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LETTER TO THE EDITOR

WILEY

Susceptibility of different cell lines to the novel canine coronavirus CCoV-HuPn-2018

Dear Editor,

Over the past few decades, we have witnessed the emergence of numerous novel viruses within the family Coronaviridae. These have included the swine acute diarrhea syndrome coronavirus (SADS-CoV), the canine respiratory coronavirus (CRCoV), the feline coronavirus serotype II (FCoV-II), and the latest severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1–5] Coronaviruses have increased opportunities for mutation and spill-over due to the frequent recombination and mutation events during replication, which helps them generate new viral threats. In fact, it is understood that all currently recognized human coronaviruses, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2, are zoonotic in origin.[6,7] However, evidence for canine and feline coronaviruses spilling over to humans has been sparse.

In a recent study evaluating a molecular diagnostic assay for coronaviruses, our team found evidence of canine coronavirus (CCoV) in eight patients hospitalized with pneumonia in Sarawak, Malaysia between 2017 and 2018.[8] Further analysis and viral isolation were then conducted in canine fibroblast tumor cells (A72). Among the eight samples, one specimen yielded a viral isolate, which was characterized by complete genome sequencing. The identified virus was a novel canine–feline recombinant alphacoronavirus (genotype II) that was named CCoV-HuPn-2018.[9]

We sought to assess the receptivity of different animal and human cell lines to the novel canine coronavirus CCoV-HuPn-2018 in comparison to another canine coronavirus, CCoV-UCD1 and a seasonal human coronavirus, HCoV-229E. The studied cell lines included adenocarcinomic human alveolar basal epithelial cells (A549), the human lung fibroblast cell line (MRC-5), Madin–Darby canine kidney (MDCK) cells, African green monkey kidney epithelial cells (VeroE6), pig testis cells (ST), and mink lung epithelial cells (Mv1Lu). A72 cells were used as a positive control for the CCoVs.

In 24-well plates, monolayers of MDCK, ST, A549, MRC.5, and A72 cells were inoculated with the two canine coronaviruses, CCoV-HuPn-2018 and CCoV-UCD1. CCoV-HuPn-2018 was also inoculated in Mv1Lu and VeroE6 cells. The human coronavirus HCoV-229E was inoculated in monolayers of MDCK, ST, A549 and MRC.5 cells. Median tissue culture infectious dose (TCID50) was calculated for each virus using the Reed–Muench method,[10] and inoculations were conducted at a multiplicity of infection (MOI) of 0.1. Cells were then incubated for 1 h at 37°C and 5% CO_2, except A72 cells which were incubated without CO_2. Following the incubation, virus was removed, and cells were washed once with phosphate-buffered saline (PBS), then fresh infection media containing 2% fetal bovine serum was added. Cells were monitored for cytopathic effect (CPE) every 24 h. Cells and supernatant were harvested at 0-, 40-, 72-, and 192-h postinoculation. RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Inc., Valencia, CA) and screened with a real-time reverse transcription polymerase chain reaction (qRT-PCR) assay specific for the virus.[9,11] Virus culture was considered positive when the cycle threshold (Ct)[7] value was at least 2 points below the 0-h inoculum and CPE was present.

CPE was observed 40-h postinoculation in A72 cells inoculated with CCoV-HuPn-2018 and CCoV-UCD1 and confirmed with qRT-PCR (Table 1). No increase in the viral replication was observed in MDCK, ST, A549, MRC.5, Mv1Lu, and VeroE6 cells even after 192-h postinoculation, suggesting that these cell lines are not permissive for CCoV-HuPn-2018 and CCoV-UCD1 (Figure S1).

CPE was observed in MRC5 cells inoculated with HCoV-229E beginning at 72-h postinoculation. This observation was also confirmed by qRT-PCR as Ct values were significantly lower than the original result. MDCK, ST, and A549 cells were monitored up to 192-h postinoculation, and no CPE was observed in these cells nor were positive qRT-PCR results detected.

The ability of the CCoVs to form CPE in A72 cells and the HCoV-229E to infect MRC.5 cells has been previously described.[9,12,13] Our experiments suggest that the studied human lung cells are not receptive for CCoV-HuPn-2018 infection and replication, despite their expression of APN receptors. However, previous studies have suggested that some coronaviruses are resistant to cell culture.[14,15] Additionally, permissiveness of various cell lines to coronavirus infection in vitro does not always recapitulate the in vivo tissue and host.[16] In vitro infection of this novel CCoV in human cell lines is challenging and requires further understanding of the virus pathogenesis and infection initiation in the human respiratory system.

TABLE 1 Susceptibility of cells to CCoV-HuPn-2018 as assessed by cytopathic effect and qRT-PCR

Cell line	Species	Cell type	CPE	Quantitative PCR Ct (hours 0, 72, 192)
A549	Human	Lung carcinoma epithelium	–	24.7, 27, 29.9
MRC-5	Human	Fetal lung fibroblast	–	24.0, 30.7, 33.4
MDCK	Canine	Kidney epithelium	–	24.6, 29.6, 33.1
A72[a]	Canine	Tumor fibroblast	+	25.4, 17.7, 16.4
Vero E6	African green Monkey	Kidney epithelium	–	25.3, 28.7, 31.3
ST	Swine	Fetal testes	–	24.2, 28.5, 31.7
Mv1Lu	Mink	Lung epithelium	–	25.2, 28.1, 31.0

Abbreviations: CPE, cytopathic effect; Ct, cycle threshold; qRT-PCR, quantitative reverse transcription polymerase chain reaction.
[a]CPE was observed 40-h postinoculation in this cell line.

KEYWORDS

alphacoronavirus, canine coronavirus, CCoV-HuPn-2018, cell lines, receptivity

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Gregory Gray: Conceptualization; funding acquisition; supervision. **Anfal Abdelgadir:** Investigation. **Anastasia Vlasova:** Conceptualization; investigation; methodology; supervision.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Clinical Infectious Diseases

BRIEF REPORT

Isolation of a Novel Recombinant Canine Coronavirus From a Visitor to Haiti: Further Evidence of Transmission of Coronaviruses of Zoonotic Origin to Humans

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We isolated a novel coronavirus from a medical team member presenting with fever and malaise after travel to Haiti. The virus showed 99.4% similarity with a recombinant canine coronavirus recently identified in a pneumonia patient in Malaysia, suggesting that infection with this virus and/or recombinant variants occurs in multiple locations.

Keywords. coronavirus; coronavirus: canine; coronavirus: zoonotic; coronavirus: recombinant; human coronavirus infection.

In March 2017, members of a medical team from University of Florida who had recently returned from a "mission trip" to Haiti presented with mild fever and malaise. Zika virus (ZIKV) was circulating in Haiti at the time, and because of concerns that their illnesses might represent ZIKV infection, freshly collected urine samples were obtained from team members and screened for ZIKV, in keeping with previously described methods [1]. All samples tested negative by reverse transcription polymerase chain reaction (RT-PCR) for ZIKV. However, at that time our routine procedure included efforts to isolate ZIKV from all diagnostic specimens, and consequently deidentified aliquots of the urine samples were subsequently inoculated onto Vero E6 and LLC-MK2 cells, which are susceptible and permissive for ZIKV.

Twenty urine samples from team members were screened. Samples from six patients produced cytopathic effects (CPE) in cell culture within 14 days of inoculation of cell lines; an example is shown for LLC-MK2 cells inoculated with urine sample Z19 (Figure 1). When aliquots of cell culture media from Vero E6 cells at 16 days post-inoculation were inoculated onto MDCK cells, CPE were noted the following day (Supplementary Figure 1). To determine whether the viruses causing CPE were of possible respiratory origin, cell culture media was tested using a GenMark eSensor XT-8 RVP system (eSensor RVP; GenMark Diagnostics, Inc., Carlsbad, California, USA) instrument [2]. Unexpectedly, the 6 samples tested showed mixed low signals for 3 of the 4 seasonal endemic human coronaviruses (threshold signal [nA] value above 3, the generally accepted positive cutoff, seen for Betacoronavirus OC43 [4 of 6 samples tested] and Alphacoronaviruses 229E [4 of 6 samples tested] and NL65 [2 of 6 samples]) (Supplementary Table 1). After follow-up RT-PCR tests of the cell culture media using species-specific coronavirus RT-PCR tests failed to establish an identity, an unbiased amplification and sequencing approach was attempted [3, 4].

As material extracted from Madin-Darby canine kidney (MDCK) cells culture media corresponding to sample Z19 appeared to have the highest virus yield based on the extent of CPE formed, RNA from this sample was purified and subjected to Sanger sequencing. Initial sequence analyses of a 2558 bp amplicon (Supplementary Figure 2) generated using an unbiased RT-PCR amplification method [5] indicated 97% (2475/2561) nucleotide (nt) identity to a porcine coronavirus, transmissible gastroenteritis virus (TGEV) strain Purdue P115 (Genbank Accession no. DQ811788.1), leading to the assumption that the virus was TGEV. However, primers based on TGEV did not effectively amplify or failed to amplify other sections of the virus' genome, suggesting that it was a different coronavirus. After the publication of Vlasova et al [6], primers that targeted parts of the RdRp gene and spike protein on the genome sequence they discovered (canine coronavirus isolate CCoV-HuPn-2018, GenBank MW591993.2) were tested and were found to produce PCR amplicons. This prompted us to focus efforts on amplifying the virus sequence of our isolate using canine coronavirus primers.

