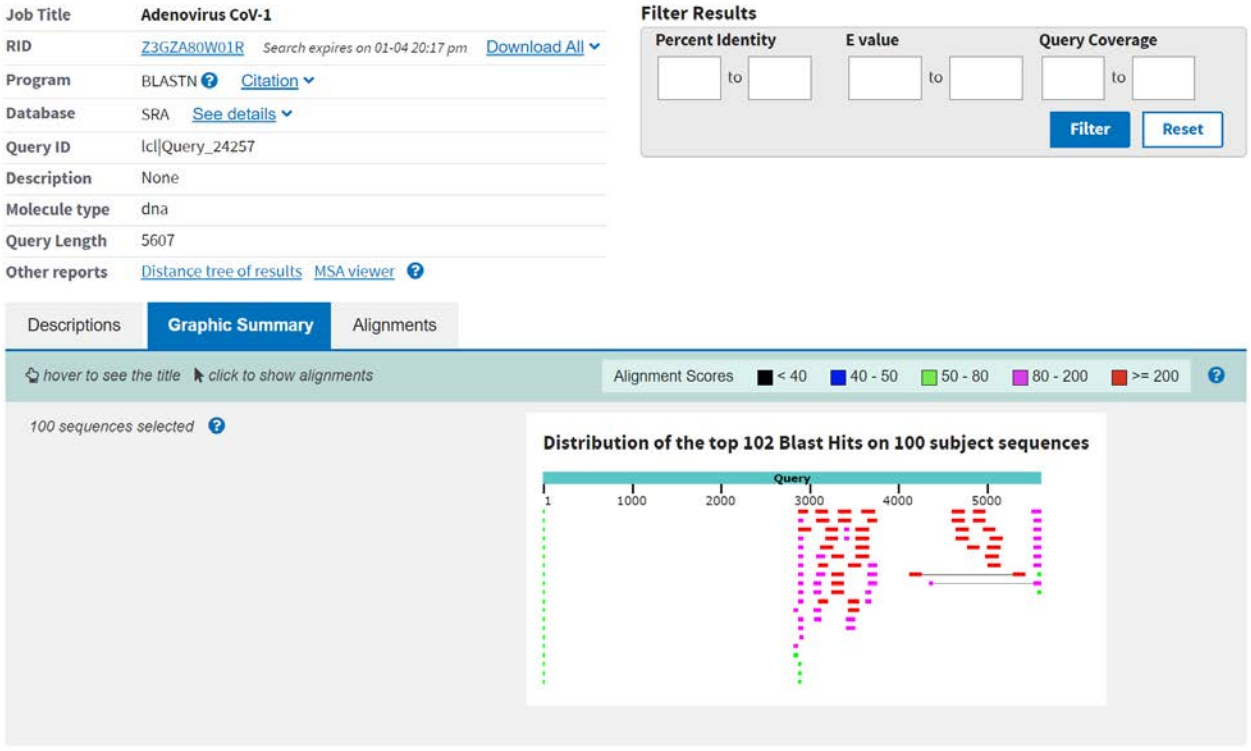


534-4573 contiguous (4040 nts)

