United States Department of State

Washington, D.C. 20520

March 22, 2024

Case No. FL-2022-00062

Mr. Gary Ruskin
U.S. Right to Know
4096 Piedmont Avenue, #963
Oakland, CA 94611

Dear Mr. Ruskin:

As we noted in our letter dated February 9, 2024, we are processing your request for material under the Freedom of Information Act ("FOIA"), 5 U.S.C. § 552. The Department of State ("Department") has identified an additional 19 responsive records subject to the FOIA. We have determined all 19 records may be released in part.

An enclosure explains the FOIA exemptions and other grounds for withholding material. Where we have made redactions, the applicable FOIA exemptions are marked on each record. Where applicable, the Department has considered the foreseeable harm standard when reviewing these records and applying FOIA exemptions. All non-exempt material that is reasonably segregable from the exempt material has been released and is enclosed.

We will keep you informed as your case progresses. If you have any questions, your attorney may contact Assistant United States Attorney Stephanie Johnson at stephanie.johnson5@usdoj.gov or (202) 252-7874. Please refer to the case number, FL-2022-00062, and the civil action number, 22-cv-01130, in all correspondence about this case.

Sincerely,

Diamonece Hickson
Chief, Litigation and Appeals Branch
Office of Information Programs and Services

Enclosures: As stated.

The Freedom of Information Act (5 USC 552)

FOIA Exemptions

(b)(1) Information specifically authorized by an executive order to be kept secret in the interest of national defense or foreign policy. Executive Order 13526 includes the following classification categories:

1.4(a) Military plans, systems, or operations
1.4(b) Foreign government information
1.4(c) Intelligence activities, sources or methods, or cryptology
1.4(d) Foreign relations or foreign activities of the US, including confidential sources
1.4(e) Scientific, technological, or economic matters relating to national security, including defense against transnational terrorism
1.4(f) U.S. Government programs for safeguarding nuclear materials or facilities
1.4(g) Vulnerabilities or capabilities of systems, installations, infrastructures, projects, plans, or protection services relating to US national security, including defense against transnational terrorism
1.4(h) Weapons of mass destruction

(b)(2) Related solely to the internal personnel rules and practices of an agency

(b)(3) Specifically exempted from disclosure by statute (other than 5 USC 552), for example:

ARMSEXP	Arms Export Control Act, 50a USC 2411(c)
CIA PERS/ORG	Central Intelligence Agency Act of 1949, 50 USC 403(g)
EXPORT CONTROL	Export Administration Act of 1979, 50 USC App. Sec. 2411(c)
FS ACT	Foreign Service Act of 1980, 22 USC 4004
INA	Immigration and Nationality Act, 8 USC 1202(f), Sec. 222(f)
IRAN	Iran Claims Settlement Act, Public Law 99-99, Sec. 505

(b)(4) Trade secrets and confidential commercial or financial information

(b)(5) Interagency or intra-agency communications forming part of the deliberative process, attorney-client privilege, or attorney work product

(b)(6) Personal privacy information

(b)(7) Law enforcement information whose disclosure would:
(A) interfere with enforcement proceedings
(B) deprive a person of a fair trial
(C) constitute an unwarranted invasion of personal privacy
(D) disclose confidential sources
(E) disclose investigation techniques
(F) endanger life or physical safety of an individual

(b)(8) Prepared by or for a government agency regulating or supervising financial institutions

(b)(9) Geological and geophysical information and data, including maps, concerning wells

Other Grounds for Withholding

NR Material not responsive to a FOIA request excised with the agreement of the requester

From: "DiNanno, Thomas G" (b)(6) @state.gov>
To: (b)(6) @state.gov>
CC: Gross, Laura J (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: RE: in the office - Gain of function—from F ord
Date: Sun, 6 Dec 2020 15:37:45 +0000

we need to identify an additional dedicated BW analyst/detailee ASAP.

On December 6, 2020 at 10:21:43 AM EST, (b)(6) @state.gov> wrote:
Tom,

I'm in the office. Are you or Gibbs coming in?

(b)(6)

(b)(6)

Chief of Staff
Bureau of Arms Control, Verification and Compliance
U.S. Department of State
HST Room 5950

Office: (b)(6)
Cell:

OpenNet: (b)(6) @state.gov
ClassNet: @state.sgov.gov
JWICS: (b)(6) @state.ic.gov

From: DiNanno, Thomas G (b)(6) @state.gov>
Sent: Friday, December 4, 2020 7:21 PM
To: Asher, David (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(
(b)(6) @state.gov>; Feith, David (b)(6) @state.gov>
Subject: Re: Gain of function—from Ford

(b)(5)

—- let's discuss

On December 4, 2020 at 7:11:32 PM EST, Asher, David (b)(6) @state.gov> wrote:
Chris will get a polite but stern retort from me....any thoughts on this, please let me know—all to be treated in confidence. Again, there is an almost impossible line to determine between syn-bio offense and defense but when you see huge gain of function attempts involved and no attempt to protect a likely spillover you must address intentions and causation. We urgently need (b)(6) analysis of the Defense One article on bio-war as well as any high side corroboration.

From: Gibbs, Jeffrey J (b)(6) @state.gov>
Sent: Friday, December 4, 2020 12:27 PM
To: Asher, David (b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(
(b)(6) @state.gov>; Feith, David (b)(6) @state.gov>
Subject: Re: Gain of function—from Ford

This sounds like "we need prove beyond any doubt, reasonable or not."

Jeff Gibbs
Senior Adviser AVC
SSD/AVC
c: (b)(6)

From: Asher, David (b)(6) @state.gov>
Sent: Friday, December 4, 2020 11:25 AM
To: DiNanno, Thomas G (b)(6) @state.gov>
Cc: Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6)
(b)(6) @state.gov>; (b)(6) @state.gov>; Feith, David (b)(6) @state.gov>
Subject: Fw: Gain of function—from Ford

From: Ford, Christopher A (b)(6) @state.gov>
Sent: Friday, December 4, 2020 10:36 AM
To: Asher, David <(b)(6) @state.gov>
Subject: Re: Gain of function

Dear David:

Sorry for being slow in replying, but I'm out of town and wanted to do your comment justice. I appreciate the message, and for for taking the time to put together yesterday's briefing (though I was a little surprised to hear that AVC had been working for so long on this project without them telling me anything about it). As I told Tom in an earlier message, I was impressed by the depth and detail of the presentation, and very much want to make sure we get this issue right.

Anyway, I look forward to continuing the conversation to assess the strength of the argument and especially to engaging others whose technical knowledge exceeds my own. On the points you raised, however — and after sniffing around at least a bit —- let me offer some tentative thoughts in response to the points you raised:

(b)(5)

(b)(5)

So let's continue this when I'm back next week. (I return Tuesday morning.)

Thanks again,

— Chris

From: David Asher <(b)(6)
Sent: Thursday, December 3, 2020 5:10 AM
To: Ford, Christopher A
Subject: Gain of function

Chris,

(b)(5)

(b)(5)

Best regards,

David

https://www.thelancet.com/action/showPdf?pii=S1473-3099%2818%2930006-9

Below: Nature commentary pointing out the futility, waste, and opportunity costs associated projects pursued by Ecohealth, WIV, NIAID, et al, in the name of "predicting the next outbreak". Though they don't address the grave hazards, and BW dual use issues, involved with the gain of function work in WIV's prediction research, they laid out other important fundamental flaws with Ecohealth and WIV's approach. The authors go on to make the more compelling case for better bio surveillance instead.https://www.nature.com/articles/d41586-018-05373-w

COMMENT

07 JUNE 2018

Pandemics: spend on surveillance, not prediction

Trust is undermined when scientists make overblown promises about disease prevention, warn Edward C. Holmes, Andrew Rambaut and Kri(stian G. Andersen.

The resurgence of Ebola virus in the Democratic Republic of the Congo this May is a stark reminder that no amount of DNA sequencing can tell us when or where the next virus outbreak will appear. More genome sequence data were obtained for the 2013–16 Ebola epidemic than for any other single disease outbreak. Still, health workers in Mbandaka, the country's northwestern provincial capital, arescrambling to contain a growing number of cases.

Over the past 15 years or so, outbreaks caused by viruses such as Ebola, SARS and Zika have cost governments billions of US dollars. Combined with a perception among scientists, health workers and citizens that responses to outbreaks have been inadequate,this has fuelled what seems like a compelling idea. Namely, that if researchers can identify the next pandemic virus before the first case appears, communities could drastically improve strategies for control, and even stop a virus from taking hold1,2. Indeed, since 2009, the US Agency for International Development has spent US$170 million on evaluating the "feasibility of preemptively mitigating pandemic threats"1.

Various experts have flagged up problems with this approach (including the three of us)3,4. Nonetheless, an ambitious biodiversity-based approach to outbreak prediction — theGlobal Virome Project— was announced in February this year, with its proponents soliciting $1.2 billion in funding from around the world(see 'High stakes'). They estimate that other mammals

and birds contain 1.67 million unknown viruses from the families of viruses that are most likely to jump to humans, and will use the funding to conduct a genomic survey of these unknown viruses, with the aim of predicting which might infect people1.

Sources: NIH; Global Virome Project

Broad genomic surveys of animal viruses will almost certainly advance our understanding of virus diversity and evolution. **In our view, they will be of little practical value when it comes to understanding and mitigating the emergence of disease.**

We urge those working on infectious disease to focus funds and efforts on a much simpler and more cost-effective way to mitigate outbreaks — proactive, real-time surveillance of human populations.

The public has increasingly questioned the scientific credibility of researchers working on outbreaks. In the 2013–16 Ebola epidemic, for instance, the international response was repeatedlycriticized for being too slow. And during the 2009 H1N1 influenza epidemic, people asked whether the severity of the virus had been overblown, and if the stockpiling of pharmaceuticals was even necessary5. Making promises about disease prevention and control that cannot be kept will only further undermine trust.

Forecasting fallacy

Supporters of outbreak prediction maintain that if biologists genetically characterize all of the viruses circulating in animal populations (especially in groups such as bats and rodents that have previously acted as reservoirs for emerging viruses), they can determine which ones are likely to emerge next, and ultimately prevent them from doing so. With enough data, coupled with artificial intelligence and machine learning, they argue, the process could be similar to predicting the weather6.

Reams of data are available to train models to predict the weather. By contrast, it is exceedingly rare for viruses to emerge and cause outbreaks. Around 250 human viruses have been described, and only a small subset of these have caused major epidemics this century.

Advocates of prediction also argue that it will be possible to anticipate how likely a virus is to emerge in people on the basis of its sequence, and by using knowledge of how it interacts with cells (obtained, for instance, by studying the virus in human cell cultures).

This is misguided. Determining which of more than 1.6 million animal viruses are capable of replicating in humans and transmitting between them would require many decades' worth of laboratory work in cell cultures and animals. Even if researchers managed to link each virus genome sequence to substantial experimental data, all sorts of other factors determine whether a virus jumps species and emerges in a human population, such as the distribution and density of animal hosts. Influenza viruses have circulated in horses since the 1950s and in dogs since the early 2000s, for instance7. These viruses have not emerged in human populations, and perhaps never will — for unknown reasons.

In short, there aren't enough data on virus outbreaks for researchers to be able to accurately predict the next outbreak strain. Nor is there a good enough understanding of what drives viruses to jump hosts, making it difficult to construct predictive models.

Biodiversity-based prediction also ignores the fact that viruses are not fixed entities. New variants of RNA viruses appear every day. This speedy evolution means that surveys would need to be done continuously to be informative. The cost would dwarf the proposed $1.2-billion budget for one-time sequencing.

Even if it were possible to identify which viruses are likely to emerge in humans, thousands of candidates could end up being identified, each with a low probability of causing an outbreak. What should be done in that case? Costs would skyrocket if vaccines and therapeutics were proposed for even a handful of these.

Screen and sequence

Currently, the most effective and realistic way to fight outbreaks is to monitor human populations in the countries and locations that are most vulnerable to infectious disease. This can be done by local clinicians, health workers in non-governmental organizations such as Médecins Sans Frontières (MSF; also known as Doctors Without Borders), and global institutions such as the World Health Organization (WHO).

We advocate the detailed screening of people who are exhibiting symptoms that cannot easily be diagnosed. Such tests should use the latest sequencing technologies to characterize all the pathogens that have infected an individual — the human 'infectome'8. To track previous infections, investigators should also assess each person's immune response, by analysing components of their blood using broad-scale serology9.

Emerging diseases are commonly associated with population expansions — when people encroach on habitats occupied hy animals — as well as with environmental disturbances and climate change. Deforestation, for instance, can promote human interactions with animals that carry new threats, and can increase encounters with new vector species such as ticks and mosquitoes10. Animal die-offs, for example that of bar-headed geese (*Anser indicus*) at Lake Qinghai in China in 2005 (which was caused by the H5N1 influenza virus), can also flag problem regions or emerging pathogens. Surveillance efforts should therefore focus on communities that live and work in such environments.

Identifying which pathogen is causing an outbreak is no longer the bottleneck it once was. It took researchers two years to determine HIV as the cause of AIDS in the early 1980s using microscopy and other techniques. By contrast, in 2012 it took only weeks for investigators using genomic technologies to discover the coronavirus that caused Middle East respiratory syndrome (MERS).

Rapid identification of viruses can be achieved only if such technologies — and the people trained to use them — are globally available, including in resource-limited regions where the risk of outbreaks might be higher.Thankfully, relevant capacity-building programmes are now beginning to be established, such as the Human Heredity and Health in Africa (H3Africa) Initiative, run by the UK Wellcome Trust and the US National Institutes of Health11.

Once an emerging outbreak virus has been identified, it needs to be analysed quickly to establish what type it is; which molecular mechanisms (such as receptor type) enable it to jump between individuals; how it spreads through human populations; and how it affects those infected. In other words, at least four kinds of analysis are needed: genomic, virological, epidemiological and clinical. And the data must be passed to key stakeholders, from researchers and health workers on the ground to international agencies such as the WHO and the MSF. Data must be kept as free of restrictions as possible, within the constraints of protections of patient privacy and other ethical issues.

This will best be achieved through an established global network of highly trained local researchers, such as the WHO Global Outbreak Alert and Response Network (GOARN). Real-time tools for reconstructing and tracking outbreaks at the genomic level, such as portable sequencing devices, are improving fast8. Information gathered during recent outbreaks has

quickly had tangible impacts on public-health decisions, largely owing to data generation and analysis by many research teams within days of people being infected12.

For instance, in the 2013–16 Ebola epidemic, genome sequencing of the virus proved that a person could sexually transmit the disease more than a year after becoming infected. This prompted the WHO to increase its recommended number of tests for persistent infection in survivors of the disease.

Ultimately, the challenge is to link genomic, clinical and epidemiological data within days of an outbreak being detected, including information about how people in an affected community are interacting. Such an open, collaborative approach to tackling the emergence of infectious disease is now possible. This is partly thanks to technology, but is mainly due to a shift in perception about the importance of this approach. At least in genomic epidemiology, there is a growing move towards real-time, open-access data and analysis, aided by the use of preprint servers and wikis such as Virological (http://virological.org). This type of collaborative effort can complement the work of agencies including the WHO and the MSF, which focus predominantly on providing information, isolating those who have been infected, and so on.

So far, researchers have sampled little of the viral universe. Surveys of animals will undoubtedly result in the discovery of many thousands of new viruses. These data will benefit studies of diversity and evolution, and could tell us whether and why some pathogens might jump species boundaries more frequently than others. But, given the rarity of outbreaks and the complexity of host–pathogen interactions, it is arrogant to imagine that we could use such surveys to predict and mitigate the emergence of disease.

New viruses will continue to emerge unexpectedly. There is a lot we can and must do to be better prepared.

*Nature***558**, 180-182 (2018)
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David L. Asher, Ph.D
Senior Fellow
Hudson Institute
1201 Pennsylvania Avenue, NW
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o. | c(b)(6)

https://www.hudson.org/experts/1299-david-asher

Sender: "DiNanno, Thomas G" (b)(6) @state.gov>

Recipient: (b)(6) @state.gov>; Gross, Laura J (b)(6) @state.gov>; (b)(6) @state.gov>

From: Evelyn Farkas (b)(6)
To: David Asher (b)(6)
CC: Laura Gross (b)(6) @state.gov>
Subject: Re: Great meeting you Thanks Ev!
Date: Wed, 18 Nov 2020 07:36:59 -0500

Thank you, Laura and David! I'm glad two smart cookies (as Donald Trump would put it In another WMD context) got to meet and perhaps you can collaborate on helping our country. I hope I can join you in someway shape or form in the future.

- Evelyn

Evelyn N. Farkas, Ph.D.
@EvelynNFarkas

On Nov 17, 2020, at 11:16 PM, David Asher (b)(6) wrote:

Laura,
Such a pleasure meeting you today. Truly look forward to working together now and in the incoming admin. This is from my think tank email but you know where to find me now at State. Attached is the 2018 NAS report on synthetic biology and bio warfare. Very important. Also, an article published today in the peer reviewed WILEY scientific publication BioEssays is worth reading. https://onlinelibrary.wiley.com/journal/15211878
Based on my little research effort and interviews with world renowned experts, there is no scientific reason for anyone to be so confident regarding the exact origins of COVID 19. According to dozens of world class scientists it is really weird that a Coronavirus could skip several generations ahead into Homo sapiens and there be no detected record of the intermediate stages. Probably unprecedented in human virological/ epidemiological history but certainly possible. Anything is possible with syn bio or nature but the best cloaking mechanism—according to scientists—is to mix gain of function and natural "adverse selection" zoological and human induced rapid evolution together. Like a game show of the most dangerous contestants getting awarded by survival for being the most virulent but robust for not dying of their own acquired plague. Except perhaps one has been given peculiar genetic resistance — from a vaccine or just being stronger in the face of hyper virulence —from dying unlike the others.....Mate a couple of these types together and you, apparently, can get super natural gain of function to kill others but not die.
Despite the Obama era ban, there is plenty of public evidence of gain of function/syn-bio research in the US as in China. There is no public evidence of massive spillover related to COVID meddling in the US, Europe, or elsewhere in Asia but, sadly, there may be in the PRC—much of it posted anonymously but with details by researchers that seems quite accurate. Some of whom have conspicuously disappeared since posting.

As part of VCOG I recommend you implement a research effort with actual scientists who aren't afraid to hide and be cited on the record (I know a few and they don't all agree on the cause but do tend to concur on the possibility).
Let's discuss in different quarters in the future but I am grateful to Evelyn for introducing us.
And let's all watch the League of the Twelve Monkeys to consider what could come next from true crazies. https://en.wikipedia.org/wiki/12_Monkeys. For the brave new world, I recommend you research Prion based disease vectors, among others. https://www.defenceiq.com/air-land-and-sea-defence-services/articles/prions-as-bioweapons
All the best and stay healthy,
David
PS- I include the latest blog posts on "Writing the Future" from Twist, one of many syn-bio companies I know in my other career.

The genetic structure of SARS-CoV-2 does not rule out a laboratory origin

SARS-COV-2 chimeric structure and furin cleavage site might be the result of genetic manipulation

Rossana Segreto

Yuri Deigin

17 November 2020

https://doi.org/10.1002/bies.202000240

Abstract

Severe acute respiratory syndrome-coronavirus (SARS-CoV)-2's origin is still controversial. Genomic analyses show SARS-CoV-2 likely to be chimeric, most of its sequence closest to bat CoV RaTG13, whereas its receptor binding domain (RBD) is almost identical to that of a pangolin CoV. Chimeric viruses can arise *via* natural recombination or human intervention. The furin cleavage site in the spike protein of SARS-CoV-2 confers to the virus the ability to cross species and tissue barriers, but was previously unseen in other SARS-like CoVs. Might genetic manipulations have been performed in order to evaluate pangolins as possible intermediate hosts for bat-derived CoVs that were originally unable to bind to human receptors? Both cleavage site and specific RBD could result from site-directed mutagenesis, a procedure that does not leave a trace. Considering the devastating impact of SARS-CoV-2 and importance of preventing future pandemics, researchers have a responsibility to carry out a thorough analysis of all possible SARS-CoV-2 origins.

INTRODUCTION

Nearly a year has passed since the outbreak of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) in Wuhan, China, and its origin is still controversial. Despite the interuational research effort conducted, a natural host, either direct or intermediate, has not yet been identified. The hypothesis that the Wuhan Huanan Seafood Wholesale Market was the first source for

animal–human virus transmission has now been conclusively dismissed[i] and the few market samples that were collected showed only human-adapted SARS-CoV-2, with no traces of zoonotic predecessor strains[ii] . Almost all scientific papers published to date purport that SARS-CoV-2 has a natural origin, and the only published paper considering possible a lab origin[1] focuses on serial passage as the technique that could justify SARS-CoV-2 special adaptation to human cells. We here describe how the two main SARS-CoV-2 features, (1) the presence of a furin cleavage site missing in other CoVs of the same group and (2) an receptor binding domain (RBD) optimized to bind to human cells[2] might be the result of lab manipulation techniques such as site-directed mutagenesis. The acquisition of both unique features by SARS-CoV-2 more or less simultaneously is less likely to be natural or caused only by cell/animal serial passage.

SARS-COV-2'S CLOSEST RELATIVES ARE BAT AND PANGOLIN CORONAVIRUSES

Zhou et al.[3] from the Wuhan Institute of Virology (WIV) were the first to identify and characterize a new coronavirus (CoV), SARS-CoV-2. The genomic sequences obtained from early cases shared 79% sequence identity to the CoVs that caused severe acute respiratory syndrome (SARS-CoV) in 2002–2003 and 96.2% sequence identity to RaTG13 (MN996532), a CoV sequence detected from a *Rhinolophus affinis* bat. RaTG13 is currently the closest phylogenetic relative for SARS-CoV-2 found,[4] but its complete genomic sequence was not published before the outbreak of SARS-CoV-2 and the original sample was collected in the Yunnan province (China) by the same group of WIV researchers in 2013. Zhou et al.[3] stated to have found a match between SARS-CoV-2 and a short region of RNA-dependent RNA polymerase (RdRp) of a CoV in their database and then fully sequenced the original sample collected in 2013, which they called RaTG13.

We discovered that the RdRp of RaTG13 has 100% nucleotide identity with the sequence BtCoV/4991 (KP876546), which was identified by Ge et al.[5] in a *Rhinolophus affinis* bat in the Yunnan province in 2013, same location and year as RaTG13. BtCoV/4991 was collected in a mine colonized by bats near Tongguanzhen, Mojiang, Yunnan. The WIV researchers were invited to investigate the mine after six miners there had contracted severe pneumonia in 2012[iii] , and three of the miners have died.[6] The miners have been tasked with clearing out bat droppings in the mine, and the severity of their pneumonia correlated with the duration of exposure to the mine.[7] Four miners' samples subsequently underwent testing at WIV, where Immunoglobulin G (IgG) antibodies against SARS were identified in all samples.[8] Considering

that only about 5300 people were infected in mainland China during the SARS outbreak of 2002–2004, most of whom resided in Guandong, the odds of four miners in Yunnan retaining antibodies from the 2002–2004 SARS outbreak are negligible. On the other hand, it is possible that the SARS antibody test administered to the miners cross-reacted with a novel SARS-like bat virus that the miners had acquired at the mine. Ge et al.[5] have identified a number of CoVs in the mine, but based on the phylogenetic analysis, BtCoV/4991 was the only SARS-related strain, clearly separated from all known alpha- and beta-CoVs at that time. BtCoV/4991 was also different from other bat CoVs in the phylogenetic analysis carried out by Wang et al. in 2019.[9] Chen et al.[10] identified BtCoV/4991 as the closest sequence to SARS-CoV-2 because RaTG13 had not yet been published at that time. BtCoV/4991 and RaTG13 have been later asserted to be two different coding names of the same strain, as their original authors at WIV registered the two strains as one entry in the Database of Bat-associated Viruses (DBatVir).iv

In late July 2020, Zhengli Shi, the leading CoV researcher from WIV, in an email interview [11] asserted the renaming of the RaTG13 sample and unexpectedly declared that the full sequencing of RaTG13 has been carried out as far back as in 2018 and not after the SARS-CoV-2 outbreak, as stated in Zhou et al.[3] The reversal in WIV's stance on when exactly RaTG13 was fully sequenced could have been due to the discovery by independent researchers into the origins of SARS-CoV-2 that the filenames of the raw sequencing reads deposited by WIV on May 19, 2020v seem to indicate that sequencing for RaTG13 was done in 2017 and 2018.vi However, no formal erratum about year of sequencing and sample renaming from the authors of Zhou et al. [3] has yet appeared, or as far as is currently known, has been submitted.

The second non-human RdRp sequence closest to BtCoV/4991 (91.89% nucleotide identity) is the CoV sequence MP789 (MT084071) isolated in 2019 in a Malaysian pangolin (*Manis javanica*) from the Guangdong province (GD), China.[12] The envelope protein of MP789 shows surprisingly 100% aminoacidic identity with the corresponding protein in RaTG13, in bat-SL-CoVZXC21 (MG772934.1), in bat-SL-CoVZC45 (MG772933.1) and in some early SARS-CoV-2 isolates (e.g. YP_009724392).[13] The envelope protein of CoVs is involved in critical aspects of the viral lifecycle, such as viral entry, replication and pathogenesis.[14]

BAT COVS HAVE BEEN THOROUGHLY STUDIED AND GENETICALLY MANIPULATED

Many studies have reported that bats are natural reservoirs for a broad diversity of potentially pathogenic SARS-like CoVs.[15, 16] Some of these viruses can potentially directly infect humans[17], whereas others need to mutate their spike protein in order to effectively bind to the human

angiotensin 1-converting enzyme 2 (hACE2) receptor and mediate virus entry.[18] In order to evaluate the emergence potential of novel CoVs, researchers have created a number of chimeric CoVs, consisting of bat CoV backbones, normally unable to infect human cells, whose spike proteins were replaced by those from CoVs compatible with human ACE2. These chimeras were meant to simulate recombination events that might occur in nature.[19, 20] Such gain-of-function experiments have raised a number of biosafety concerns and stirred controversy among researchers and the general public. One of the main arguments in favor of gain-of-function studies is the need to be prepared with an arsenal of drugs and vaccines for the next pandemic.[21] By contrast, one of the main arguments against them is that the next pandemic itself could be caused by those experiments, due to the risk of lab escape.[22, 23]

In recent years, the field of corona-virology had been focused on pan-CoV therapies and vaccines, as evident from research conducted in the past 5 years,[24-27] as well as from media reports.**vii** Synthetically generating diverse panels of potential pre-emergent CoVs was declared a goal of active grants for the EcoHealth Alliance, which funded some of such research at WIV, in collaboration with laboratories in the USA and other international partners.**viii**

CREATING CHIMERIC COVS WITH NOVEL RBDS HAS GONE ON FOR DECADES

Researchers have been generating chimeric CoVs for over two decades, long before the advent of modern sequencing or genetic engineering techniques. For example, in 1999, a group from Utrecht University used targeted RNA recombination to create a "cat-and-mouse" CoV chimera: the RBDs of a feline and murine CoV were swapped, demonstrating that this exchange swapped also species tropism during *in vitro* experiments.[28]

In 2007, the Shi group at WIV created a series of "bat-man" CoV chimeric spike proteins while trying to determine what exactly confers CoVs the ability to jump from one species to another. The researchers used different segments of the spike protein of the human SARS virus to replace corresponding segments in the spike protein of a bat viral backbone. It was concluded that a relatively short region (aa 310 to 518) of the spike protein "was necessary and sufficient to convert Rp3-S into a huACE2-binding molecule,"[29] that is to provide the bat CoV spike protein with a novel ability of binding to a human ACE2 receptor.