Ultimately, 39 primer pairs covering the whole virus genome were designed for complete genome sequencing (Supplementary Table 2). Three additional primers for 5′ and 3′ Rapid Amplification of cDNA Ends (RACE) were also designed for this work; that work was accomplished using the RACE System (Invitrogen) used according to the manufacturer's

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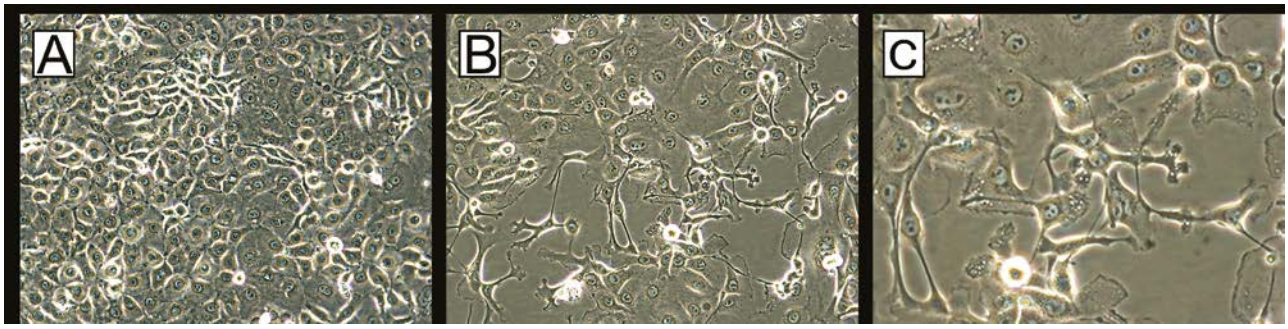

Figure 1. Virus-induced cytopathic effects in LLC-MK2 cells 16 days post-inoculation with urine specimen Z19. *A*, Mock-inoculated cells, original magnification 200×. *B*, Cells inoculated with urine, original magnification 200×. *C*, Detail from image *B*, original magnification 400×.

manual. By using the primers of Supplementary Table 2, virus genomic RNA (vgRNA) was reverse-transcribed into cDNA using an AccuScript High fidelity 1st Strand cDNA Synthesis Kit (Agilent, Santa Clara, California, USA), and PCR performed with Q5 high-fidelity DNA polymerase (New England Biolabs). Sanger sequencing was performed using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, USA). The strain has been designated HuCCoV_Z19Haiti; GenBank accession number is MZ420153. The five other samples showing CPE effects (Z03, Z04, Z11, Z12, and Z14) were only partly characterized (for verification purposes) after Z19 was fully sequenced. Primers 36F and 36R (Supplementary Table 2) were used to RT-PCR amplify 850 nt amplicons from the other 5, and after sequencing these amplicons were found to be identical to the sequence of Z19. The remaining 14 patient urine samples were negative on screening with multiple primers. Neither this virus—or other canine coronaviruses—were present in our laboratory prior to this study.

Further recombination and phylogenetic analyses were conducted as previously described in Lednicky et al [4]; details of methods are provided in Supplementary Materials. A maximum likelihood (ML) tree inferred on the full genome alignment (Figure 2A), regardless of potential recombinant genomic fragments, confirmed the close relationship between HuCCoV_Z19Haiti and CCoV-HuPn-2018, with 99.4% identity between the 2 virus strains. The second half of the HuCCoV_Z19Haiti genome, starting from gene E, showed greater divergence from CCoV-HuPn-2018 (similarity plot, Supplementary Figure 3, Supplementary Table 3). Furthermore, the HuCCoV_Z19Haiti isolate did not have the 36 nt deletion in gene N and the 228 nt deletion in ORF7b, both characteristic of the Malaysian strain. A further Blast search [7] on the NCBI database, conducted only with the genes E, M, N, and the ORF7 segment, did show a match with the Chinese canine coronavirus strain CCoV B639_ZJ_2019 [8] (Supplementary Figure 3). Fragmenting the genome of HuCCoV_Z19Haiti alignment by gene, as was done by Vlaslova et al [6], further confirmed the chimeric nature of the virus isolated in Haiti (Supplementary Figure 4). Both Spike S1 and S2 ML trees clustered HuCCoV_Z19Haiti with CCoV-HuPn-2018, although in the gene M ML tree the closest relative was the Chinese CCoV B639_ZI_2019. In the gene N phylogeny, the Haitian strain clusters with TGEV, although the bootstrap values might be too low to make a strong inference.

We identified the same pattern of recombination events reported by Vlasova et al [6] in the spike and ORF1 of the Haitian genome (Supplementary Table 4) suggesting that recombination occurred ancestrally to CCoV-HuPn-2018 and HuCCoV_Z19Haiti. The Haitian isolate, however, further diverged from the Malaysian strain through additional and multiple recombination events across the genome, notably affecting the gene E – ORF7 segment, which closely relates to CCoV B639_ZJ_2019. Further recombination events with other CCoVs overlapped to the segment originated from CCoV B639_ZJ_2019. To corroborate the recombination analysis, 5 subsets of genomic fragments were analyzed: the larger one constituted by most of the genome, minus the recombinant fragments, and 4 smaller ones constituted by the segments involved in the inferred recombination events involving HuCCoV_Z19Haiti. Recombinant segments common to CCoV-HuPn-2018 and HuCCoV_Z19Haiti were removed, as they were considered to have occurred prior to divergence; other recombinant segments, involving taxa other than the Haitian strain, were also removed from recombinant sequences. Following assessment of phylogenetic signal (Supplementary Figure 5), ML trees for each nonrecombinant fragment (Figure 2B–F) confirmed CCoV-HuPn-2018 as the major parent of the Haitian strain, and the chimeric nature of the other fragments, involving other CCoVs, as well as possibly unsampled ancestors of TGEVs.

COMMENT

Coronaviruses are known to infect a wide range of mammalian and bird species [9]. They have also long been recognized as one of the causes of the "common cold" in humans, associated with what have been termed the seasonal endemic human coronaviruses: HCoV 229E and NL63 in the genus *Alphacoronavirus*; and HCoV HKU1 and OC43 in the genus *Betacoronavirus* [10].

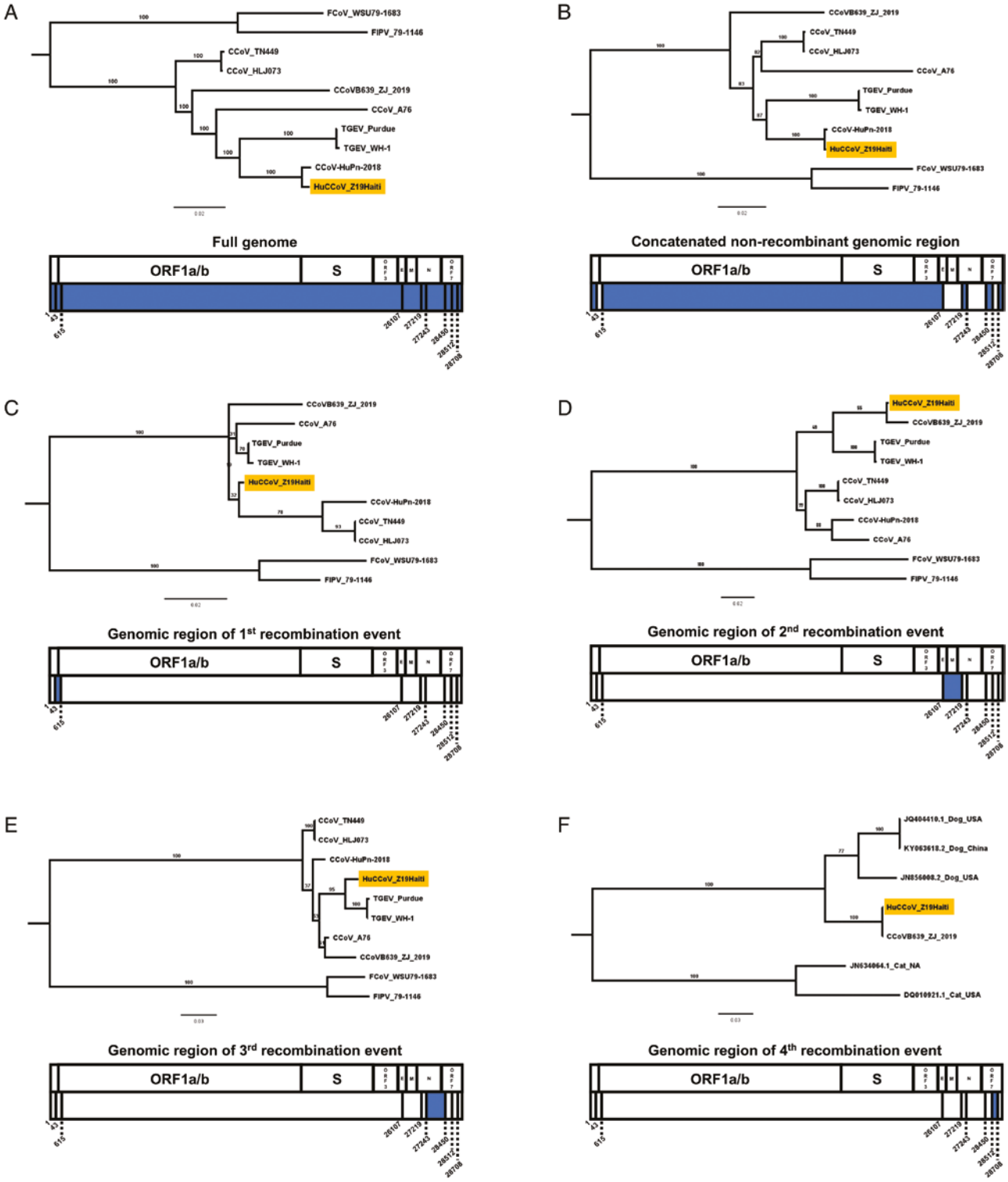

Figure 2. Maximum likelihood (ML) tree of alphacoronavirus strains. ML trees were inferred from 10 genome sequences, including CCoV-HuPn-2018 and CCoVB639_ZJ_2019, using the best fitting nucleotide substitution models as detected by Bayesian information criterion. Branches are scaled in number of nucleotide substitutions per site according to the bar below each tree. Nonparametric bootstrap values (1000 replicates) are indicated along supported branches. Haitian strain HuCCoV Z19 is highlighted. *A*, ML tree calculated using full genomes, prior to any recombination analysis. Panels *B–F* show trees inferred based on non-recombinant genomic fragments, indicated in blue in the schematic genome below each tree for clarity. Genome coordinates are based on HuCCoV Z19. *B*, ML tree calculated using non-recombinant segments of the genome. *C*, ML tree calculated using HuCCoV Z19 recombinant segment 43-615. *D*, ML tree calculated using HuCCoV Z19 recombinant segment 26107-27219. *E*, ML tree calculated using HuCCoV Z19 recombinant segment 27243-28450. *F*, ML tree calculated using HuCCoV Z19 recombinant segment 28512-28708. Segments involved in recombination events of genomes other than HuCCoV Z19 and CCoV-HuPn-2018 were replaced by gaps in the affected sequences in trees *B–F*.

However, over the past 2 decades we have seen the emergence of three coronavirus species that are highly pathogenic for humans, and which appear in each instance to have arisen from a zoonotic origin: severe acute respiratory syndrome coronavirus (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome 2 (SARS-CoV-2), all in the genus *Betacoronavirus*.