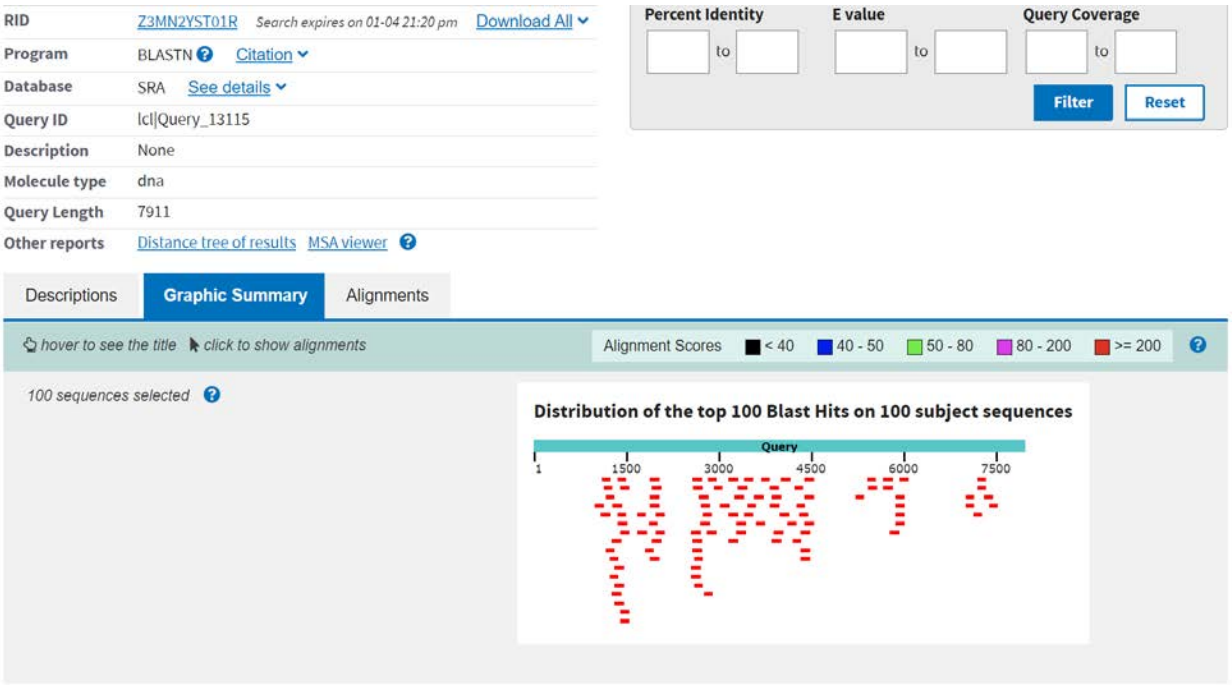
Adenovirus with CoV-1 partial sequence

534-1905 (1372 nts) contiguous

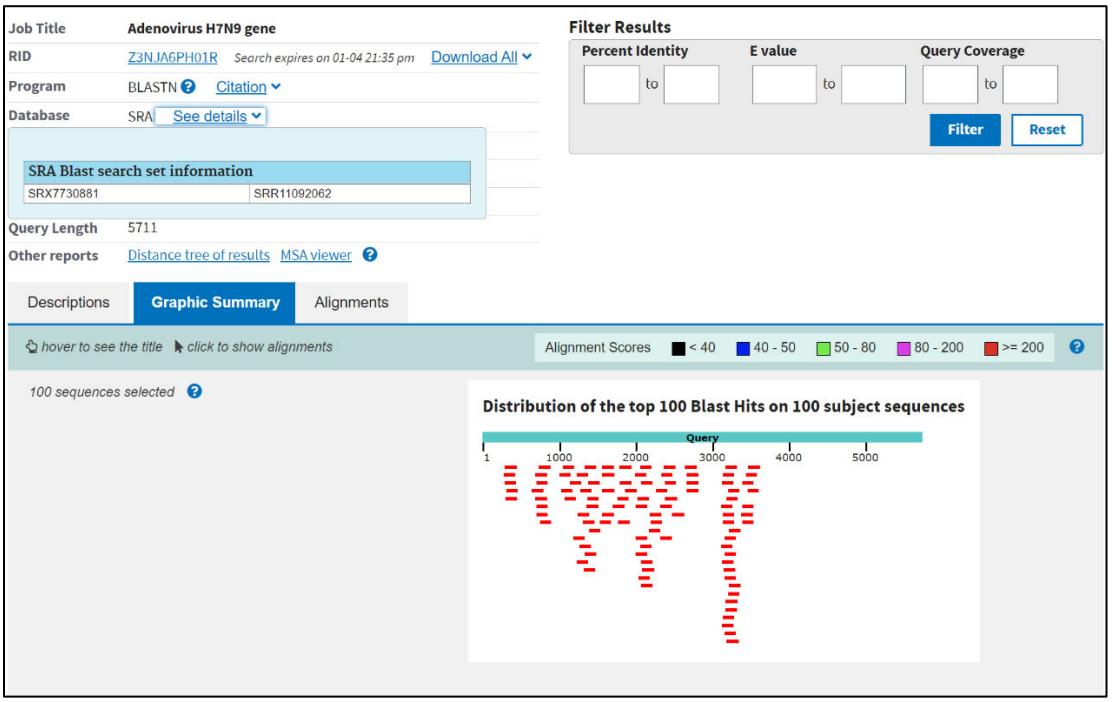




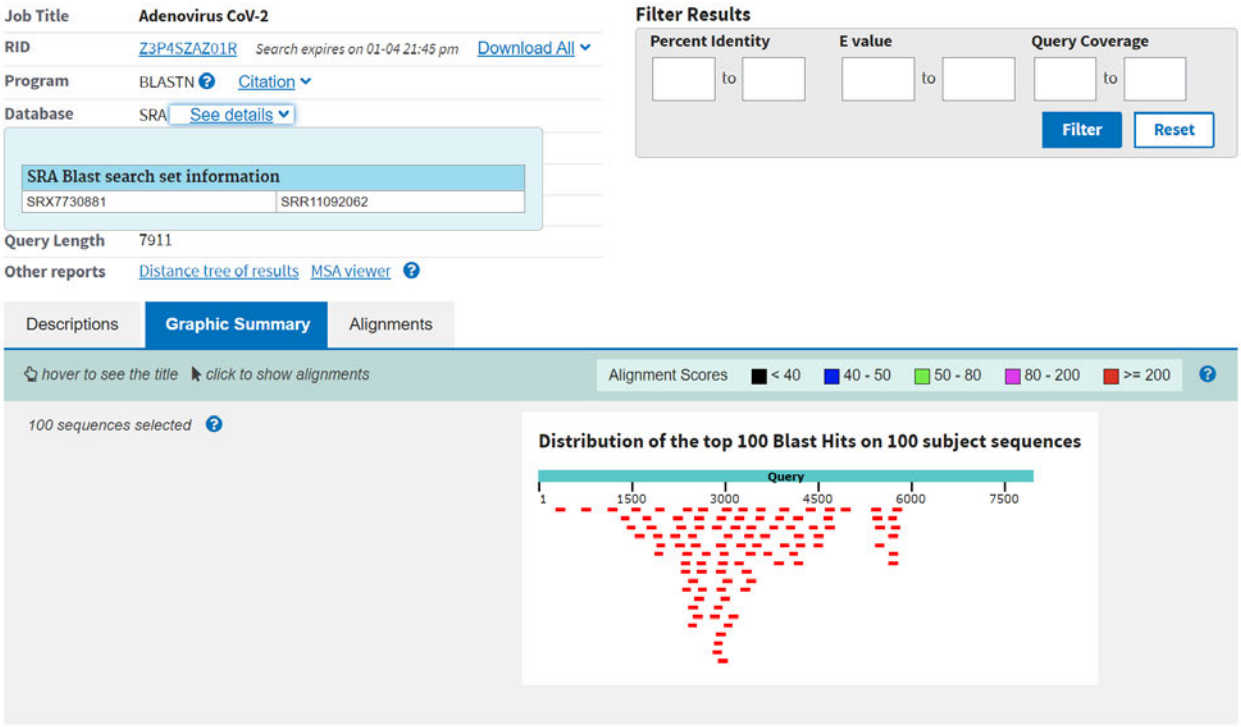
Adenovirus vaccine with CoV-2 SP:



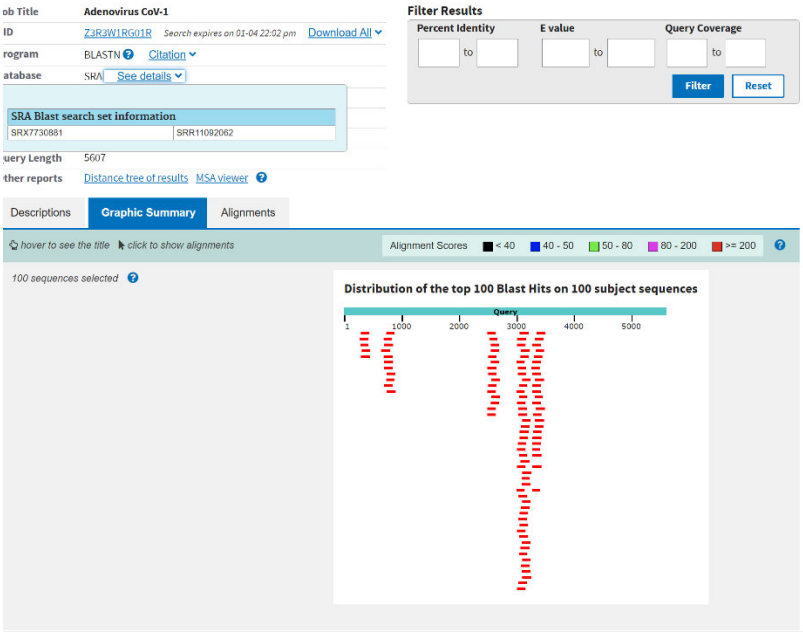
Adenovirus H7N9



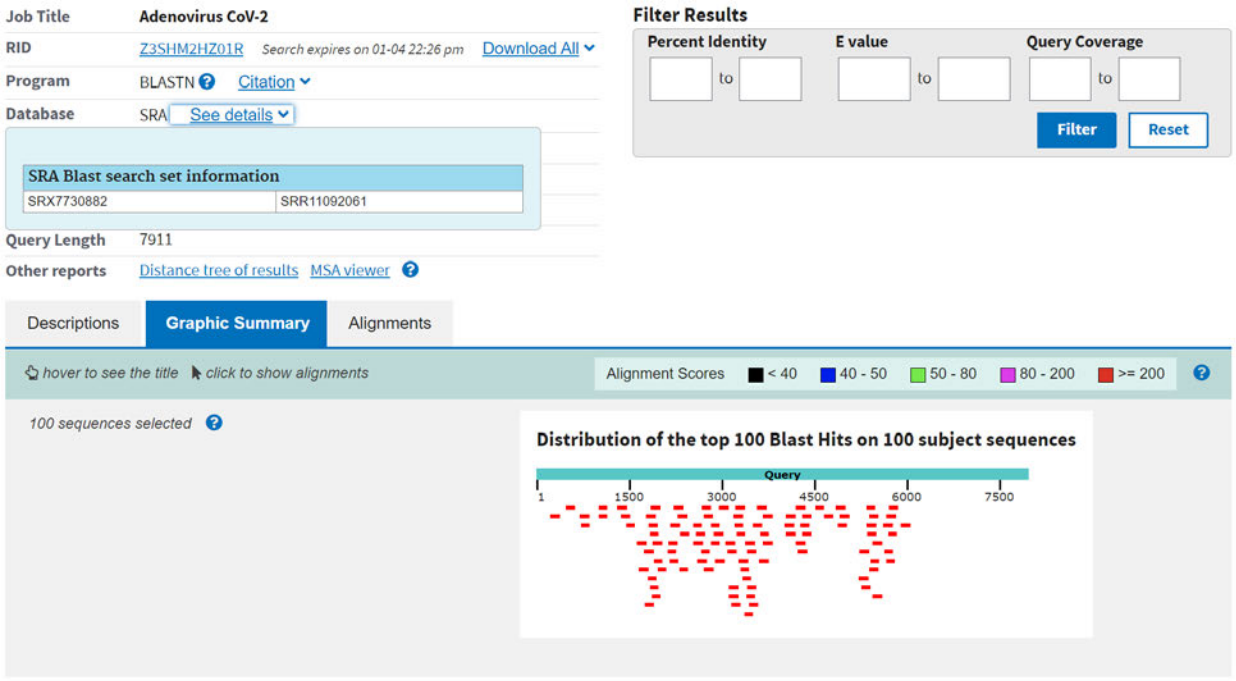
Adenovirus CoV-2



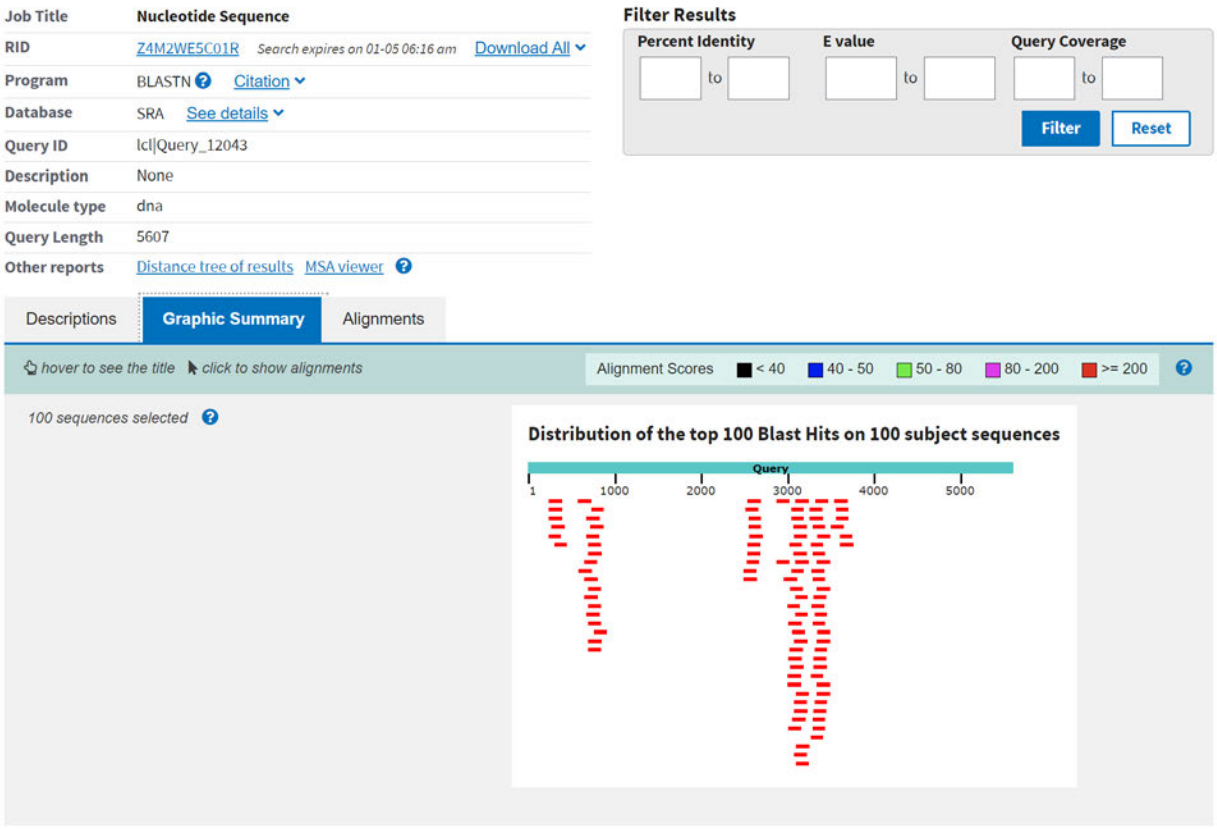
Adenovirus CoV-1



Adenovirus CoV-2



WIV-7 patient blast with Adenovirus to CoV-1



Blast analysis of early RNA seq raw reads from the Wuhan Institute of virology contain extensive reads matching "Expression vector pShuttle-SN" sequences, the same adenovirus vector used by the PLA Army for the creation of a vaccine.