In 2008, the Baric group at the University of North Carolina (UNC) took the WIV research one step further: instead of using human immunodeficiency viruses (HIV) pseudo-viruses with bat CoV spike proteins, a live chimeric CoV was created. Following the experiments of their 2007

WIV colleagues, the Baric group used a bat SARS-like CoV as a backbone and replaced its RBD with the RBD from human SARS.[30]

In 2015, the Shi and Baric groups joined forces and published probably the most famous gain-of-function virology paper, which described the creation of another synthetic chimeric virus.[19] This time the RBD of a mouse-adapted SARS backbone (SARS-MA15) was replaced by the RBD of RsSHC014, a bat strain previously isolated from Yunnan bats in 2011 by the Shi group. In 2016, the Baric group repeated their 2015 experiment using the same SARS-MA15 backbone and the RBD from Rs3367,[31] a close relative of RsSHC014 also previously found in Yunnan by WIV and renamed "WIV1" after live culturing.[17]

Probably the largest reported number of novel chimeric viruses created was described in a 2017 paper from the Shi group at WIV,[15] in which the authors reported creating eight chimeric viruses using WIV1 as a backbone and transplanting into it various RBDs from bat SARS-like viruses. These viruses were collected over a span of 5 years from the same cave near Kunming, Yunnan Province, where the Shi group originally found Rs3367 and RsSHC014. Only two of the eight live chimeric viruses were successfully rescued, and those two strains were found to possess the ability to bind to the human ACE2 receptor, as confirmed by experiments in hACE2-expressing HeLa cells and RT-PCR quantification of viral RNA.

SARS-COV-2 SHARES ITS RBD WITH A PANGOLIN COV

The possibility that pangolins could be the intermediate host for SARS-CoV-2 has long been under discussion. [32-34] The biggest divergence between SARS-CoV-2 and RaTG13 is observed in the RBD of their spike proteins.[4] Although its overall genome similarity is lower to SARS-CoV-2 than that of RaTG13, the MP789 pangolin strain isolated from GD pangolins has an almost identical RBD to that of SARS-CoV-2. Indeed, pangolin CoVs and SARS-CoV-2 possess identical amino acids at the five critical residues of the RBD, whereas RaTG13 only shares one amino acid with SARS-CoV-2.[35] ACE2 sequence similarity is higher between humans and pangolins than between humans and bats. Intriguingly, the spike protein of SARS-CoV-2 has a higher predicted binding affinity to human ACE2 receptor than to that of pangolins and bats.ix Before the SARS-CoV-2 outbreak, pangolins were the only mammals other than bats documented to carry and be infected by SARS-CoV-2 related CoV.[12] Recombination events between the RBD of CoV from pangolins and RaTG13-like backbone could have produced SARS-CoV-2 as chimeric strain. For such recombination to occur naturally, the two

viruses must have infected the same cell in the same organism simultaneously, a rather improbable event considering the low population density of pangolins and the scarce presence of CoVs in their natural populations.x Moreover, receptor binding studies of reconstituted RaTG13 showed that it does not bind to pangolin ACE2.xi

THE FURIN CLEAVAGE SITE: THE KEY DIFFERENCE BETWEEN SARS-COV-2 AND ITS CLOSEST RELATIVE RATG13

SARS-CoV-2 differs from its closest relative RaTG13 by a few key characteristics. The most striking difference is the acquisition in the spike protein of SARS-CoV-2 of a cleavage site activated by a host-cell enzyme furin, previously not identified in other beta-CoVs of lineage b[36] and similar to that of Middle East respiratory syndrome (MERS) coronavirus.[35] Host protease processing plays a pivotal role as a species and tissue barrier and engineering of the cleavage sites of CoV spike proteins modifies virus tropism and virulence.[37] The ubiquitous expression of furin in different organs and tissues have conferred to SARS-CoV-2 the ability to infect organs usually invulnerable to other CoVs, leading to systemic infection in the body.[38] Cell-cultured SARS-CoV-2 that was missing the above-mentioned cleavage site caused attenuated symptoms in infected hamsters,[39] and mutagenesis studies have confirmed that the polybasic furin site is essential for SARS-CoV-2's ability to infect human lung cells.[40]

The polybasic furin site in SARS-CoV-2 was created by a 12-nucleotide insert TCCTCGGCGGGC coding for a PRRA amino acid sequence at the S1/S2 junction (Figure 1). Interestingly, the two joint arginines are coded by two CGGCGG codons, which are rare for these viruses: only 5% of arginines are coded by CGG in SARS-CoV-2 or RaTG13, and CGGCGG in the new insert is the only doubled instance of this codon in SARS-CoV-2. The CGGCGG insert includes a *Fau*I restriction site, of which there are six instances in SARS-CoV-2 and four instances in RaTG13 (and two in MP789). The serendipitous location of the *Fau*I site could allow using restriction fragment length polymorphism (RFLP) techniques [41] for cloning [42] or screening for mutations, [43] as the new furin site is prone to deletions *in vitro*.[39, 44]

FIGURE 1

Open in figure viewerPowerPoint

Nucleotide sequence of the S protein at the S1/S2 junction in SARS-CoV-2 (NC045512.2) showing the furin cleavage site (in blue) that includes a *Faul* enzyme restriction site

A study by Zhou et al.[45] reported the discovery of a novel CoV strain RmYN02, which the authors claim exhibits natural PAA amino acid insertions at the S1/S2 cleavage site where SARS-CoV-2 has the PRRA insertion. However, upon close examination of the underlying nucleotide sequence of RmYN02 in comparison with its closest ancestors bat-SL-CoVZC45 and bat-SL-CoVZXC21, no insertions are apparent, just nucleotide mutations (Figure 2).

FIGURE 2

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Alignment of nucleotide and amino acid sequences of the S protein from bat-SL-CoVZC45 (MG772933.1) and RmYN02 at the S1/S2 junction site. No insertions of nucleotides possibly evolving in a furin cleavage site can be observed (in blue)

Therefore, SARS-CoV-2 remains unique among its beta CoV relatives not only due to a polybasic furin site at the S1/S2 junction, but also due to the four amino acid insert PRRA that had created it. The insertion causes a split in the original codon for serine (TCA) in MP789 or RaTG13 to give part of a new codon for serine (TCT) and part of the amino acid alanine (GCA) in SARS-CoV-2 (Figure 3).

FIGURE 3

Open in figure viewerPowerPoint

Alignment of nucleotide and amino acid sequences of the S protein from RaTG13 (MN996532), MP789 (MT084071) and SARS-CoV-2 (NC045512.2) at the S1/S2 site. The common nucleotides and amino acids are given in black, SARS-CoV-2 unique nucleotides and amino acids in red, RaTG13 unique nucleotides and amino acids in green and common nucleotides and amino acids in SARS-CoV-2 and RaTG13 that differ in MP789 in blue. The codon forserine (TCA) in RaTG13 and MP789 is split in SARS-CoV-2 to give part of a new codon forserine (TCT) and part of the amino acidalanine (GCA)

The insertion of the furin cleavage site in SARS-CoV-2 is not in frame with the rest of the sequence, when compared with the MP789 and the RaTG13 sequences (Figure 3). Therefore, it is possible to exclude that such insertion could have originated by polymerase slippage or by releasing and repriming, because insertion mutations generated by these mechanisms have been postulated to maintain the reading frame of the viral sequence.[46] The possibility that the furin cleavage site could have been acquired by recombination has been recently questioned by Seyran et al.,[47] because the SARS-CoV-2 spike protein seems to lack any further recombination event in contrast with the recombination model of other CoVs.

CRITIQUE OF "THE PROXIMAL ORIGIN OF SARS-COV-2"

Due to the broad-spectrum of research conducted over almost 20 years on bat SARS-CoVs justified by their potential to spill over from animal to human,[48] a possible synthetic origin by laboratory engineering of SARS-CoV-2 cannot be excluded. The widely cited article of Andersen et al.[2] stated that SARS-CoV-2 has most likely a natural origin. The main argument brought by the authors is that the high-affinity binding of the SARS-CoV-2 spike protein to hACE2 could not have been predicted by models based on the RBD of SARS-CoV. Based on the structural analysis conducted by Wan et al.,[49] SARS-CoV-2 has the potential to recognize hACE2 more efficiently than the SARS-CoV, which emerged in 2002. Moreover, generation of CoV chimeric strains has recently demonstrated that bat CoV spikes can bind to the hACE2 receptor with more plasticity than previously predicted.[15] All amino acids in the RBD have been extensively analyzed and new models to predict ACE2 affinity are available.[50] In this regard, BatCoV Rs3367 (99.9% identity to WIV1) has been shown to share with SARS-CoV-2 four out of six critical residues in the RBD. Considering that WIV1 was shown to directly bind to hACE2, the same assumption could easily have been made about SARS-CoV-2 RBD.[51]

As described above, creation of chimeric viruses has been carried out over the years with the purpose of studying the potential pathogenicity of bat CoVs for humans. In this context, SARS-CoV-2 could have been synthesized by combining a backbone similar to RaTG13 with the RBD of CoV similar to the one recently isolated from pangolins[12], because the latter is characterized by a higher affinity with the hACE2 receptor. Such research could have aimed to identify pangolins as possible intermediate hosts for bat-CoV potentially pathogenic for humans. Subsequent serial cell or animal passage, as described by Sirotkin & Sirotkin [1] could have provided the perfect adaptation of the RBD to the hACE2.

Regarding the furin cleavage site, Andersen et al.[2] state that "the functional consequence of the polybasic cleavage site in SARS-CoV-2 is unknown." New studies from several groups have lately identified this activation site as possibly enabling the virus to spread efficiently between humans and attack multiple organs.[52] Experiments on proteolytic cleavage of CoV spike proteins have been recently suggested as future key studies to understand virus transmissibility in different hosts.[50]

Andersen et al.[2] also state, based on the work of Almazan et al.[53] that "the genetic data irrefutably show that SARS-CoV-2 is not derived from any previously used virus backbone." In the last 6 years before the outbreak of SARS-CoV-2 the number of potential bat backbones has been undeniably increased by several bat CoV screenings, last but not least bringing RaTG13 to scientific attention in January 2020. Other possible backbones could, as well, still wait for publication.

Andersen et al.[2] affirm that "the acquisition of both the polybasic cleavage site and predicted O-linked glycans also argues against culture-based scenarios." Methods for insertion of a polybasic cleavage site in infectious bronchitis CoV are given in Cheng et al.[54] and resulted in increased pathogenicity. Concerning the predicted O-linked glycans around the newly inserted polybasic site, it should be noted that this prediction was not confirmed by Cryo-EM inquiry into the SARS-CoV-2 spike glycoprotein.[55] Nevertheless, while it is true that O-linked glycans are much more likely to arise under immune selection, they could be added in the lab through site-directed mutagenesis[56] or arise in the course of *in vivo* experiments, for example, in BLT-L mice with human lung implants and autologous human immune system[57] or in mice expressing the hACE2 receptor.[31] To overcome problems of bat CoV isolation, experiments based on direct inoculation of bat CoV in suckling rats have been carried out.[58] Humanized mice, ferrets, primates and/or other animals with similar ACE2 conformation could have all been used for serial passage experiments, as described in detail by Sirotkin and Sirotkin.[1]

Andersen et al.[2] also state that "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." It should not be excluded that such experiments could have been aborted due to the SARS-CoV-2 outbreak, before a possible publication of the results or that the results were never intended to be published.

It is important to mention that RaTG13 and the pangolin CoV sequences from smuggled pangolins confiscated in the GD province in March 2019, and to which most of published papers supporting a natural origin of SARS-CoV-2 refer,[2] have recently been questioned as to the accuracy of their assembly data**xii** and require further analyses to prove their correctness.[**xiii** ·**xiv**] It should also be noted that *in vitro* receptor binding studies of reconstituted RaTG13 yielded some peculiar results.[xii] The most surprising observation was that RaTG13, unlike SARS-CoV-2, is unable to bind ACE2 in *R. macrotis* bats, a close relative of RaTG13's purported host, *R. affinis*[59] (whose ACE2 receptor has not yet been tested). At the same time, RaTG13 was observed to bind hACE2[60], but not as well as ACE2 of rats and mice, to which SARS-CoV-2 did not bind at all. Is it possible that just as SARS-MA15 was a mouse-adapted strain of SARS, RaTG13 is actually a mouse-adapted version of a CoV extracted from the Mojiang cave, rather than a strain obtained from a bat fecal swab? Unfortunately, the RaTG13 sample has been exhausted and it is no longer available for external examination,[11] which is unfortunate given a number of inconsistencies in its sequencing raw data. Also, the status and availability of the Mojiang miners' samples remain as well an open and highly relevant question.

Several samples from the miners have been collected[7, 8] and likely stored, and it would be of great value to test them for the presence of SARS-CoV-2-like CoVs.

Another open question is the reason for modification and subsequent deletion of WIV's own viral database. In May 2020, several media outlets have reported that the change tracking system of WIV's internal database showed that the database was renamed from "Wildlife-borne viral pathogen database" to "Bat and rodent-borne viral pathogen database," and its description was edited to replace instances of "wild animal" by "bat and rodent"; in addition, mention of "arthropod vectors" was deleted.**xv** The database description reported that it contained over 60 Mb of data in structured query language (SQL) format, but at as of early May 2020 the download link no longer worked.**xvi** Subsequently, the database page was taken down in its entirety but its snapshot is still available on Web Archive.**xvii** It is possible that other international CoV labs might have downloaded the SQL archive of the WIV database before it was taken down, in which case such groups should make those data publicly available.

HOW COULD THE VIRUS HAVE ESCAPED FROM A LAB?

The leak of highly dangerous pathogens from laboratories is not a rare event and occurrences have been documented in several countries. The most notable lab leak known is the 1977 H1N1 lab escape from China that caused a worldwide pandemic.[61] The most recent one is the November 2019 outbreak of brucellosis that occurred in two research centers in Lanzhou, China, infecting over 100 students and staff members.[62] Several lab escapes of the first SARS virus have been reported as well: in the summer of 2003 in Singapore,[63] then in December 2003 in Taiwan,**xviii** and in the spring of 2004 twice in China.**xix**

Concerns about WIV's lab safety were raised in 2018 by U.S. Embassy officials after visiting the Institute and having an interview with Zhengli Shi. The lab auditors summarized their worries in subsequent diplomatic cables to Washington.**xx** Chinese experts have also raised concerns about lab safety in their own country, lamenting that "lab trash can contain man-made viruses, bacteria or microbes" and that "some researchers discharge laboratory materials into the sewer after experiments without a specific biological disposal mechanism."**xxi**

American labs have also had their share of safety issues. Recently, research operations in the Biosafety level (BSL)-4 United States Army Medical Research Institute of Infectious Diseases (USAMRIID) facility in Fort Detrick were interrupted in August 2019 following safety

violations, in particular, relating to the disposal of infective materials.**xxii** Other US labs have been cited for safety issues as well.[22]

A number of scenarios causing SARS-CoV-2 to leak from a lab can be hypothesized. For example, an infected animal could have escaped from a lab or it could have scratched or bitten a worker (a concern raised in 2017 about the establishment of a BSL-4 primate vaccine testing facility in Kunming, Yunnan[64]), or a researcher could have accidentally stuck themselves with inoculate (as happened in two cases in Russia**xxiii**). Until 2020, CoVs were not considered particularly deadly or virulent. SARS-like CoVs did not require BSL-4 and could be manipulated under BSL-2 and BSL-3[42] conditions, making an accidental leak more likely. Aerosol experiments with CoVs[65] could result in lab leak as well, because a failure in the equipment used could go unnoticed for a long time before infection of lab workers is detected. Finally, the virus could potentially have leaked through the sewage system if proper waste disposal and/or decontamination procedures were not followed.

CONCLUSIONS AND OUTLOOK

On the basis of our analysis, an artificial origin of SARS-CoV-2 is not a baseless conspiracy theory that is to be condemned[66] and researchers have the responsibility to consider all possible causes for SARS-CoV-2 emergence. The insertion of human-adapted pangolin CoV RBD obtained by cell/animal serial passage and furin cleavage site could arise from site-directed mutagenesis experiments, in a context of evolutionary studies or development of pan-CoV vaccines or drugs. A recent article in Nature[67] affirms that a laboratory origin for SARS-CoV-2 cannot be ruled out, as researchers could have been infected accidentally, and that gain-of-function experiments resulting in SARS-CoV-2 could have been performed at WIV. Genetic manipulation of SARS-CoV-2 may have been carried out in any laboratory in the world with access to the backbone sequence and the necessary equipment and it would not leave any trace. Modern technologies based on synthetic genetics platforms allow the reconstruction of viruses based on their genomic sequence, without the need of a natural isolate.[68]

A thorough investigation on strain collections and research records in all laboratories involved in CoV research before SARS-CoV-2 outbreak is urgently needed. Special attention should be paid to strains of CoVs that were generated in virology laboratories but have not yet been published, as those possibly described in the deleted WIV database. Because finding a possible natural host could take years, as with the first SARS,[67] or never succeed, equal priority should be given to investigating natural and laboratory origins of SARS-CoV-2.

Xiao Qiang, a research scientist at Berkeley, recently stated: "To understand exactly how this virus has originated is critical knowledge for preventing this from happening in the future."[lxxii]

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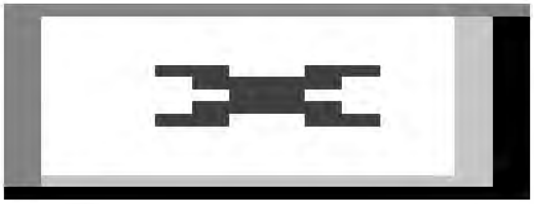

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PROBLEMS & PARADIGMS Prospects & Overviews www.bioessays-journal.com Might SARS-CoV-2 Have Arisen via Serial Passage through an Animal Host or Cell Culture? A potential explanation for much of the novel coronavirus' distinctive genome Karl Sirotkin* and Dan Sirotkin Despite claims from prominent scientists that SARS-CoV-2 indubitably emerged naturally, the etiology of this novel coronavirus remains a pressing and open question: Without knowing the true nature of a disease, it is impossible for clinicians to appropriately shape their care, for policy-makers to correctly gauge the nature and extent of the threat, and for the public to appropriately modify their behavior. Unless the intermediate host necessary for completing a natural zoonotic jump is identified, the dual-use gain-of-function research practice of viral serial passage should be considered a viable route by which the novel coronavirus arose. The practice of serial passage mimics a natural zoonotic jump, and o□ers explanations for SARS-CoV-2's distinctive spike-protein region and its unexpectedly high a□nity for angiotensin converting enzyme (ACE2), as well as the notable polybasic furin cleavage site within it. Additional molecular clues raise further questions, all of which warrant full investigation into the novel coronavirus's origins and a re-examination of the risks and rewards of dual-use gain-of-function research. 1. Introduction To date, the origins of SARS-CoV-2 remain in doubt, and its behavior enigmatic: It has been reported that "the virus acts like no microbe humanity has ever seen." [1] Although based on sequence analysis many prominent virologists and other eminent scientists have concluded that the novel coronavirus causing the current pandemic was not designed or manipulated in a laboratory and was the result of a natural zoonotic jump, [2] this assertion fails to fully account for all possible origins of two unique genomic characteristics found in SARS-CoV-2, and ignores the long history of serial passage as a method to manipulate viral genomes.Thelong-standingpracticeofserialpassageisaformof gain-of-function research that forces zoonosis between species, and requires the same molecular adaptations necessary for a natural zoonotic jump to occur within a laboratory, leaving the Dr. K. Sirotkin, D. Sirotkin Karl Sirotkin LLC, [redacted] (b)(6)
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/bies.202000091 DOI: 10.1002/bies.202000091 same genetic signatures behind as a natural jump but occurring in a much shorter period of time. The genetic signatures in question includes two distinctive features possessed by SARS-CoV-2's spike-protein: the unique sequence in the receptor binding domain (RBD), a region known to be critical for SARS-CoV-2's utilization of human angiotensin converting enzyme (ACE2), which is the cell surface receptor used by both SARS-CoV and SARS-CoV-2 for fusion with target cells and subsequent cell entry. The second feature is the presence of a polybasic furin cleavage site, which is also known as a multibasic cleavage site (MBS)—a four amino acid insertion with limited sequence flexibility—within the coronavirus's novel spike-protein, that is not found in SARS-CoV or other lineage B coronaviruses. This furin cleavage site, which is poly or multibasic by definition since its composed of multiple basic amino acids, is an important virulence feature observed to have been acquired by fusion proteins of avian influenza viruses and Newcastle Disease Virus either grown under experimental conditions or isolated from commercial animal farms—settings that mimic the conditions of serial laboratory passage. In fact, no influenza virus with a furin cleavage site has ever been found in nature, [3] and it is a feature that has been thoroughly investigated in the literature since it appears to allow the influenza viruses that carry it to

establish a systemic multiorgan infection using di□erent cell types including nerve cells, [3] is correlated with high pathogenicity, and also plays a key role in overcoming the species barrier. [4] More generally, despite the fact that not all serially passed viruses have demonstrated an increase in pathogenicity, the fact remains that every highly pathogenic avian influenza virus, defined by having a furin cleavage site, has either been found on commercial poultry farms that create the pseudo-natural conditions necessary for serial passage, or created in laboratories with gain-of-function serial passage experiments. [3] Although they only emerge under artificial conditions in influenza viruses, these furin cleavage sites are found within several branches of the coronavirus family tree. However SARSCoV-2 is the only lineage B coronavirus found with one, and the only other coronaviruses known to have them are only at most 60% identical to this novel coronavirus. [5] An intriguing BioEssays 2020, 2000091 © 2020 Wiley Periodicals LLC 2000091 (1 of 7) www.advancedsciencenews.com www.bioessays-journal.com clinical correlate is that furin cleavage sites within influenza viruses are associated with lymphopenia in infected mice, and with neurological conditions following replication in the brains of ferrets, [6] both of which are clinical manifestations observed in hospitalized patients infected by SARS-CoV-2 and su□ering from COVID-19. [1] This indicates that furin cleavage sites may be an example of the convergent evolution that dominates virus–host interactions, since viral proteins evolve convergently and often accumulate many of the same linear motifs that mediate many functionally diverse biophysical interactions in order to manipulate complex host processes. [7] It is possible that this novel coronavirus gained its furin cleavage site through recombination in an intermediate host species, however there are also two laboratory processes that may have imbued SARS-CoV-2 with its furin cleavage site which will be discussed below. Without incorporating the historical and biological implications of serial viral passage either through lab animals in vivo or through cell cultures in vitro, it is impossible to comprehensively evaluate whether SARS-CoV-2 is the result of a laboratory leak or a natural zoonotic jump. Moreover, despite the published consensus being that SARS-CoV-2 arose naturally, because these publications universally ignore the scenario of the widely used practice of laboratory serial passage, this latter scenario deserves a thorough investigation. Especially since serial passage through a live animal host simply forces the same molecular processes that occur in nature to happen during a zoonotic jump, and in vitro passage through cell culture mimics many elements of this process—and neither necessarily leaves any distinguishing genetic traces. 2. The History of Viral Serial Passage The dual-use gain-of-function research tool of serial passage was first applied to a strain of H1N1 Swine Flu, a variant of the pandemic influenza virus that was genetically modified before it either leaked out of a Soviet lab or was introduced as part of an attenuated vaccine trial in 1977. Although no one has ever taken responsibility for the introduction of this virus, it would become the first known example of a virus created by serial passage leaving a lab, which was later determined due to its inexplicable genetic distance from any known sister strain. [8] This extra distance would be expected since serial passages artificially accelerates genetic divergence between taxa, resulting in the accumulation of genetic distance at a much faster rate than it occurs in a natural setting. Then in 1979, just 2 years after the introduction of this modified H1N1 Swine Flu, a di□erent Soviet lab leaked weaponized anthrax out through an improperly maintained exhaust filter, and Soviet authorities convincingly blamed the deaths on contaminated local meat. This cover up withstood a formal inquiry conducted in 1986, and was not revealed to be a fabrication until 1992, when an analysis of dispersion patterns revealed that the victims were not those working with the supposedly contaminated meat, but instead all lived downwind from the Sverdlovsk weapons lab and its

improperly maintained exhaust vent. Therefore, there is a history of denying laboratory leaks on the commercial meat industry that dates back about 40 years, an e□ective excuse that provided the Soviets with an alibi that held up for nearly 2 decades. The Soviet strain of serially passaged H1N1 Swine Flu was likely being developed as part of a vaccine program, one of the humane goals of gain-of-function research that exist alongside riskier and more troublesome ones like developing bioweapons. Its emergence ignited the debate between the risks and rewards of dual-use gain-of-function research—causing it to became the poster virus for the dangers this protocol posed. [8] This debate would largely fade in the decades that followed, until two separate teams used genetic manipulation followed by serial passage between ferrets to create mammal-transmissible H5N1 Bird Flu strains of influenza virus in 2011 that had the gain-of-function of being transmissible by aerosol. The first team was led by Dr. Ron Fouchier and conducted at the Erasmus Medical Center in the Netherlands, and demonstrated that as few as five mutations prior to serial passage were su□cient to create a modified strain of the H5N1 Bird Flu that could be transmitted by aerosol while remaining highly lethal. [9] The creation of this highly virulent strain that was said by a reporter to be able to "make the deadly 1918 pandemic look like a pesky cold," [10] and was contentious enough to cause the scientists working on them to prepare for a media storm [11] —a storm that rolled in on the back of a second similar experiment. Instead of only tweaking the H5N1 Bird Flu in a few places before serial passage, Dr. Yoshihiro Kawaoka of the Universities of Tokyo and Wisconsin used genetic engineering to combine genes from the H1N1 Swine Flu as well as the H5N1 Bird Flu to create a chimeric virus that was then serially passed through ferrets, creating another airborne virus with potentially pandemic properties. [12] Both experiments created a modified genome that appeared to be the result of natural, albeit accelerated, selection since the process of serial passage forces the mutations selected for in natural zoonotic jumps, and masks the direct genetic engineering done on the viruses. These experiments were viewed by many as being su□ciently dangerous that they should not be published, [13] however they were both eventually released with certain methodological and sequence details left out. In the years that followed, gain-of-function serial passage through ferrets was used to increase the virulence of the H7N1 Bird Flu as well as allowing for its aerosol transmission without first introducing any mutations. [14] Additionally, the H1N1 Bird Flu was also found to become airborne and increase in virulence after in vivo passage through swine. [15,16] And although serial passage in the laboratory does not invariably increase viral pathogenicity, highly pathogenic influenza viruses all contain furin cleavage sites, [16] which only emerge after serial passage in laboratories or pseudo-naturally on commercial animal farms. The process of sequential passage through animal hosts or cell cultures leaves a genome that appears natural and not purposefully manipulated since it e□ectively mimics the natural process of zoonosis, and leaves a genome that appears to be the result of natural selection so long as its relationship to related strains of virus is ignored. However, the artificial generations added by forced serial passage creates the artificial appearance of evolutionary distance, which was the characteristic of the H1N1 Swine Flu Soviet leak in the 1970s that lead researchers BioEssays 2020, 2000091 © 2020 Wiley Periodicals LLC 2000091 (2 of 7)
www.advancedsciencenews.com www.bioessays-journal.com to conclude it had been constructed in a lab, and is exactly what is found with SARS-CoV-2, which is distant enough from any other virus that it has been placed in its own clade. [17] 2.1. Serial Passage and Its Molecular Signatures Although serial passage mimics many of the natural zoonotic processes that occur during a natural zoonotic jump, because serial passage artificially condenses a natural phenomenon into a small temporal window, some subtle di□erences can be found. In addition to