Our group has recently reported isolation of a porcine deltacoronavirus (PDCoV) from children in Haiti presenting with fever and gastrointestinal complaints, with genomic and evolutionary analyses suggesting that human infections were the result of at least 2 independent zoonoses of distinct viral lineages that acquired a common mutational signature in the *nsp15* and the *spike* glycoprotein genes by convergent evolution [4]. As noted above, Vlasova et al reported isolation of an *Alphacoronavirus* of apparent canine origin, with evidence of recombination with a feline coronavirus, from patients with pneumonia in Malaysia [6]. We report here identification of a coronavirus of canine origin which is closely related to the Malaysian virus reported by Vlasova et al, albeit isolated in this instance from a visitor to Haiti, and with a further recombinational history. Samples were deidentified after initial screening by RT-PCR for Zika, limiting our ability to obtain detailed clinical and epidemiological information on specific infected individuals; however, all members of the group reported mild fever and malaise, and all recovered uneventfully. Our data highlight the potential among coronaviruses for rapid evolution combined with frequent recombination events, leading to periodic emergence of strains capable of crossing species barriers into human populations. In many instances such strains would appear to be of low virulence for humans, as reflected in our work with PDCoV and now CCoV-Haiti; however, the potential for such strains to carry or acquire genes capable of causing severe disease in humans remains of clear concern.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Clinical Infectious Diseases

MAJOR ARTICLE

Novel Canine Coronavirus Isolated from a Hospitalized Patient With Pneumonia in East Malaysia

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Background. During the validation of a highly sensitive panspecies coronavirus (CoV) seminested reverse-transcription polymerase chain reaction (RT-PCR) assay, we found canine CoV (CCoV) RNA in nasopharyngeal swab samples from 8 of 301 patients (2.5%) hospitalized with pneumonia during 2017–2018 in Sarawak, Malaysia. Most patients were children living in rural areas with frequent exposure to domesticated animals and wildlife.

Methods. Specimens were further studied with universal and species-specific CoV and CCoV 1-step RT-PCR assays, and viral isolation was performed in A72 canine cells. Complete genome sequencing was conducted using the Sanger method.

Results. Two of 8 specimens contained sufficient amounts of CCoVs as confirmed by less-sensitive single-step RT-PCR assays, and 1 specimen demonstrated cytopathic effects in A72 cells. Complete genome sequencing of the virus causing cytopathic effects identified it as a novel canine-feline recombinant alphacoronavirus (genotype II) that we named CCoV–human pneumonia (HuPn)–2018. Most of the CCoV-HuPn-2018 genome is more closely related to a CCoV TN-449, while its S gene shared significantly higher sequence identity with CCoV-UCD-1 (S1 domain) and a feline CoV WSU 79-1683 (S2 domain). CCoV-HuPn-2018 is unique for a 36-nucleotide (12–amino acid) deletion in the N protein and the presence of full-length and truncated 7b nonstructural protein, which may have clinical relevance.

Conclusions. This is the first report of a novel canine-feline recombinant alphacoronavirus isolated from a human patient with pneumonia. If confirmed as a pathogen, it may represent the eighth unique coronavirus known to cause disease in humans. Our findings underscore the public health threat of animal CoVs and a need to conduct better surveillance for them.

Keywords. canine coronavirus; novel alphacoronavirus; pneumonia: zoonotic disease; East Malaysia.

Human coronaviruses (HCoVs) associated with common colds (HCoV-229E and HCoV-OC43) were initially identified in the mid-1960s, and 2 more, HCoV-NL63 and HCoV-HKU1, were described in 2004 and 2005, respectively [1–3]. The emergence of severe acute respiratory syndrome (SARS) coronavirus (CoV) in 2002–2003 and Middle East respiratory syndrome CoV in 2012 demonstrated that CoVs can cause severe to fatal disease [4]. Evidence suggests that bats are likely to be the original source of SARS-CoV and Middle East respiratory syndrome CoV [5, 6]. The most recent and notable CoV-related threat is represented by the coronavirus disease 2019 pandemic caused by SARS-CoV-2 [7]. While the origin of SARS-CoV-2 is still debated [8], it is thought to have emerged via a spillover event originating at a Chinese wet market. Thus, zoonotic CoVs pose a major threat to human health, with different animals serving as natural reservoirs or intermediate hosts to CoVs transmittable to humans [9, 10]. However, the potential threat represented by cats and dogs or their CoVs has been sparsely studied.

Different genotypes (I, II) of canine CoVs (CCoVs) of *Alphacoronavirus 1* species cause moderate-to-severe enteric disease in dogs [11]. CCoV-II circulation has been confirmed in dogs since 1971, and CCoV-I was discovered about 3 decades later [12, 13]. Transmissible gastroenteritis virus (TGEV), CCoV-II, and feline CoV (FCoV) II have reportedly originated from CCoV-I and FCoV-I through gene loss and recombination [14]. Similarly to FCoVs, CCoV-I strains do not grow or grow poorly in cell culture and their cellular receptor is unknown, while CCoV-II strains grow readily in culture using aminopeptidase N as a cellular receptor [15]. This emphasizes the complex evolution of CCoVs/*Alphacoronavirus 1* species and their ability to infect different hosts, inducing variable clinical disease. It has

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been demonstrated that another CoV, using aminopeptidase N as a cellular receptor, porcine deltacoronavirus, can infect cells of unusually broad species origin, including human and chicken [16].

Previous studies documenting CCoV in human patients with pneumonia in Sarawak [17] and FCoV-like CoVs in human patients with acute respiratory symptoms in Arkansas [18] represent the only evidence that *Alphacoronavirus 1* species may infect and be associated with a clinical disease in humans. Here we report isolation, complete genome sequencing and molecular analysis of a CCoV virus from one of the patients with pneumonia.

METHODS

Sample Source, Screening, and Cell Culture Isolation

Eight of 301 nasopharyngeal swab (NPS) specimens from hospitalized patients with pneumonia (2017–2018 at Sibu and Kapit Hospitals, Sarawak, Malaysia) were previously confirmed to contain CCoV using a seminested reverse-transcription polymerase chain reaction (RT-PCR) assay and Sanger sequencing (Table 1 and Supplementary Table 1) [17]. The 8 patients with pneumonia all came from Sibu Hospital (Table 1). Seven (87.5%) were aged <5 years, 4 were infants, and most were from Sarawak's indigenous ethnic groups, who typically live in rural or suburban longhouses or villages. Seven of the patients (87.5%) had evidence of a viral coinfection (Table 1). All bacterial blood cultures were negative, and all patients were hospitalized for 4–6 days and recovered.

RNA Extraction and RT-PCR

RNA was extracted from suspended NPS samples using the 5X MagMAX Viral Isolation Kit (Applied Biosystems). Because 1-step RT-PCR is less sensitive than nested or seminested RT-PCR, further characterization was conducted using 1-step RT-PCR assays to ensure no contamination. A Qiagen 1-step RT-PCR kit was used (primers and cycling protocols provided in Supplementary Table 2). Amplicons generated with CCoV-N-F/CCoV-N-R primers were gel extracted using the QIAquick Gel Extraction Kit (Qiagen) and sequenced using the Sanger method at the Molecular and Cellular Imaging Center (MCIC) at the Ohio Agricultural Research and Development Center, The Ohio State University, Wooster.

Virus Isolation in A72 Cell Culture and Transmission Electron Microscopy

Canine fibroblast tumor (A72) cells (received from Alfonso Torres, Cornell College of Veterinary Medicine) were maintained and used for sample inoculation, as described elsewhere [22]. Serially diluted NPS fluids (1:10–1:10 000) were used to inoculate the A72 monolayers. After 72 hours the infected cells and medium were harvested and used for RNA extraction with the RNEasy Mini Kit (Qiagen). Immune transmission electron microscopy (I-TEM) was conducted as described elsewhere,

Table 1. Demographic and Clinical Characteristics of 8 Patients With Molecular Evidence of Canine Coronavirus in Nasopharyngeal Swab Specimens

ID	Sex/Age/Ethnicity	Town/Housing Type (No. of Cohabitants at Home)	Underlying Condition/Medication	Known Exposure to Animals	Highest Oxygen Support During Admission	Duration of Hospital Stay	Other Concomitant Pathogens Detected[a]
1090	Male/13.5 m/Iban	Sibu/unknown (2)	Preschool wheeze/inhaled budesonide	No	No information	No information	Adenovirus
1116	Male/9.5 m/Iban	Sibu/longhouse (9)	Glucose-6-phosphate dehydrogenase deficiency/oral amoxycillin	No	Nasal prong oxygen (1 L/min)	5 Complete days	Adenovirus
1126	Female/2.5 y/Iban	Bintulu/longhouse (9)	None	No	No information	No information	Parainfluenza virus 3
1128	Female/11 m/Iban	Sibu/longhouse (7)	None	Yes (cats)	Nasal prong oxygen (1 L/min)	5 Complete days	Parainfluenza virus 3
1131	Female/4.5 y/Chinese	Sibu/townhouse (4)	None	Yes (cats and dogs)	No information	No information	Influenza A
1153[b]	Male/5.5 m/Melanau	Daro/village (10)	None/oral ampicillin and cloxacillin	No	No information	No information	Rhinovirus C
1157	Female/10 m/Bidayuh	Julau/longhouse (4)	Preschool wheeze/inhaled fluticasone	No	Nasal prong oxygen (1 L/min)	6 Complete days	Adenovirus
2062	Female/375 y/Iban	Sibu/staff quarter (15)	Bronchial asthma/inhaled fluticasone	Yes (dogs)	Nasal prong oxygen (3 L/min)	4 Complete days	None

Abbreviation: ID, patient identifier.

[a]Patients' nasopharyngeal swab specimens were studied with molecular assays for adenovirus, human enterovirus, influenza A, B, C, and D, respiratory syncytial virus A and B, parainfluenza viruses 1, 2, 3, and 4, and rhinovirus [19–21].

[b]Canine coronavirus–human pneumonia–2018 was isolated from sample 1153.

using polyclonal anti-CCoV guinea pig serum (BEI Resources; NR-2727); the I-TEM images were captured at the MCIC [23].

Complete Genome Sequencing With the Sanger Method

The viral RNA was converted into complementary DNA (cDNA) using a SuperScript III cDNA synthesis kit (Invitrogen). Forty-two primer pairs (Supplementary Table 3) covering the whole genome were designed based on the sequence of CCoV, strain TN-449, the most closely related strain, as determined by The Basic Local Alignment Search Tool (BLAST) nucleotide (BLASTn) analysis of the partial N gene sequence of the newly identified CCoV for which the complete genome was available. Using these primers and Platinum Taq (Invitrogen), 12 amplicons (1.7–3.6 kb) were generated and purified using the QIAquick Gel Extraction Kit and sequenced with 3× coverage, using the Sanger dideoxy method with a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems), at the MCIC and at the James Comprehensive Cancer Center Shared Genomics Core, The Ohio State University, Columbus. After the initial analysis/sequence assembly, 7 additional primer pairs were designed, based on the newly generated sequences, to close the remaining gaps (Supplementary Table 3). The fragments were amplified and sequenced as described above. The 5' and 3' genomic ends were amplified using the 5' and 3′ RACE System for Rapid Amplification of cDNA Ends (Invitrogen), according to the manufacturer's instructions.