Following the 2003 SARS epidemic, Liu et al. developed an adenoviral expression vector of a truncated S1 subunit of SARS-CoV spike protein that resulted in specific humoral immune responses against SARS-CoV in rats.[141] This same vector was used to create the CoV-2 adenovirus vector vaccine.[142]

In order to test the hypothesis that CoV-2 began in the PLA Hospital as a vaccine challenge clinical trial that went awry, RNA-Seq raw reads from nasopharyngeal specimens of Wuhan COVID patients were blasted against the published genome sequence of the SARS-CoV-1 vaccine (GenBank AY862402.1). I used the SARS-CoV-1 vaccine because the PLA CoV-2 vaccine has not been published.

Nt Sequence	Function
1-990	Adeno virus genes
991-2506	Truncated N-terminus of SARS-CoV-1 Spike Protein
2507-5607	Adeno virus genes

The expected result would be the finding of RNA-Seq sequence raw reads that were homologous to the two Adenovirus regions but only partially homologous (about 80%) to the SARS-CoV-1 regions.

Eleven entries were found on GenBank of SRA data for RNA-Seq of early COVID-19 patients from Wuhan that were sequenced at either the WIV or the Hubei Provincial Center for Disease Control and Prevention (Hubei CDC). These entries are in the Text-Table below.

[141] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114075/
[142] Chinese patent, attached herein.

1. **RNA-Seq** of Homo sapiens: hCov-19 infected patients Bronchoalveolar **lavage** uid
   1 ILLUMINA (Illumina MiSeq) run: 5.8M spots, 1.7G bases, 634.3Mb downloads
   Accession: SRX8032203

2. **RNA-Seq** of Homo sapiens: hCov-19 infected patients Bronchoalveolar **lavage** uid
   1 ILLUMINA (Illumina MiSeq) run: 5.2M spots, 1.6G bases, 583.4Mb downloads
   Accession: SRX8032202

3. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina MiSeq) run: 5.2M spots, 1.5G bases, 772.9Mb downloads
   Accession: SRX7730887

4. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina MiSeq) run: 5.2M spots, 1.5G bases, 768.3Mb downloads
   Accession: SRX7730886

5. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina MiSeq) run: 8.3M spots, 2.2G bases, 1.2Gb downloads
   Accession: SRX7730885

6. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 3000) run: 38.5M spots, 11.5G bases, 7.1Gb downloads
   Accession: SRX7730884

7. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 3000) run: 29.7M spots, 8.9G bases, 5.6Gb downloads
   Accession: SRX7730883

8. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 3000) run: 34.3M spots, 10.3G bases, 6.4Gb downloads
   Accession: SRX7730882

9. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
   1 ILLUMINA (Illumina HiSeq 1000) run: 61.3M spots, 18.4G bases, 11.4Gb downloads
   Accession: SRX7730881

10. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
    1 ILLUMINA (Illumina HiSeq 3000) run: 67.1M spots, 20.1G bases, 12.6Gb downloads
    Accession: SRX7730880

11. **RNA-Seq** of Homo sapiens: bronchoalveolar **lavage** fluid
    1 ILLUMINA (Illumina MiSeq) run: 3.6M spots, 1G bases, 548.1Mb downloads
    Accession: SRX7730879

The WIV entry with the greatest read depth, Number 10 above, is described below:

SRX7730880: **RNA-Seq of Homo sapiens: bronchoalveolar lavage fluid**
1 ILLUMINA (Illumina HiSeq 3000) run: 67.1M spots, 20.1G bases, 12.6Gb downloads

Design: Total RNA was extracted from bronchoalveolar lavage fluid using the QIAamp Viral RNA Mini Kit (50) following the manufacturers instructions. An RNA library was then constructed using the MGIEasy RNA Library Prep Set (96 RXN) (Cat. No.: 1000006384). Paired-end (150 bp sequencing of the RNA library was performed on the MGISEQ-2000RS platform .

Submitted by: Wuhan Institute of Virology, Chinese Academy of Sciences

Study: Severe acute respiratory syndrome coronavirus 2 Raw sequence reads
PRJNA605983 • SRP249613 • All experiments • All runs
show Abstract