the inexplicable genetic distance from its sister strains, which screams out for an intermediate relative to complete the phylogenetic picture, SARS-CoV-2 has a remarkably strong a□nity for spike-protein binding to ACE2—some 10– 20 times higher than SARS-CoV's. [18] That a□nity may have emerged after mutational events either in an intermediate natural host or after a zoonotic jump into humans that theoretically could have occurred earlier than the first documented infection, which would give it time to increase that significantly. So logically, it could also have emerged via selection after serial passage through laboratory cell cultures or laboratory animals as well. And regarding the second distinctive feature found in the novel coronavirus: If other viruses have been observed to acquire furin cleavage sites by passage under experimental laboratory conditions, then such a mechanism is theoretically possible for SARSCoV-2 as well. [2] In the case of influenza viruses like those mentioned above, their gain-of-function furin cleavage sites are thought to be a result of two di□erent molecular processes. The first is either nucleotide insertions or substitutions that are able to be rescued and then eventually selected for due to the high multiplicity of infection found in serial passage protocols. [19] And the second is the recombination of multiple viral RNAs inside a host cell, [20] which may also include additional viruses introduced through accidental laboratory co-infections. Unlike influenza viruses, serial passage through ferrets has not been recorded in the literature for coronaviruses. However, since several branches of coronavirus have furin cleavage sites, a molecular pathway for their emergence must exist and may reemerge during serial passage. Several factors weigh into the probability that coronaviruses can gain furin cleavage sites following serial passage: The frequency of evolutionary motifs meant to deal with virus–host interactions that are often shared between viruses, the observations that when the infectious bronchitis coronavirus (IBV) coronavirus is serially passed through chickens it developed notable mutations along its spike-protein genes, [21] and the fact that when a lineage A bovine coronavirus was subject to in vitro serial passage through cell lines, a 12nucleotide insert found within only a small minority of the pooled viruses spike-protein region was strongly selected for and quickly emerged as the dominate strain. [22] These findings all point to the possibility that SARS-CoV-2 may have gained its furin cleavage site the same way influenza viruses do—through the in vivo serial passage between the live hosts that presents the immune challenges and intense selective pressure necessary for the recombination and mutations that lead to its emergence to occur. And just like influenza viruses are only able to preserve their furin cleavages in artificial environments since the heightened virulence they impart kills their hosts before they can propagate in a natural setting, based on the known taxonomy lineage B coronaviruses do not appear to be able to support furin cleavages in nature. There is no doubt that the acquisition of the furin cleavage site was one of the key adaptations that enable SARS-CoV-2 to e□ciently spread in the human populations compared to other lineage B coronaviruses, and provides a gain-of-function. [23] In addition to the possibility of obtaining a furin cleavage site through natural recombination in a secondary host or through serial passage either in a laboratory or on a commercial farm, one could have been spliced directly into the novel coronavirus's backbone in a laboratory using classic recombinant DNA technology that has been available for nearly 20 years. This allows for the removal of the restriction site junctions that are the telltale sign of direct genetic manipulation and permits reassembly without introducing nucleotide changes—creating a virus without any evidence of manipulation using the aptly named "No See'm technology." [24] So although the entire spike-protein RBD was not assembled from scratch, it is certainly plausible that the 12-nucleotide-long furin cleavage site could have been spliced directly into SARS-CoV-2. Furin cleavages already have been successfully spliced into other

coronaviruses, including the IBV, [25] and even into SARS-CoV, where it increased cell-to-cell fusion in in vitro experiments that only examined only the spike-protein's function, which would presumably heighten its infectivity in vivo. [26] Moreover, when a furin cleavage site was introduced to the IBV coronavirus spike-protein via recombination, just like influenza viruses hosting this feature, it appeared to impart it with increased lethality as well as inflict neurological symptoms that had never previously been reported in studies of the murine IBV coronavirus. [25] The presence of this cleavage site also increased damage to the respiratory and urinary systems, paralleling SARSCoV-2 systemic multiorgan symptoms—especially reports that infection with the novel coronavirus not only targets the lungs where it binds to ACE2 receptors, but also the entire cardiovascular system, [27] the nervous system, [28] and our kidneys as well. [29] It might be more than a coincidence that the Vero cells often used in serial passage are derived from kidney epithelial cells extracted from African green monkeys, which have ACE2 receptors very similar to those found in humans and would be shared by the humanized mice that are also used for serial passage research. 2.2. Natural Origin, or Gain-of-Function Lab Escape? Gain-of-function research on bat-borne coronaviruses has been ongoing for nearly a decade everywhere from the University of North Carolina to theWuhan's Institute of Virology, which is supported by related facilities such as Wuhan's Center for Disease Control and Prevention as well as Wuhan University. A coronavirus that targets the ACE2 receptor like SARS-CoV-2 was first isolated from a wild bat in 2013 by a team out of Wuhan. This research was funded in part by EcoHealth Alliance, [30] and set the stage for the manipulation of bat-borne coronavirus genomes that target this receptor and can become airborne. Many more viruses have been collected in Wuhan over the years, and one BioEssays 2020, 2000091 © 2020 Wiley Periodicals LLC 2000091 (3 of 7) www.advancedsciencenews.com www.bioessays-journal.com research expedition captured as many as 400 wild viruses, [31] which were added to a private repository that has since grown to over 1500 strains of virus, [32] meaning that the Wuhan Center for Disease Control and Prevention has a massive catalogue of largely undisclosed viruses to draw from for experiments. And in subsequent years, EcoHealth Alliance received funding for project proposals outlining gain-of-function research to be done in Wuhan, hoping to use cell cultures and humanized mice as well as "[spike]-protein sequence data, infectious clone technology, in vitro and in vivo infection experiments and analysis of receptor binding" [33] to manipulate bat coronavirus genomes—all of which are consistent with the wet-work that would be needed to engineer this novel coronavirus in a laboratory. But for whatever reason, the Wuhan Institute of Virology has refused to release the lab notebooks of its researchers, which are ubiquitous in even the simplest laboratories and are expected to be meticulously detailed given the sensitive and delicate work that takes place in BSL-4 research labs intent on documenting their intellectual property, despite the fact that these notebooks would likely be enough to exonerate the lab from having any role in the creation of SARS-CoV-2. [34] Although it does not prove a laboratory origin, another gainof-function experiment demonstrates one possible step along the way to engineering SARS-CoV-2: the synthetic reconstruction of the SARS coronavirus to impart this virus with a high a□nity for ACE2. This involved isolating a progenitor coronavirus from civets and then serially passing it through mammalian ACE2 receptor-expressing cells—serial passage through host cell lines instead of entire hosts, which imparted a strong a□nity for ACE2, [35] and another novel strain of coronavirus that was also presumably airborne. A few years after this study, more gainof-function research was performed that involved the creation of a chimeric bat-borne coronavirus by directly manipulating the bat coronavirus spike-protein gene, [36] which created a coronavirus so virulent that it evoked the following dire warning from SimonWain-

Hobson, a virologist with the Pasteur Institute in Paris: "If the [new] virus escaped, nobody could predict the trajectory." [37] Although SARS-CoV-2's e□cient solution for ACE2 binding has been accurately described as something that could not be intentionally engineered nucleotide-by-nucleotide, [2] it could well be selected for after serial passage through ferrets or cell cultures in a lab. The only origin for the SARS-CoV-2 spike-protein RBD that the sequence data excludes is the deliberate manufacturing and introduction of the entire SARS-CoV spike-protein RBD sequence to create SARS-CoV-2. Otherwise, there are no genetic data to distinguish among natural and engineered possibilities at the present time. 2.3. Ferreting Out the Signs of Serial Passage Curiously, studies examining SARS-CoV-2's infectivity in ferrets found that it spreads readily among them, and also appears airborne in that animal model. [38] This lends support to the idea that ferrets may have been used for serial passage since viruses typically take a significant many months if not years to acclimate enough to spread at all among any new species, nonetheless become airborne, which requires further mutations. This relationship was further supported by reports out of the Netherlands that the novel coronavirus had spread among thirteen di□erent mink farms there, and also to at least one farm in Denmark [39] and to another in Spain where 87% of the mink were infected. [40] Minks are a closely related subspecies of ferret that can produce fertile o□spring together, and so the fact that not only did the virus spread to fifteen di□erent farms in three countries, but also appears to have spread from minks into farm workers [41] indicates that accidental commercial serial passage through minks could have played a role in its creation, as an alternative to laboratory ferrets. Nevertheless, regardless of where any possible serial passage occurred, the fact that SARS-CoV-2 spreads from humans to minks and then back to humans demonstrates a high a□nity for both species, despite neither nominally being a natural reservoir. Further support for the possibility that serial passage through lab ferrets or throughout mink farms played a role in the genesis of this novel coronavirus is provided by a preprint that notes the obvious ease with which it passes through the air between ferrets, since SARSCoV-2 was transmitted through the air to three out of four indirect recipient ferrets monitored for airborne passage of the novel coronavirus. [42] It seems reasonable to think that SARS-Cov-2's apparent a□nity for ferrets and minks should lead to an investigation of mink farms in the Hubei province were the novel coronavirus was discovered, since a viable pathway for its emergence could be infected bats defecating on commercial mink farms, which would loosely parallel the emergence of MERS-CoV from herds of camels following putative fecal contamination by local bats. [43] The prospect that serial passage through lab animals or on commercial farms may have played a role in the creation of SARS-CoV-2 is also raised by an April 2020 preprint, which appears to have been retracted after Chinese authorities implemented the censorship of any papers relating to the origins of the novel coronavirus. [44] This paper found that coronaviruses that target the ACE2 receptor bind with ferret cells more tightly than any other species except the tree shrew, which only scored about 2% higher. Tree shrews have also been used for serial viral passage, and have been promoted as a preferable animal host for laboratory experimentation since they are cheaper, smaller, easier to handle, and closer to humans evolutionarily and physiologically than ferrets. [45] However, one does not exclude the other as a possible host, and a recent preprint examining SARS-CoV's binding a□nity in humans raises additional questions about its initial emergence. It found that the novel coronavirus appears to be far more adapted to human ACE2 receptors than those found in bats, which is unexpected given that bats are the virus's assumed source, and which lead the lead research to observe that SARS-CoV-2 was perfectly adapted to infect humans since its first contact with us, and had no apparent need to for any adaptive evolution at all. [46] Although the novel

coronavirus also appears to have a high a□nity for the pangolin ACE2 receptor, [47] phylogenetic analysis of the neutral sites that best determine shared heritage [48] and a distinctive amino acid sequence both indicate that pangolins are unlikely to have served as an intermediate host, [47] so this a□nity is likely due to the convergent motifs that often mark viral evolution and not shared heritage. The unexpected immediate BioEssays 2020, 2000091 © 2020 Wiley Periodicals LLC 2000091 (4 of 7) www.advancedsciencenews.com www.bioessays-journal.com a□nity for humans was also reflected by another preprint, which observed that SARS-CoV-2 appeared just as adapted to humans at the very start of its epidemic as SARS-CoV was in the latest stages of its emergence, [49] an unexpected finding since viruses are expected to mutate substantially as they acclimate to a new species. [50] SARS-CoV-2's muddled origins are made even more Gordian by a study published March 2018 that examined people who live in villages about a kilometer away from bat caves. This study revealed that only 2.7% of those villagers had antibodies indicating any past exposure to bat coronaviruses. The authors also sampled people living in Wuhan, and found no evidence of exposure to SARS-CoV-like coronaviruses at all. [51] This means there is very little serological evidence of any exposure to these coronaviruses even in Chinese villagers living in close proximity to bat caves, and at the epicenter of the current outbreak—no previous exposure was found at all. These data do not support the idea that SARS-CoV-2 was circulating in humans prior to the outbreak began inWuhan in the early winter or fall of 2019, making a zoonotic jump even more unlikely since natural jumps leave wide serological footprints in their new host populations as early variants of a prospective virus make limited and unsuccessful jumps into individuals of the new host species, a trial-and-error that must occur before mutations that allow adaptation to a new host species are selected. [50] However these results do not rule out a much earlier jump into humans somewhere outside Hubei province, an alternative that is awaiting empirical support. Taken together, the available evidence does not point definitively toward a natural origin for SARS-CoV-2, rather, much of it is more consistent with what would be found if the novel coronavirus had arisen from serial passage of a "precursor" progenitor virus in a lab, or from bats infecting a commercial mink farm somewhere in China, which would also provide the conditions for serial passage. However, more evidence is requiredbeforeaconclusivejudgementcanbemadeonewayor the other. Further research around SARS-CoV-2's a□nity to ferrets and minks, as well as other possible intermediate hosts seems warranted, and certainly the examination of all past gain-of-function serial passage research by the scientific community at large should occur to determine what other definitive genomic signatures serial passage leaves besides the creation of furin cleavage sites, in case more of those can be found in this novel coronavirus. Two additional unique genomic signature are already being researched, as one preprint indicates that SARS-CoV-2 possesses a genomic region not found in other coronaviruses that appears to cloak the novel coronavirus from white blood cells, a characteristic also found with HIV. [52] And the second preprint identifies a region on the spike-protein gene found in no other bat-borne coronavirus that is nearly identical to superantigenic and neurotoxic motifs found in some bacteria, which may contribute to the immune overreaction that leads to the Kawasakilike multisystem inflammatory syndrome in children, and cytokine storms in adults. [53] Given the unique traits found in SARS-CoV-2 and all the open questions there still are around its emergence, until either a natural or laboratory origin is conclusivelydemonstratedbothavenuesshouldberobustlyinvestigated by the scientific community. 3. Conclusions and Outlook The history of gain-of-function research is one of science's most significant and troubling, especially since the Nuremberg Code, research scientists' Hippocratic

Oath, dictates that experiments that could endanger human life should only occur if the potential humanitarian benefits significantly outweigh the risks. [54] It seems ill-advised to rule out the possibility that gain-of-function techniques such as serial passage may have played a role in the creation of SARS-CoV-2 until more definitive data are collected, and when the Center for Arms Control and Non-Proliferation has calculated that the odds that any given potential pandemic pathogen might leak from a lab could be better than one in four. [55] The release of the H1N1 Swine Flu in 1977 first initiated the discussion about the moral and physical hazards involved with dual-use gain-of-function research, and it was the creation of extraordinarily virulent H5N1 Bird Flu strains—using the same technique of serial passage through an animal host in a lab—that contributed to the NIH imposing a moratorium on dual-use gainof-function research from 2014 until 2017, after which it was relaxed explicitly to allow influenza strains as well as coronaviruses to he studied. This moratorium was meant to limit "the potential to create, transfer, or use an enhanced potential pandemic pathogen." [56] However, just as an increased pace of research into influenza vaccines increased the odds that a leak would occur leading up to the 1977 release of H1N1 Swine Flu, which is the most often cited as originating from a laboratory leak, [8] it would follow that an increased pace of research into coronaviruses over the past few years would have increased the odds that a lab leak of one would occur; after all, these viruses were pinpointed back in 2006 as a viable vector for an HIV vaccine [57] and research into a pan-coronavirus vaccine has been ongoing for decades. And whether or not gain-of-function research is determined to have played a role in SARS-CoV-2's emergence, the fact that it creates opportunities for pandemic viruses to leak out of labs calls for a re-examination of the moratorium against this practice, because the emergence of this novel coronavirus has demonstrated that the international public health community is not prepared to handle the leak of a pandemic virus. Furthermore, none of the gain-of-function research conducted since 2014 has provided humanity with any tools at all to fight back against the ongoing pandemic caused by this novel coronavirus. Acknowledgements The authors would like to thank Philip Murphy M.D. for encouragement and helpful suggestions. Conflict of Interest The authors declare no conflict of interest. Keywords coronavirus, COVID-19, gain-of-function, intermediate host, pandemic, SARS-CoV-2, serial passage, virology BioEssays 2020, 2000091 © 2020 Wiley Periodicals LLC 2000091 (5 of 7) www.advancedsciencenews.com www.bioessays-journal.com Received: April 22, 2020 Revised: July 13, 2020 Published online: [1] M. Wadman, J. Couzin-Frankel, J. Kaiser, C. Matacic, Science 2020. [2] K. G. Andersen, A. Rambaut, W. I. Lipkin, E. C. Holmes, R. F. Garry, Nat. Med. 2020, 26, 450. [3] D. M. Tscherne, A. Garcia-Sastre, J. Clin. Invest. 2011, 121,6. [4] R. Salomon, J. Franks, E. A. Govorkova, N. A. Ilyushina, H. L. Yen, D. J. Hulse-Post, J. Humberd, M. Trichet, J. E. Rehg, R. J. Webby, R. G. Webster, E. Ho□mann, J. Exp. Med. 2006, 203, 689. [5]C.Wu,Y.Yang,Y.Liu,P.Zhang,Y.Wang,H.Li,Q.Wang,Y.Xu, Li Mingxue, M. Zheng, L. Chen, Furin, a Potential Therapeutic Target for COVID-19, 8 Apr. 2020, 22:46, fairdomhub.org/publications/ 539. [6] A. L. Suguitan, Jr., Y. Matsuoka, Y. F. Lau, C. P. Santos, L. Vogel, L. I. Cheng, M. Orandle, K. Subbarao, J. Virol. 2012, 86, 2706. [7] S. Garamszegi, E. A. Franzosa, Y. Xia, PLoS Pathog. 2013, 9, e1003778. [8] M. Rozo, G. Kwik Gronvall, MBio 2015, 6. https://doi.org/10.1128/ mbio.01013-15. [9] S. Herfst, E. J. Schrauwen, M. Linster, S. Chutinimitkul, E. de Wit, V. J. Munster, E. M. Sorrell, T. M. Bestebroer, D. F. Burke, D. J. Smith, G. F. Rimmelzwaan, A. D. Osterhaus, R. A. Fouchier, Science 2012, 336, 1534. [10] C. Zimmer, Dallas News 2019, www.dallasnews.com/opinion/ commentary/2011/12/31/carl-zimmer-should-scientists-selfcensor-their-h5n1-research/. [11] M. Enserink, Science 2017, 11. www.sciencemag.org/news/2011/11/ scientists-brace-media-storm-around-controversial-flu-

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From: "Asher, David" (b)(6) @state.gov>
To: (b)(6) @state.gov>; Gibbs, Jeffrey J <(b)(6) @state.gov>; (b)(6) @state.gov>
CC: DiNanno, Thomas G <(b)(6) @state.gov>; (b)(6) @state.gov>; Feith, David (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: NIH discussion
Date: Wed, 23 Dec 2020 18:15:10 +0000

(b)(6) For a discussion with **NIH** scientific researchers, what papers do you all think we should send over? This includes earlier analysis from Dr. Quay.
Also, can we get both Dr. Quay and Livermore researchers on the line when we have the discussion so we can (b)(5)
(b)(6)
It is a terrible time to try to put together a call and it may be that this can't happen until after the **New** Year.
By then — assuming so things come out — it may be an easier time to have a serious discussion.

From: Asher, David (b)(6) @state.gov>
Sent: Wednesday, December 23, 2020 1:05 PM
To: (b)(6) (NIH/OD) [E] (b)(6) @nih.gov>
Cc: DiNanno, Thomas G (b)(6) @state.gov>
Subject: Re: Scientific murder board

This won't be a murder board or inquiry, though we would like offer to have one or two State consulting scientists available to explain their analysis and get feedback. We mainly just want to hear NIH scientists analysis surrounding the origins issue—I assume you have a variety of hypotheses, given the lack of data from the PRC. I'll send some papers for them to respond to. If there is a way to do this next week for an hour — on MS teams — it would be helpful, given a deadline we face right after the **New** Year. I assume you are looped in what is going on with the IC.
David

From: (b)(6) (NIH/OD) [E] (b)(6) @nih.gov>
Sent: Wednesday, December 23, 2020 9:37 AM
To: Asher, David (b)(6) @state.gov>
Cc: DiNanno, Thomas G (b)(6) @state.gov>
Subject: Re: Scientific murder board

Hey David,

Okay, I'll see who can talk to your folks about theories of transmission. What is the format of the conversation ((b)(said something about a murder board?), who else is involved, and what is your

timeline? It's been a long and grueling year over here and I'm trying to let the scientists have a little time with families this week.

Also, can you send over the journal articles you reference? I can have them take a look ahead of time so the conversation can be more productive. They may have other articles to share.

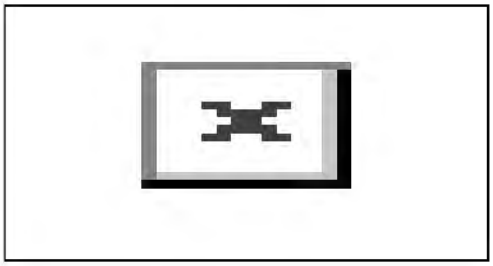
Take care,
(b)(6)

From: "Asher, David" (b)(6) @state.gov>
Date: Tuesday, December 22, 2020 at 8:21 PM
To: (b)(6) @nih.gov>
Cc: "DiNanno, Thomas G" (b)(6) @state.gov>
Subject: Re: Scientific murder board

(b)(6)

(b)(5)

(b)(5)



Op-Ed: COVID-19 lesson: Diseases can be ideal biological weapons - Los Angeles Times

The devastation COVID-19 has wrought on the U.S. population is staggering. Yet the risks it poses to our national security are also chilling: Diseases are, in many terrible ways, ideal weapons.

www.latimes.com

All the best and hope we can set up a conference call soon with your experts and see if they can review some unclassified information published in peer reviewed journals as well as material independently submitted by scientific authorities questioning the solidity of the natural origins issue and providing alternative hypotheses.

David

PS- I copy our senior official in AVC, Tom DiNanno who leads all WMD Treaty compliance. Tom is a good friend and we can have an off the record, personal discussion as well tomorrow or

after Christmas on the best way to approach this sensitive terrain appropriately, sensitively, and carefully.

David L. Asher, Ph.D
NSRI Strategic Advisor
Bureau of Arms Control, Verification and Compliance (AVC)
US Department of State
(b)(6)
JWICS: (b)(6) @state.ic.gov
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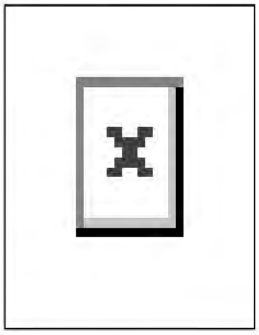

Biodefense in the Age of Synthetic Biology - The National Academies Press

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www.nap.edu

From: (b)(6) NIH/OD) [E] (b)(6) @nih.gov>
Sent: Tuesday, December 22, 2020 6:44 PM
To: Asher, David (b)(6) @state.gov>
Subject: Scientific murder board

Hey David,

I've now connected with a few different folks at NIH as a result of our conversation. While I think there are a number of scientists who can add significant insight to the issues you raise, I think it's going to be tricky to find one scientist who can meet all of your needs.

Can you tell me more about the goals of the murder board and I can work on finding the right person to whom you should extend the invitation?

Thanks!
(b)(6)

Sender: "Asher, David" (b)(6) @state.gov>
(b)(6) @state.gov>;
Gibbs, Jeffrey J (b)(6) @state.gov>;
(b)(6) @state.gov>;
Recipient: DiNanno, Thomas G (b)(6) @state.gov>;
(b)(6) @state.gov>;
Feith, David (b)(6) @state.gov>;
(b)(6) @state.gov>

From: "Feith, David"
To: Matthew Pottinger (b)(6)
Subject: Fwd: RE: your input.....one last time
Date: Thu, 3 Dec 2020 12:44:30 +0000

FYSA as discussed.

---------- Forwarded message ----------
From: Feith, David <(b)(6) @state.gov>
Date: December 2, 2020 at 9:49:00 AM EST
Subject: RE: your input.....one last time
To: Asher, David <(b)(6) @state.gov>,Gibbs, Jeffrey J <(b)(6) @state.gov>,DiNanno, Thomas G <(b)(6) @state.gov>
Cc: (b)(6) @state.gov>(b)(6) @state.gov>,Stilwell, David R <(b)(6) @state.gov>,Switzer, Bryan R (Rick) <(b)(6) @state.gov>,Keshap, Atul (b)(6) .@state.gov>

Team, thanks on all. I'd be happy to join a 1pm meeting. A/S Stilwell (cc'ed) will be out of town.

I would say though, reiterating our discussion from last week, that EAP thinks we are best served by a continued cautious approach. On the evidence, I think Pease lays it out nicely in the email attached.(b)(5)
(b)(5)

(b)(5) it would likely be unwise to pursue them before bringing the domestic and international public along, which has not happened. (As ever, we can also expect strong views in the interagency.)

Sorry for the quick take here. Keen to speak further.

--
David Feith
Deputy Assistant Secretary
Bureau of East Asian and Pacific Affairs (EAP)
U.S. Department of State
(b)(6)
(b)(6) @state.gov

~~SENSITIVE BUT UNCLASSIFIED~~

From: Asher, David <(b)(6) @state.gov>
Sent: Wednesday, December 2, 2020 8:43 AM
To: Gibbs, Jeffrey J (b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>; Feith, David (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>; Stilwell, David R (b)(6) @state.gov>; Switzer, Bryan R (Rick) (b)(6) @state.gov>; Keshap, Atul <(b)(6) @state.gov>
Subject: Fw: your input.....one last time
Importance: High

Team,

Matt, apparently, Read both Jeff and my "essays' with interest. Not what more we can do but EAP FO (Stilwell/ Keshap) can call Ford right now and demand he take tough and immediate measures). We are seeing Ford at 1pm — unless Tom object's—I recommend top level ISN, EAP and S/P involvement—one last that we all be in the same room. Perhaps AVC conference room is best due to size and Anthony, Ivan, and maybe Matt can join from the WH. Then we might get a serious decision....

I think the Secretary and Principals should (b)(5)
(b)(5)

David

From: Pottinger, Matthew F. EOP/WHO <(b)(6)
Sent: Wednesday, December 2, 2020 5:43 AM
To: Asher, David (b)(6) @state.gov>
Subject: Re: your input.....one last time

David, thanks for this.
Sent from my iPhone

On Dec 1, 2020, at 8:46 PM, Asher, David (b)(6) @state.gov> wrote:

Matt, Won't bother you again on this but there really needs to be a clear decision on allegations derived from fact and implications from WH. I don't think punting is an intelligent decision. See private comments from an esteemed colleague. All the best from Gibson Island, David (b)(6) *(NSTS)*

Here are my thoughts on the matter. Dave & (b)(6) please feel free to embellish and confirm my draft of the first answer.

(b)(5)

(b)(5)

From: Asher, David <(b)(6) @state.gov>
Sent: Tuesday, December 1, 2020 4:41 PM
To: Yu, Miles (b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>; Feith, David <(b)(6) @state.gov>; (b)(6) 2@state.gov>
Cc: Gibbs, Jeffrey J <(b)(6) @state.gov>; Switzer, Bryan R (Rick) (b)(6) @state.gov>; (b)(6) S@state.gov>; (b)(6) @state.gov>; Stilwell, David R (b)(6) @state.gov>; Keshap, Atul (b)(6) @state.gov>; Matthew Pottinger (b)(6) Kanapathy, Ivan <(b)(6) (b)(6) (b)(6) @state.gov>
Subject: Re: your input

Colleagues,

(b)(5)

(b)(5)

Asher

Executive Order 13382 of June 28, 2005

Blocking Property of Weapons of Mass Destruction Proliferators and Their Supporters

By the authority vested in me as President by the Constitution and the laws of the United States of America, including the International Emergency Economic Powers Act (50 U.S.C. 1701 et

seq.) (IEEPA), the National Emergencies Act (50 U.S.C. 1601 et seq.), and section 301 of title 3, United States Code,

I, George W. Bush, President of the United States of America, in order to take additional steps with respect to the national emergency described and declared in Executive Order 12938 of November 14, 1994, regarding the proliferation of weapons of mass destruction and the means of delivering them, and the measures imposed by that order, as expanded by Executive Order 13094 of July 28, 1998, hereby order:

Section 1. (a) Except to the extent provided in section 203(b)(1), (3), and (4) of IEEPA (50 U.S.C. 1702(b)(1), (3), and (4)), or in regulations, orders, directives, or licenses that may be issued pursuant to this order, and notwithstanding any contract entered into or any license or permit granted prior to the effective date of this order, all property and interests in property of the following persons, that are in the United States, that hereafter come within the United States, or that are or hereafter come within the possession or control of United States persons, are blocked and may not be transferred, paid, exported, withdrawn, or otherwise dealt in:

(i) the persons listed in the Annex to this order;

(ii) any foreign person determined by the Secretary of State, in consultation with the Secretary of the Treasury, the Attorney General, and other relevant agencies, to have engaged, or attempted to engage, in activities or transactions that have materially contributed to, or pose a risk of materially contributing to, the proliferation of weapons of mass destruction or their means of delivery (including missiles capable of delivering such weapons), including any efforts to manufacture, acquire, possess, develop, transport, transfer or use such items, by any person or foreign country of proliferation concern;

(iii) any person determined by the Secretary of the Treasury, in consultation with the Secretary of State, the Attorney General, and other relevant agencies, to have provided, or attempted to provide, financial, material, technological or other support for, or goods or services in support of, any activity or transaction described in paragraph (a)(ii) of this section, or any person whose property and interests in property are blocked pursuant to this order; and

(iv) any person determined by the Secretary of the Treasury, in consultation with the Secretary of State, the Attorney General, and other relevant agencies, to be owned or controlled by, or acting or purporting to act for or on behalf of, directly or indirectly, any person whose property and interests in property are blocked pursuant to this order.