Sequence Assembly and Analysis

Raw sequences were trimmed to remove low-quality reads and amplicon-primer linkers. Each open reading frame (ORF) was analyzed using Viral Genome ORF Reader (VIGOR4) to predict viral protein sequences. The annotated CCoV genome was submitted to GenBank (accession no. MW591993). The alignments were further analyzed using the Sequence Manipulation Suite (SMS; version 2) (https://www.bioinformatics.org/sms2/) to determine nucleotide identities between the reference and newly generated sequences. Sequence alignment and phylogenetic analysis were performed using the ClustalW method and the maximum-likelihood method with the general time-reversible nucleotide substitution model and bootstrap tests of 1000 replicates with MEGA X software. The CoV genomes for reference strains from GenBank used in the phylogenetic analyses are listed in (Table 2). The Recombinant Identification Program (RIP; http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html) was used to identify recombination points within the CCoV–human pneumonia (HuPn)–2018 genome, with a window size of 400 and a confidence threshold of 90%. Glycosylation prediction was conducted using the NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/).

RESULTS

RT-PCR and Partial Sequencing of CCoV

Samples from 2 of the 8 patients from whom CCoV was earlier detected were positive in universal and CCoV-specific 1-step RT-PCR assays (Supplementary Table 2). This result could be due to differences in the quantity or integrity of CCoV in samples collected at variable time points after infection. According to the BLASTn search, the sequences obtained for both samples using CCoV-N-F/CCoV-N-R primers shared the highest nucleotide identity (96.31%) with several CCoV strains, including TN-449 and HLJ-073 (listed in Table 2). We selected the TN-449 sequence to design sequencing primers covering the complete genome (Supplementary Table 3).

CCoV Replication in A72 Canine Cells

While 8 CCoV-positive NPS samples were inoculated into A72 cells, only 1 sample (sample 1153; Table 1) produced cytopathic effects in the cells (Supplementary Figure 1). The A72 cell–passaged material (P1) was inoculated into A72 cells again, and cytopathic effects were observed within the same time frame (P2). RNA extracted from both P1 and P2 tested CCoV positive; RNA extracted from P1 was used for complete genome sequencing. This virus was visualized using I-TEM (Figure 1) and is referred to as CCoV-HuPn-2018 throughout.

Genomic Organization of CCoV-HuPn-2018

The assembled viral genome was 29 083/29 351 nucleotides long (owing to differences in length between the two 7b forms), excluding the poly(A) tail. The genomic organization and gene order were typical of other *Alphacoronavirus 1* species: ORF1a1b, spike (S), ORF3a, ORF3b, ORF3c, envelope (E), membrane (M), nucleocapsid (N), ORF7a and ORF7b (Supplementary Figure 2 and Table 3). The structural and nonstructural proteins (NSPs) were flanked by 5′ and 3' untranslated regions (UTRs) with a 3′ poly(A) tail.

The 5' UTR consisted of 313 nucleotides, including the leader sequence (nucleotides 1–94) and the conserved core 5-CU(T)AAAC-3 (nucleotides 95–100) of the transcription regulatory sequence (TRS) that controls the messenger RNA synthesis during the subgenomic RNA discontinuous transcription. Similar TRS signals preceded 5 genes: S (nucleotide 20 335), 3a (24 787), E (25 866); M (26 156), N (26 951), and 7a/b (28 072) (Table 3). There were no TRS signals in front of 3b/3c and 7b, suggesting that they may be expressed from polycistronic messenger RNAs. The 3' end of the viral genome consists of a 275-nucleotide 3' UTR, followed by the poly(A) tail. The 20 061 nucleotides following the 5' UTR were occupied by the replicase gene encoding for 2 large polyproteins, polyproteins 1a and 1b, with polyprotein 1ab synthesized through ribosomal slippage at position 12 33, as reported for the highly related CCoV TN-449.

The SMS analysis demonstrated that the genome was mostly similar to CCoV strains TN-449, HLJ-073, and A76 and the TGEV Purdue strain, sharing 93.31%, 91.744%, 90.63% and 91.47% nucleotide identity, respectively, followed by FCoV/feline infectious peritonitis virus (FIPV) strains (83.96%–84.58% nucleotide identity) (Table 2). This suggests

Table 2. Identity Between Canine Coronavirus–Human Pneumonia–2018 and *Alphacoronavirus 1* Reference Strains for Complete Genomic Sequence and Genes for Structural Proteins

			Nucleotide Identity to CCoV-HuPn-2018, %						
Alphacoronavirus 1	Strain	Accession No.	Complete Genome	S	S1	S2	E	M	N
CCoV-IIa	TN-449	JQ404410.1	93.31[a]	93.42[a]	73.22	95.20	93.57	95.08	93.42
CCoV-IIa	HLJ-073	KY063618.2	91.74	93.33	73.32	95.20	93.17	95.08	93.33
CCoV-IIc	A76	JN856008.2	90.63	93.77	53.80	85.42	95.18[a]	97.08[a]	93.77[a]
CCoV	UCD-1	AF116248.1	NA	NA	99.19[a]	NA	NA	NA	NA
TGEV	Purdue (virulent)	DQ811789.2	91.47	92.12	90.93	94.59	93.98	92.65	92.12
FCoV-II	WSU 79-1683	JN634064.1	84.58	74.91	72.80	97.13[a]	93.68	86.25	74.91
FCoV-II/FIPV	79-1146	DQ010921.1	84.04	75.5	73.04	95.04	79.92	81.77	75.5

Abbreviations: CCoV, canine coronavirus; CCoV-HuPn-2018, CCoV–human pneumonia 2018; E, envelope; FCoV, feline coronavirus; FIPV, feline infectious peritonitis virus; HuPN, human pneumonia; M, membrane; N, nucleocapsid; NA, not available; S, spike; TGEV, transmissible gastroenteritis virus.
[a]Highest nucleotide identity between CCoV-HuPn-2018 and given strain.

that CCoV-HuPn-2018 represents a novel strain within the *Alphacoronavirus 1* species.

Similar to the complete genome, CCoV-HuPn-2018 ORF1ab region shared the highest nucleotide identity with those of TN-449 (95.84%), HLJ-073 (95.70%), and A76 (95.40%), followed by other CCoV (89%–94.28%), various TGEV (92.6%–94.49%), and FCoV (82.08%–85.84%) strains. Furthermore, while the full-length S gene of CCoV-HuPn-2018 shared the highest nucleotide identity with CCoV TN-449 (93.42%), its S1 domain was nearly identical to that of CCoV UCD-1 (for which only the S1 sequence is available), sharing 99.19% nucleotide identity, higher than for any other genomic region (Table 2). The S2 domain of CCoV-HuPn-2018 shared the highest identity (97.13%) with FCoV WSU 79-1683, providing additional evidence of the recombinant (feline-canine, canine-TGEV) nature of most CCoV S genes [24]. The remaining 3 genes, encoding for structural proteins E, M, and N, shared the highest nucleotide identities (95.18%, 97.08%, and 93.77%), respectively, with CCoV A76 (Table 2).

Phylogenetic Analysis

Phylogenetic analysis of complete genome sequences demonstrated that the novel CCoV-HuPn-2018 formed a monophyletic branch with CCoV, TGEV, FCoV strains, and swine enteric CoV (TGEV with porcine epidemic diarrhea virus recombinant S gene) (Figure 2A). Furthermore, the full-length S gene of the CCoV-HuPn-2018 was closely related to CCoV strains and TGEV Purdue (Figure 2B), while its S1 and S2 domains were most closely related to CCoV UCD-1 and FCoV WSU 79-1683, respectively (Figure 2C and 2D). Phylogenetic analysis of the E gene confirmed the close relation between CCoV-HuPn-2018 and CCoV A76; however, owing due to the high level of conservation of this gene, all of the analyzed *Alphacoronavirus 1* strains, except FIPV 79-1146, formed a tight cluster (Figure 2E). The M and N gene phylogenetic analysis confirmed that N and M genes were highly similar between CCoV-HuPn-2018 and CCoV A76, followed by other CCoVs and TGEV, while FCoVs formed separate clusters supporting a higher degree of divergence in this genomic region, evident from SMS analysis (Figure 2F and 2G and Table 2).

Recombination Analysis

Potential recombination break points between the background CCoV and TGEV strains were present throughout the ORF1ab, resulting in the short regions sharing more similarity with HLJ-073, A76, and the TGEV Purdue strain (Figure 3A). In addition, while the first two-thirds of the ORF1ab was relatively dissimilar between the CCoV-HuPn-2018 and FCoV WSU 79-1683/FIPV 79-1146, the similarity was greater (and comparable to that in CCoV/TGEV strains) in the last third, with multiple recombination break points (Figure 3A). The 3' end of the genome downstream from the S gene was most similar between CCoV-HuPn-2018 and CCoV strain A76. While the S2 domain shared the highest similarity with that of FCoV WSU 79-1683, the sequence similarity between the CCoV-HuPn-2018 and all the background sequences in the hypervariable S1 region was low. Thus, this finding is consistent with the SMS and phylogenetic analysis results and indicates the recombinant nature of this strain (Figure 3A).

The S gene RIP analysis revealed the presence of the recombination point at approximately 2 kb, with the S2 domain being highly similar to FCoV WSU 79-1683, as noted above (Figure 3B and 3C). The S1 domain RIP analysis allowed us to include the CCoV UCD-1 S1 domain in the analysis and confirm that it indeed shared the highest similarity with the CCoV-HuPn-2018 S1. These observations confirmed that the novel strain carries a recombinant CCoV/FCoV S protein.