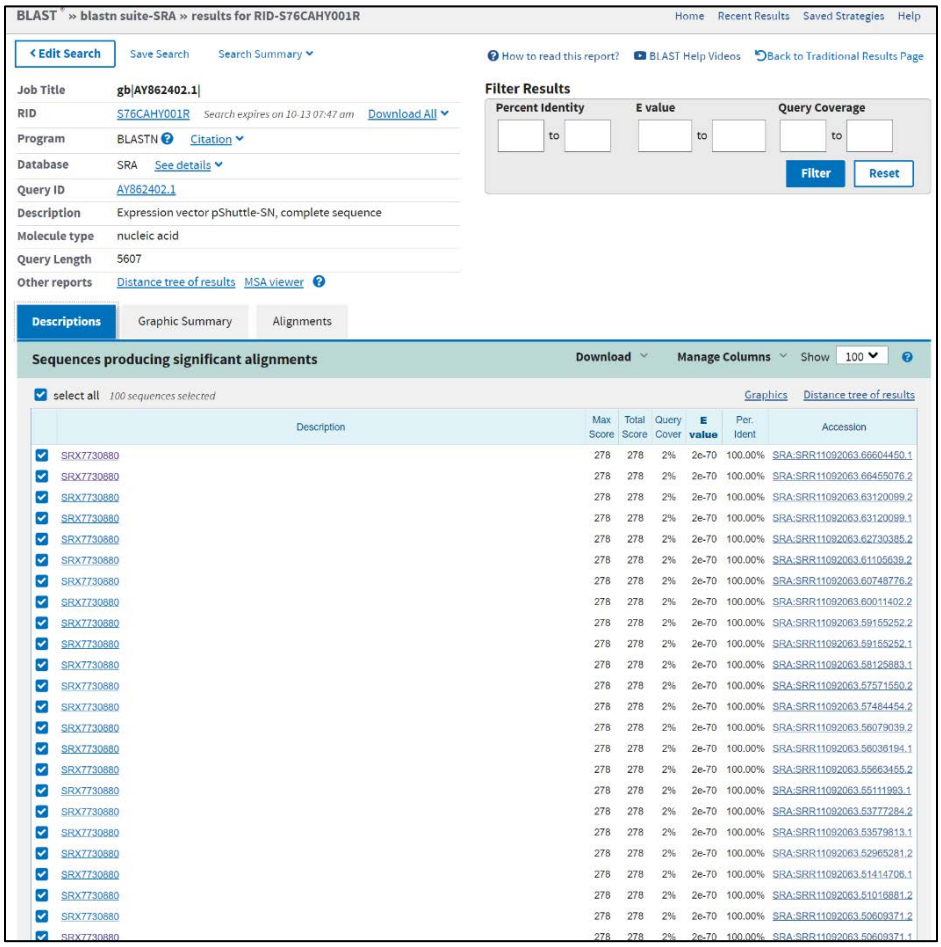
Sample:
SAMN14082196 • SRS6151291 • All experiments • All runs
Organism: Severe acute respiratory syndrome coronavirus 2

Library:
Name: WIV02-2
Instrument: Illumina HiSeq 3000
Strategy: RNA-Seq
Source: METAGENOMIC
Selection: RANDOM
Layout: PAIRED

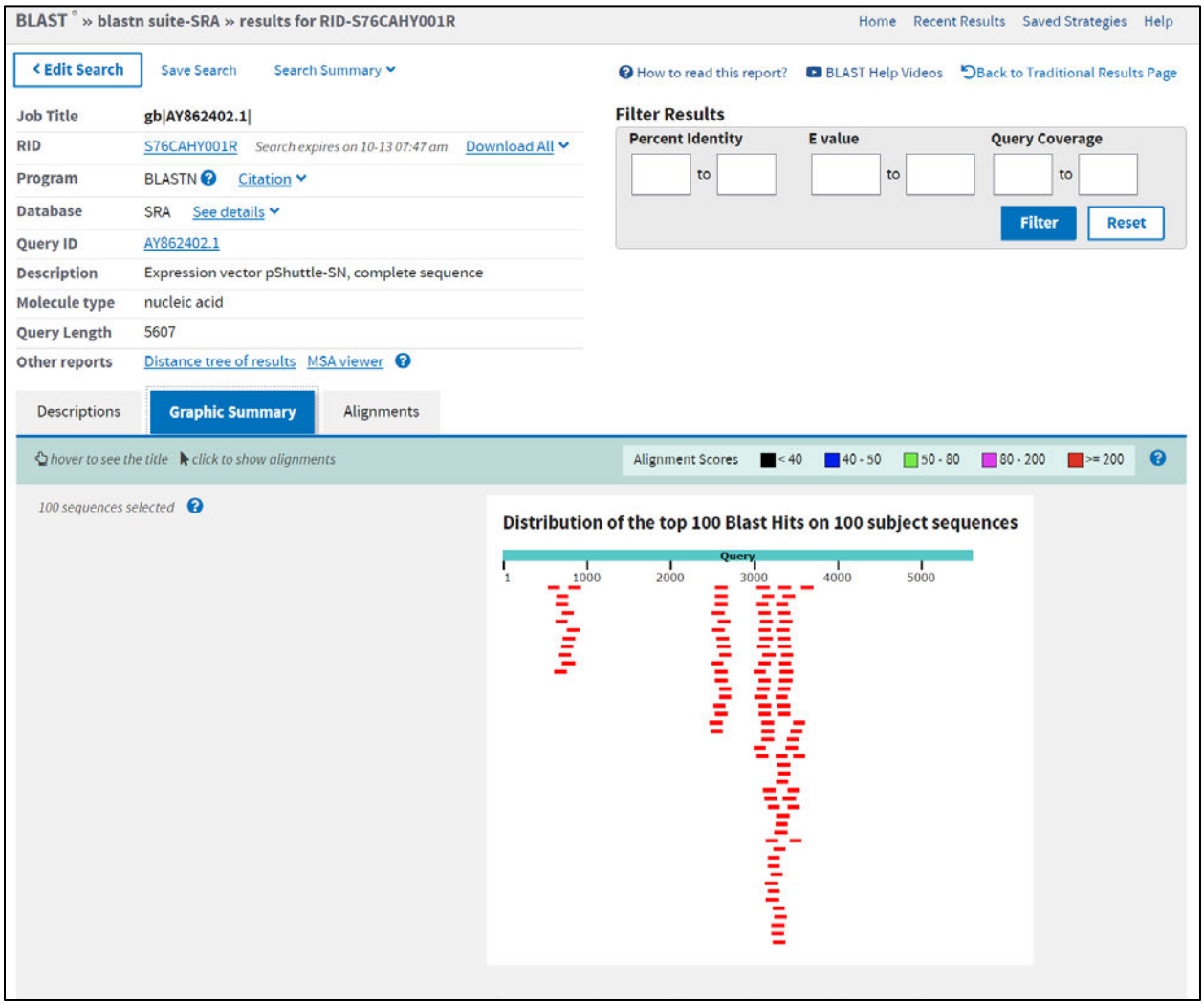
Runs: 1 run, 67.1M spots, 20.1G bases, 12.6Gb

Run	# of Spots	# of Bases	Size	Published
SRR11092063	67,083,195	20.1G	12.6Gb	2020-02-16