(b) Any transaction or dealing by a United States person or within the United States in property or interests in property blocked pursuant to this order is prohibited, including, but not limited to, (i) the making of any contribution or provision of funds, goods, or services by, to, or for the benefit of, any person whose property and interests in property are blocked pursuant to this order, and (ii) the receipt of any contribution or provision of funds, goods, or services from any such person.

(c) Any transaction by a United States person or within the United States that evades or avoids, has the purpose of evading or avoiding, or attempts to violate any of the prohibitions set forth in this order is prohibited.

(d) Any conspiracy formed to violate the prohibitions set forth in this order is prohibited.

Sec. 2. For purposes of this order:

(a) the term ``person'' means an individual or entity;

(b) the term ``entity'' means a partnership, association, trust, joint venture, corporation, group, subgroup, or other organization; and

(c) the term ``United States person'' means any United States citizen, permanent resident alien, entity organized under the laws of the United States or any jurisdiction within the United States (including foreign branches), or any person in the United States.

Sec. 3. I hereby determine that the making of donations of the type of articles specified in section 203(b)(2) of IEEPA (50 U.S.C. 1702(b)(2)) by, to, or for the benefit of, any person whose property and interests in property are blocked pursuant to this order would seriously impair my ability to deal with the national emergency declared in Executive Order 12938, and I hereby prohibit such donations as provided by section 1 of this order.

Sec. 4. Section 4(a) of Executive Order 12938, as amended, is further amended to read as follows:

``Sec. 4. Measures Against Foreign Persons.

(a) Determination by Secretary of State; Imposition of Measures. Except to the extent provided in section 203(b) of the International Emergency Economic Powers Act (50 U.S.C. 1702(b)), where applicable, if the Secretary of State, in consultation with the Secretary of the Treasury, determines that a foreign person, on or after November 16, 1990, the effective date of Executive Order 12735, the predecessor order to Executive Order 12938, has engaged, or attempted to engage, in activities or transactions that have materially contributed to, or pose a risk of materially contributing to, the proliferation of weapons of mass destruction or their means of delivery (including missiles capable of delivering such weapons), including any efforts to manufacture, acquire, possess, develop, transport, transfer, or use such items, by any person or foreign country of proliferation concern, the measures set forth in subsections (b), (c), and (d) of this section shall be imposed on that foreign person to the extent determined by the Secretary of State, in consultation with the implementing agency and other relevant agencies. Nothing in this section is intended to preclude the imposition on that foreign person of other measures or sanctions available under this order or under other authorities."

Sec. 5. For those persons whose property and interests in property are blocked pursuant to section 1 of this order who might have a constitutional presence in the United States, I find that because of the ability to transfer funds or other assets instantaneously, prior notice to such persons of measures to be taken pursuant to this order would render these measures ineffectual. I therefore determine that for these measures to be effective in addressing the national emergency declared in Executive Order 12938, as amended, there need be no prior notice of a listing or determination made pursuant to section 1 of this order.

Sec. 6. The Secretary of the Treasury, in consultation with the Secretary of State, is hereby authorized to take such actions, including the promulgation of rules and regulations, and to employ all powers granted to the President by IEEPA as may be necessary to carry out the purposes of this order. The Secretary of the Treasury may redelegate any of these functions to other officers and agencies of the United States Government, consistent with applicable law. All agencies of the United States Government are hereby directed to take all appropriate measures within their authority to carry out the provisions of this order and, where appropriate, to advise the Secretary of the Treasury in a timely manner of the measures taken.

Sec. 7. The Secretary of the Treasury, in consultation with the Secretary of State, is hereby authorized to determine, subsequent to the issuance of this order, that circumstances no longer warrant the inclusion of a person in the Annex to this order and that the property and interests in property of that person are therefore no longer blocked pursuant to section 1 of this order.

Sec. 8. This order is not intended to, and does not, create any right or benefit, substantive or procedural, enforceable at law or in equity by any party against the United States, its

departments, agencies, instrumentalities, or entities, its officers or employees, or any other person.
Sec. 9. (a) This order is effective at 12:01 a.m. eastern daylight time on June 29, 2005.
(b) This order shall be transmitted to the Congress and published in the Federal Register.
[signed:] George W. Bush
THE WHITE HOUSE,

June 28, 2005.

ANNEX

Korea Mining Development Trading Corporation
Tanchon Commercial Bank
Korea Ryonbong General Corporation
Aerospace Industries Organization
Shahid Hemmat Industrial Group
Shahid Bakeri Industrial Group
Atomic Energy Organization of Iran
Scientific Studies and Research Center

Sender: "Feith, David"
Recipient: Matthew Pottinger (b)(6)

From: (b)(6) @state.gov>

To: Gibbs, Jeffrey J (b)(6) @state.gov>;
Yu, Miles (b)(6) @state.gov>;
Asher, David (b)(6) @state.gov>;
DiNanno, Thomas G (b)(6) @state.gov>;
Feith, David (b)(6) @state.gov>;
Switzer, Bryan R (Rick) (b)(6) @state.gov>;
(b)(6) @state.gov>

CC: (b)(6) @state.gov>

Subject: RE: your input

Date: Wed, 2 Dec 2020 13:19:54 +0000

Jeff & Dave – Enjoyed both drafts. Time permits only two quick observations/inputs from Jeff's piece:

(b)(5)

(b)(5)

-----Original Message-----
From: Gibbs, Jeffrey J <(b)(6) @state.gov>
Sent: Tuesday, December 1, 2020 5:38 PM
To: Yu, Miles (b)(6) @state.gov>; Asher, David (b)(6) @state.gov>; (b)(6)
(b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>; Feith, David <(b)(6) @state.gov>;
Switzer, Bryan R (Rick) <(b)(6) @state.gov>; (b)(6) @state.gov>
Subject: RE: your input

Here are my thoughts on the matter. Dave & (b)(6) please feel free to embellish and confirm my draft of the first answer.

(b)(5)

Page 049

Withheld pursuant to exemption

(b)(5)

(b)(5)

Jeff Gibbs
Senior Advisor
AVC Bureau
Department of State
(b)(6)

SBU - DELIBERATIVE PROCESS

-----Original Message-----
From: Yu, Miles <(b)(6) @state.gov>
Sent: Tuesday, December 1, 2020 3:01 PM
To: Asher, David <(b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6)
(b)(6) @state.gov>; DiNanno, Thomas G <(b)(6) @state.gov>; Feith, David (b)(6) @state.gov>;
Switzer, Bryan R (Rick) <(b)(6) @state.gov>; (b)(6) @state.gov>
Subject: your input

Colleagues,
The Secretary will be interviewed by a news organization later this week and he needs some talking points for the following potential questions. While working on my own TPs sheet, I realized that it's better to post the list of potential questions to you all for your sage input.

I think this group is well positioned to answer the first question, which is very crucial and it has to be precise, safe and backlash-free.

Please give me your best shot on these TPs for all questions, which do not have to be perfect and comprehensive, 2-3 bullets (except for the first one, which could be as long as it needs be) for each will suffice. And send them back to me tomorrow, COB. All info for the TPs should be unclass material.

You are welcome to provide additional potential questions with TPs attached.

Thanks for your help.

Miles

--Where the virus came from
--Whether there is a link between authoritarianism and an ability (or rather inability) to control diseases like this. We're interested in China and Iran --How the virus has affected the balance of power in the world --Whether any states have taken advantage of the virus or have an interest in it continuing to affect their geopolitical rivals --The international political implications of access to vaccines --America's continued role as the preeminent superpower in the coming years

Dr. M. Miles Yu
Policy Planning Staff
Office of the Secretary of State
Washington, DC
(b)(6)

~~SBU - DELIBERATIVE PROCESS~~

Sender: (b)(6) @state.gov>

Recipient: Gibbs, Jeffrey J <(b)(6) @state.gov>;
Yu, Miles <(b)(6) @state.gov>;
Asher, David (b)(6) @state.gov>;
DiNanno, Thomas G <(b)(6) @state.gov>;
Feith, David <(b)(6) @state.gov>;
Switzer, Bryan R (Rick) (b)(6) @state.gov>;
(b)(6) @state.gov>;
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Subject: FW: Re: AVC Scientific Panel Discussion on COVID, Thursday, January 7, at 5:30 pm EST

Date: Wed, 6 Jan 2021 21:44:30 +0000

Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 1 of 77 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 2 of 77 The cumulative circumstantial evidence that SARS-CoV-2 came from a laboratory is beyond a reasonable doubt Evidence of adenovirus vaccine experimentation by the Wuhan Institute of Virology in hospitalized COVID-19 patients in December 2019 is documented Executive Summary. The one-year anniversary of the COVID-19 pandemic records 1.85 million deaths, 85.5 million confirmed cases, 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 public understanding and consensus of 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 allow it to infect human cells and to make it more infectious, and then it was released, probably accidentally, in Wuhan, China. For most of 2019 this theory was considered a crackpot idea but in the last few weeks there has been more media attention on the possibility that the Wuhan Institute of Virology, in central Wuhan, may have been the source of the laboratory genetic manipulation and subsequent leak. Given the majority bias in favor of a zoonosis and the massive effort undertaken by China to find an animal source, for political reasons, one can assume that any evidence in favor of a natural origin, no matter how trivial, would be widely disseminated. This provides a potential evidence bias in favor of a natural origin which isn't quantified but should be kept in mind. This also becomes important when evidence can be used to support a laboratory origin that has been directly provided by leading Chinese scientists themselves, like Dr. Zhengli Shi, head of coronavirus research at the Wuhan Institute of Virology, by the Chinese government, or by powerful and vocal, pro-natural origin scientists, like Dr. Peter Daszak, of the NYC-based NGO, EcoHealth Alliance. The report uses Bayesian inference, a common statistical tool in which Bayes' theorem, a wellknown 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 has begun to be used in the law. The starting probability for the zoonotic or natural hypothesis was set at 98.8% with the laboratory origin set at 1.2%. Each piece of new evidence for or against each hypothesis is then Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 3 of 77 used to adjust the probabilities. If evidence favors a natural origin the math adjusts upward the probability of a natural origin, and so on. The final probability in this report of a laboratory origin for CoV-2 was 98.9% with a corresponding probability of zoonotic origin as 1.1%. This exceeds most academic law school discussions of quantifying 'beyond a reasonable doubt' in legal terms. The report contains the detailed quantitative basis for the statistics and can be referred to if necessary. The following Text-Table summarizes the 21 pieces of evidence that were examined in this analysis

and the change in probabilities of the origin for each step: The summary which follows will simply be a review and discussion of the evidence in the context of the two hypotheses. Evidence Zoonotic Origin Laboratory Origin Initial State 98.8% 1.2% Lack of evidence of prior seroconversion in China 95.0% 5.0% Lack of posterior diversity 66.0% 34.0% Lack of furin cleavage sites in any other sarbecovirus 17.7% 82.3% Rare useage of -CGG- single codons & no CGG-CGG pairs 2.6% 96.9% Routine use of CGG in laboratory codon optimization, including Daszak & Shi 1.1% 98.8% Spike Protein receptor binging region (200 amino acids) optimized for humans 1.1% 98.9% Whole genome analysis shows pre-adaption of CoV-2 1.1% 98.9% The finding of CoV-2 in Barcelona wastewater in early 2019 was an artifact 1.1% 98.9% Shi and the WHO comment early on that CoV-2 seemed to begin with a single patient 1.1% 98.9% Mammalian biodiversity between Yunnan and Hubei is limited, reducing candidates for intermediate host 1.1% 98.9% The ancestor of CoV-2 can only obtain a furin site from other subgenera viruses but recombination is limited/non-existent between subgenera 1.1% 98.9% Canvas of 410 animals shows humans and primates are the best, bats are the worst, for ACE2-Spike Protein interaction 1.1% 98.9% A government requested review of samples collected from a mineshaft may have caused the COVID-19 pandemic 1.1% 98.9% The Hunan Seafood Market was not the source of the pandemic 1.1% 98.9% Line 2 of the Wuhan Metro System is the likely conduit of the pandemic and is the subway line used by WIV employees 1.1% 98.9% Feral and domestic cats are not the intermediate host 1.1% 98.9% Extraodinary pre-adaption for the use of human tRNA is observed 1.1% 98.9% Evidence of Lax and disregard of laboratory safety protocols and regulations in China 1.1% 98.9% Previous SARS-CoV-1 laboratory accidents 1.1% 98.9% Shi and Daszak use Wuhan residents as negative control for zoonotic coronavirus exposure 1.1% 98.9% Appendix Information Evidence that Dr. Shi has published contrived data, making the credibility of everything she says suspect Evidence for and against RaTG13 as the direct precursor of CoV-2. I have not made up my mind on this important Remarkable evidence of the synthetic Adenovirus vector vaccine in patients sequenced at the WIV Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 4 of 77 A zoonosis has at least three elements, a host, a virus, and the human population. With some viruses there is often 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 kinds of bat species. 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 and still waiting, despite a much larger effort inside China. For both of these pandemics a bat species reservoir host was also identified. Based on the genome sequence of CoV-2, Dr. Shi and Daszak have proposed that the reservoir host for CoV-2 is the intermediate horseshoe bat (Rhinolophus affinis), which lives 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 it would be the distance and difference between the Everglades of Florida and New York City. The intermediate horseshow bat isn't found in Hubei province making a direct bat-to-human transmission improbable. Experiments in three independent laboratories also demonstrate that CoV-2 has changed genetically so much that it can no longer infect any bat species tested. So, while the leading US coronavirus expert, Dr.

Ralph Baric of The University of North Carolina stated in early 2020 that CoV-2 may have jumped into the human population directly from bats without an intermediate host, this hypothesis is no longer viable. For the zoonosis hypothesis to be advanced, it is now required to find an intermediate host. In December 2019 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. This 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 for SARS-CoV-1 or the camel markets for MERS. The Chinese authorities closed the market on December 31, 2019 after performing extensive environmental sampling and sanitation. But by May, 2020 Gao Fu, Director of the Chinese CDC, announced that the market was not the source of CoV-2 as all of the animal specimens were negative for CoV-2. And while SARSCoV-1 was found in 100% of farmed civets when tested, CoV-2 was different. In July 2020 Dr. Shi reported that extensive testing of farmed animals in Hubei Province failed to find CoV-2. For about six months the pangolin, a scaly anteater, was suspected to be the intermediate host but finally Dr. Daszak had to report that CoV-2 was not found in pangolins in the wild or from the (illegal) market trade. Domestic and feral cats were also ruled out as a possible source. A comprehensive computer-based screen of 410 different animals reported the remarkable finding that the best hosts were primates (or primate cells) and included the favorite laboratory coronavirus host, the VERO monkey cell culture, and that all bats were the worst host. At the time of the writing of this report there is not even a working hypothesis of what is the intermediate host. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 5 of 77 A zoonosis has a number of characteristic properties that can allow identification as a zoonotic infection even in the absence of finding an intermediate host. None of these properties are found for CoV-2. They all have in common the principle that when nature uses evolution to allow a virus to move from, for example, a bat host to a camel host to a human host, it is a hit and miss, slow process. After all, evolution is 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 virus spent months and years jumping from the intermediate host into humans, not having all of the best mutations needed to be aggressive, grow, and then spread, but enough to cause an infection and an immune response. The hallmark evidence of this 'practice' in host jumping is in the stored or archived human blood specimens from before the epidemic, where one can find antibodies to the eventual epidemic virus. For SARS-CoV-1 and MERS, about 0.6% of people in the region where the epidemic began show signs of an infection in archived blood. With CoV-2, this seroconversion, as it is called, has never been found, including in over 500 specimens reported by the WHO. Because this is such a potent signal of a zoonosis and because we believe that China has over 100,000 stored specimens from Wuhan taken before 2020, the lack of reports of seroconversion, the silence from China on this, speaks volumes. Another hallmark of this same, slow natural process 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 the hosts living in close proximity. During this time, they would accumulate a background of genetic mistakes, mutations. Usually about one mistake every two weeks. When the final chip falls and a mutation happens allowing the jump into humans, the virus with that new mutation also jumps around in 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 descendants. In a true zoonosis

the family tree doesn't pass back through the first patient but instead meets together in an ancestor months or years earlier. This is called posterior diversity and is an easy genetic test to perform. With CoV-2, every one of the more than 200,000 virus genomes sequenced an be traced back to the first genomic cluster and patient, who was seen at the People's Liberation Army (PLA) Hospital about one mile from the Wuhan Institute of Virology. CoV-2 has the genetic signature of one pure virus sequence infecting one human; that is the one and only jump into the human population ever seen. This lack of posterior diversity has been reported by Dr. Shi, the WHO, and other prominent virologists; they just never take the evidence to the proper inference. The virus in a zoonosis also contains the signatures of the gradual changes and adaptions it made in the protein key, the Spike Protein, it uses to unlock our cells and cause infection. With SARSCoV-1 the first jump into humans had less than one-third of all the changes it would develop by the time it became an epidemic. With CoV-2 it was almost perfectly adapted to the human lock, with only a 0.5% improvement possible. The new strain that began in the UK was one of the 0.5% improvements for the virus. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 6 of 77 Since with CoV-2 we have no evidence from stored blood that it was quietly practicing on humans in the community it is surprising that when it finds its first person, it has perfected to 99.5% its human attack ability. If this adaption couldn't have happened in the community, the only place it could have done this adaption work is in a laboratory, by what is called serial passage, repeatedly giving the virus a chance to practice on humanized mice or VERO cells. A related study of which of dozens of protein manufacturing tools CoV-2 uses (called tRNAs) shows the same uncanny adaption to the human tools with no evidence that the tools from other potential intermediate hosts would be suitable. The evidence presented makes a strong case that CoV-2 did not come from nature but is there affirmative evidence that it came from a laboratory? The answer is yes. 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. No other coronavirus in the same subgenera have a furin cleavage site, as they are called. 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 more deadly is to give it a furin site in the laboratory. At least eleven gain-of-function experiments, adding a furin site to make a virus more deadly, are published in the open literature. This has caused a flurry of Chinese papers trying to show a natural furin site in a related virus (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 viruses infect the same host and then make a mistake in copying their genetic material, and swap sequences. These hypothetical methods fail because the viruses that have furin sites are found in different host bats, in different regions of China, and even with these barriers, in the lab they are too far apart to recombine. But it gets worse for the zoonosis theory. The gene sequence for the furin site in CoV-2 is a very rare set of codons, three letter words, that are never used together by coronaviruses in nature but are always used together by scientists in the laboratory when they want to add amino acids that code for the furin site. When scientists want to add an arginine codon to a coronavirus, they invariably use the word, CGG, but coronaviruses in nature rarely (<1%) use this codon. So, there is no example of a furin protein site in nature that could be introduced into CoV-2 by recombination, there is no example of the

particular gene sequence for the furin protein site of CoV-2 being used to code for anything in nature, but this particular coding is exactly what Dr. Shi and others have used in published experiments to insert genetic material. 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 short of showing the furin site, the one she had introduced, seemingly not wanting to call attention to her handywork. She apparently failed to realize that an accomplished but innocent virologist, finding the first furin site in this class of viruses apparently coming from nature, would have featured the Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 7 of 77 presence of the furin site prominently and would also predict from her experience what it would foretell for the world due to its aggressive nature. Dr. Shi has denied the virus came from her lab, but she now created a record of multiple examples of obfuscation, half-truths, contrived specimens, genetic sequences taken from thin air, 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 in Shanghai was, "Could this have come from our lab?" 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 coronaviruses she had in her building. So the day the pandemic began in Wuhan she chose to cover up her work at the expense of transparency and cooporation. 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, to the top military medical research doctor, General Chen Wei, being placed in charge of the WIV, and many more, it is clear an event occurred sometime in late 2019 that is most consistent with a laboratory escape. The Asian region has a two-decade record of a little over one laboratory-acquired infection per year. After the first SARS-CoV-1 patient and the epidemic was ended, SARS-CoV-2 jumped six more times into the human population, all from laboratories, with two in China. The last smallpox death was a secretary two floors above a research lab in England, who contracted it through the ventilation system. Over and over again there is a history and record of laboratory acquired infections that provides the background for considering what happened here. 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 Ben Wei has been involved in vaccine research since joining the PLA 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." In this context, genetic sequence evidence of an adenovirus vaccine used and developed by the Chinese has been found in five ICU patients from a Wuhan hospital in December 2019 who also had SARS-CoV-2 in their throat swab specimens. The Wuhan Institute of Virology conducted the sequencing on these specimens. This would be consistent with a vaccine challenge trial. There is evidence of an emerging H7N9 influenza component as well, as if this was a universal vaccine program. 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. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 8 of 77 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 but would lead the world to regulate the raw materials of such bombs and to sanction sovereign nations who attempted to violate the rules. This had followed on the contribution of chemistry to human killing in the form of chemical warfare during World War I, in which 100,000 were killed, and which 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 either physics or 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. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 9 of 77 The cumulative circumstantial evidence that SARS-CoV-2 came from a laboratory is beyond a reasonable doubt A two-hypothesis, Bayesian analysis was conducted to determine the origin of the SARS-CoV-2. 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. The most unusual evidence presented, which has not been fully reconciled, is the finding of adenovirus vaccine vector sequence data in human nasopharyngeal lavage specimens taken the end of December from ICU patients at Wuhan Jinyintan Hospital and sequenced at the Wuhan institute of Virology. A high priority of current research is understanding why these patients had vaccine vector sequences, as if from a nasally administered vaccine, and what the vaccine was directed against (it is not directed to Spike Protein from SARS-CoV-1 nor from the codon optimized SARS-Cov-2 Spike Protein). This data is contained in the Appendix. Introduction. 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," 1 that transmission from laboratories was a major source of zoonotic disease. The Figure below from the Daszak paper shows this important relationship (green arrow): 1 https://www.nature.com/articles/srep14830 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 10 of 77 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 (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: Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 11 of 77 Clearly, before the beginning of the pandemic, Daszak, a member of both the WHO and Lancet teams being sent to China to explore the origin of CoV-2, could entertain the possibility of a laboratory created virus escaping into the human population/community. The purpose of this analysis is to use a Bayesian Network approach to the collected evidence that is available to provide likelihoods of the alternative hypotheses as to the origin of SARS-CoV-2. The analysis will also include certain prior probabilistic conclusions to help set the initial state before the proprietary evidence is used. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 12 of 77 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 acquired in 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 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. 2 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: 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%. 2 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6073996/ 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021

@2021. Steven C. Quay, MD, PhD Page 13 of 77 Independent prior analyses: Rootclaim. The next data that will be used is a recent analysis published on the Rootclaim website. 3 Three hypotheses below were analyzed through a series of evidence statements and the probabilities that each was the origin of SARS-CoV-2 determined: As can be seen, the highest likelihood probability is a lab escape. 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: As can be seen, the starting point assumed an 82% probability of a zoonotic origin. This starting point is a reasonable value. 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. 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 3 https://www.rootclaim.com/analysis/what-is-the-source-of-covid-19-sars-cov-2 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% 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% Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 14 of 77 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." 4 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." 5 "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. " 6 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: 7 4 https://www.sciencemag.org/sites/default/files/Shi%20Zhengli%20Q%26A.pdf 5 https://www.phe.gov/s3/dualuse/Pages/GainOfFunction.aspx 6 https://www.phe.gov/s3/dualuse/Pages/default.aspx 7 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 15 of 77 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 8 and is included herein in its entirety. For the sake of brevity, the zoonotic origin evidence was based primarily of 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. Using a simple online calculator 9 the mean of these three value sets is 94.5%, the standard deviation is + 3.8%, and the 95% confidence interval is + 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%. 8 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. 9 https://www.calculator.net/standard-deviationcalculator.html?numberinputs=91%2C+94%2C+98.6&ctype=s&x=48&y=19 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% Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 16 of 77 1. General approach of this analysis 10 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. 11 Some sources place the economic damage at $21 trillion USD. 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. 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. For evidence that cannot be quantified, the decision was made to treat these as quantitative outcomes with a 51% to 49% value with respect to the 'winning' hypothesis. This has the effect of increasing that hypothesis by 1.04. This is related to the legal standard of the 'preponderance of the evidence.' Because of the overall nature of the analyses here, all likelihoods are carried forward at the 'one significant figure' level, with standard rounding rules applied. 10 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. 11 https://www.worldometers.info/coronavirus/coronavirus-cases/ Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 17 of 77 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 18 of 77 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 19 of 77 • 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% Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 20 of 77 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021.