Structural/Nonstructural Protein Analysis

The S protein comprised 1448 amino acids, similar to other CCoV II strains and shorter than S proteins of CCoV I characterized elsewhere [25]. Twenty-nine potential glycosylation sites were predicted in the S protein of the newly identified CCoV-HuPn-2018 (Supplementary Figure 3*A*), similar to

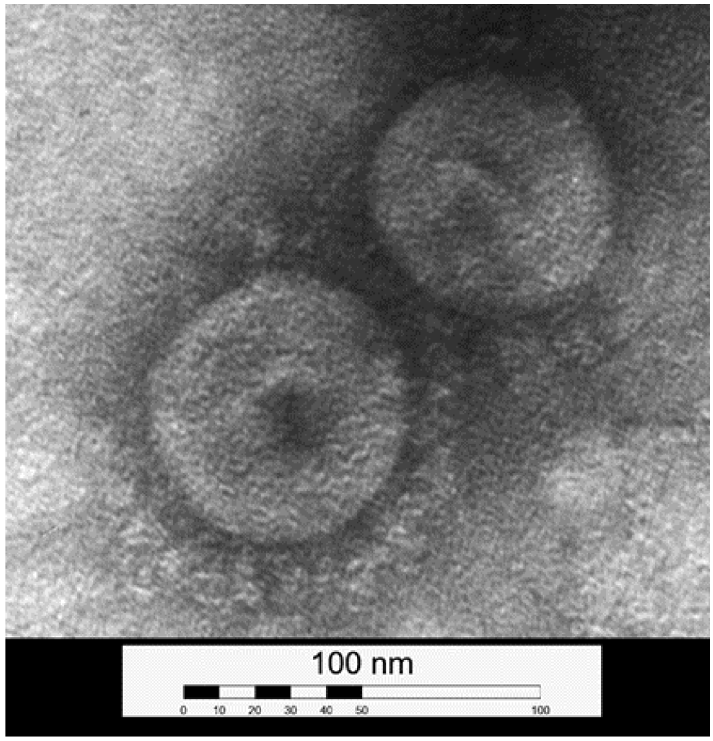

Figure 1. Immune transmission electron microscopic image of canine coronavirus (CCoV)–human pneumonia (HuPn)–2018 from an A72 cell culture. The sample was incubated with anti–CCoV guinea pig serum, leading to the specific viral antibody aggregates. Scale bar represents 100 nm.

findings in other CCoVs [25]. Unlike CCoV I, some FCoV and all betacoronaviruses and gammacoronaviruses, the characteristic multibasic motif (RRXRR)–furin recognition site was absent in the S protein of CCoV-HuPn-2018, suggesting that the virus carries an uncleaved S protein, similarly to most other alphacoronaviruses [15]. Thus, this novel strain shares more similarities with CCoV-II strains.

Surprisingly, there were no unique deletions or insertions in the S protein of CCoV-HuPn-2018. There were also a total of 5 amino acid differences between CCoV-HuPn-2018 and CCoV UCD-1 in the S1 domain; however, these amino acids were identical to those found in the TGEV Purdue S1 and were not unique.

The E protein was 81 amino acids long and did not contain any N-glycosylation sites, whereas 3 N-glycosylated residues have been predicted in each the 261–amino acid M and the 370–amino acid N proteins (Supplementary Figure 3*B* and 3*C*), similar to findings in several other FCoV/CCoV strains. While no evidence of recombination was observed for E, M or N proteins, the N protein contained a unique 12–amino acid deletion within the SR-rich region (located between amino acids 164 and 177 for other CCoV strains). The presence of this deletion was confirmed in the original NSP samples 1116 and 1153.

The 3 ORFs, 3a, 3b, and 3c, between the S and E genes encoded for proteins with sizes of 71, 71, and 244 amino acids, respectively. ORF3, previously found in CCoV I genomes only [14, 25], was not present in the new strain. The 3' end accessory protein gene 7a encoded for 101 amino acids, while there were at least 2 forms of 7b: full-length (213 amino acids) and the one with a 227-nucleotide deletion (leading to a frame shift and premature truncation of the putative protein).

DISCUSSION

A previous study identified 8 patients with pneumonia who had molecular evidence of CCoV in their NPS specimens [17]. Partial sequencing and BLASTn analysis suggested that these were closely related but distinct CCoV variants (Supplementary Table 1). The 8 patients with pneumonia were mainly children living in longhouses or villages in rural or suburban areas, where domestic animal and jungle wildlife exposure with the family is common.

In the current study, we confirmed the presence of CCoV with different, less sensitive 1-step RT-PCR assays in 2 specimens, grew a virus in A72 cells from 1 specimen, and conducted a complete genome sequence analysis of the CCoV. Our results demonstrated that CCoV-HuPn-2018 is a novel canine-feline–like recombinant strain with a unique N. To our knowledge, this is the first report suggesting that a CCoV without major genomic rearrangements or adaptive modifications in the S protein might replicate in association with pneumonia in a human host.

Table 3. Complete Genome, Individual Gene Length, and Other Characteristics of Canine Coronavirus–Human Pneumonia–2018

Genomic Region or ORF No.	Coding Sequence	Length, Nucleotides	Putative TRS Start		Protein Name	Protein Size, Amino Acids	Note
			Nucleotide Position	Sequence			
5′ UTR	No	313	No	No	No	No	Similar to other CCoVs
3′ UTR	No	275	No	No	No	No	
ORF1b	314–20 374	20 061	90	TCGAA*CTAAACGAAAT*	Pp1ab	6686	Putative ribosomal slippage is at position 12 339
ORF2	20 371–24 717	4347	20 335	GTTA*CTAAACTTTG*	S	1448	Recombinant structure with the S1 domain most closely related to CCoV UCD-1 and the S2 domain most closely related to FCoV WSU 79-1683
ORF3a	24 820–25 035	216	24 787	AGAA*CTAAACTTATG*	3a	71	Only 1 TRS before 3a was found; 3a, 3b, and 3c are likely to be expressed from polycistronic mRNAs
ORF3b	24 980–25 195	216	No	No	3b	71	
ORF3c	25 192–25 926	735	No	No	3c	244	
ORF4	25 913–26 158	246	25 866	GGTT*CTAAACGAAAT*	E	81	No unique features
ORF5	26 169–26 954	786	26 156	TGAA*CTAAACAAAAT*	M	261	
ORF6	26 967–28 079	1113	26 951	ATAA*CTAAACTTCTA*	N	370	Contains 36-nucleotide deletion in middle region
ORF7a	28 084–28 389	306	28 072	CGAA*CTAAACGAATG*	7a	101	Only 1 TRS before 7a is located; 7b is expressed from polycistronic mRNAs
ORF7b	28 394–28 808/29 035	415/642	No	No	7b	34/213	Truncated, likely nonfunctional; contains an out-of-frame 227-nucleotide deletion close to its 5′ end, followed by premature stop codons and full-length forms

Abbreviations: CCoV, canine coronavirus; FCoV, feline coronavirus; mRNAs, messenger RNAs; ORF, open reading frame; TRS, transcription regulatory sequence; UTR, untranslated region.

The conducted analyses demonstrated that the newly identified CCoV-HuPn-2018 was most closely related to CCoV TN-449, while its S1 and S2 domains shared the highest nucleotide identity with CCoV UCD-1 and FCoV WSU 79-1683, respectively. These findings are suggestive of the recombinant nature of this strain, similar to many previously characterized CCoVs [24]. Phylogenetic and recombinational analyses confirmed that CCoV-HuPn-2018 was only distantly related to other *Alphacoronavirus* species, including HCoVs (229E and NL63) and bat CoVs, and likely originated via multiple recombination events between different *Alphacoronavirus 1* strains, but not other alphacoronaviruses. The ability of the novel strain to replicate in A72 canine cells, the absence of ORF3, the higher overall similarity with CCoV-II strains (TN-449 and HLJ-073), and the lack of the furin cleavage site between S1 and S2 domains suggest that the strain belongs to CCoV genotype II [25].

The unique feature not found in any other known CCoVs and *Alphacoronavirus 1* species—namely, the 12–amino acid deletion in the middle portion of the N protein—was confirmed in both original NSP samples, 1153 and 1116. While insertions or deletions in the N protein are not found among the known *Alphacoronavirus 1* strains, the deletion of the SR-rich domain within the middle region of SARS-CoV N protein reportedly resulted in dramatic changes in its cellular localization soon after its zoonotic transmission [26]. Thus, similar to SARS-CoV, CCoV-HuPn-2018 possesses some unique genetic features suggestive of recent zoonotic transmission. Notably, such N protein rearrangements are characteristic of SARS-CoV/SARS-CoV-2 with higher case fatality rates [27].

While SARS-CoV and FCoV NSP7b was not essential for viral replication in vitro and in vivo experiments, its deletion or truncation may be associated with attenuated phenotype [28]. Disruption in the expression of the NSPs after zoonotic transmission of SARS-CoV was reported previously, suggesting that it may represent an adaptive mechanism [29]. Finally, deletions unique to FIPVs were found in ORFs 3c and/or 7b and were hypothesized to be responsible for the shift from enteric (FCoV) to FIPV phenotype and increased pathogenicity [30]. The ability of CCoV to evolve quickly through frequent recombination events and induce disease of variable severity is even more concerning, given that these data indicating that circulating CCoV may already be transmittable to humans.

The current study had a number of limitations. First, we have not met recognized standards of causality, such as Koch postulates or Bradford Hill criteria. Second, we recognize that the detected CCoVs could only be "carried" in some of the 8 patients' airways, not causing disease. However, identification of (1) FCoV-like CoVs in influenza-negative patients with acute respiratory symptoms in Arkansas and (2) porcine deltacoronavirus in children in Haiti further emphasizes that *Alphacoronavirus 1* species may be infectious or pathogenic to humans [18, 31].

In conclusion, we recovered and characterized a novel recombinant CoV, CCoV-HuPn-2018, from a hospitalized patient