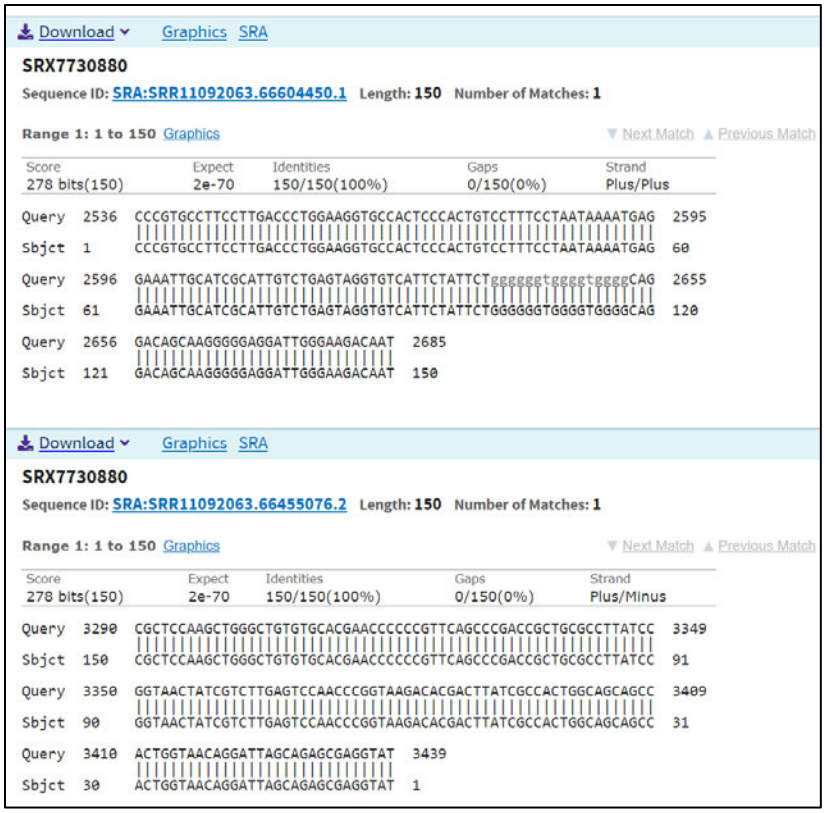
Unexpectedly, over 100 sequences producing significant alignment were identified:



A graphical display of the alignments shows they are not in the Spike Protein region (961 to 2507) of the adenovirus vector but outside of those regions.



An examination of individual reads shows 100% homology over the entire 150 nt segments and outside of the Spike Protein region. The first set of reads are immediately downstream of the Spike Protein segment. The other read is from the 5' boundary of the Adenovirus vector with the Spike Protein region.



Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.66604450.1 Length: 150 Number of Matches: 1

Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Plus

```
Query  2536  CCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAG  2595
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     CCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAG  60

Query  2596  GAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTggggggtggggtggggCAG  2655
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    GAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG  120

Query  2656  GACAGCAAGGGGGAGGATTGGGAAGACAAT  2685
              |||||||||||||||||||||||||||||
Sbjct  121   GACAGCAAGGGGGAGGATTGGGAAGACAAT  150
```

Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.66455076.2 Length: 150 Number of Matches: 1

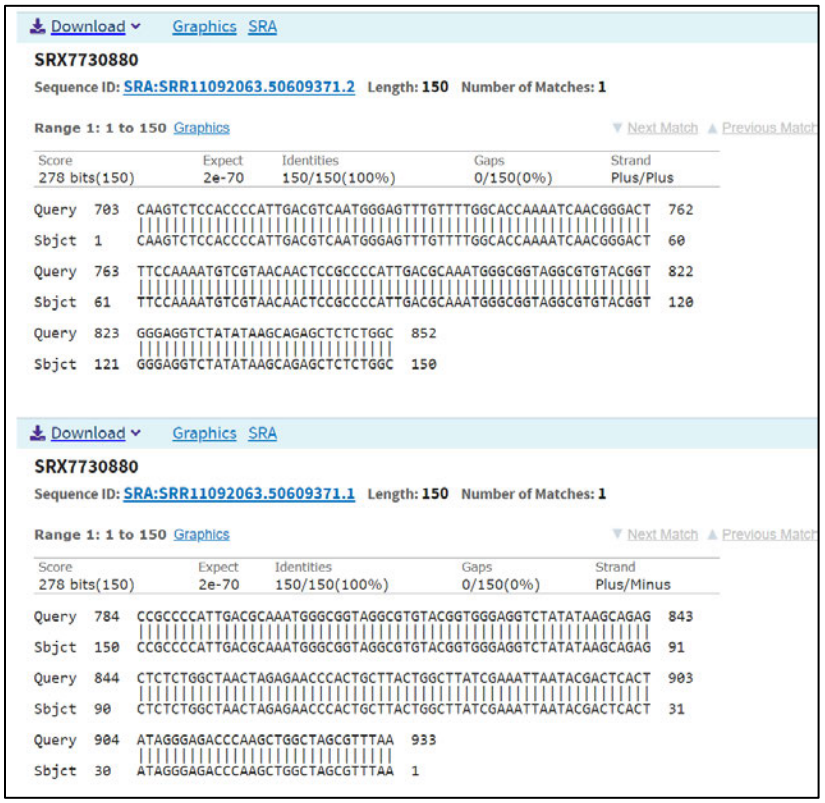
Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Minus

```
Query  3290  CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCC  3349
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150   CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCC  91

Query  3350  GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC  3409
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90    GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC  31

Query  3410  ACTGGTAACAGGATTAGCAGAGCGAGGTAT  3439
              |||||||||||||||||||||||||||||
Sbjct  30    ACTGGTAACAGGATTAGCAGAGCGAGGTAT  1
```



Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.50609371.2 Length: 150 Number of Matches: 1

Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Plus

```
Query  703  CAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT  762
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT  60

Query  763  TTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGT  822
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGT  120

Query  823  GGGAGGTCTATATAAGCAGAGCTCTCTGGC  852
             |||||||||||||||||||||||||||||
Sbjct  121  GGGAGGTCTATATAAGCAGAGCTCTCTGGC  150
```

Download Graphics SRA

SRX7730880

Sequence ID: SRA:SRR11092063.50609371.1 Length: 150 Number of Matches: 1

Range 1: 1 to 150 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
278 bits(150)	2e-70	150/150(100%)	0/150(0%)	Plus/Minus