Steven C. Quay, MD, PhD Page 21 of 77 • 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 • 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 > 91% Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 22 of 77 Here, the negative predictive value (NPV) represents the probability that a CoV-2 is not a zoonosis, given the negative seroconversion findings. Confidence: 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 TextTable below. Adjusted likelihood: Zoonotic origin (95%) and laboratory origin (5%) Evidence or process Zoonotic Origin (ZO) Laboratory Origin Starting likelihood 0.988 0.012 Negative predictive value of lack of seroconversion 0.91 Reduced by 90% confidence 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 0.988/4.6 = 0.215 Normalize this step of analysis 0.215/(0.215 + 0.012) = 0.947 0.012/(0.215 + 0.012) = 0.053 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 23 of 77 Evidence: Lack of posterior diversity for SARS-CoV-2 compared to MERS and SARSCoV-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-tohuman infection and so are attributed to an independent intermediate host-to-human infection. 12 • That is about 54% non-human-to-human transmission. • 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. 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. 12 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/ Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 24 of 77 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 TextTable contains such data. The study of MERS noted above was published in 2013 in Lancet 13 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 14 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 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 13 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3898949/ 14 https://www.sciencedirect.com/science/article/pii/S1567134820301829 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 25 of 77 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 15 compared to CoV-2 on the

right 16 . 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. 15 https://pubmed.ncbi.nlm.nih.gov/14585636/ 16 https://nextstrain.org/ Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 26 of 77 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. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 27 of 77 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 man, either because of a new "enabling mutation" or for a non-domestic species, a chance encounter, and Source Zero and Patient Zero met 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 begins to be so 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 directly descendants but cousins and only descended from an earlier virus, who 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. They 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. . Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 28 of 77 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 17 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 2019nCoV 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% Confidence: 95% (a one in 20 chance this is wrong) 17 https://www.gisaid.org/ Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 29 of 77 Below is the impact of the pack of posterior diversity on the likelihood of a zoonotic versus laboratory origin Adjusted likelihood: Zoonotic origin (66%) and laboratory origin (34%) Evidence or process Zoonotic Origin (ZO) Laboratory Origin Starting likelihood 0.947 0.053 Negative predictive value of lack of posterior diversity 0.95 Reduced by 95% confidence 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 0.947/9 = 0.105 Normalize this step of analysis 0.105/(0.105 + 0.053) = 0.66 0.053/(0.105 + 0.053) = 0.34 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 30 of 77 Evidence and Motive for laboratory genetic 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 which 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. 18 Another database of 2956 genomes of sarbecovirus strains sequences shows that none have a furin site. 19 This is a highly significant finding with a probability that sarbecovirus has a furin site in the wild of one in about 985. 20 It has been known since 1994 that viral glycoproteins can be cleaved by secreted proteases, including furin. 21 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. 22 The first

paper using furin inhibitors to define a role for an FCS in coronavirus-cell fusion was published in 2004. 23 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. 18 https://academic.oup.com/bioinformatics/article/36/11/3552/5766118 19 https://academic.oup.com/database/advance-article/doi/10.1093/database/baaa070/5909701 20 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. 21 https://www.ncbi.nlm.nih.gov/pubmed/8162439 22 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7172898/pdf/main.pdf 23 https://www.ncbi.nlm.nih.gov/pubmed/15141003 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 31 of 77 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 syndromecoronavirus 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, 24 was reported by Tse et al. 2014. The mechanism of the FCS acquisition here was 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 was described for the insertion of arginine. The insert generates a canonical 20 AA furin site sequence. In 2011 Tian et al. 25 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′). 24 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911587/ 25 https://www.nature.com/articles/srep00261 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD,

PhD Page 32 of 77 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 26 , 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. Confidence. 95% confidence (only a one in 20 chance this is wrong). Below is the calculation of the Bayesian adjustment. Adjusted likelihood. Zoonotic origin (17.7%), laboratory origin (82.3%). 26 https://academic.oup.com/database/advance-article/doi/10.1093/database/baaa070/5909701 A S Y Q T Q T N S P R R A R S V A S Q S P14 P13 P12 P11 P10 P9 P8 P7 P6 P5 P4 P3 P2 P1 P1' P2' P3' P4' P5' P6' AA obeys furin substrate rules Solvent accessible Small polar, hydrophylic Positive charge, small, aliphatic Small residue Arginine, cleavage site S or T for glycosylation Aliphatic/hydrophobic Evidence or process Zoonotic Origin (ZO) Laboratory Origin Starting likelihood 0.66 0.34 Negative predictive value of a lack of furin sites in sarbecovirus genomes 0.95 Reduced by 95% confidence 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 0.66/9 = 0.073 Normalize this step of analysis 0.073/(0.073 + 0.34) = 0.177 0.34/(0.34 + 0.073) = 0.823 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 33 of 77 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, 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 be used as 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. 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. 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 34 of 77 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 x 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, 27 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 27 https://www.ncbi.nlm.nih.gov/pubmed/22889422 0 0.5 1 1.5 2 2.5 TAT CAG ACT CAG ACT AAT TCT CCT CGG CGG GCA CGT AGT GTA GCT AGT CAA TCC ATC N Q T Q T N S P R R A R S V A S Q S I RSCU Ave = 1.15 (red) AA/Codon Codon Bias in Furin Cleavage Sequence Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 35 of 77 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. 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 SARSCoV-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. 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 36 of 77 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 x 10 -5 . Therefore, the frequency of finding the center half of the SARS-CoV2 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 1 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 in the wild somewhere. The fact that 10 of the 12 nts are either G or C coupled with the documented bias against GC suggests this search will futile. Furin PBCS sequence Beta Coronavirus Total Arginine Dimers Anywhere CGGCGG in Spike Protein *

CGGCGG Anywhere in genome * CCGCCG Anywhere in genome SRRKRRS Human CoV-HKU1 GenBank: KF686346.1 12 0 0 0 KRRSRRA 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 PRSVRS MERS-CoV NCBI Reference Sequence: NC_019843.3 21 0 0 0 NRRSRGA 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. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 37 of 77 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. Confidence. 95% confidence (only a one in 20 chance this is wrong). Below is the calculation of the Bayesian adjustment. Adjusted likelihood. Zoonotic origin (2.6%), laboratory origin (96.9%). Evidence or process Zoonotic Origin (ZO) Laboratory Origin Starting likelihood 0.177 0.823 Negative predictive value of the absence of the -CGG-CGG- pair in any coronavirus in nature 0.95 Reduced by 95% confidence 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 0.177/9 = 0.022 Normalize this step of analysis 0.022/(0.022 + 0.823) = 0.026 0.823/(0.823 + 0.026) = 0.969 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 38 of 77 Evidence. Laboratory codon optimization uses CGG for laboratory insertions 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 crossspecies 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 28 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, 29 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 codonoptimized 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 28 http://patft.uspto.gov/netacgi/nphParser?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 29 https://www.ncbi.nlm.nih.gov/pubmed/15367630 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 39 of 77 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 SARSCoV 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 AngiotensinConverting 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 40 of 77 QuikChange mutagenesis (Stratagene) 30 Identification of murine CD8 T cell epitopes in codonoptimized 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, 31 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 32 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. 30 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." 31 From the Wuhan Institute of Virology; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7125963/ 32 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5382765/ Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 41 of 77 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. Confidence: 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: Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.8%). Evidence or process Zoonotic Origin (ZO) Laboratory Origin (LO) Starting likelihood 0.026 0.969 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 0.969 x 2.378 = 2.304 Normalize this step of analysis 0.026/(2.304 + 0.026) = 0.011 2.303/(0.026 + 2.304) = 0.988 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 42 of 77 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. 33 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. • 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 34 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. • 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 show 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. 35 33 https://www.nature.com/articles/s41591-020-0820-9 34 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. 35 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. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 43 of 77 • 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. 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." Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 44 of 77 Evidence given by Andersen: Reference 7 in the Andersen paper above is a Ralph Baric paper 36 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 2019nCoV 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 wrong, and CoV-2 binds the ACE2 receptor about ten-times better than SARS-CoV (year 2002). 37 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 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." 36 https://jvi.asm.org/content/94/7/e00127-20 37 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 45 of 77 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. 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 be 'strong evidence' of natural selection. An alternative and comprehensive analysis in another paper: 38 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

were able to create 3804 of the potential variants or 99.6% of the possible variants. It is probably 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 was also 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 38 https://www.cell.com/action/showPdf?pii=S0092-8674%2820%2931003-5 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 46 of 77 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. 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. 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 optimized as the original virus. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 47 of 77 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 SARSCoV-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 fir that matter) were being selected for. 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. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 48 of 77 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 is maximized. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 49 of 77 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. 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. Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). Evidence or process Zoonotic Origin (ZO) Laboratory Origin (LO) Starting likelihood 0.011 0.988 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 0.988 = 1.028 Normalize this step of analysis 0.011/(0.011 + 1.028) = 0.011 1.028/(0.011 + 1.028) = 0.989 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 50 of 77 Evidence. Whole genome comparison of human adaption of CoV-2 compared to SARSCoV-1 is consistent with a "pre-adaption" of CoV-2 to the human host A paper 39 entitled, "SARS-CoV-2 is well adapted for humans. What does this mean for

reemergence?" 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 near future re-emergence. [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. 39 https://www.biorxiv.org/content/10.1101/2020.05.01.073262v1 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 51 of 77 The adjusted likelihoods are shown in the following table. Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). Evidence or process Zoonotic Origin (ZO) Laboratory Origin (LO) Starting likelihood 0.011 0.989 This is 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 0.989 = 1.030 Normalize this step of analysis 0.011/(0.011 + 1.030) = 0.011 1.030/(0.011 + 1.030) = 0.989 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 52 of 77 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" 40 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-CoV2 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). 41 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. 40 https://www.medrxiv.org/content/10.1101/2020.06.13.20129627v1.full.pdf 41 https://twitter.com/quay_dr/status/1340572543548227585/photo/1 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 53 of 77 Evidence: WHO and Dr. Shi have spoken of the

singular nature the beginning of COVID19 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…" 42 By February 3, 2020, when the final version of this paper was published, this sentence had been deleted. 43 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." 44 The evidence is more consistent with a single introduction in a laboratory accident like the lack of posterior diversity and seroconversion reported earlier. This evidence will not be used to adjust probabilities but is included because it could be a form of party admissions of unfavorable facts. 42 RaTG13 paper as a preprint 43 RaTG13 final Nature paper 44 WHO document page 2 of 12 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 54 of 77 Evidence. Mammalian biodiversity and bat species differences between Yunnan and Hubei Provence are significant and are not supportive of 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. They 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 their 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," 45 in which they make a number of observations that are relevant to this analysis. It should be remembered that multiple, strong, public statements over many months by both lead authors that SARS-CoV-2 is a natural zoonosis have been made. Yunnan and Hubei Provinces have very dissimilar mammalian diversity Quoting from the Methods section of the paper: "Defining zoogeographic regions in China Hierachical clustering was used to define zoogeographic regions within China by clustering provinces with similar mammalian diversity45. 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 database84 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. Hunan and Jiangxi, clustering with the SO provinces in our dendrogram, were included within 45 https://www.nature.com/articles/s41467-020-17687-

3#Sec19 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 55 of 77 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 China85. 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. 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. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 56 of 77 Shi 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 is 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 confidence adjustment. Because of the rule on the use of significant figures, the likelihood does not change. Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). Evidence or process Zoonotic Origin (ZO) Laboratory Origin (LO) Starting likelihood 0.011 0.989 This data from Shi & Daszak disfavors a ZO 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 57 of 77 Evidence: The ancestor of SARS-CoV-2 can 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. 46 • 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. 4748 • 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. 49 The phylogeny below shows the problem of host incompatibility for beta coronaviruses (from reference 69): 46 https://www.sciencedirect.com/science/article/pii/S1873506120304165#f0015 47 file:///C:/Users/Steven%20Quay/Desktop/journal.pgen.1009272.pdf 48

https://academic.oup.com/mbe/advance-article/doi/10.1093/molbev/msaa281/5955840 49 https://www.nature.com/articles/s41467-020-17687-3#Sec2 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 58 of 77 • 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 only outward and not inward, as required by the above, admittedly, convoluted process. The data Figure is shown here: • Daszak and Shi also observed outward host switches from Rhinolophus at the genera level as well, also against a hypothesis for furin-site acquisition: Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 59 of 77 • 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: 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 SARSCoV-2, is located. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 60 of 77 • Independent evidence documents that Hubei province does not have the bat species needed for SARS-CoV-2 reservoir host 50 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 (49%) will be used. There will be no confidence adjustment. The results from the calculations are shown below. Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). 50 file:///C:/Users/Steven%20Quay/Desktop/Zhangetal2009.pdf Evidence or process Zoonotic Origin (ZO) Laboratory Origin (LO) Starting likelihood 0.011 0.989 This data from Shi & Daszak and the 'furin sites are everywhere' paper are disfavored 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 61 of 77 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. 51 • 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 SARSCoV-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." 52 • The following two Tables are taken from the paper and are organized according to ACE2 SARS-CoV-2 affinity, from highest to lowest: 51 https://www.pnas.org/content/117/36/22311 52 https://wwwnc.cdc.gov/eid/article/26/12/20-2308_article Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 62 of 77 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 63 of 77 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 preadapted 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 64 of 77 WIV in GoF research. 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. Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). Evidence or process Zoonotic Origin (ZO) Laboratory Origin (LO) Starting likelihood 0.011 0.989 A study of 410 animal ACE2 receptors shows CoV2 binds best to humans and other primates and worst to bat species 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 65 of 77 Evidence: Did a Review of Samples Collected from a Mineshaft Cause the COVID-19 Pandemic? 53 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 allege 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 also proposes that the chance of identifying the outbreak may have been reduced by the issuance of new influenza guidance in November 2019. It is a meticulous sourced analysis. It purposely avoids the question of whether SARS-CoV-2 was being grown or manipulated in the laboratory. This will not be used to adjust

the likelihoods. Current likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). 53 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 SARSCoV-2. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 66 of 77 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: 54 "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 stalls operators were trading live wild animals including chipmunks, foxes, racoons, wild boar, giant salamanders, hedgehogs, sika deer, among 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 in the market, 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. 55 This will not be used to adjust the likelihoods. Current likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). 54 https://drive.google.com/file/d/1rx0W2efbE0R1Aq-lALWTqD22VsWbTlO-/view 55 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1212604/ Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 67 of 77 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 500,000 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 56 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 are also 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. 57 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 highspeed 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. 56
https://zenodo.org/record/4119263#.X-rszNgzbOg 57
https://mp.weixin.qq.com/s/LXTfDmsQLf3qZnu_S_MxcA ;
https://thehill.com/policy/international/china/531935-study-shows-wuhan-coronavirus-cases-may-have-been-10times-higher Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 68 of 77 In a separate paper by Quay and Lee from May 2020, now accepted for publication in Epidemics, 58 they 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. While of little probative value, this 50-second video 59 from Rep. Steven Smith's (R-GA) Twitter account is a concise summation of this evidence: the speaker is Peter Daszak, at 17-seconds it shows a crowded Wuhan Metro Station with a Line 2 sign overhead, and then at 25-seconds it shows Drs. Daszak and Shi looking at a computer screen inside the Wuhan Institute of Virology. 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 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 confidence adjustment. The results from the calculations are shown below. Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). 58
https://www.researchgate.net/publication/341742303_COVID19_May_Have_Have_Reached_United_States_in_January_2020_05272020 59 https://twitter.com/i/status/1264742199754756097 Evidence or process Zoonotic Origin (ZO) Laboratory Origin (LO) Starting likelihood 0.011 0.989 The finding of Line 2 as the likely geoorigin for CoV-2 and the fact it services the WIV this evidence favors a LO 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 69 of 77 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 60 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 (1.1%), laboratory origin (98.9%). 60 https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1817796 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 70 of 77 Evidence: The extraordinary pre-adaption of SARS-CoV-2 for human cells is demonstrated by a paper looking at a tRNA adaption index. 61 "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 and chicken. In terms of the tAI, humans show the highest translational adaptation among all others, followed by chicken, 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 to humans of SARS-CoV-2 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 (1.1%), laboratory origin (98.9%). 61
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1008450#pcbi.1008450.s004 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 71 of 77 Evidence: Evidence of Lax and disregard of laboratory safety protocols and regulations in China A collection 62 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). The laboratories involved including Chemistry labs, Biolabs, Computer labs as well as Physics and Engineering labs. From these first-handed documentation, we obtained evidence of relaxed safety regulations and frequent breach of such regulations, with reasons ranging from poor training/education on lab safety, chronic ignorance of safety rules to intentional breach 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, damage to property to lab-acquired infection and escape of in-lab pathogens. With consequences from personal-level to institution-level. Here is the reference to the State Department cables concerning safety concerns at the WIV. 63 The following document shows that in June 2019, the Chinese CDC was soliciting for the removal of 25-years of solid and liquid medical waste. The total 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 is now removed from the internet. Having 25 years of toxic waste on site shows a level of lab safety disregard that is staggering. I do not think this is directly linked to CoV-2 origin but 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 originally thought to originate. 62
https://zenodo.org/record/4307879#.X-yUo9gzbOh 63
https://foia.state.gov/Search/Results.aspx?caseNumber=F-2020-05255 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021

@2021. Steven C. Quay, MD, PhD Page 72 of 77 This will not be used to adjust the likelihoods. Current likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 73 of 77 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." If you don't know what you are looking for this, "especially during disposal," is a bit of an odd qualifier. Other evidence elsewhere 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 (1.1%), laboratory origin (98.9%). Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 74 of 77 Evidence: The Good, the Bad and the Ugly: a review of SARS Lab Escapes 64 In 2003–04, in the wake of the SARS epidemics, there were multiple cases of laboratory acquired infection (LAI) with SARS in 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" 65 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. Adjusted likelihood: Zoonotic origin (1.1%), laboratory origin (98.9%). 64 https://gillesdemaneuf.medium.com/the-good-the-bad-and-the-ugly-a-review-of-sars-lab-escapes898d203d175d 65 https://www.usatoday.com/story/news/2015/05/29/some-recent-us-lab-incidents/25258237/ 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 Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 75 of 77 Evidence: Drs. Shi and Daszak use Wuhan residents as negative controls for zoonotic coronavirus seroconversion 66 "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 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 (1.1%), laboratory origin (98.9%). 66 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6178078/ Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 76 of 77 The Appendix contains the following information which was determined to be important to the overall investigation into the origin of CoV-2 but which did not become part of the Bayesian analysis: • Evidence that Dr. Shi has published contrived data, making the credibility of everything she says suspect. Specifically: o 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 laboratoryderived synthetic biology o 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 • Evidence for and against RaTG13 as the direct precursor of CoV-2. I have not made up my mind on this important hypothesis o To establish a precursor-product relationship for RaTG13 and CoV-2 a relative simple process must be proposed to make approximately 1140 nt changes in the 30,000 nt genome o Evidence in favor of the hypothesis: While the nucleotide sequence data show these coronaviruses are only 96.2% homologous a comparison of their amino acid homology indicates they are 98.8% identical and as similar as the Civet SARS-CoV-1 and human SARS-CoV-1 About 26% of the entire genomes contain only synonymous mutations without any non-synonymous mutations, a highly improbably outcome in nature but an easy exercise in the laboratory to introduce. The motivation would be to obscure the closeness of the two genomes without worrying about introducing detrimental mutations. This represents about 200 of the nt differences There are two restriction enzyme sites in RaTG13 that begin at the receptor binding domain and end 3' to the furin cleavage site that use the 'No See 'Em' technology developed and patented by Ralph Baric, a Dr. Shi and WIV collaborator. Shi has used these enzymes herself. As expected for the technology, the sites are lost in CoV-2. However, they are not the "pureform" of the Baric technology, are less hidden, and so I would be surprised if Shi did this less robust approach. Nonetheless, the likelihood these sites are there by chance is infinitesimal. Bayesian Analysis of SARS-CoV-2 Origin – Rev. 2 CONFIDENTIAL

Steven C. Quay, MD, PhD 6 January 2021 @2021. Steven C. Quay, MD, PhD Page 77 of 77 □ CoV-2 and RaTG13 share a >100 nt insertion in the ORF1ab gene found no where else in sarbecoviruses. A very strange fact and significantly greater than the 12-nt furin site that has caught so much attention. I spent a day or so probing the function of the site, I believe it is nsp3 (from memory), but didn't find a smoking gun to warrant deeper work. Should be returned to. □ It is part of the nine viruses found in the Yunnan cave where miners died of a coronavirus-like illness. o Evidence against RaTG13 □ My proof that it did not come from the bat feces specimen as reported by Shi is troublesome for an hypothesis it is the critical precursor virus □ To my knowledge no has grown it and examination of its Spike Protein by numerous groups comes to the unlikely conclusion it will bind to ACE2 of most species or grow in a lab culture. □ Peter Daszak, who has said many things proven to be false, nonetheless has described RaTG13 as a "composite sequence" a term used for a really mixed specimen where metagenomics are used to obtain a "genome sequence" which in reality was pieced together artificially by the computers running the analysis □ I can reduce the 1140 nt difference to about 600 with two steps, the No See 'Em insertion of the CoV-2 RBD in the Spike Protein and using a synonymous mutation algorithm to create artificial phylogenetic distance. But a simple method of closing that 600 nt, mostly non-synonymous mutations, has not been identified. □ Shi collected nine beta coronaviruses in the mine but has published the sequence of only RaTG13. She voluntarily published RaTG13. It seems more likely that she would publish a virus close to CoV-2 to establish the bat origin in the medical field (the RaTG13 paper title was "A pneumonia outbreak associated with a new coronavirus of probable bat origin) but not publish the actual virus she used for the construction of CoV-2, in the unlikely event a 'bullet proof' connection that she hadn't thought of could be found. • Remarkable evidence of the synthetic Adenovirus vector vaccine in patients sequenced at the WIV o More work will be focused on this to establish what the immunogen is and to further this proof.

From: "Asher, David" (b)(6) @state.gov>

To: Christopher Yeaw (b)(6) ;
(b)(6) @state.gov>

CC: David Asher <(b)(6)
(b)(6) @state.gov>;
Gibbs, Jeffrey J (b)(6) @state.gov>

Subject: Fw: My intention to resign from advising AVC as a consultant

Date: Thu, 4 Feb 2021 16:13:41 +0000

Chris-
Despite what I sent Eliot (who is not my boss) I never submitted the resignation letter to Alexandra. I decided that I should a finish up a few remaining research tasks. I also hope to receive notice in writing requesting I cease work on the contract (and perhaps explaining why—for the record). I never did a thing from a political perspective and was not a "political" contractor. I was simply trying to help effect a proper professional investigation, first into CAEP — China's main nuclear and asymmetrical weapons network of companies, and then into the WIV and COV19 origins. The COV 19 declassification, which I don't understand why anyone would see as counterproductive to helping the WHO and others assess the origins issue, was handled by EAP, who cleared it with Chris Ford and the other U/S as well Chris Park, etc. Above my/our pay grade.
I continued to work from home on the contract until January 29th, trying to help Pease and Josh dig in deeper on matters with the WIV and COVID origins and join in some LE related discussions on CAEP and the PRC's illicit activities related CW. That is the last day I worked remotely and I do not intend to work or bill after.
In addition, I would like to request a private out brief before I formally depart. In addition to Alexandra, others from the FO, of course, should attend. Trust me, it will be 100% positive and supportive—(b)(5)

(b)(5)

I will be very supportive AVC from the Hudson Institute. At Hudson I plan to write an issue brief about COVID and its consequences/implications. The credibility of the international treaty architecture is threatened by the failure to identify the origins of COV19 (which is why there still needs to be a much more significant and sweeping intelligence driven investigation conducted by the USG, with support from allies and partners, not just the WHO whitewash).
I appreciate keeping me dormantly in the NSRI/State system until I get a new contract elsewhere—hopefully very soon.
It has been an honor to work with the AVC FO team and I hope someday we will have the opportunity to again work together as well as stay in touch
Best regards,
David

(b)(6)
Cell (b)(6)
From: Asher, David
Sent: Monday, January 25, 2021 11:30 PM
To: Kang, Eliot <(b)(6)@state.gov>
Subject: My intention to resign from advising AVC as a consultant

Eliot,
Not surprisingly, (b)(5)
(b)(5)
(b)(5) I sincerely tried to help advance the mission of T, not disrupt it. (b)(5)
(b)(5)

For the record, getting the COVID story out was NOT the view of Donald Trump. In fact, I suspect the previous President had a hand in deflecting proper focus in the government into the natural or "super natural" causal pathway of COV 19 and its release because it might prove embarrassing that some USG funding for whatever reasons went into the WIV's Coronavirus R&D efforts. There are good reasons why I never voted for Trump, including the fact I knew him a bit from NY real estate finance. The man was and is a total jerk who deserves to be doubly impeached.

So even as I step away from working as a mere State contractor, my support for the T mission of advancing non proliferation and arms control under President Biden is unabated. We just need to get our heads around the power of synthetic biology and massive destructive potential of viral BW vectors (b)(5)
(b)(5)

No matter what, I promise to support you all from the Hudson Institute. Among many other matters, we will be looking hard into the pros and cons of gain of function research as well as the need to develop a global bio surveillance capability to protect against natural as well as man made pathogens. If State ever wants my confidential input, just give me a call. Also, I really hope to privately help Phil and you enhance your CP network and intel analytical fusion capabilities, especially around smart sanctions. The little NSRI team, especially Mike Pease, could be incredibly helpful to ISN and I highly encourage you to bring them under your wing.

I will send a formal, far shorter and less expressive note to Alexandra wishing her great success.

All the best,

David

(b)(6)

PS- If possible, I would appreciate State keeping my SCI clearance active for a short period of time so that I can move it to a place where my knowledge and experience can remain relevant to USG deep fight. I will not bill any more hours to the Department and stay out of the building while I transition, if acceptable. If not, I can come by and turn in my creds and read out ASAP.

Sender: "Asher, David" (b)(6) @state.gov>

Recipient: Christopher Yeaw (b)(6) ;
(b)(6) @state.gov>;
David Asher <(b)(6)
(b)(6) @state.gov>;
Gibbs, Jeffrey J <(b)(6) @state.gov>

From: "Gross, Laura J" (b)(6) @state.gov>
To: Paulopol, Andreea I (b)(6) @state.gov>
Subject: Fw: in the office - Gain of function—from F ord
Date: Thu, 10 Dec 2020 19:06:03 +0000

found it - see below. Best - Laura
From: DiNanno, Thomas G (b)(6) @state.gov>
Sent: Sunday, December 6, 2020 10:37 AM
To: (b)(6) @state.gov>
Cc: Gross, Laura J (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: RE: in the office - Gain of function—from Ford

(b)(5)

On December 6, 2020 at 10:21:43 AM EST, (b)(6) @state.gov> wrote:
Tom,

I'm in the office. Are you or Gibbs coming in?

(b)(6)

(b)(6)

Chief of Staff
Bureau of Arms Control, Verification and Compliance
U.S. Department of State
HST Room S9S0

(b)(6)

OpenNet: (b)(6) @state.gov
ClassNet: (b)(6) @state.sgov.gov

JWICS: (b)(6) @state.ic.gov

From: DiNanno, Thomas G <(b)(6) @state.gov>
Sent: Friday, December 4, 2020 7:21 PM
To: Asher, David <(b)(6) @state.gov>; Gibbs, Jeffrey J <(b)(6) @state.gov>
Cc: Pease, Michael <(b)(6) @state.gov>; <(b)(6) @state.gov>; <(b)(6) @state.gov>; Feith, David <(b)(6) @state.gov>
Subject: Re: Gain of function—from Ford

(b)(5)

On December 4, 2020 at 7:11:32 PM EST, Asher, David <(b)(6) @state.gov> wrote:
Chris will get a polite but stern retort from me....any thoughts on this, please let me know—all to be treated in confidence. Again, there is an almost impossible line to determine between syn-bio offense and defense but when you see huge gain of function attempts involved and no attempt to protect a likely spillover you must address intentions and causation. We urgently need (b)(6) analysis of the Defense One article on bio-war as well as any high side corroboration.

From: Gibbs, Jeffrey J <(b)(6) @state.gov>
Sent: Friday, December 4, 2020 12:27 PM
To: Asher, David <(b)(6) @state.gov>; DiNanno, Thomas G <(b)(6) @state.gov>
Cc: Pease, Michael <(b)(6) @state.gov>; <(b)(6) @state.gov>; <(b)(6) @state.gov>; Feith, David <(b)(6) @state.gov>
Subject: Re: Gain of function—from Ford

This sounds like (b)(5)

Jeff Gibbs
Senior Adviser AVC
SSD/AVC
(b)(6)

From: Asher, David <(b)(6) @state.gov>
Sent: Friday, December 4, 2020 11:25 AM
To: DiNanno, Thomas G <(b)(6) @state.gov>
Cc: Gibbs, Jeffrey J <(b)(6) @state.gov>; Pease, Michael <(b)(6) @state.gov>; <(b)(6) @state.gov>; <(b)(6) @state.gov>; Feith, David <(b)(6) @state.gov>
Subject: FW: Gain of function—from Ford

From: Ford, Christopher A (b)(6) @state.gov>
Sent: Friday, December 4, 2020 10:36 AM
To: Asher, David (b)(6) @state.gov>
Subject: Re: Gain of function

Dear David:

Sorry for being slow in replying, but I'm out of town and wanted to do your comment justice. I appreciate the message, and for for taking the time to put together yesterday's briefing (though I was a little surprised to hear that AVC had been working for so long on this project without them telling me anything about it). As I told Tom in an earlier message, I was impressed by the depth and detail of the presentation, and very much want to make sure we get this issue right.

Anyway, I look forward to continuing the conversation to assess the strength of the argument and especially to engaging others whose technical knowledge exceeds my own. On the points you raised, however — and after sniffing around at least a bit —- let me offer some tentative thoughts in response to the points you raised:

(b)(5)

(b)(5)

So let's continue this when I'm back next week. (I return Tuesday morning.)

Thanks again,

— Chris

From: David Asher (b)(6) @hudson.org>
Sent: Thursday, December 3, 2020 5:10 AM
To: Ford, Christopher A
Subject: Gain of function

Chris,

It is interesting that (b)(6) quoted Dr. Andersen regarding the natural and apparently "obvious" zoonotic origin or COVID-19 —an increasingly debatable conclusion, including based on the presentation I provided. His colleague then defended the proposition that gain of function research is commonplace—included into pathogens? It is precisely this gain of function research that of all people, Dr. Andersen personally trashed in Nature in 2018 (see below). Does this everyday GOF research include work on super biological pathogens like COV 19—several

generations ahead of what nature could produce, based on history? What is State's official policy on supporting gain of function research into pathogens with super spreader characteristics like COV 19? Did we actually help support the WIV? (b)(5)
(b)(5)

Sorry to drop names and places yesterday but I actually have a bit of on the ground experience with several of the most suspect entities in China and elsewhere. (b)(5)
(b)(5)

Best regards,

David

https://www.thelancet.com/action/showPdf?pii=S1473-3099%2818%2930006-9

Below: Nature commentary pointing out the futility, waste, and opportunity costs associated projects pursued by Ecohealth, WIV, NIAID, et al, in the name of "predicting the next outbreak". Though they don't address the grave hazards, and BW dual use issues, involved with the gain of function work in WIV's prediction research, they laid out other important fundamental flaws with Ecohealth and WIV's approach. The authors go on to make the more compelling case for better bio surveillance instead.https://www.nature.com/articles/d41586-018-05373-w

COMMENT

07 JUNE 2018

Pandemics: spend on surveillance, not prediction

Trust is undermined when scientists make overblown promises about disease prevention, warn Edward C. Holmes, Andrew Rambaut and Kri(stian G. Andersen.