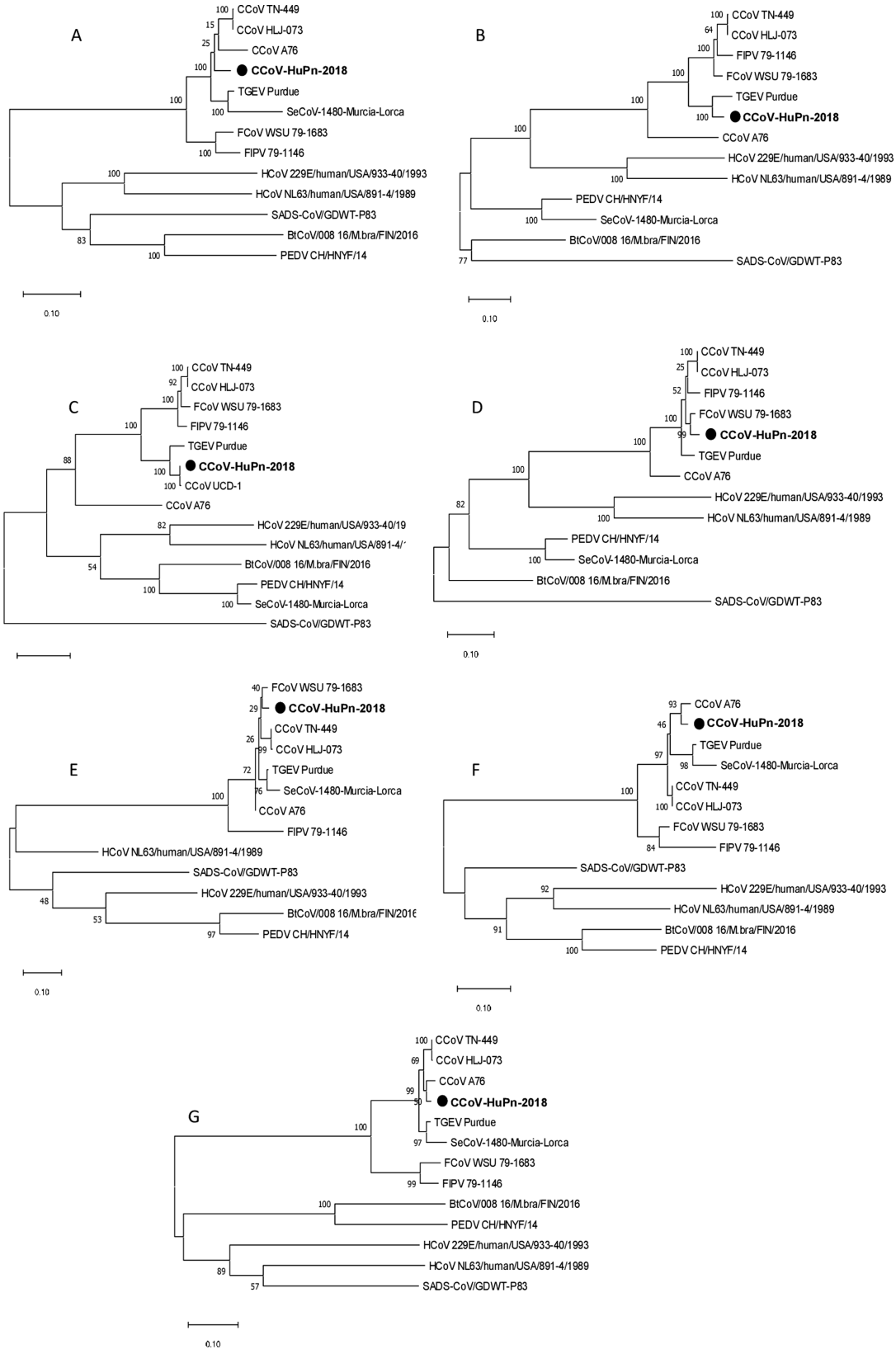

Figure 2. Phylogenetic tree based on complete genome (*A*), S gene (*B*), S1 (*C*), S2 domain (*D*), E gene (*E*), M gene (*F*) and N gene (*G*) sequences of the canine coronavirus (CCoV)–human pneumonia (HuPn)–2018 viral isolate and other *Alphacoronavirus* species. Bootstrap values are represented at key nodes. Scale bar indicates nucleotide substitutions per site. The evolutionary history was inferred using the maximum likelihood method and the general time-reversible model. This analysis involved 13 nucleotide sequences. Evolutionary analyses were conducted using MEGA X software. Black circles represent the newly identified viral isolate, CCoV-HuPn-2018. Abbreviations: BtCoV, bat coronavirus (CoV); FCoV, feline CoV; FIPV, feline infectious peritonitis virus; HCoV, human CoV; PEDV, porcine epidemic diarrhea virus; SADS-CoV, swine acute diarrhea syndrome CoV; SeCoV, Swine enteric CoV; TGEV, transmissible gastroenteritis virus.

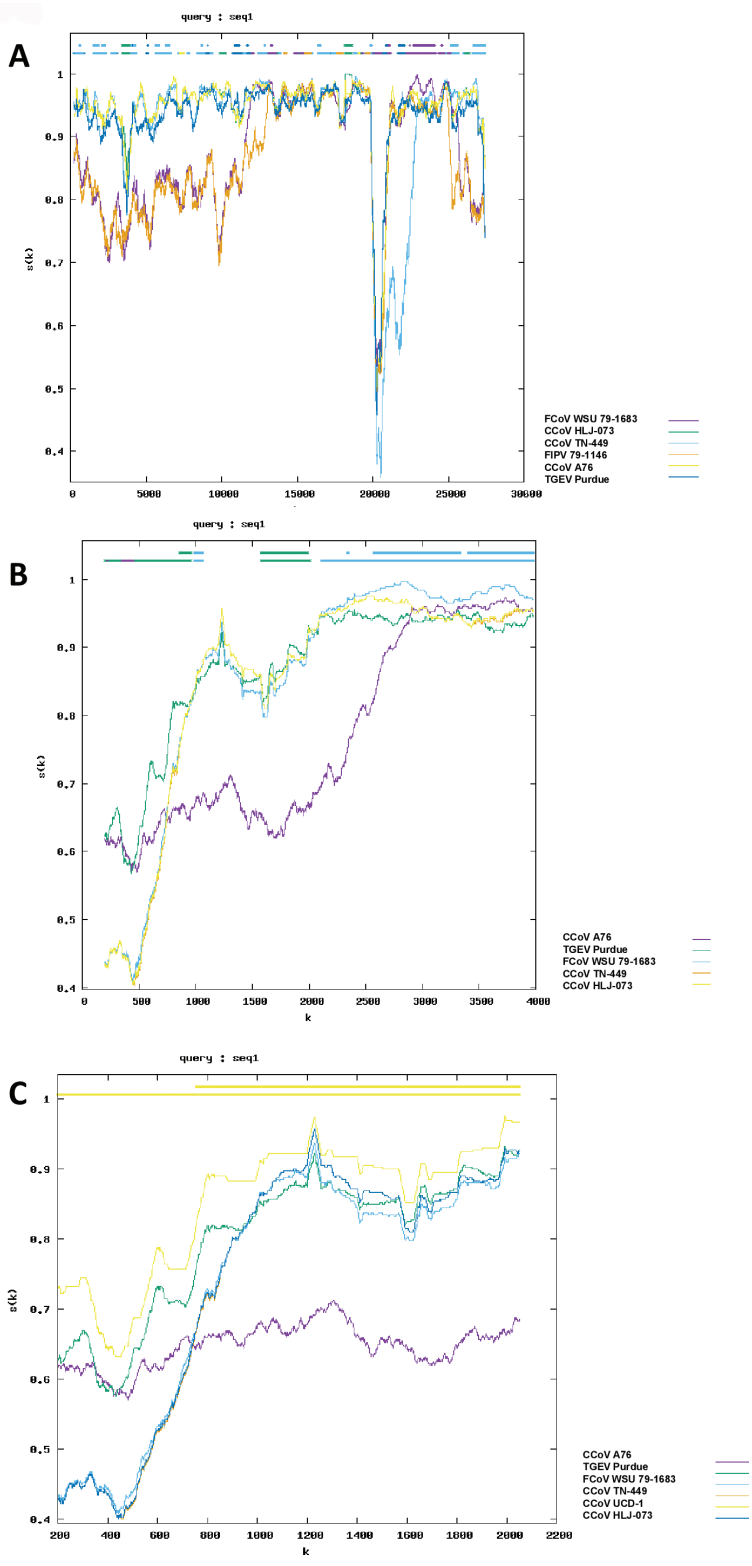

Figure 3. Recombinational analysis of the canine coronavirus (CCoV)–human pneumonia (HuPn)–2018 complete genome (*A*), S1 (*B*) and S2 (*C*) domains. At each position of the window, the query sequence CCoV-HuPn-2018 was compared with background sequences for 6 strains shown in the legend on the right. The x-axes represent the length of the sequence, and the y-axes, the similarity value (Similarity = Match Fraction = 1 - distance). The two bars on the top of the graph represent the "best match" (lower bar), and the significance of this match (upper bar). The "best match" sequence is the background sequence with the highest similarity to the query. The upper bar is also colored at a position when the best match is significantly better than the second match. Arrows represent potential recombination break points. Abbreviations: FCoV, feline coronavirus; FIPV, feline infectious peritonitis virus; TGEV, transmissible gastroenteritis virus.

with pneumonia. While possessing some unique characteristics likely suggestive of a recent zoonotic transmission, this novel strain with recombinant CCoV UCD-1/FCoV WSU 79-1683 S protein shares multiple genomic features of widespread CCoV-II. Further studies are needed to investigate CCoV prevalence, seroprevalence, and pathogenic potential in humans. Additional studies should be conducted to evaluate the biological relevance of the observed deletion in the N protein.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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The study—source of nasopharyngeal swab samples—has received a scientific review, and all procedures followed were in accordance with the ethical standards of the Malaysian Ministry of Health's Medical Research and Ethics Committee (protocol NMRR-17-316-34395), the Duke University Health System Institutional Review Board, the Duke-NUS Medical School Ethical Review Board, and the Naval Medical Research Center–Asia Human Research Protection Program (HRPO no. W911QY-16-D-0058).

Author Contributions. A. N. V. designed, oversaw, and provided financial support for the experiments on canine coronavirus (CCoV)–human pneumonia (HuPn)–2018 characterization and sequences, sequenced parts of the genome, analyzed the data, and wrote the manuscript. A. D. conducted most of the experiments on CCoV-HuPn-2018 cell culture isolation and Sanger sequencing. D. D. conducted some of the experiments on Sanger sequencing. L. X. screened 301 samples and identified the 8 samples positive for coronavirus/CCoV. T. H. T. and J. S. Y. L. coordinated sample collection, obtained ethical clearance, and collected and processed the patient demographic data. L. J. S. critically revised the manuscript draft. G. C. G. led the original studies, oversaw the new study, provided financial support, and revised the manuscript.

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From:	Gray, Gregory C. on behalf of Gray, Gregory C. <gcgray@UTMB.EDU>
To:	Tseng, Chien-Te K.; Ralph Baric (rbaric@email.unc.edu); Vlasova, Anastasia; Menachery, Vineet; eric.laing@usuhs.edu; Perlman, Stanley; Lednicky,John
Cc:	Marushchak, Lyudmyla; Pulscher, Laura
Subject:	Research discussions regarding CCoV-HuPn-2018
Date:	Friday, May 12, 2023 6:21:39 AM
Attachments:	Susceptibility of different cell lines to the novel canine (IORV 2021 Abdelqadir).pdf Isolation of a Novel Recombinant Canine Coronavirus from a Visitor to Haiti (Clin Infect Dis 2021 Lednicky).pdf Novel Canine Coronavirus Isolated from a Hospitalized Patient With Pneumonia in East Malaysia (Clin Infect Dis 2022 Vlasova).pdf image001.png

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Dear Kent, Ralph, Anastasia, Vineet, Eric, Stan, and John

To avoid duplicated efforts a number of us thought we might want to hold a Zoom meeting to discuss research plans regarding CCoV-HuPn-2018 and HuCCoV_Z19Haiti (see attached). I know some cloning and serologic assay development work is underway.