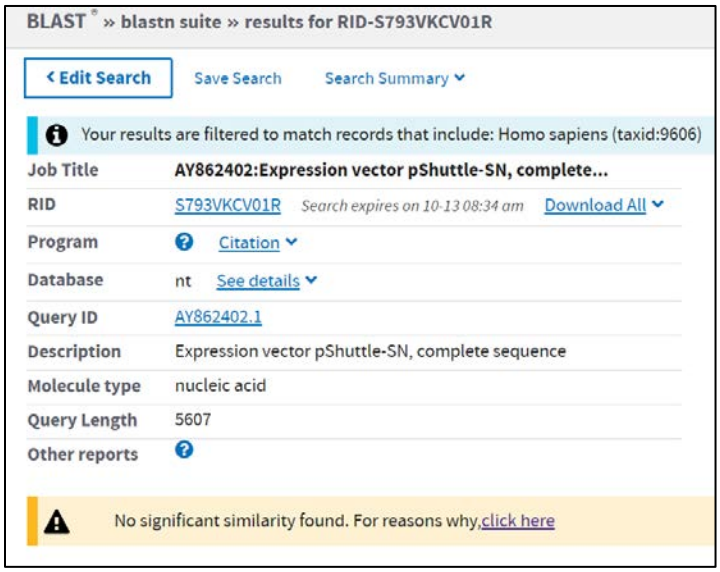
```
Query  784  CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAG  843
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150  CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAG  91

Query  844  CTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACT  903
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90   CTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACT  31

Query  904  ATAGGGAGACCCAAGCTGGCTAGCGTTTAA  933
             |||||||||||||||||||||||||||||
Sbjct  30   ATAGGGAGACCCAAGCTGGCTAGCGTTTAA  1
```

To test if this was the actual SARS-CoV-1 vaccine vector and had been given to the patients as an desperate attempt to create immunity during an infection, the Spike Protein region of the vaccine was blasted against the above sample, looking for a near 100% homology. The only reads were a 38 nt segment of 1482-1518, with one gap, as expected. The absence of long reads for the SARS-CoV-1 Spike Protein establishes that this vaccine was not a CoV-1 vaccine.

To test if the homology seen between lavage specimens of patients in Wuhan with the CoV-1 Adenovirus vaccine was due to homology with human sequencies, the Expression vector itself was blasted against *Homo sapien* sequencies, but no matches were found, as shown below.



From: Su, Lishan
To: Liu, Shan-Lu; Lu, Shan
Subject: FW: article query
Date: Thursday, May 21, 2020 12:53:09 PM

---

fyi

---Lishan

From: Jim <johnstonmdjd@gmail.com>
Date: Thursday, May 21, 2020 at 12:15 PM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: Re: article query

Thank you very much for your prompt reply. That certainly helps provide some clarification. I work in global health, so frequently asked questions regarding this pandemic, and the literature as well as media often have conflicting or misleading reports or theories - so I appreciate your explanations.
kind regards,
jim

On Thu, May 21, 2020 at 10:17 AM Su, Lishan <lishan_su@med.unc.edu> wrote:

> Hi Jim:
>
> Thank you for your interest in our recent commentary, and for your questions.
> Please see my answers/comments below each of the specific questions.
> Best regards and stay well!
>
> ---Lishan
>
> **From:** Jim <johnstonmdjd@gmail.com>
> **Date:** Thursday, May 21, 2020 at 1:55 AM
> **To:** "Su, Lishan" <lishan_su@med.unc.edu>
> **Subject:** RE: article query
>
> Lishan Su,
>
> I read with interest your recent article in Emerging Microbes and Infections, and had a few questions.
>
> If I understand correctly, you state that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. Do you have any evidence to support that conclusion?
>
> ---We simply point out the fact that this new SARS-CoV-2 is genetically distinct from all known

coronaviruses, from bats or other hosts, but cannot rule out the possibility that it was originated by recombination between two coronaviruses from bats or other hosts. It is more a speculation than a conclusion.

Additionally, you correctly note from reference 7 that the Wuhan Institute of Virology was in fact constructing chimeric CoV capable of infecting mice and particular devastating in vitro to human cells. Your statement 'this claim lacks any scientific basis' is confusing, as I suspect you are saying that this particular chimeric CoV differs from the current SARS-CoV-2?

---The chimeric virus study was carried out by the Baric group at the University of North Carolina, using spike or S gene sequences from one of the bat-derived SARS-like coronaviruses (SL-COVs) previously published by a group from Wuhan Institute of Virology. The bat-derived SL-COV S gene was cloned into the mouse-adapted SARS-COV. It should be clarified that the chimeric virus was no more pathogenic or devastating than the original SARS-COV in human cells in vitro. In fact, it is less pathogenic than the original mouse adapted SARS-COV in mice. Based on the genetic make-up (or sequences), the chimeric virus is genetically unrelated to the SARS-CoV-2 with >6,000 nucleotide differences across the whole genome. We therefore state that this particular chimeric CoV differs from the current SARS-CoV-2.

But that would not suggest that the referenced study has no scientific basis. Is that correct - because I assume if the Wuhan Institute of Virology reported that they created the chimeric CoV then we can accept that they in fact did so - or does your statement mean the 2015 report was false, inaccurate or not scientific?

---Correct. The findings in the 2015 report are scientifically sound. As stated above, the chimeric virus was constructed by Dr. Ralph Baric's group at the University of North Carolina, using S gene sequences from one of the bat-derived SARS-like coronaviruses previously published by a Wuhan Institute of Virology group. In other words, the Wuhan group was only involved in providing the bat SL-COV S gene sequence.

I notice you did not list any conflicts although the journal seems to be directed by the Shanghai Shangyixun Cultural Communications Company. Were there any associated financial or non-financial incentives or directives?

--- The authors have no conflict to declare. EMI is published by Taylor & Francis, part of a global company called Informa based in UK, although Shanghai Shangyixun Cultural Communications Company (SSCC) is the owner of the journal. Neither Informa nor Shanghai Shangyixun Cultural Communications Company (SSCC) has any influence on the content or the publication of the article.

Thank you for your help.

kind regards,
j johnston

From: Lu, Shan
To: Su, Lishan; Liu, Shan-Lu
Subject: RE: article query
Date: Thursday, May 21, 2020 9:03:18 AM

I felt that this is an "honest" question but somehow stuck.