The resurgence of Ebola virus in the Democratic Republic of the Congo this May is a stark reminder that no amount of DNA sequencing can tell us when or where the next virus outbreak will appear. More genome sequence data were obtained for the 2013–16 Ebola epidemic than for any other single disease outbreak. Still, health workers in Mbandaka, the country's northwestern provincial capital, arescrambling to contain a growing number of cases.

Over the past 15 years or so, outbreaks caused by viruses such as Ebola, SARS and Zika have cost governments billions of US dollars. Combined with a perception among scientists, health workers and citizens that responses to outbreaks have been inadequate,this has fuelled what seems like a compelling idea. Namely, that if researchers can identify the next pandemic virus before the first case appears, communities could drastically improve strategies for control, and even stop a virus from taking hold1,2. Indeed, since 2009, the US Agency for International Development has spent US$170 million on evaluating the "feasibility of preemptively mitigating pandemic threats"1.

Various experts have flagged up problems with this approach (including the three of us)3,4. Nonetheless, an ambitious biodiversity-based approach to outbreak prediction — theGlobal Virome Project— was announced in February this year, with its proponents soliciting $1.2

billion in funding from around the world(see 'High stakes'). They estimate that other mammals and birds contain 1.67 million unknown viruses from the families of viruses that are most likely to jump to humans, and will use the funding to conduct a genomic survey of these unknown viruses, with the aim of predicting which might infect people1.

Sources: NIH; Global Virome Project

Broad genomic surveys of animal viruses will almost certainly advance our understanding of virus diversity and evolution. **In our view, they will be of little practical value when it comes to understanding and mitigating the emergence of disease.**

We urge those working on infectious disease to focus funds and efforts on a much simpler and more cost-effective way to mitigate outbreaks — proactive, real-time surveillance of human populations.

The public has increasingly questioned the scientific credibility of researchers working on outbreaks. In the 2013–16 Ebola epidemic, for instance, the international response was repeatedlycriticized for being too slow. And during the 2009 H1N1 influenza epidemic, people asked whether the severity of the virus had been overblown, and if the stockpiling of pharmaceuticals was even necessary5. Making promises about disease prevention and control that cannot be kept will only further undermine trust.

Forecasting fallacy

Supporters of outbreak prediction maintain that if biologists genetically characterize all of the viruses circulating in animal populations (especially in groups such as bats and rodents that have previously acted as reservoirs for emerging viruses), they can determine which ones are likely to emerge next, and ultimately prevent them from doing so. With enough data, coupled with artificial intelligence and machine learning, they argue, the process could be similar to predicting the weather6.

Reams of data are available to train models to predict the weather. By contrast, it is exceedingly rare for viruses to emerge and cause outbreaks. Around 250 human viruses have been described, and only a small subset of these have caused major epidemics this century.

Advocates of prediction also argue that it will be possible to anticipate how likely a virus is to emerge in people on the basis of its sequence, and by using knowledge of how it interacts with cells (obtained, for instance, by studying the virus in human cell cultures).

This is misguided. Determining which of more than 1.6 million animal viruses are capable of replicating in humans and transmitting between them would require many decades' worth of laboratory work in cell cultures and animals. Even if researchers managed to link each virus genome sequence to substantial experimental data, all sorts of other factors determine whether a virus jumps species and emerges in a human population, such as the distribution and density of animal hosts. Influenza viruses have circulated in horses since the 1950s and in dogs since the early 2000s, for instance7. These viruses have not emerged in human populations, and perhaps never will — for unknown reasons.

In short, there aren't enough data on virus outbreaks for researchers to be able to accurately predict the next outbreak strain. Nor is there a good enough understanding of what drives viruses to jump hosts, making it difficult to construct predictive models.

Biodiversity-based prediction also ignores the fact that viruses are not fixed entities. New variants of RNA viruses appear every day. This speedy evolution means that surveys would need to be done continuously to be informative. The cost would dwarf the proposed $1.2-billion budget for one-time sequencing.

Even if it were possible to identify which viruses are likely to emerge in humans, thousands of candidates could end up being identified, each with a low probability of causing an outbreak. What should be done in that case? Costs would skyrocket if vaccines and therapeutics were proposed for even a handful of these.

Screen and sequence

Currently, the most effective and realistic way to fight outbreaks is to monitor human populations in the countries and locations that are most vulnerable to infectious disease. This can be done by local clinicians, health workers in non-governmental organizations such as Médecins Sans Frontières (MSF; also known as Doctors Without Borders), and global institutions such as the World Health Organization (WHO).

We advocate the detailed screening of people who are exhibiting symptoms that cannot easily be diagnosed. Such tests should use the latest sequencing technologies to characterize all the pathogens that have infected an individual — the human 'infectome'8. To track previous infections, investigators should also assess each person's immune response, by analysing components of their blood using broad-scale serology9.

Emerging diseases are commonly associated with population expansions — when people encroach on habitats occupied hy animals — as well as with environmental disturbances and climate change. Deforestation, for instance, can promote human interactions with animals that carry new threats, and can increase encounters with new vector species such as ticks and mosquitoes10. Animal die-offs, for example that of bar-headed geese (*Anser indicus*) at Lake Qinghai in China in 2005 (which was caused by the H5N1 influenza virus), can also flag problem regions or emerging pathogens. Surveillance efforts should therefore focus on communities that live and work in such environments.

Identifying which pathogen is causing an outbreak is no longer the bottleneck it once was. It took researchers two years to determine HIV as the cause of AIDS in the early 1980s using microscopy and other techniques. By contrast, in 2012 it took only weeks for investigators using genomic technologies to discover the coronavirus that caused Middle East respiratory syndrome (MERS).

Rapid identification of viruses can be achieved only if such technologies — and the people trained to use them — are globally available, including in resource-limited regions where the risk of outbreaks might be higher.Thankfully, relevant capacity-building programmes are now beginning to be established, such as the Human Heredity and Health in Africa (H3Africa) Initiative, run by the UK Wellcome Trust and the US National Institutes of Health11.

Once an emerging outbreak virus has been identified, it needs to be analysed quickly to establish what type it is; which molecular mechanisms (such as receptor type) enable it to jump between individuals; how it spreads through human populations; and how it affects those infected. In other words, at least four kinds of analysis are needed: genomic, virological, epidemiological and clinical. And the data must be passed to key stakeholders, from researchers and health workers on the ground to international agencies such as the WHO and the MSF. Data must be kept as free of restrictions as possible, within the constraints of protections of patient privacy and other ethical issues.

This will best be achieved through an established global network of highly trained local researchers, such as the WHO Global Outbreak Alert and Response Network (GOARN). Real-time tools for reconstructing and tracking outbreaks at the genomic level, such as portable sequencing devices, are improving fast8. Information gathered during recent outbreaks has

quickly had tangible impacts on public-health decisions, largely owing to data generation and analysis by many research teams within days of people being infected12.

For instance, in the 2013–16 Ebola epidemic, genome sequencing of the virus proved that a person could sexually transmit the disease more than a year after becoming infected. This prompted the WHO to increase its recommended number of tests for persistent infection in survivors of the disease.

Ultimately, the challenge is to link genomic, clinical and epidemiological data within days of an outbreak being detected, including information about how people in an affected community are interacting. Such an open, collaborative approach to tackling the emergence of infectious disease is now possible. This is partly thanks to technology, but is mainly due to a shift in perception about the importance of this approach. At least in genomic epidemiology, there is a growing move towards real-time, open-access data and analysis, aided by the use of preprint servers and wikis such as Virological (http://virological.org). This type of collaborative effort can complement the work of agencies including the WHO and the MSF, which focus predominantly on providing information, isolating those who have been infected, and so on.

So far, researchers have sampled little of the viral universe. Surveys of animals will undoubtedly result in the discovery of many thousands of new viruses. These data will benefit studies of diversity and evolution, and could tell us whether and why some pathogens might jump species boundaries more frequently than others. But, given the rarity of outbreaks and the complexity of host–pathogen interactions, it is arrogant to imagine that we could use such surveys to predict and mitigate the emergence of disease.

New viruses will continue to emerge unexpectedly. There is a lot we can and must do to be better prepared.

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doi:https://doi.org/10.1038/d41586-018-05373-w

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Sender: "Gross, Laura J" (b)(6) state.gov>
Recipient: Paulopol, Andreea I (b)(6) @state.gov>

From: "Paulopol, Andreea I" (b)(6) @state.gov>
To: Meda22 (meda22@aol.com) <meda22@aol.com>
Subject: FW: For Review: Draft Article 5 re China BWC compliance—serial passage as another techni que that might have been applied
Date: Mon, 23 Nov 2020 22:44:56 +0000

~~SENSITIVE BUT UNCLASSIFIED~~

From: Asher, David (b)(6) @state.gov>
Sent: Sunday, November 22, 2020 1:12 PM
To: Gross, Laura J (b)(6) @state.gov>; Paulopol, Andreea I (b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>
Cc: Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: Re: For Review: Draft Article 5 re China BWC compliance—serial passage as another technique that might have been applied

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7435492/

https://en.wikipedia.org/wiki/Serial_passage

Serial passage - Wikipedia

Serial passage refers to the process of growing bacteria or a virus in iterations. For instance, a virus may be grown in one environment, and then part of that virus can be removed and put into a new environment.

en.wikipedia.org

Bioessays. 2020 Aug 12 : 2000091.
doi: 10.1002/bies.202000091 [Epub ahead of print]

PMCID: PMC7435492
PMID: 32786014

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

Karl Sirotkin [1] and Dan Sirotkin [1]

Go to:

1. Introduction

To date, the origins of SARS-CoV-2 remain in doubt, and its behavior enigmatic: It has been reported that "the virus acts like no microbe humanity has ever seen."[1] Although based on sequence analysis many prominent virologists and other eminent scientists have concluded that the novel coronavirus causing the current pandemic was not designed or manipulated in a laboratory and was the result of a natural zoonotic jump,[2] this assertion fails to fully account for all possible origins of two unique genomic characteristics found in SARS-CoV-2, and ignores the long history of serial passage as a method to manipulate viral genomes. The long-standing practice of serial passage is a form of gain-of-function research that forces zoonosis between species, and requires the same molecular adaptations necessary for a natural zoonotic jump to occur within a laboratory, leaving the same genetic signatures behind as a natural jump but occurring in a much shorter period of time.

The genetic signatures in question includes two distinctive features possessed by SARS-CoV-2's spike-protein: the unique sequence in the receptor binding domain (RBD), a region known to be critical for SARS-CoV-2's utilization of human angiotensin converting enzyme (ACE2), which is the cell surface receptor used by both SARS-CoV and SARS-CoV-2 for fusion with target cells and subsequent cell entry. The second feature is the presence of a polybasic furin cleavage site, which is also known as a multibasic cleavage site (MBS)—a four amino acid insertion with limited sequence flexibility—within the coronavirus's novel spike-protein, that is not found in SARS-CoV or other lineage B coronaviruses. This furin cleavage site, which is poly or multibasic by definition since its composed of multiple basic amino acids, is an important virulence feature observed to have been acquired by fusion proteins of avian influenza viruses and Newcastle Disease Virus either grown under experimental conditions or isolated from commercial animal farms—settings that mimic the conditions of serial laboratory passage. In fact, no influenza virus with a furin cleavage site has ever been found in nature,[3] and it is a feature that has been thoroughly investigated in the literature since it appears to allow the influenza viruses that carry it to establish a systemic multiorgan infection using different cell types including nerve cells,[3] is correlated with high pathogenicity, and also plays a key role in overcoming the species barrier.[4] More generally, despite the fact that not all serially passed viruses have demonstrated an increase in pathogenicity, the fact remains that every highly pathogenic avian influenza virus, defined by having a furin cleavage site, has either been found on commercial poultry farms that create the pseudo-natural conditions necessary for serial passage, or created in laboratories with gain-of-function serial passage experiments.[3]

Although they only emerge under artificial conditions in influenza viruses, these furin cleavage sites are found within several branches of the coronavirus family tree. However SARS-CoV-2 is the only lineage B coronavirus found with one, and the only other coronaviruses known to have them are only at most 60% identical to this novel coronavirus.[5] An intriguing clinical correlate

is that furin cleavage sites within influenza viruses are associated with lymphopenia in infected mice, and with neurological conditions following replication in the brains of ferrets,[6] both of which are clinical manifestations observed in hospitalized patients infected by SARS-CoV-2 and suffering from COVID-19.[1] This indicates that furin cleavage sites may be an example of the convergent evolution that dominates virus–host interactions, since viral proteins evolve convergently and often accumulate many of the same linear motifs that mediate many functionally diverse biophysical interactions in order to manipulate complex host processes.[7] It is possible that this novel coronavirus gained its furin cleavage site through recombination in an intermediate host species, however there are also two laboratory processes that may have imbued SARS-CoV-2 with its furin cleavage site which will be discussed below.

Without incorporating the historical and biological implications of serial viral passage either through lab animals in vivo or through cell cultures in vitro, it is impossible to comprehensively evaluate whether SARS-CoV-2 is the result of a laboratory leak or a natural zoonotic jump. Moreover, despite the published consensus being that SARS-CoV-2 arose naturally, because these publications universally ignore the scenario of the widely used practice of laboratory serial passage, this latter scenario deserves a thorough investigation. Especially since serial passage through a live animal host simply forces the same molecular processes that occur in nature to happen during a zoonotic jump, and in vitro passage through cell culture mimics many elements of this process—and neither necessarily leaves any distinguishing genetic traces.

Go to:

2. The History of Viral Serial Passage

The dual-use gain-of-function research tool of serial passage was first applied to a strain of H1N1 Swine Flu, a variant of the pandemic influenza virus that was genetically modified before it either leaked out of a Soviet lab or was introduced as part of an attenuated vaccine trial in 1977. Although no one has ever taken responsibility for the introduction of this virus, it would become the first known example of a virus created by serial passage leaving a lab, which was later determined due to its inexplicable genetic distance from any known sister strain.[8] This extra distance would be expected since serial passages artificially accelerates genetic divergence between taxa, resulting in the accumulation of genetic distance at a much faster rate than it occurs in a natural setting.

Then in 1979, just 2 years after the introduction of this modified H1N1 Swine Flu, a different Soviet lab leaked weaponized anthrax out through an improperly maintained exhaust filter, and Soviet authorities convincingly blamed the deaths on contaminated local meat. This cover up withstood a formal inquiry conducted in 1986, and was not revealed to be a fabrication until 1992, when an analysis of dispersion patterns revealed that the victims were not those working with the supposedly contaminated meat, but instead all lived downwind from the Sverdlovsk weapons lab and its improperly maintained exhaust vent. Therefore, there is a history of denying laboratory leaks on the commercial meat industry that dates back about 40 years, an effective excuse that provided the Soviets with an alibi that held up for nearly 2 decades.

The Soviet strain of serially passaged H1N1 Swine Flu was likely being developed as part of a vaccine program, one of the humane goals of gain-of-function research that exist alongside riskier and more troublesome ones like developing bioweapons. Its emergence ignited the debate

between the risks and rewards of dual-use gain-of-function research—causing it to became the poster virus for the dangers this protocol posed.[8]

This debate would largely fade in the decades that followed, until two separate teams used genetic manipulation followed by serial passage between ferrets to create mammal-transmissible H5N1 Bird Flu strains of influenza virus in 2011 that had the gain-of-function of being transmissible by aerosol. The first team was led by Dr. Ron Fouchier and conducted at the Erasmus Medical Center in the Netherlands, and demonstrated that as few as five mutations prior to serial passage were sufficient to create a modified strain of the H5N1 Bird Flu that could be transmitted by aerosol while remaining highly lethal.[9] The creation of this highly virulent strain that was said by a reporter to be able to "make the deadly 1918 pandemic look like a pesky cold,"[10] and was contentious enough to cause the scientists working on them to prepare for a media storm[11]—a storm that rolled in on the back of a second similar experiment.

Instead of only tweaking the H5N1 Bird Flu in a few places before serial passage, Dr. Yoshihiro Kawaoka of the Universities of Tokyo and Wisconsin used genetic engineering to combine genes from the H1N1 Swine Flu as well as the H5N1 Bird Flu to create a chimeric virus that was then serially passed through ferrets, creating another airborne virus with potentially pandemic properties.[12] Both experiments created a modified genome that appeared to be the result of natural, albeit accelerated, selection since the process of serial passage forces the mutations selected for in natural zoonotic jumps, and masks the direct genetic engineering done on the viruses. These experiments were viewed by many as being sufficiently dangerous that they should not be published,[13] however they were both eventually released with certain methodological and sequence details left out.

In the years that followed, gain-of-function serial passage through ferrets was used to increase the virulence of the H7N1 Bird Flu as well as allowing for its aerosol transmission without first introducing any mutations.[14] Additionally, the H1N1 Bird Flu was also found to become airborne and increase in virulence after in vivo passage through swine.[15 , 16] And although serial passage in the laboratory does not invariably increase viral pathogenicity, highly pathogenic influenza viruses all contain furin cleavage sites,[16] which only emerge after serial passage in laboratories or pseudo-naturally on commercial animal farms.

The process of sequential passage through animal hosts or cell cultures leaves a genome that appears natural and not purposefully manipulated since it effectively mimics the natural process of zoonosis, and leaves a genome that appears to be the result of natural selection so long as its relationship to related strains of virus is ignored. However, the artificial generations added by forced serial passage creates the artificial appearance of evolutionary distance, which was the characteristic of the H1N1 Swine Flu Soviet leak in the 1970s that lead researchers to conclude it had been constructed in a lab, and is exactly what is found with SARS-CoV-2, which is distant enough from any other virus that it has been placed in its own clade.[17]

2.1. Serial Passage and Its Molecular Signatures

Although serial passage mimics many of the natural zoonotic processes that occur during a natural zoonotic jump, because serial passage artificially condenses a natural phenomenon into a small temporal window, some subtle differences can be found. In addition to the inexplicable genetic distance from its sister strains, which screams out for an intermediate relative to

complete the phylogenetic picture, SARS-CoV-2 has a remarkably strong affinity for spike-protein binding to ACE2—some 10–20 times higher than SARS-CoV's.[18] That affinity may have emerged after mutational events either in an intermediate natural host or after a zoonotic jump into humans that theoretically could have occurred earlier than the first documented infection, which would give it time to increase that significantly. So logically, it could also have emerged via selection after serial passage through laboratory cell cultures or laboratory animals as well. And regarding the second distinctive feature found in the novel coronavirus: If other viruses have been observed to acquire furin cleavage sites by passage under experimental laboratory conditions, then such a mechanism is theoretically possible for SARS-CoV-2 as well.[2]

In the case of influenza viruses like those mentioned above, their gain-of-function furin cleavage sites are thought to be a result of two different molecular processes. The first is either nucleotide insertions or substitutions that are able to be rescued and then eventually selected for due to the high multiplicity of infection found in serial passage protocols.[19] And the second is the recombination of multiple viral RNAs inside a host cell,[20] which may also include additional viruses introduced through accidental laboratory co-infections.

Unlike influenza viruses, serial passage through ferrets has not been recorded in the literature for coronaviruses. However, since several branches of coronavirus have furin cleavage sites, a molecular pathway for their emergence must exist and may reemerge during serial passage. Several factors weigh into the probability that coronaviruses can gain furin cleavage sites following serial passage: The frequency of evolutionary motifs meant to deal with virus–host interactions that are often shared between viruses, the observations that when the infectious bronchitis coronavirus (IBV) coronavirus is serially passed through chickens it developed notable mutations along its spike-protein genes,[21] and the fact that when a lineage A bovine coronavirus was subject to in vitro serial passage through cell lines, a 12-nucleotide insert found within only a small minority of the pooled viruses spike-protein region was strongly selected for and quickly emerged as the dominate strain.[22] These findings all point to the possibility that SARS-CoV-2 may have gained its furin cleavage site the same way influenza viruses do—through the in vivo serial passage between the live hosts that presents the immune challenges and intense selective pressure necessary for the recombination and mutations that lead to its emergence to occur. And just like influenza viruses are only able to preserve their furin cleavages in artificial environments since the heightened virulence they impart kills their hosts before they can propagate in a natural setting, based on the known taxonomy lineage B coronaviruses do not appear to be able to support furin cleavages in nature.

There is no doubt that the acquisition of the furin cleavage site was one of the key adaptations that enable SARS-CoV-2 to efficiently spread in the human populations compared to other lineage B coronaviruses, and provides a gain-of-function.[23] In addition to the possibility of obtaining a furin cleavage site through natural recombination in a secondary host or through serial passage either in a laboratory or on a commercial farm, one could have been spliced directly into the novel coronavirus's backbone in a laboratory using classic recombinant DNA technology that has been available for nearly 20 years. This allows for the removal of the restriction site junctions that are the telltale sign of direct genetic manipulation and permits reassembly without introducing nucleotide changes—creating a virus without any evidence of manipulation using the aptly named "No See'm technology."[24] So although the entire

spike-protein RBD was not assembled from scratch, it is certainly plausible that the 12-nucleotide-long furin cleavage site could have been spliced directly into SARS-CoV-2. Furin cleavages already have been successfully spliced into other coronaviruses, including the IBV,[25] and even into SARS-CoV, where it increased cell-to-cell fusion in in vitro experiments that only examined only the spike-protein's function, which would presumably heighten its infectivity in vivo.[26]

Moreover, when a furin cleavage site was introduced to the IBV coronavirus spike-protein via recombination, just like influenza viruses hosting this feature, it appeared to impart it with increased lethality as well as inflict neurological symptoms that had never previously been reported in studies of the murine IBV coronavirus.[25] The presence of this cleavage site also increased damage to the respiratory and urinary systems, paralleling SARS-CoV-2 systemic multiorgan symptoms—especially reports that infection with the novel coronavirus not only targets the lungs where it binds to ACE2 receptors, but also the entire cardiovascular system,[27] the nervous system,[28] and our kidneys as well.[29] It might be more than a coincidence that the Vero cells often used in serial passage are derived from kidney epithelial cells extracted from African green monkeys, which have ACE2 receptors very similar to those found in humans and would be shared by the humanized mice that are also used for serial passage research.

2.2. Natural Origin, or Gain-of-Function Lab Escape?

Gain-of-function research on bat-borne coronaviruses has been ongoing for nearly a decade everywhere from the University of North Carolina to the Wuhan's Institute of Virology, which is supported by related facilities such as Wuhan's Center for Disease Control and Prevention as well as Wuhan University. A coronavirus that targets the ACE2 receptor like SARS-CoV-2 was first isolated from a wild bat in 2013 by a team out of Wuhan. This research was funded in part by EcoHealth Alliance,[30] and set the stage for the manipulation of bat-borne coronavirus genomes that target this receptor and can become airborne. Many more viruses have been collected in Wuhan over the years, and one research expedition captured as many as 400 wild viruses,[31] which were added to a private repository that has since grown to over 1500 strains of virus,[32] meaning that the Wuhan Center for Disease Control and Prevention has a massive catalogue of largely undisclosed viruses to draw from for experiments. And in subsequent years, EcoHealth Alliance received funding for project proposals outlining gain-of-function research to be done in Wuhan, hoping to use cell cultures and humanized mice as well as "[spike]-protein sequence data, infectious clone technology, in vitro and in vivo infection experiments and analysis of receptor binding"[33] to manipulate bat coronavirus genomes—all of which are consistent with the wet-work that would be needed to engineer this novel coronavirus in a laboratory. But for whatever reason, the Wuhan Institute of Virology has refused to release the lab notebooks of its researchers, which are ubiquitous in even the simplest laboratories and are expected to be meticulously detailed given the sensitive and delicate work that takes place in BSL-4 research labs intent on documenting their intellectual property, despite the fact that these notebooks would likely be enough to exonerate the lab from having any role in the creation of SARS-CoV-2.[34]

Although it does not prove a laboratory origin, another gain-of-function experiment demonstrates one possible step along the way to engineering SARS-CoV-2: the synthetic reconstruction of the

SARS coronavirus to impart this virus with a high affinity for ACE2. This involved isolating a progenitor coronavirus from civets and then serially passing it through mammalian ACE2 receptor-expressing cells—serial passage through host cell lines instead of entire hosts, which imparted a strong affinity for ACE2,[35] and another novel strain of coronavirus that was also presumably airborne. A few years after this study, more gain-of-function research was performed that involved the creation of a chimeric bat-borne coronavirus by directly manipulating the bat coronavirus spike-protein gene,[36] which created a coronavirus so virulent that it evoked the following dire warning from Simon Wain-Hobson, a virologist with the Pasteur Institute in Paris: "If the [new] virus escaped, nobody could predict the trajectory."[37]

Although SARS-CoV-2's efficient solution for ACE2 binding has been accurately described as something that could not be intentionally engineered nucleotide-by-nucleotide,[2] it could well be selected for after serial passage through ferrets or cell cultures in a lab. The only origin for the SARS-CoV-2 spike-protein RBD that the sequence data excludes is the deliberate manufacturing and introduction of the entire SARS-CoV spike-protein RBD sequence to create SARS-CoV-2. Otherwise, there are no genetic data to distinguish among natural and engineered possibilities at the present time.

2.3. Ferreting Out the Signs of Serial Passage

Curiously, studies examining SARS-CoV-2's infectivity in ferrets found that it spreads readily among them, and also appears airborne in that animal model.[38] This lends support to the idea that ferrets may have been used for serial passage since viruses typically take a significant many months if not years to acclimate enough to spread at all among any new species, nonetheless become airborne, which requires further mutations.