If you are available and want to join us, this link will show you proposed times. All you need to do is click on when is good for you... http://whenisgood.net/85dtpnx

Thanks much!

Greg

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Rev. 11/17/22				PPT?	Presentation #	Code	Session / Presentation Title	Speaker
THURSDAY - NOV 17, 2022								
Day of Congress	**Begin**	**End**	**Room**			**Code**	**Workshops (Pre-Congress)**	
					W1		**Developing the Role of the ID pharmacist**	
11/17/22	8:00	12:00	Room 304 / 305		**W1.1**	**W1**	**Developing the Role of the ID pharmacist**	Sasheela - Introduction
11/17/22	8:00	12:00	Room 304 / 305		**W1.2**	**W1**	What does antimicrobial stewardship look like where you are? Narratives on pharmacist and nurse roles from around the world	Esmita Charani
11/17/22	8:00	12:00	Room 304 / 305		**W1.3**	**W1**	The role of community pharmacists in antimicrobial stewardship – current practices and opportunities for improvement	Oluchi Mbamalu
11/17/22	8:00	12:00	Room 304 / 305		**W1.4**	**W1**	Current practice in Malaysia and how to develop a strategy for greater pharmacist involvement in AMS	Petrik Periyasamy
11/17/22	8:00	12:00	Room 304 / 305		**W1.6**	**W1**	Pharmacist led quality improvement initiatives in AM	Noor Shamas
11/17/22	8:00	12:00	Room 304 / 305		**W1.7**	**W1**	Role of AMS pharmacist in Malaysia	Rahela ambaras khan
11/17/22	13:00	17:00	Room 302 / 303		**W2**	**W2**	**Innovations in Rapid Diagnostics**	***Regional Focus***
11/17/22	13:00	17:00	Room 302 / 303		**W2.1**	**W2**	Taking an Idea from the Lab to Commercialization	Jesus Rodriguez Manzano
11/17/22	13:00	17:00	Room 302 / 303		**W2.2**	**W2**	R&D Challenges and Emerging Diagnostic Technologies	Travis Schlappi
11/17/22	13:00	17:00	Room 302 / 303		**W2.3**	**W2**	Integration/Deployment of Diagnostic Tools into the Clinic with Particular Focus in Asian Countries	Sophie Yacoub
11/17/22	13:00	17:00	Room 304 / 305		**W3**	**W3**	**Misinformation: Responding to Social Media with Science**	
11/17/22	13:00	17:00	Room 304 / 305		**W3.1**	**W3**		Enrique Castro-Sanchez
11/17/22	13:00	17:00	Room 304 / 305		**W3.2**	**W3**		Edson C. Tandoc Jr
11/17/22	13:00	17:00	Room 304 / 305		**W3.3**	**W3**		Edmund Lee Wei Jian
								Seth Seet Kai
FRIDAY - NOV 18, 2022								
							MEET-THE-EXPERT sessions	
11/18/22	8:00	9:00	Room 304 / 305		**M1**	**M1**	**Urinary tract infections - Updates in prevention and management**	
11/18/22	8:00	9:00	Room 304 / 305		**M1.1**	**M1**	cUTI and resistance of gram negative bacteria	Sally Roberts
11/18/22	8:00	9:00	Room 304 / 305		**M1.2**	**M1**	Preventing Urinary tract infections in adults	Ting Soo Chow
11/18/22	8:00	9:00	Room 302 / 303		**M2**	**M2**	**Meet-the-Editors**	
11/18/22	8:00	9:00	Room 302 / 303		**M2.1**	**M2**	IJID	Shui Shan Lee
11/18/22	8:00	9:00	Room 302 / 303		**M2.2**	**M2**	IJID One Health	Lucille Blumberg
11/18/22	8:00	9:00	Room 302 / 303		**M2.3**	**M2**	The Lancet Digital Health	Rupa Sarkar
11/18/22	8:00	9:00	Room 306		**M3**	**M3**	**Bacterial Infections - Meet the expert**	
11/18/22	8:00	9:00	Room 306		**M3.1**	**M3**	The utility of randomised controlled trials in optimal treatment of bacteraemia	David Lye
11/18/22	8:00	9:00	Room 306		**M3.2**	**M3**	Clinical evidence for therapy of gram-negative bacterial infections	Sasheela Ponnampalavanar

			Room 306		M3.3	M3	Precision Antibiotic Dosing for Treatment of Severe GN Infections	Jason Roberts
11/18/22	9:15	10:00	CH2		P1	P1	**PLENARY I - National COVID-19 Responses**	
11/18/22	9:15	10:00	CH2		P1.1	P1	Moderator: **Alison Holmes**	
11/18/22	9:15	10:00	CH2		P1.2	P1	Moderator: Chen Chien Jen	
11/18/22	9:15	10:00	CH2		P1.3	P1	Malaysia's COVID-19 Response	Norhayati Rusli
11/18/22	9:15	10:00	CH2		P1.4	P1	Ireland's COVID-19 Response	Mary Horgan
11/18/22	9:15	10:00	CH2		P1.5	P1	Barbado's COVID-19 Response	Corey Forde
							PARALLEL SYMPOSIA- Morning	
11/18/22	10:30	12:00	Banquet Hall		S1	S1	**One Health session: Serosurveillance of High Consequence Zoonotic Viruses at the Human-Animal Interface**	
11/18/22	10:30	12:00	Banquet Hall		S1.1	S1	**Co-Chair: Latiffah Hassan**	
11/18/22	10:30	12:00	Banquet Hall		S1.2	S1	Zoonoses at a One Health interface In South Africa- it's the little five not the big five!	Lucille Blumberg
11/18/22	10:30	12:00	Banquet Hall		S1.3	S1	Insight of zoonotic viruses at human-animal interfaces in Cambodia	Veasna Duong
11/18/22	10:30	12:00	Banquet Hall		S1.4	S1	COVID-19: a multi-host pandemic	Pablo Beldomenico
11/18/22	10:30	12:00	Banquet Hall		S1.5	S1	Bat antibody dynamics in time: what can we learn about pathogen dynamics from multiplexed Luminex serological assays	Eric Laing
11/18/22	10:30	12:00	CH3		S2	S2	**Clinical management of hard to treat infections (case-based discussions) – (in collaboration with MSIDC)**	
11/18/22	10:30	12:00	CH3		S2.1	S2	**Co-Chair: Sasheela**	
11/18/22	10:30	12:00	CH3		S2.2	S2	**Co-Chair: Paul Tambyah**	
11/18/22	10:30	12:00	CH3		S2.3	S2	Management of (recurrent or) persistent MRSA infection	Paul Tambyah
11/18/22	10:30	12:00	CH3		S2.4	S2	Optimising treatment for carbapenemase producing Enterobacterales	David Lye
11/18/22	10:30	12:00	CH3		S2.5	S2	Drug resistant enteric pathogens	Priscilla Rupali
	10:30	12:00	CH3		S2.6	S2	Issues to consider for MDR-Acinetobacter baumannii treatment	Anucha Apisarnthanarak
11/18/22	10:30	12:00	CH1		S3	S3	**Infectious disease innovations for a digital world**	
11/18/22	10:30	12:00	CH1		S3.1	S3	**Co-chairs: Rupa Sarkar**	
11/18/22	10:30	12:00	CH1		S3.2	S3	**Co-chairs: Sean Wasserman**	
11/18/22	10:30	12:00	CH1		S3.3	S3	**Getting to better pandemic and epidemic preparedness**	Philip AbdelMalik
11/18/22	10:30	12:00	CH1		S3.4	S3	Spatial epidemiology and malaria elimination strategies	Iqbal Elyazar
11/18/22	10:30	12:00	CH1		S3.5	S3	Digital Publishing- Preprints and Open Access Publishing: What Happens Next?	Gonzalo Bearman
11/18/22	10:30	12:00	Room 304 / 305		Oral1	Oral1	**Oral Abstracts Session 1- Genomics and Infectious Diseases**	
11/18/22	10:30	12:00	Room 304 / 305		Oral1	Oral1	**Co-Chair: Kalisvar Marimuthu**	

11/18/22	10:30	12:00	Room 304 / 305		**Oral1.1**	**Oral1**	IDENTIFYING THE NEXT MUTATION OF CONCERN - EXPLOITING GENOMICS FOR TRACKING SARS-COV-2 MUTATIONS AND THEIR BIOLOGICAL IMPACT IN CANADA	Dr. Muhammad Zohaib Anwar
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.2**	**Oral1**	RESISTOME PROFILES AND GENOME DYNAMICS OF MULTI-DRUG RESISTANT SHIGELLA SPP. ISOLATED IN BANGLADESH	Mr. Asaduzzaman Asad
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.3**	**Oral1**	GENETIC VARIATION IN PENICILLIN-BINDING GENES 1A, 2B, AND 2X OF STREPTOCOCCUS PNEUMONIAE CAUSING INVASIVE PNEUMOCOCCAL DISEASE IN INDIAN CHILDREN	Dr. MUTHUMEENAKSHI BHASKARAN
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.4**	**Oral1**	A DECADE OF STUDY ON K.PNEUMONIAE CAPSULAR TYPE DISTRIBUTION IN INDIA – REVEALS HIGH DIVERSITY AND ITS IMPLICATION IN VACCINE DEVELOPMENT	Dr. Nagaraj Geetha
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.5**	**Oral1**	DIVERSE GENETIC BACKGROUND OF MULTIDRUG RESISTANT PSEUDOMONAS AERUGINOSA CIRCULATING IN INDIA	Dr. Nagaraj Geetha
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.6**	**Oral1**	THE GENOMIC POPULATION STUDY OF BLOODSTREAM ASSOCIATED ESCHERICHIA COLI IN 2020 IN SOUTHWEST, UK	Ms. Winnie Lee
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.7**	**Oral1**	GENOMIC ANALYSIS TO UNDERSTAND NON-TYPHOIDAL SALMONELLA CARRIAGE: SALMONELLA AGONA – THE BUG THAT WON'T GO AWAY	Ms. Winnie Lee
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.8**	**Oral1**	GENOMIC DIVERSITY AND RESISTOME PROFILING OF MULTI-DRUG RESISTANT SALMONELLA ENTERICA SUBSP. ENTERICA ISOLATED IN BANGLADESH	Dr. Suraia Nusrin
11/18/22	10:30	12:00	Room 304 / 305		**Oral1.9**	**Oral1**	A PROSPECTIVE CLINICAL STUDY ON THE USE OF A NON-INVASIVE WEARABLE DEVICE AND NEURAL NETWORK MODELS FOR PATIENTS WITH DENGUE	Bernard Hernandez and Chanh Ho Quang
11/18/22	10:30	12:00	Room 302 / 303		**S4**	**S4**	**HIV - Hot Topics**	
11/18/22	10:30	12:00	Room 302 / 303		**S4.1**	**S4**	**Co-chairs: Adeeba Kamarulzaman**	
11/18/22	10:30	12:00	Room 302 / 303		**S4.2**	**S4**	**Co-chairs: Nittaya Phanuphak nittaya.p@ihri.org**	
11/18/22	10:30	12:00	Room 302 / 303		**S4.3**	**S4**	HIV Pre-Exposure Prophylaxis (PrEP) Updates for the Infectious Disease (ID) Physician	Raja Iskandar Raja Azwa
11/18/22	10:30	12:00	Room 302 / 303		**S4.4**	**S4**	Updates on ART	Adeeba Kamarulzaman
11/18/22	10:30	12:00	Room 302 / 303		**S4.5**	**S4**	Self Testing in HIV	Vu Ngoc Bao
	10:30	12:00	Room 302 / 303		**S4.6**	**S4**	A people centred health systems approach to living long with HIV	Jeffrey Lazarus
11/18/22	10:30	12:00	Theatre		**PD1**	**PD1**	**Policy Discussion - Health systems resilience**	
11/18/22	10:30	12:00	Theatre		**PD1.1**	**PD1**	Moderator: **Christopher Lee**	
11/18/22	10:30	12:00	Theatre		**PD1.2**	**PD1**	TBD	Corey Forde
11/18/22	10:30	12:00	Theatre		**PD1.3**	**PD1**	ID training and capacity building	Marc Mendelson
							WORKING GROUP	
11/18/22	12:30	13:45	Room 304 / 305		**W4**	**W4**	**Guide to Infection Control Working Group**	
11/18/22	12:30	13:45	Room 304 / 305		**W4.1**	**W4**	The Guide- Last 2 years in review	Gonzalo Bearman
11/18/22	12:30	13:45	Room 304 / 305		**W4.2**	**W4**	Fungal Outbreaks: Implications for IPC	Anucha Apisarnthanarak