See my comments below.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Thursday, May 21, 2020 8:03 AM
To: Liu, Shan-Lu <liu.6244@osu.edu>; Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Fwd: article query

FYI
-Lishan

From: Jim <johnstonmdjd@gmail.com>
Sent: Thursday, May 21, 2020 1:54:34 AM
To: Su, Lishan <lishan_su@med.unc.edu>
Subject: RE: article query

Lishan Su,

I read with interest your recent article in Emerging Microbes and Infections, and had a few questions.

If I understand correctly, you state that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. Do you have any evidence to support that conclusion?

Did you guys stated explicitly on this as "recombinant CoV"? Or it is only an evolution of some bat CoV to become a human one?

Additionally, you correctly note from reference 7 that the Wuhan Institute of Virology was in fact constructing chimeric CoV capable of infecting mice and particular devastating in vitro to human cells. Your statement 'this claim lacks any scientific basis' is confusing, as I suspect you are saying that this particular chimeric CoV differs from the current SARS-CoV-2?

1. You should point that it was UNH but not WIV made the chimeric CoV. 2) yes, your paper only try to say that the new SARS-CoV-2 is not the same from the early Chimeric CoV published.

But that would not suggest that the referenced study has no scientific basis. Is that correct - because I assume if the Wuhan Institute of Virology reported that they created the chimeric CoV then we can accept that they in fact did so - or does your statement mean the 2015 report was false, inaccurate or not scientific?

If the reader understand the answer above, he should understand what you meant is that the claim of the CURRENT SARS-CoV-2 is a chimeric lacking scientific evidence.

I notice you did not list any conflicts although the journal seems to be directed by the Shanghai Shangyixun Cultural Communications Company. Were there any associated financial or non-financial incentives or directives?

The authors has no conflict to declare. Shanghai Shangyixun Cultural Communications Company (SSCC) is the owner of journal but EMI is published by Taylor & Francis, part of a big global company called Informa. Why don't you ask any conflicts with T&F or Informa? Only because SSCC is based in China and T&F/Infoma based in UK?

Thank you for your help.

kind regards,
j johnston

From: Lu, Shan
To: "Su, Lishan"; Liu, Shan-Lu
Subject: RE: article query
Date: Thursday, May 21, 2020 10:48:43 AM

Well done. No change from me.

From: Su, Lishan <lishan_su@med.unc.edu>
Sent: Thursday, May 21, 2020 10:46 AM
To: Liu, Shan-Lu <liu.6244@osu.edu>
Cc: Lu, Shan <Shan.Lu@umassmed.edu>
Subject: Re: article query

He seems to be serious with reasonable questions and deserves a response.
See my draft responses, and about coi based on Shan Lu's information. Let me know your thoughts.
Thanks,

Hi Jim:

Thank you for your interest in our recent commentary, and for your questions.
Please see my answers/comments below each of the specific questions.

---Lishan

From: Jim <johnstonmdjd@gmail.com>
Date: Thursday, May 21, 2020 at 1:55 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: article query

Lishan Su,

I read with interest your recent article in Emerging Microbes and Infections, and had a few questions.

If I understand correctly, you state that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. Do you have any evidence to support that conclusion?

---We simply point out the fact that this new SARS-CoV-2 is genetically distinct from all known coronaviruses, from bats or other hosts, but cannot rule out the possibility that it was originated between two coronaviruses from bats and other hosts. It is more a speculation than a conclusion.

Additionally, you correctly note from reference 7 that the Wuhan Institute of Virology was in fact constructing chimeric CoV capable of infecting mice and particular devastating in vitro to human cells. Your statement 'this claim lacks any scientific basis' is confusing, as I suspect you are saying that this particular

chimeric CoV differs from the current SARS-CoV-2?

---The chimeric virus study was carried out by the Baric group at the University of North Carolina, using S gene sequences from one of the bat-derived SARS-like coronaviruses previously published by a group from Wuhan Institute of Virology. The bat-derived SL-COV S gene was cloned into the mouse-adapted SARS-COV. It should be clarified that the chimeric virus was no more pathogenic than the mouse adapted SARS-COV in mice or in human cells in vitro. Based on the genetic make-up (or sequences), the chimeric virus is genetically unrelated to the SARS-CoV-2 with >5000 nucleotide differences. We therefore conclude that this particular chimeric CoV differs from the current SARS-CoV-2.

But that would not suggest that the referenced study has no scientific basis. Is that correct - because I assume if the Wuhan Institute of Virology reported that they created the chimeric CoV then we can accept that they in fact did so - or does your statement mean the 2015 report was false, inaccurate or not scientific?

---Correct. The findings in the 2015 report are scientifically sound. As stated above, the chimeric virus was constructed by Dr. Ralph Baric's group at the University of North Carolina, using S gene sequences from one of the bat-derived SARS-like coronaviruses previously published by Wuhan Institute of Virology group. In other words, the Wuhan group was only involved in providing the bat SL-COV S gene sequence.

I notice you did not list any conflicts although the journal seems to be directed by the Shanghai Shangyixun Cultural Communications Company. Were there any associated financial or non-financial incentives or directives?

--- The authors have no conflict to declare. EMI is published by Taylor & Francis, part of a global company called Informa based in UK, although Shanghai Shangyixun Cultural Communications Company (SSCC) is the owner of the journal. Neither Informa nor Shanghai Shangyixun Cultural Communications Company (SSCC) has any influence on the content or the publication of the article.

Thank you for your help.

kind regards,
j johnston

From: Liu, Shan-Lu
To: Su, Lishan
Cc: Lu, Shan
Subject: Re: article query
Date: Thursday, May 21, 2020 10:52:06 AM

Great response, nothing to add from me.

Thanks.

Shan-Lu

From: "Su, Lishan" <lishan_su@med.unc.edu>
Date: Thursday, May 21, 2020 at 10:46 AM
To: Shan-Lu Liu <liu.6244@osu.edu>
Cc: "Lu, Shan" <Shan.Lu@umassmed.edu>
Subject: Re: article query

He seems to be serious with reasonable questions and deserves a response.
See my draft responses, and about coi based on Shan Lu's information. Let me know your thoughts.
Thanks,

Hi Jim:

Thank you for your interest in our recent commentary, and for your questions.
Please see my answers/comments below each of the specific questions.

---Lishan

From: Jim <johnstonmdjd@gmail.com>
Date: Thursday, May 21, 2020 at 1:55 AM
To: "Su, Lishan" <lishan_su@med.unc.edu>
Subject: RE: article query

Lishan Su,

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j johnston