This relationship was further supported by reports out of the Netherlands that the novel coronavirus had spread among thirteen different mink farms there, and also to at least one farm in Denmark[39] and to another in Spain where 87% of the mink were infected.[40] Minks are a closely related subspecies of ferret that can produce fertile offspring together, and so the fact that not only did the virus spread to fifteen different farms in three countries, but also appears to have spread from minks into farm workers[41] indicates that accidental commercial serial passage through minks could have played a role in its creation, as an alternative to laboratory ferrets. Nevertheless, regardless of where any possible serial passage occurred, the fact that SARS-CoV-2 spreads from humans to minks and then back to humans demonstrates a high affinity for both species, despite neither nominally being a natural reservoir. Further support for the possibility that serial passage through lab ferrets or throughout mink farms played a role in the genesis of this novel coronavirus is provided by a preprint that notes the obvious ease with which it passes through the air between ferrets, since SARS-CoV-2 was transmitted through the air to three out of four indirect recipient ferrets monitored for airborne passage of the novel coronavirus.[42] It seems reasonable to think that SARS-Cov-2's apparent affinity for ferrets and minks should lead to an investigation of mink farms in the Hubei province were the novel coronavirus was discovered, since a viable pathway for its emergence could be infected bats defecating on commercial mink farms, which would loosely parallel the emergence of MERS-CoV from herds of camels following putative fecal contamination by local bats.[43]

The prospect that serial passage through lab animals or on commercial farms may have played a role in the creation of SARS-CoV-2 is also raised by an April 2020 preprint, which appears to

have been retracted after Chinese authorities implemented the censorship of any papers relating to the origins of the novel coronavirus.[44] This paper found that coronaviruses that target the ACE2 receptor bind with ferret cells more tightly than any other species except the tree shrew, which only scored about 2% higher. Tree shrews have also been used for serial viral passage, and have been promoted as a preferable animal host for laboratory experimentation since they are cheaper, smaller, easier to handle, and closer to humans evolutionarily and physiologically than ferrets.[45] However, one does not exclude the other as a possible host, and a recent preprint examining SARS-CoV's binding affinity in humans raises additional questions about its initial emergence. It found that the novel coronavirus appears to be far more adapted to human ACE2 receptors than those found in bats, which is unexpected given that bats are the virus's assumed source, and which lead the lead research to observe that SARS-CoV-2 was perfectly adapted to infect humans since its first contact with us, and had no apparent need to for any adaptive evolution at all.[46]

Although the novel coronavirus also appears to have a high affinity for the pangolin ACE2 receptor,[47] phylogenetic analysis of the neutral sites that best determine shared heritage[48]and a distinctive amino acid sequence both indicate that pangolins are unlikely to have served as an intermediate host,[47] so this affinity is likely due to the convergent motifs that often mark viral evolution and not shared heritage. The unexpected immediate affinity for humans was also reflected by another preprint, which observed that SARS-CoV-2 appeared just as adapted to humans at the very start of its epidemic as SARS-CoV was in the latest stages of its emergence,[49] an unexpected finding since viruses are expected to mutate substantially as they acclimate to a new species.[50] SARS-CoV-2's muddled origins are made even more Gordian by a study published March 2018 that examined people who live in villages about a kilometer away from bat caves. This study revealed that only 2.7% of those villagers had antibodies indicating any past exposure to bat coronaviruses. The authors also sampled people living in Wuhan, and found no evidence of exposure to SARS-CoV-like coronaviruses at all.[51]

This means there is very little serological evidence of any exposure to these coronaviruses even in Chinese villagers living in close proximity to bat caves, and at the epicenter of the current outbreak—no previous exposure was found at all. These data do not support the idea that SARS-CoV-2 was circulating in humans prior to the outbreak began in Wuhan in the early winter or fall of 2019, making a zoonotic jump even more unlikely since natural jumps leave wide serological footprints in their new host populations as early variants of a prospective virus make limited and unsuccessful jumps into individuals of the new host species, a trial-and-error that must occur before mutations that allow adaptation to a new host species are selected.[50] However these results do not rule out a much earlier jump into humans somewhere outside Hubei province, an alternative that is awaiting empirical support.

Taken together, the available evidence does not point definitively toward a natural origin for SARS-CoV-2, rather, much of it is more consistent with what would be found if the novel coronavirus had arisen from serial passage of a "precursor" progenitor virus in a lab, or from bats infecting a commercial mink farm somewhere in China, which would also provide the conditions for serial passage. However, more evidence is required before a conclusive judgement can be made one way or the other.

Further research around SARS-CoV-2's affinity to ferrets and minks, as well as other possible intermediate hosts seems warranted, and certainly the examination of all past gain-of-function

serial passage research by the scientific community at large should occur to determine what other definitive genomic signatures serial passage leaves besides the creation of furin cleavage sites, in case more of those can be found in this novel coronavirus. Two additional unique genomic signature are already being researched, as one preprint indicates that SARS-CoV-2 possesses a genomic region not found in other coronaviruses that appears to cloak the novel coronavirus from white blood cells, a characteristic also found with HIV.[52] And the second preprint identifies a region on the spike-protein gene found in no other bat-borne coronavirus that is nearly identical to superantigenic and neurotoxic motifs found in some bacteria, which may contribute to the immune overreaction that leads to the Kawasaki-like multisystem inflammatory syndrome in children, and cytokine storms in adults.[53] Given the unique traits found in SARS-CoV-2 and all the open questions there still are around its emergence, until either a natural or laboratory origin is conclusively demonstrated both avenues should be robustly investigated by the scientific community.

Go to:

3. Conclusions and Outlook

The history of gain-of-function research is one of science's most significant and troubling, especially since the Nuremberg Code, research scientists' Hippocratic Oath, dictates that experiments that could endanger human life should only occur if the potential humanitarian benefits significantly outweigh the risks.[54] It seems ill-advised to rule out the possibility that gain-of-function techniques such as serial passage may have played a role in the creation of SARS-CoV-2 until more definitive data are collected, and when the Center for Arms Control and Non-Proliferation has calculated that the odds that any given potential pandemic pathogen might leak from a lab could be better than one in four.[55]

The release of the H1N1 Swine Flu in 1977 first initiated the discussion about the moral and physical hazards involved with dual-use gain-of-function research, and it was the creation of extraordinarily virulent H5N1 Bird Flu strains—using the same technique of serial passage through an animal host in a lab—that contributed to the NIH imposing a moratorium on dual-use gain-of-function research from 2014 until 2017, after which it was relaxed explicitly to allow influenza strains as well as coronaviruses to be studied. This moratorium was meant to limit "the potential to create, transfer, or use an enhanced potential pandemic pathogen."[56] However, just as an increased pace of research into influenza vaccines increased the odds that a leak would occur leading up to the 1977 release of H1N1 Swine Flu, which is the most often cited as originating from a laboratory leak,[8] it would follow that an increased pace of research into coronaviruses over the past few years would have increased the odds that a lab leak of one would occur; after all, these viruses were pinpointed back in 2006 as a viable vector for an HIV vaccine[57] and research into a pan-coronavirus vaccine has been ongoing for decades.

And whether or not gain-of-function research is determined to have played a role in SARS-CoV-2's emergence, the fact that it creates opportunities for pandemic viruses to leak out of labs calls for a re-examination of the moratorium against this practice, because the emergence of this novel coronavirus has demonstrated that the international public health community is not prepared to handle the leak of a pandemic virus. Furthermore, none of the gain-of-function research conducted since 2014 has provided humanity with any tools at all to fight back against the ongoing pandemic caused by this novel coronavirus.

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Conflict of Interest

The authors declare no conflict of interest.

Go to:

Acknowledgements

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Notes

Sirotkin K., Sirotkin D., Might SARS-CoV-2 Have Arisen via Serial Passage through an Animal Host or Cell Culture?. BioEssays 2020, 2000091 10.1002/bies.202000091 [PMC free article][PubMed] [CrossRef]

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From: Gross, Laura J (b)(6) @state.gov>
Sent: Friday, November 20, 2020 3:43 PM
To: Paulopol, Andreea I (b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>
Cc: Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) @state.gov>; Asher, David (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: Re: For Review: Draft Article 5 re China BWC compliance

Hi all - I know that I am a bit behind on the background of this effort, having only been briefed this week. However, wouldn't it make sense first to have a draft document that explains concerns prior to taking this step? My understanding is that David and Michael are still pulling together their draft slide deck. During my conversation with them, we also agreed they would develop some questions for the IC. I recommend we first have a written "theory of the case" prior to taking this step, which I would characterize as moving forward to try it. Best - Laura

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From: Paulopol, Andreea I (b)(6) @state.gov>
Sent: Friday, November 20, 2020 2:43 PM
To: DiNanno, Thomas G (b)(6) @state.gov>
Cc: Gross, Laura J (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) (b)(6) @state.gov>; Asher, David (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: Re: For Review: Draft Article 5 re China BWC compliance

AA/S DiNanno—

Resending per our separate email just now. And again, in order for us to report something under Article 5, we need to either issue a diplomatic inquiry and citing Article 5 to the them and follow by with the same info by a NV to the BWC ISU or just the ISU if you want to single no direct dialogue. But an action needs to be taken under 5 in order to report some action for the Compliacne Report.

Happy to discuss further.

Thanks,
Andreea

SENSITIVE BUT UNCLASSIFIED

From: Paulopol, Andreea I
Sent: Friday, October 30, 2020 5:02 PM
To: DiNanno, Thomas G (b)(6) @state.gov>
Cc: Gross, Laura J (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) (b)(6) @state.gov>; Asher, David (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: For Review: Draft Article 5 re China BWC compliance

AA/S DiNanno—

Per discussions, I'm attaching a draft approach re China BWC compliance under Article 5, along with a US working paper that I spearheaded last year for the 2019 MX5 meeting. I've also included the Chinese CBMs for 2019 and partial translation of their 2020 CBM, which I reported on back in May in the DAR below. Please note that their CBMs are password protected on the restricted BWC Implementation Support Unit side. Also added here are Chinese statements from 2019 BWC meeting and most recent one from the UNFC.

Again, while Article 5 has been invoked only once by Cuba, please note that Article 6 has never been tested before. Whichever way we go, we will need to request downgrades for information.

Welcome your review and happy to answer any questions.

Thanks,
Andreea

SENSITIVE BUT UNCLASSIFIED

From: Paulopol, Andreea I
Sent: Friday, May 29, 2020 1:48 PM
To: AVC-CBW-DL <AVC-CBW-DL2@state.gov>
Cc: (b)(6) @state.gov>
Subject: DARs re Chinese CBMs and C-19 RFI

(U//FOUO) **Update on Chinese BWC CBMs re COVID-19**: CBW (Paulopol) reviewed the partial translation of restricted access of Chinese BWC CBMs which acknowledge "a new coronavirus pneumonia outbreak appeared in Wuhan, Hubei Province in December 2019" and notes that "the outbreak has spread to 31 provinces." It further highlights that "beginning on March 6, 2020, the number of new cases in mainland China dropped to below 100, beginning on March 12, the number of new cases dropped to single digits, and beginning March 13, the number of imported cases was greater

than the number of new cases in China." There could be questions about how reliably they are diagnosing cases, however, the statement is probably correct, though the choice to highlight this in their CBMs, seems political in nature. The CBMs also note that the Wuhan Institute of Virology was the one that first processed the unknown virus in a moderate risk (BSL-2) laboratory before moving to high-risk (BSL-4) laboratory and indicates that the "source of the virus is pending confirmation by scientific research."

(~~SBU~~) **COVID-19 Request for Information**: DOD (IC) released its COVID-19 request for information from industry and academia on seven mission areas that also includes combating the spread among the items listed. It's been reported that over 3300 submissions have been received up to now. Providing the link for more details and awareness: https://www.afwerx.af.mil/coronavirus.html

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~~SENSITIVE BUT UNCLASSIFIED~~

~~SENSITIVE BUT UNCLASSIFIED~~

(b)(6)

From: "Paulopol, Andreea I" (b)(6) @state.gov>
To: (b)(6)
Subject: Fw: Evaluation of the Yan report—(b)(5)
(b)(5)
Date: Mon, 26 Oct 2020 01:43:41 +0000

Sent from my BlackBerry 10 smartphone.

From: Asher, David (b)(6) @state.gov>
Sent: Sunday, October 25, 2020 12:32 PM
To: Paulopol, Andreea I
Cc: (b)(6) (b)(6) ; DiNanno, Thomas G; (b)(6)
Subject: Re: Evaluation of the Yan report—(b)(5)
(b)(5)

From: Paulopol, Andreea I (b)(6) @state.gov>
Sent: Tuesday, October 20, 2020 3:40 PM
To: Asher, David <AsherD@state.gov>
Cc: (b)(6) @state.gov> (b)(6) @state.gov>
Subject: RE: Evaluation of the Yan report

(b)(5)

~~SENSITIVE BUT UNCLASSIFIED~~

From: Asher, David (b)(6) @state.gov>
Sent: Tuesday, October 20, 2020 11:12 AM
To: Paulopol, Andreea I (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: Re: Evaluation of the Yan report

You definitely should read it. Quite an indictment of incompetence—willful blindness on a huge screw up.

Rumor I heard from credible sources is US researchers who had worked in WIV might be the authors of the anonymous report but this is just a rumor. Have you been in contact with the FBI? I am trying to find a POC there. Suspect they may know....

We spoke last night with two leading bio-informatic researchers involved in frontlines of the state of California COVID effort who did not see signs of genetic tinkering or even culturing — and they used single cell PCR and cultured the virus to identify a pattern. When cultured there apparently is a lot of mutation. Same with "original" SARs. However, they did rule out lab based

adverse selection research on the most potent sample. Or in VIVO research (scary). These sources don't have clearances but are highly regarded in their field.

As an investigator, the theory of anonymous remains at the forefront in my mind because it comports with most the published facts but it remains a theory of the case. Please let us know your thoughts—including on the sensitive report Mike gave you. Communicate views on other systems.

From: Paulopol, Andreea I (b)(6) @state.gov>
Sent: Monday, October 19, 2020 4:23 PM
To: Asher, David (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: RE: Evaluation of the Yan report

I have not, but see that Michael may have found something.

Why anonymous and do you know where they published this report?

From: Asher, David (b)(6) @state.gov>
Sent: Friday, October 16, 2020 6:23 PM
To: Paulopol, Andreea I (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>
Subject: Re: Evaluation of the Yan report

Have you seen the attached anonymous report? Was there a review of its findings? Can we see that? Thanks!

From: Asher, David (b)(6) @state.gov>
Sent: Friday, October 16, 2020 5:57 PM
To: Paulopol, Andreea I (b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>; (b)(6) @state.gov>; Yu, Miles (b)(6) @state.gov>
Subject: Re: Evaluation of the Yan report

Andrea,

Thanks for your personal and professional analysis. Much to discuss when we next meet in the SCIF.

Here is the draft one page research proposal I received from Professor Muller earlier today. Interested in everyone's thoughts.

(b)(5)

(b)(5) Prof Muller is great at running these projects and getting other famous scientists with specific domain expertise — who otherwise wouldn't take the time —- to contribute. I think we should have him perform independent research with a small team of virologists, biowarfare scientists, and epidemiologists. (b)(6)

(b)(6) if AAS DiNanno wishes for him to undertake the effort.

Have a nice weekend and stay safely away from COVID!

David

———

Proposed Program to examine the SARS Cov-2 Origin

(these are not necessarily in time sequence; some could be done in parallel)

Part 1: Academic review.

Goal: determine merit in analysis by Yan et al.

Requirements: 2 or (preferably) 3 experts in genomics/virology, working independently

Challenges: finding truly objective experts.

any guilt on China is likely to result in black-listing by the Chines (much viral work is done in collaboration with China)

many academics fear a hardening of relations with China

commercial scientists also fear hardening relations

Technical issues:

1. China claims natural mutation of related RaTG13virus.

Is it plausibly natural? Compelling?

Was the analysis properly published?

Can we rule out fraud?

Is it possible that the sequence was manufactured as a precursor to SARS CoV-2?

2. Synthesis of SAR-CoV-2 from ZC45 or ZXC21. Is method described in Yan paper correct?

Part 2. Identification of the geographic source

Study Quai paper. Can we out bat origin in wet market?

Bring together all intelligence information about Wuhan lab.
Study China response to outbreak.

Part 3. Laboratory work. Use Yan method (or other) to synthesize SARS CoV-2.
Can it be done quickly, as Yan says?

Part 4. Independent analysis of complete Cov-2 sequence.
Determine if there is any indications that it is bioengineered?
Is the spike protein similarity or identical to the SARS 2003?
If similar, is it "too" similar? Could it have been created independently (or just lab copies)?

Part 5. Examine all evidence that China had produced a. vaccine by mid 2020.
(Personally, I consider this to be the *smoking gun*.)

Part 6. International study. Reviews all unclassified results from Parts 1-5.
Perhaps have classified review with UK, others?
This is tricky, because it potentially brings in political considerations in the choice of participations. That must be avoided.

From: Paulopol, Andreea I (b)(6) @state.gov>
Sent: Friday, October 16, 2020 5:31 PM
To: DiNanno, Thomas G (b)(6) @state.gov>
Cc: (b)(6) @state.gov>; (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>; Asher, David (b)(6) @state.gov>; (b)(6) @state.gov>; Yu, Miles (b)(6) @state.gov>
Subject: Evaluation of the Yan report

Dear AA/S DiNanno—

Giving our meeting yesterday with Prof Muller, and separately with David Asher, I thought to circle back to provide some comments and takes on the lines of data and issues raised in these recent meetings.

With respect to genomic sequences, somewhat similar coronavirus (CoV) sequences have been previously identified. With the amount of surveillance done for coronaviruses in bats, it makes sense that similar sequences have been identified or collected and many SARS-like bat CoVs sequences have been described before.

There are some notable features that make the virus more contagious:

1. SARS-CoV-2 spikes bind to human ACE2: A number of the Spike proteins and receptor binding domains from these SARS-like bat CoVs have been shown to use ACE2 (an entry receptor) and even though they were identified in bats, the sequence was sufficient for infection of human cells in culture. (https://www.nature.com/articles/nm.3985; note this paper also describes some of the original reverse genetics for swapping out Spike and RBDs). Furthermore, there are now 7 known human coronaviruses, 6 of which have suspected bat origins with an intermediate host, either more direct like SARS-CoV in civets or further apart like MERS-CoV circulating in camels for ~20-30 years before the first identified human case, so being able to have human infectivity from an animal reservoir is not odd.

2. Presence of a furin cleavage site: Furin cleavage sites are not present in SARS-CoV-1, but they can be found in other CoVs including ones that infect humans (HCoV-OC43, HCoV-HKU1, and MERS-CoV). Other studies have identified furin cleavage sites (although a different combination of amino acid residues) in SARS-like CoVs. (https://pubmed.ncbi.nlm.nih.gov/32416074/). Finally, a recent pre-print from quite a few well-known and well-respected laboratories have evaluated the possible consequences of the furin cleavage site. They found that in Vero E6 cells (the standard cell type for growing coronaviruses and used for synthetic biology approaches to generate virus from infectious clones), the virus without a furin cleavage site replicates better and that when SARS-CoV-2 isolates from humans are grown in this cell line they quickly lose the furin cleavage site. The furin cleavage site does appear to confer an advantage for virus replication in respiratory cell lines (Calu-3 cells) and is required for more severe disease in a hamster model of SARS-CoV-2 infection while not significantly altering virus replication dynamics (https://www.biorxiv.org/content/10.1101/2020.08.26.268854v1).

Other notes to keep in mind, coronaviruses love to undergo homologous recombination to diversify their genomes, this means there can be large chunks that are swapped between different CoVs to create a new strain or virus, thus seeing large portions of the genome that are similar to previously identified CoVs and other portions that are different or are more similar to another CoV is not particularly extraordinary. Reference of note: this paper covers some additional data on the evolutionary origins of the SARS-CoV-2 lineage responsible for the COVID-19 pandemic if of interest to anyone (https://www.nature.com/articles/s41564-020-0771-4).

From a compliance perspective, you will recall that under former A/S Poblete, we brought China BW back into the unclassified report because of our compliance concerns. Apparently we are not alone about those concerns. You will see that the Yan report has been met with a lot of concern from other policy security experts and scientists. Unfortunately, the Yan report presents inappropriate, misleading, and inaccurate scientific statements—but Gigi and Nancy went through it carefully. Their analysis is available at:

Publication page: https://www.centerforhealthsecurity.org/our-work/publications/in-response-yan-et-al-preprint-examinations-of-the-origin-of-sars-cov-2
PDF: https://www.centerforhealthsecurity.org/our-work/pubs_archive/pubs-pdfs/2020/200921-in-response-yan.pdf

I thought this background might be useful as you consider other evaluations of the Yan report —which may or may not shed more light.

Hope this helps and happy to answer questions.

Andreea

Physical Scientist
Office of Chemical and Biological Weapons Affairs
Bureau of Arms Cantral, Compliance and Verification
U.S. Department of State
2201 C Street, N.W.
Washingtan, DC 20S20
Desk: (b)(6)
(b)(6) *@state.gov*

~~SENSITIVE BUT UNCLASSIFIED~~

Sender: "Paulopol, Andreea I" (b)(6)
Recipient: (b)(6)

From: (b)(6) @state.gov>
To: EAP-FO-Office-DL <EAP-FO-Office-DL@state.gov>
CC: EAP-P-Office-DL <EAP-P-Office-DL@state.gov>; EAP-CM-Office-DL <EAP-CM-Office-DL@state.gov>
Subject: FW: Ensuring a Transparent, Thorough Investigation of CO VID-19's Origin
Date: Sat, 16 Jan 2021 01:34:34 +0000

From: U.S. Department of State <usstatebpa@public.govdelivery.com>
Sent: Friday, January 15, 2021 7:38 PM
To: EAP-Press <EAP-Press@state.gov>
Subject: Ensuring a Transparent, Thorough Investigation of COVID-19's Origin

You are subscribed to Secretary's Remarks for U.S. Department of State. This information has recently been updated, and is now available.

Ensuring a Transparent, Thorough Investigation of COVID-19's Origin

01/15/2021 07:23 PM EST

Michael R. Pompeo, Secretary of State

The United States has repeatedly called for a transparent and thorough investigation into the origin of COVID-19. Understanding the origin of this pandemic is essential for global public health, economic recovery, and international security.

To assist the vital work of the World Health Organization (WHO) investigative team that arrived in China this week, the United States government is today sharing new information concerning the activities inside China's government laboratories in 2019.

In particular, we urge the WHO to press the government of China to address the following:

1. **Illnesses at the Wuhan Institute of Virology (WIV):** The United States 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.

2. **WIV Research on "RaTG13" and "gain of function":** Starting in at least 2016, WIV researchers studied RaTG13, the bat coronavirus identified by the WIV in January 2020 as its closest sample to SARS-CoV-2 (96.2% similar). Since the outbreak, the WIV has not been transparent nor consistent about its work with RaTG13 or other similar viruses, including possible "gain of function" experiments to enhance transmissibility or lethality.

3. **Secret WIV Links to Military Research:** Despite the WIV presenting itself as a civilian institution, the WIV has collaborated on publications and secret projects with China's military. The WIV has engaged in classified research, including laboratory animal experiments, on behalf of the Chinese military since at least 2017.

The COVID-19 pandemic was avoidable. Any responsible country would have invited world health investigators to Wuhan within days of an outbreak. China instead refused offers of help – including from the United States – and punished brave Chinese doctors, scientists, and journalists who tried to alert the world to the dangers of the virus. Beijing continues today to withhold vital information that scientists need to protect the world from this deadly virus, and the next one.

The United States reiterates the importance of unfettered access to virus samples, lab records and personnel, eyewitnesses, and whistleblowers to ensure the credibility of the WHO's final report. Until the CCP allows a full and thorough accounting of what happened in Wuhan, it is only a matter of time until China births another pandemic and inflicts it on the Chinese people, and the world.

Fact Sheet: Activity at the Wuhan Institute of Virology

(b)(6)

From: "Feith, David" (b)(6) @state.gov>
To: (b)(6)
Subject: FW: Articles on COVID origins
Date: Thu, 17 Dec 2020 01:24:01 +0000

~~SENSITIVE BUT UNCLASSIFIED~~

From: Asher, David (b)(6) @state.gov>
Sent: Wednesday, December 16, 2020 8:19 PM
To: Feith, David (b)(6) @state.gov>; (b)(6); (b)(7)(B) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>
Subject: Re: Articles on COVID origins

Attached NAS report is key. See the media coverage and congressional hearings as well. The dangers of GOF with virology were well discussed and observed. People at State were consulted and should be held accountable. How any R&D into GOF for Corona was permitted with the WIV, which was well known to do "other government work" is ridiculous but so was the DTRA and DARPA support to PRC via Eco Health Alliance as well as NIAID/NIH to WIV directly.....State MED also seems to have been involved based on the FOIA'd emails.

https://sites.nationalacademies.org/PGA/PGA_160392

Media Coverage: Gain of Function Research

Media Coverage Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop (April 2015) Read Online Free Buy the book or Download the Free PDF June 3, 2016 Gain-of-Function Oversight

sites.nationalacademies.org

From: Feith, David (b)(6) @state.gov>
Sent: Wednesday, December 16, 2020 4:26 PM
To: Asher, David (b)(6) @state.gov>; (b)(6) @state.gov>; DiNanno, Thomas G (b)(6) @state.gov>; (b)(6) @state.gov>; (b)(6) @state.gov>; Gibbs, Jeffrey J (b)(6) @state.gov>
Subject: Articles on COVID origins

Team – what are your quick favorite open-source references on COVID origins? Looking for a collection of 5-6 to have on hand to share with others.

Initial ideas:

1. Boston Magazine: "Could COVID-19 Have Escaped from a Lab? The world's preeminent scientists say a theory from the Broad Institute's Alina Chan is too wild to be believed. But when the theory is about the possibility of COVID being man-made, is this science or censorship?" (https://www.bostonmagazine.com/news/2020/09/09/alina-chan-broad-institute-coronavirus/)
2. BioEssays Wiley (attached): The genetic structure of SARS-CoV-2 does not rule out a laboratory origin: SARS-COV-2 chimeric structure and furin cleavage site might be the result of genetic manipulation.
3. NYT: "As it praised Beijing, the World Health Organization concealed concessions to China and may have sacrificed the best chance to unravel the virus's origins. Now it's a favorite Trump attack line." (https://www.nytimes.com/2020/11/02/world/who-china-coronavirus.html)

But otherwise I'm drawing blanks. There must be other good reporting out there on basics of WIV suspicions, gain of function risks, etc…

Thanks.

--
David Feith
Deputy Assistant Secretary
Bureau of East Asian and Pacific Affairs (EAP)
U.S. Department of State
(b)(6)
(b)(6) @state.gov

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~~SENSITIVE BUT UNCLASSIFIED~~

~~SENSITIVE BUT UNCLASSIFIED~~

Sender: "Feith, David" (b)(6) @state.gov>
Recipient: (b)(6)

From: "Asher, David" <(b)(6)@state.gov>
To: DiNanno, Thomas G (b)(6)@state.gov>
CC: (b)(6)@state.gov>; (b)(6)@state.gov>; Stilwell, David R (b)(6)@state.gov>; Feith, David (b)(6)@state.gov>; (b)(6)@state.gov>; (b)(6)@state.gov>; (b)(6)@state.gov>; (b)(6)@state.gov>; Gross, Laura J (b)(6)@state.gov>; Yu, Miles (b)(6)@state.gov>; (b)(6)@state.gov>; (b)(6)@state.gov>; Keshap, Atul <(b)(6)@state.gov>
Subject: FRaTG13 (shared in confidence)—
Date: Sun, 29 Nov 2020 22:58:17 +0000

Tom,
The attached note from Dr. Quay seems important. I asked Dr. Quay to respond specifically to Anderson et al who were among the early proponents that COVID 19, undoubtedly, was of natural zoonotic origin. This assertion in various forms gets repeated like is serious scientific fact based truth—when it may be the opposite based on some of the very evidence they put forward.
Bizarrely Anderson et al also were among the main proponents of the view that Gain of Function for virological spread prediction was a waste of money (see below). This said, Anderson et al never contemplate that someone could genetically engineer a bio threat vector with the exact characteristics they observe as "natural." Since many of us have dealt with unconventional warfare and weapons designed to scare, maim, destroy economic resilience, etc the type of analysis presented by Quay resonates from that perspective. Like IEDS and mines, the most effective weapons in UW are hiding and plain site. Same rules apply to BW, in theory. This genetic sequence analysis doesn't confirm BW research as a possible origin but it does further highlight that the COVID 19 vector could have been bio-engineered for unknown reasons and somehow got out out into the wild. So Quay's independent analysis does seem to conform with Segreto and
Deigin. https://onlinelibrary.wiley.com/doi/epdf/10.1002/bies.202000240

Prospects&Overviews ThegeneticstructureofSARS-CoV-2doesnotruleouta laboratoryorigin

2of9 SEGRETOANDDEIGIN adaptation to human cells. We here describe how the two main SARS-CoV-2features,(1)thepresenceofafurincleavagesitemissinginother ...

onlinelibrary.wiley.com

(b)(5)

Thanks.
David
From: Steven Quay <(b)(6)
Sent: Sunday, November 29, 2020 8:24 AM
To: Asher, David <(b)(6) @state.gov>
Subject: Re: Fw: RaTG13 (shared in confidence)

David
Here is my response to the Andersen argument that CoV-2 was not ideal for the receptor binding and so should have come from nature. The facts show the exact opposite.
Regards, Steve

On Sun, 29 Nov 2020 at 03:45, Asher, David <(b)(6) @state.gov> wrote:
Steve, Here are the same authors laying out why COV-19 had to be natural. Have you considered a response letter? David
https://www.nature.com/articles/s41591-020-0820-9.pdf
From: Asher, David <(b)(6) @state.gov>
Sent: Saturday, November 28, 2020 2:32 PM
To: Steven Quay <(b)(6)
Subject: Re: Fw: RaTG13 (shared in confidence)

Below: Noture commentary pointing out the futility, waste, and opportunity costs associated projects pursued by Ecohealth, WIV, NIAID, et ol, in the nome of "predicting the next outbreak". Though they don't address the grave hazards, and BW dual use issues, involved with the goin of function work in WIV's prediction reseorch, they loid out other important fundomental flaws with Ecohealth and WIV's approach. The authors go on to make the more compelling cose for better bio surveillance instead.https://www.nature.com/orticles/d41586-018-05373-w

COMMENT

07 JUNE 2018

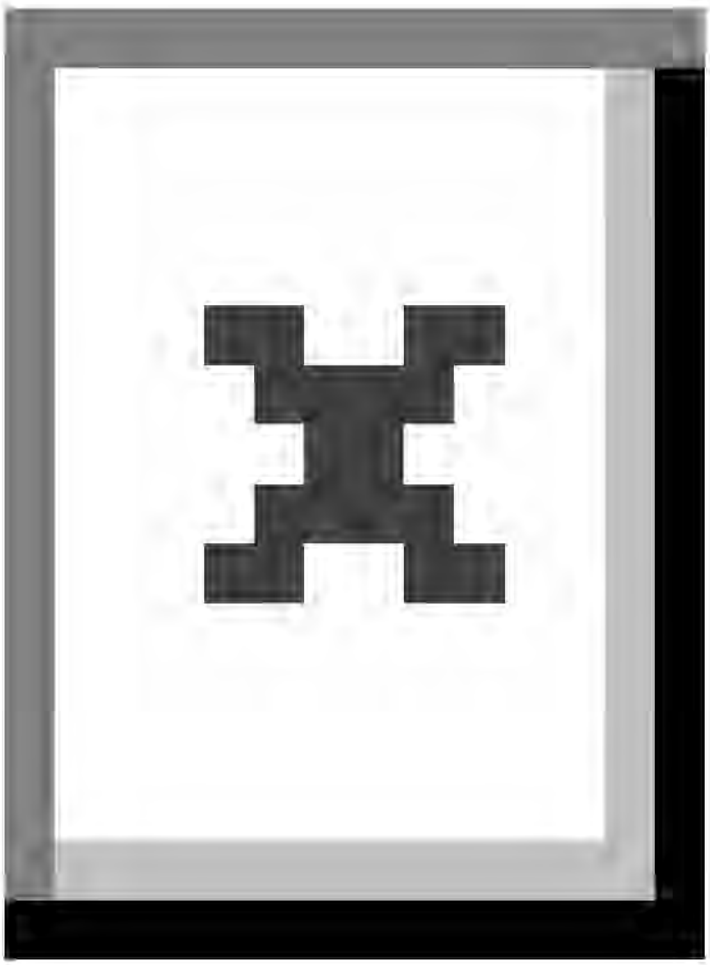
Pandemics: spend on surveillance, not prediction

Trust is undermined when scientists make overblown promises about disease prevention, warn Edward C. Holmes, Andrew Rambaut and Kristian G. Andersen.

The resurgence of Ebola virus in the Democratic Republic of the Congo this May is a stark reminder that no amount of DNA sequencing can tell us when or where the next virus outbreak will appear. More genome sequence data were obtained for the 2013–16 Ebola epidemic than for any other single disease outbreak. Still, health workers in Mbandaka, the country's northwestern provincial capital, arescrambling to contain a growing number of cases.

Over the past 15 years or so, outbreaks caused by viruses such as Ebola, SARS and Zika have cost governments billions of US dollars. Combined with a perception among scientists, health workers and citizens that responses to outbreaks have been inadequate,this has fuelled what seems like a compelling idea. Namely, that if researchers can identify the next pandemic virus before the first case appears, communities could drastically improve strategies for control, and even stop a virus from taking hold[1,2]. Indeed, since 2009, the US Agency for International Development has spent US$170 million on evaluating the "feasibility of preemptively mitigating pandemic threats"[1].

Various experts have flagged up problems with this approach (including the three of us)[3,4]. Nonetheless, an ambitious biodiversity-based approach to outbreak prediction — the [redacted] — was announced in February this year, with its proponents soliciting $1.2 billion in funding from around the world(see 'High stakes'). They estimate that other mammals and birds contain 1.67 million unknown viruses from the families of viruses that are most likely to jump to humans, and will use the funding to conduct a genomic survey of these unknown viruses, with the aim of predicting which might infect people[1].



Sources: NIH; Global Virome Project

Broad genomic surveys of animal viruses will almost certainly advance our understanding of virus diversity and evolution. **In our view, they will be of little practical value when it comes to understanding and mitigating the emergence of disease.**

We urge those working on infectious disease to focus funds and efforts on a much simpler and more cost-effective way to mitigate outbreaks — proactive, real-time surveillance of human populations.

The public has increasingly questioned the scientific credibility of researchers working on outbreaks. In the 2013–16 Ebola epidemic, for instance, the international response was repeatedlycriticized for being too slow. And during the 2009 H1N1 influenza epidemic, people asked whether the severity of the virus had been overblown, and if the stockpiling of pharmaceuticals was even necessary[5]. Making promises about disease prevention and control that cannot be kept will only further undermine trust.

Forecasting fallacy

Supporters of outbreak prediction maintain that if biologists genetically characterize all of the viruses circulating in animal populations (especially in groups such as bats and rodents that have previously acted as reservoirs for emerging viruses), they can determine which ones are likely to emerge next, and ultimately prevent them from doing so. With enough data, coupled with artificial intelligence and machine learning, they argue, the process could be similar to predicting the weather[6].

Reams of data are available to train models to predict the weather. By contrast, it is exceedingly rare for viruses to emerge and cause outbreaks. Around 250 human viruses have been described, and only a small subset of these have caused major epidemics this century.

Advocates of prediction also argue that it will be possible to anticipate how likely a virus is to emerge in people on the basis of its sequence, and by using knowledge of how it interacts with cells (obtained, for instance, by studying the virus in human cell cultures).

This is misguided. Determining which of more than 1.6 million animal viruses are capable of replicating in humans and transmitting between them would require many decades' worth of laboratory work in cell cultures and animals. Even if researchers

managed to link each virus genome sequence to substantial experimental data, all sorts of other factors determine whether a virus jumps species and emerges in a human population, such as the distribution and density of animal hosts. Influenza viruses have circulated in horses since the 1950s and in dogs since the early 2000s, for instance[7]. These viruses have not emerged in human populations, and perhaps never will — for unknown reasons.

In short, there aren't enough data on virus outbreaks for researchers to be able to accurately predict the next outbreak strain. Nor is there a good enough understanding of what drives viruses to jump hosts, making it difficult to construct predictive models.

Biodiversity-based prediction also ignores the fact that viruses are not fixed entities. New variants of RNA viruses appear every day. This speedy evolution means that surveys would need to be done continuously to be informative. The cost would dwarf the proposed $1.2-billion budget for one-time sequencing.

Even if it were possible to identify which viruses are likely to emerge in humans, thousands of candidates could end up being identified, each with a low probability of causing an outbreak. What should be done in that case? Costs would skyrocket if vaccines and therapeutics were proposed for even a handful of these.

Screen and sequence

Currently, the most effective and realistic way to fight outbreaks is to monitor human populations in the countries and locations that are most vulnerable to infectious disease. This can be done by local clinicians, health workers in non-governmental organizations such as Médecins Sans Frontières (MSF; also known as Doctors Without Borders), and global institutions such as the World Health Organization (WHO).

We advocate the detailed screening of people who are exhibiting symptoms that cannot easily be diagnosed. Such tests should use the latest sequencing technologies to characterize all the pathogens that have infected an individual — the human 'infectome'[8]. To track previous infections, investigators should also assess each person's immune response, by analysing components of their blood using broad-scale serology[9].

Emerging diseases are commonly associated with population expansions — when people encroach on habitats occupied by animals — as well as with environmental disturbances and climate change. Deforestation, for instance, can promote human interactions with animals that carry new threats, and can increase encounters with new vector species such as ticks and mosquitoes[10]. Animal die-offs, for example that of bar-headed geese (*Anser*

indicus) at Lake Qinghai in China in 2005 (which was caused by the H5N1 influenza virus), can also flag problem regions or emerging pathogens. Surveillance efforts should therefore focus on communities that live and work in such environments.

Identifying which pathogen is causing an outbreak is no longer the bottleneck it once was. It took researchers two years to determine HIV as the cause of AIDS in the early 1980s using microscopy and other techniques. By contrast, in 2012 it took only weeks for investigators using genomic technologies to discover the coronavirus that caused Middle East respiratory syndrome (MERS).

Rapid identification of viruses can be achieved only if such technologies — and the people trained to use them — are globally available, including in resource-limited regions where the risk of outbreaks might be higher.Thankfully, relevant capacity-building programmes are now beginning to be established, such as the Human Heredity and Health in Africa (H3Africa) Initiative, run by the UK Wellcome Trust and the US National Institutes of Health[11].

Once an emerging outbreak virus has been identified, it needs to be analysed quickly to establish what type it is; which molecular mechanisms (such as receptor type) enable it to jump between individuals; how it spreads through human populations; and how it affects those infected. In other words, at least four kinds of analysis are needed: genomic, virological, epidemiological and clinical. And the data must be passed to key stakeholders, from researchers and health workers on the ground to international agencies such as the WHO and the MSF. Data must be kept as free of restrictions as possible, within the constraints of protections of patient privacy and other ethical issues.

This will best be achieved through an established global network of highly trained local researchers, such as the WHO Global Outbreak Alert and Response Network (GOARN). Real-time tools for reconstructing and tracking outbreaks at the genomic level, such as portable sequencing devices, are improving fast[8]. Information gathered during recent outbreaks has quickly had tangible impacts on public-health decisions, largely owing to data generation and analysis by many research teams within days of people being infected[12].

For instance, in the 2013–16 Ebola epidemic, genome sequencing of the virus proved that a person could sexually transmit the disease more than a year after becoming infected. This prompted the WHO to increase its recommended number of tests for persistent infection in survivors of the disease.

Ultimately, the challenge is to link genomic, clinical and epidemiological data within days of an outbreak being detected, including information about how people in an affected community are interacting. Such an open, collaborative approach to tackling the emergence of infectious disease is now possible. This is partly thanks to technology, but is mainly due to a shift in perception about the importance of this approach. At least in genomic epidemiology, there is a growing move towards real-time, open-access data and analysis, aided by the use of preprint servers and wikis such as Virological (http://virological.org). This type of collaborative effort can complement the work of agencies including the WHO and the MSF, which focus predominantly on providing information, isolating those who have been infected, and so on.

So far, researchers have sampled little of the viral universe. Surveys of animals will undoubtedly result in the discovery of many thousands of new viruses. These data will benefit studies of diversity and evolution, and could tell us whether and why some pathogens might jump species boundaries more frequently than others. But, given the rarity of outbreaks and the complexity of host–pathogen interactions, it is arrogant to imagine that we could use such surveys to predict and mitigate the emergence of disease.

New viruses will continue to emerge unexpectedly. There is a lot we can and must do to be better prepared.

*Nature***558**, 180-182 (2018)

doi:https://doi.org/10.1038/d41586-018-05373-w

From: Steven Quay (b)(6)
Sent: Thursday, November 26, 2020 4:03 AM
To: Asher, David <(b)(6) @state.gov>
Subject: Re: Fw: RaTG13 (shared in confidence)

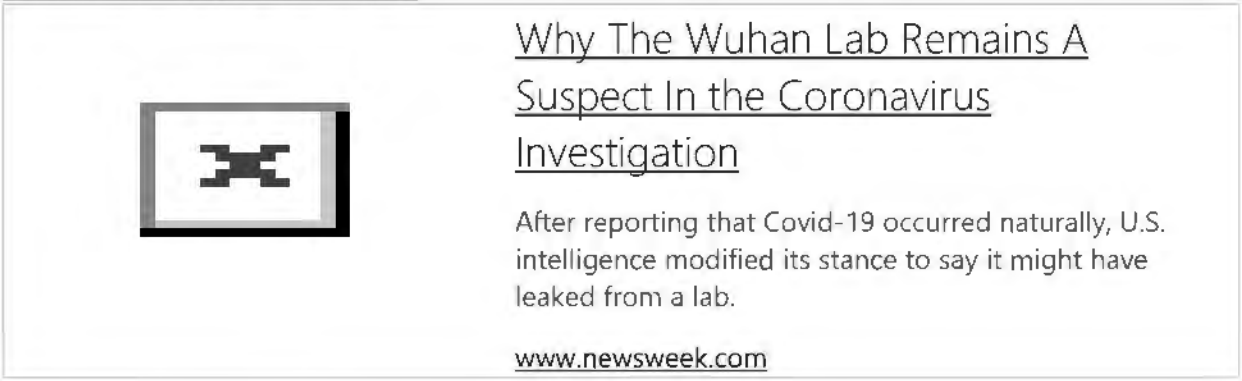
David-
Tank you for your kind words. We will be camping in the mountains of Taiwan until Saturday and I'm not sure of Internet access but please feel free to send me things. I hope you can have a happy Thanksgiving in some fashion this year.
Regards, Steve

On Wed, Nov 25, 2020, 11:31 PM Asher, David <(b)(6) @state.gov> wrote:
Steve,
Very helpful! Thank you.
We are working hard on some specific potential courses of actions against WIV and PRC.
Please let me know if Dr. Lai has offered follow on introductions to other AS scientists with experience working on Coronaviruses, including with WIV. Also, if Dr. Baric responds please

confidentially fill us in. Do Taiwanese researchers have any direct samples obtained from Wuhan—if so, from when? We also are interested in any lab notebooks or other info on what was going on internally, including staff that may have fallen ill in Nov-Dec.
You are superb scientific detective and scientific researcher. A rare combination!
We are indebted for your insight and assistance,
David
From: Steven Quay (b)(6)
Sent: Wednesday, November 25, 2020 9:15 AM
To: Asher, David <(b)(6) @state.gov>
Cc: Lawrence Remmel <(b)(6)
Subject: Re: Fw: RaTG13 (shared in confidence)

David-
See answers attached. Regards, Steve

On Wed, 25 Nov 2020 at 14:56, Asher, David <(b)(6) @state.gov> wrote:
Steve,
Please let us know your thoughts. Are there other alternative pathways you have found beyond RaTG13?
Thanks.
David
https://www.newsweek.com/controversial-wuhan-lab-experiments-that-may-have-started-coronavirus-pandemic-1500503



From: Feith, David <(b)(6) @state.gov>
Sent: Tuesday, November 24, 2020 7:29 PM
To: Asher, David <(b)(6) @state.gov>; (b)(6) @state.gov>; Switzer, Bryan R (Rick) (b)(6) @state.gov>
Subject: RE: RaTG13

With Q&A attached...

~~SENSITIVE BUT UNCLASSIFIED~~

From: Feith, David
Sent: Tuesday, November 24, 2020 7:25 PM

To: Asher, David (b)(6) @state.gov>;(b)(6) @state.gov>; Switzer, Bryan R (Rick) (b)(6) @state.gov>
Subject: RaTG13

When WIV said in January/February 2020 that RaTG13 was the closest sample they could find to SARS-CoV-2, what history of their RaTG13 research did WIV provide?

WIV said that RaTG13 was found in the Yunnan cave in 2013, but did WIV say they had done experiments with it in the years after 2013? Or did WIV say/suggest that RaTG13 had effectively stayed in the freezer until December 2019/January 2020, after the SARS-CoV-2 outbreak?

It seems that WIV's original Nature article of Feb. 3, 2020 didn't include this history: https://www.nature.com/articles/s41586-020-2012-7. After public challenges, WIV published an addendum just last week, on Nov. 17 2020: https://www.nature.com/articles/s41586-020-2951-z. Shi Zhengli also gave an interview to Science published July 31 (https://science.sciencemag.org/content/369/6503/487?rss=1); the full Q&A attached includes her statement that WIV "didn't isolate this virus" (page 5). Does that suggest WIV didn't do any research involving RaTG13 before the SARS-CoV-2 outbreak?

Appreciate any thoughts. Thanks.

--
David Feith
Deputy Assistant Secretary
Bureau of East Asian and Pacific Affairs (EAP)
U.S. Department of State
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(b)(6) @state.gov

~~SENSITIVE BUT UNCLASSIFIED~~

~~SENSITIVE BUT UNCLASSIFIED~~

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(b)(6) @state.gov>;
(b)(6) @state.gov>;
Keshap, Atul (b)(6) @state.gov>

From: "Feith, David" (b)(6) @state.gov>
To: Steven Quay, MD, PhD (b)(6)
Subject: Ensuring a Transparent, Thorough Investigation of COVID- 19's Origin; Activity at the Wuhan Institute of Virolo gy
Date: Sat, 16 Jan 2021 00:55:11 +0000

Dr. Quay, thanks for your tireless work. Hope this is of interest.

All best,
David

--
David Feith
Deputy Assistant Secretary
Bureau of East Asian and Pacific Affairs (EAP)
U.S. Department of State
(b)(6)
(b)(6) @state.gov

https://www.state.gov/ensuring-a-transparent-thorough-investigation-of-covid-19s-origin/

Ensuring a Transparent, Thorough Investigation of COVID-19's Origin
Michael R. Pompeo
January 15, 2021

The United States has repeatedly called for a transparent and thorough investigation into the origin of COVID-19. Understanding the origin of this pandemic is essential for global public health, economic recovery, and international security.

To assist the vital work of the World Health Organization (WHO) investigative team that arrived in China this week, the United States government is today sharing new information concerning the activities inside China's government laboratories in 2019.

In particular, we urge the WHO to press the government of China to address the following:

1. Illnesses at the Wuhan Institute of Virology (WIV): The United States 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.

2. WIV Research on "RaTG13" and "gain of function": Starting in at least 2016, WIV researchers studied RaTG13, the bat coronavirus identified by the WIV in January 2020 as its closest sample to SARS-CoV-2 (96.2% similar). Since the outbreak, the WIV has not been transparent nor

consistent about its work with RaTG13 or other similar viruses, including possible "gain of function" experiments to enhance transmissibility or lethality.

3. Secret WIV Links to Military Research: Despite the WIV presenting itself as a civilian institution, the WIV has collaborated on publications and secret projects with China's military. The WIV has engaged in classified research, including laboratory animal experiments, on behalf of the Chinese military since at least 2017.

The COVID-19 pandemic was avoidable. Any responsible country would have invited world health investigators to Wuhan within days of an outbreak. China instead refused offers of help – including from the United States – and punished brave Chinese doctors, scientists, and journalists who tried to alert the world to the dangers of the virus. Beijing continues today to withhold vital information that scientists need to protect the world from this deadly virus, and the next one.

The United States reiterates the importance of unfettered access to virus samples, lab records and personnel, eyewitnesses, and whistleblowers to ensure the credibility of the WHO's final report. Until the CCP allows a full and thorough accounting of what happened in Wuhan, it is only a matter of time until China births another pandemic and inflicts it on the Chinese people, and the world.

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https://www.state.gov/fact-sheet-activity-at-the-wuhan-institute-of-virology/

Fact Sheet: Activity at the Wuhan Institute of Virology
Office of the Spokesperson
January 15, 2021

For more than a year, the Chinese Communist Party (CCP) has systematically prevented a transparent and thorough investigation of the COVID-19 pandemic's origin, choosing instead to devote enormous resources to deceit and disinformation. Nearly two million people have died. Their families deserve to know the truth. Only through transparency can we learn what caused this pandemic and how to prevent the next one.

The U.S. government does not know exactly where, when, or how the COVID-19 virus—known as SARS-CoV-2—was transmitted initially to humans. We have not determined whether the outbreak began through contact with infected animals or was the result of an accident at a laboratory in Wuhan, China.

The virus could have emerged naturally from human contact with infected animals, spreading in a pattern consistent with a natural epidemic. Alternatively, a laboratory accident could resemble a natural outbreak if the initial exposure included only a few individuals and was compounded by asymptomatic infection. Scientists in China have researched animal-derived coronaviruses under conditions that increased the risk for accidental and potentially unwitting exposure.

The CCP's deadly obsession with secrecy and control comes at the expense of public health in China and around the world. The previously undisclosed information in this fact sheet, combined with open-source reporting, highlights three elements about COVID-19's origin that deserve greater scrutiny:

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.
- Accidental infections in labs have caused several previous virus outbreaks in China and elsewhere, including a 2004 SARS outbreak in Beijing that infected nine people, killing one.
- The CCP has prevented independent journalists, investigators, and global health authorities from interviewing researchers at the WIV, including those who were ill in the fall of 2019. Any credible inquiry into the origin of the virus must include interviews with these researchers and a full accounting of their previously unreported illness.

2. Research at the WIV:

- Starting in at least 2016 – and with no indication of a stop prior to the COVID-19 outbreak – WIV researchers conducted experiments involving RaTG13, the bat coronavirus identified by the WIV in January 2020 as its closest sample to SARS-CoV-2 (96.2% similar). The WIV became a focal point for international coronavirus research after the 2003 SARS outbreak and has since studied animals including mice, bats, and pangolins.
- The WIV has a published record of conducting "gain-of-function" research to engineer chimeric viruses. But the WIV has not been transparent or consistent about its record of studying viruses most similar to the COVID-19 virus, including "RaTG13," which it sampled from a cave in Yunnan Province in 2013 after several miners died of SARS-like illness.
- WHO investigators must have access to the records of the WIV's work on bat and other coronaviruses before the COVID-19 outbreak. As part of a thorough inquiry, they must have a full accounting of why the WIV altered and then removed online records of its work with RaTG13 and other viruses.

3. Secret military activity at the WIV:

- Secrecy and non-disclosure are standard practice for Beijing. For many years the United States has publicly raised concerns about China's past biological weapons work, which Beijing has neither documented nor demonstrably eliminated, despite its clear obligations under the Biological Weapons Convention.
- Despite the WIV presenting itself as a civilian institution, the United States has determined that the WIV has collaborated on publications and secret projects with China's military. The WIV has engaged in classified research, including laboratory animal experiments, on behalf of the Chinese military since at least 2017.
- The United States and other donors who funded or collaborated on civilian research at the WIV have a right and obligation to determine whether any of our research funding was diverted to secret Chinese military projects at the WIV.

Today's revelations just scratch the surface of what is still hidden about COVID-19's origin in China. Any credible investigation into the origin of COVID-19 demands complete, transparent access to the research labs in Wuhan, including their facilities, samples, personnel, and records.

As the world continues to battle this pandemic – and as WHO investigators begin their work, after more than a year of delays – the virus's origin remains uncertain. The United States will continue to do everything it can to support a credible and thorough investigation, including by continuing to demand transparency on the part of Chinese authorities.

Sender: "Feith, David" <(b)(6) @state.gov>
Recipient: Steven Quay, MD, PhD <(b)(6)

From: (b)(6) @state.gov>
To: DiNanno, Thomas G (b)(6) @state.gov>
(b)(6) @state.gov>;
Gibbs, Jeffrey J <(b)(6) @state.gov>;
CC: (b)(6) @state.gov>;
(b)(6) @state.gov>;
Asher, David (b)(6) @state.gov>
Subject: Emerging Issues
Date: Wed, 16 Dec 2020 23:59:22 +0000

Tom -
Reference our phonecon and previous emails, I am running into a number of puzzling issues which I believe should be considered as we look to draft a demarche. (b)(5)

(b)(5)

(b)(5)

VR/MP

Sender: (b)(6) @state.gov>

Recipient: DiNanno, Thomas G (b)(6) @state.gov>;
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(b)(6) @state.gov>;
(b)(6) @state.gov>;
Asher, David (b)(6) @state.gov>

23 June 2020

[draft paper for 2020 MX2 meeting; 27-28 August 2020]

(b)(5)

Withheld pursuant to exemption

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16 July 2020

[draft paper for 2020 MX2 meeting; 27-28 August 2020]

(b)(5)

Withheld pursuant to exemption

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14 July 2020

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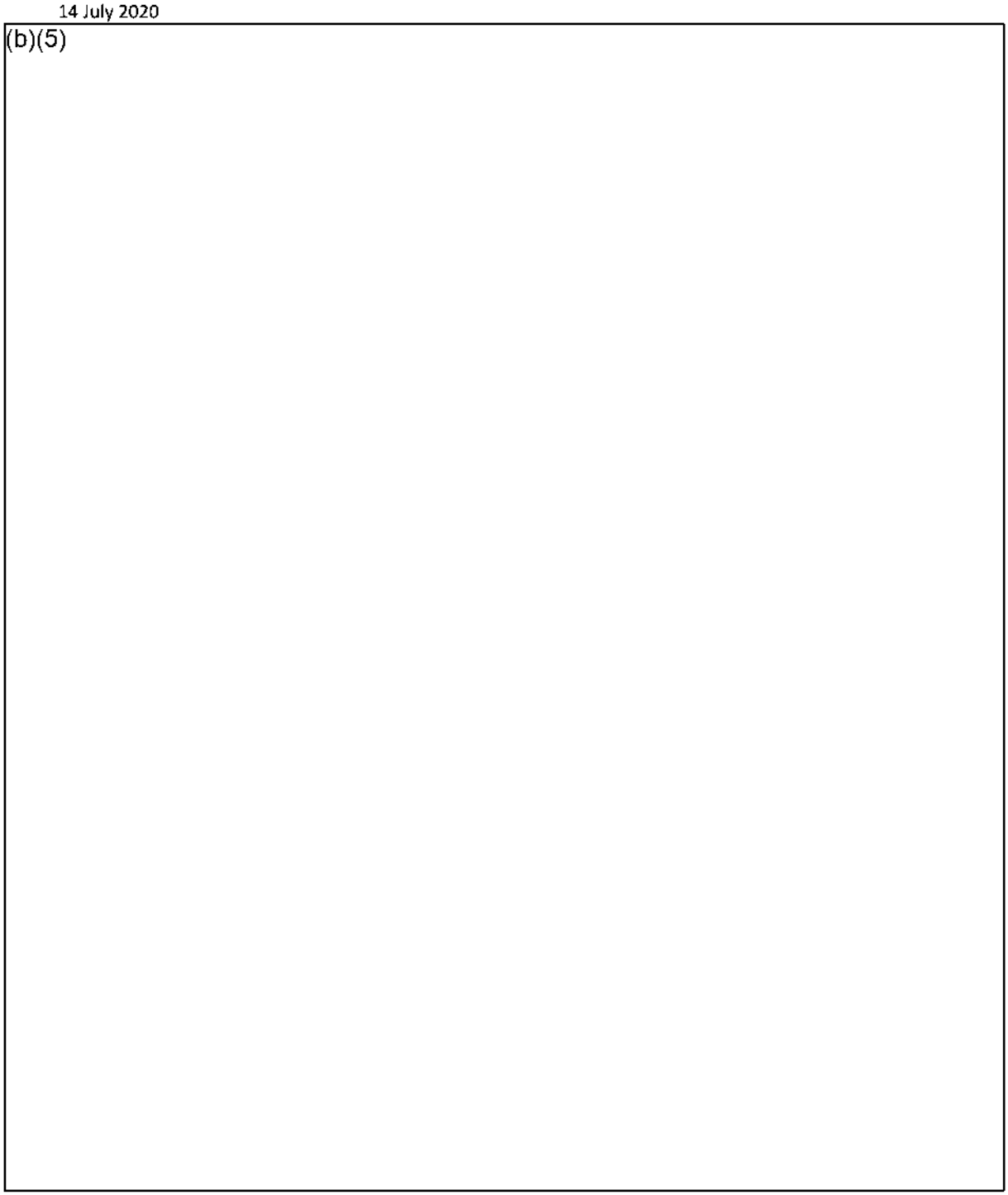

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14 July 2020

[draft paper for 2020 MX2 meeting; 27-28 August 2020]

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