							PARALLEL SYMPOSIA - Afternoon	
11/18/22	14:00	15:30	CH3		**S5**	**S5**	**Perspectives from Emerging Leaders Session**	
11/18/22	14:00	15:30	CH3		**S5.1**	**S5**	**Chair: Esmita Charani**	
11/18/22	14:00	15:30	CH3		**S5.2**	**S5**	Challenges And Solutions Towards Antimicrobial Stewardship Implementation	Afreenish Amir
11/18/22	14:00	15:30	CH3		**S5.3**	**S5**	Infectious Diseases in the COVID-19 era	Tatiana Pinto
11/18/22	14:00	15:30	CH3		**S5.4**	**S5**	One Health perspectives for responding to pandemics	**David Moore**
11/18/22	14:00	15:30	Banquet Hall		**S6**	**S6**	**Debate: Antibiotics for diarrheal disease**	
11/18/22	14:00	15:30	Banquet Hall		**S6.1**	**S6**	**Moderator: Senjuti Saha**	
11/18/22	14:00	15:30	Banquet Hall		**S6.2**	**S6**		Adebola Olayinka
11/18/22	14:00	15:30	Banquet Hall		**S6.3**	**S6**		**Suresh Kumar**
11/18/22	14:00	15:30	Room 302 / 303		**S7**	**S7**	**Respiratory infections**	
11/18/22	14:00	15:30	Room 302 / 303		**S7.1**	**S7**	**Co-chair: Bill Davis**	
11/18/22	14:00	15:30	Room 302 / 303		**S7.2**	**S7**	**Co-chair: Nicholas Feasey**	
11/18/22	14:00	15:30	Room 302 / 303		**S7.3**	**S7**	Impact of influenza in South East Asia	Yasmin Abdul Malik
11/18/22	14:00	15:30	Room 302 / 303		**S7.4**	**S7**	USA H5N1 HPAI response	Krista Kniss
11/18/22	14:00	15:30	Room 302 / 303		**S7.5**	**S7**	Pan-respiratory disease surveillance	Bill Davis
11/18/22	14:00	15:30	Room 304 / 305		**Oral2**	**Oral2**	**Oral Abstracts Session 2 - Tuberculosis & Other Mycobacterial Infections**	
11/18/22	14:00	15:30	Room 304 / 305		**Oral2**	**Oral2**	Co-Chairs: Sean Wasserman, Cynthia Chee	
11/18/22	14:00	15:30	Room 304 / 305		**Oral2.1**	**Oral2**	CARE SEEKING AND DELAYS IN CASE OF DRUG-RESISTANT TUBERCULOSIS PATIENTS IN BANGLADESH	Dr. Md Zulqarnine Ibne Noman
11/18/22	14:00	15:30	Room 304 / 305		**Oral2.2**	**Oral2**	HEALTH SYSTEM RELATED BARRIERS TO MULTIDRUG-RESISTANT TUBERCULOSIS (MDR-TB) CARE IN AN INDIAN SETTING: FROM PATIENTS' PERSPECTIVE	Mrs. Jayshri Deepak Jagtap
11/18/22	14:00	15:30	Room 304 / 305		**Oral2.3**	**Oral2**	COMBINATION OF LIGAND-BASED PHARMACOPHORE MODELLING, MOLECULAR DYNAMICS, AND DEEP LEARNING APPROACH TO IDENTIFY SELECTIVE PANK INHIBITORS AS ANTITUBERCULAR AGENTS.	Mr. Prakash Jha
11/18/22	14:00	15:30	Room 304 / 305		**Oral2.4**	**Oral2**	CENTRAL NERVOUS SYSTEM TUBERCULOSIS IMMUNOPATHOLOGY IS DRIVEN BY MATRIX DESTRUCTION WITH MATRIX METALLOPROTEINASES INHIBITION REDUCING INFLAMMATION AND IMPROVING SURVIVAL	Dr. Fei Kean Loh
11/18/22	14:00	15:30	Room 304 / 305		**Oral2.5**	**Oral2**	TUBERCULOSIS IN PATIENTS CO-INFECTED WITH VISCERAL LEISHMANIASIS AND HIV – A NEW DIAGNOSTIC AND MANAGEMENT CHALLENGE	Mr. Raman Mahajan Mahajan
11/18/22	14:00	15:30	Room 304 / 305		**Oral2.6**	**Oral2**	EXPLORING INTER-REGULATORY GENE NETWORK DERIVED CANDIDATE GENES IMPARTING RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS	Dr. Muhammad Ahsan Ahsan Naeem
11/18/22	14:00	15:30	Room 304 / 305		**Oral2.7**	**Oral2**	IMMUNOPATHOLOGY OF DIABETES MELLITUS IN PULMONARY TUBERCULOSIS IS DRIVEN BY NEUTROPHIL HYPER-INFLAMMATORY DYSFUNCTION: DATA FROM A CELLULAR MODEL AND HUMAN COHORT.	Ms. Pei Min Thong

11/18/22	14:00	15:30	Theatre		**S8**	**S8**	**Enterovirus Infections in Asia Pacific – Critical Updates (Member-proposed Symposium)**	
11/18/22	14:00	15:30	Theatre		**S8.1**	**S8**	**Co-chair: Chow Ting Soo**	
11/18/22	14:00	15:30	Theatre		**S8.2**	**S8**	**Co-chair: Robert Heyderman**	
11/18/22	14:00	15:30	Theatre		**S8.3**	**S8**	Asia-Pacific Network for Enterovirus Surveillance overview and development of enterovirus A71 vaccines	Min-Shi Lee
11/18/22	14:00	15:30	Theatre		**S8.4**	**S8**	Laboratory Diagnosis and Management of Enterovirus Infections	Yoke Fun Chan
11/18/22	14:00	15:30	Theatre		**S8.5**	**S8**	Antiviral strategies for human enteroviruses.	Justin Chu
11/18/22	14:00	15:30	CH1		**S9**	**S9**	**Dengue Updates**	
11/18/22	14:00	15:30	CH1		**S9.1**	**S9**	**Co-Chair: Sophie Yacoub**	
11/18/22	14:00	15:30	CH1		**S9.2**	**S9**	**Co-Chair: Therese Umuhoza**	
11/18/22	14:00	15:30	CH1		**S9.3**	**S9**	Innovations in Monitoring Patients with Dengue	Sophie Yacoub
11/18/22	14:00	15:30	CH1		**S9.4**	**S9**	Immune Responses & Severe Dengue	Neelika Malavige
11/18/22	14:00	15:30	CH1		**S9.5**	**S9**	New proactive paradigm for dengue surveillance using GOS traps and NS1 kits	Datin Indra
11/18/22	14:00	15:30	CH1		**S9.6**	**S9**	Dengue Vaccine Implementation Updates	Jaime Torres
11/18/22	16:00	17:00	CH2		**P2**	**P2**	**PLENARY II**	
11/18/22	16:00	17:00	CH2		**P2**	**P2**	**Stopping the next pandemic before it strikes**	
11/18/22	16:00	17:00	CH2		**P2.1**	**P2**	**Chair: Alison Holmes**	
11/18/22	16:00	17:00	CH2		**P2.2**	**P2**		Chen Chien Jen
11/18/22	16:00	17:00	CH2		**P2.3**	**P2**		Jeffrey Lazarus
SATURDAY - NOV 19, 2022								
11/19/22	8:00	9:00	Room 302 / 303				**MEET-THE-EXPERT sessions**	
11/19/22	8:00	9:00	Room 302 / 303		**M4**	**M4**	**Surgical infections and antibiotic use**	
11/19/22	8:00	9:00	Room 302 / 303		**M4.1**	**M4**		Marc Mendelson
11/19/22	8:00	9:00	Room 302 / 303		**M4.2**	**M4**		Sasheela Sri La Sri Ponnampalavanar
11/19/22	8:00	9:00	Room 304 / 305		**M5**	**M5**	**Career in Public Health/Global Health**	
11/19/22	8:00	9:00	Room 304 / 305		**M5.1**	**M5**		Wan Noraini Wan Mohamed Noor
11/19/22	8:00	9:00	Room 304 / 305		**M5.2**	**M5**		Paul Tambyah
11/19/22	8:00	9:00	Theatre		**M6**	**M6**	**Meet the ProMED Moderators**	
11/19/22	8:00	9:00	Theatre		**M6.1**	**M6**	ProMED moderators	Ghassan Matar
11/19/22	8:00	9:00	Theatre		**M6.2**	**M6**		Pablo Beldomenico
11/19/22	8:00	9:00	Theatre		**M6.3**	**M6**		Jorge Gonzalez Mendoza
11/19/22	8:00	9:00	Banquet Hall		**Oral3**	**Oral3**	**Oral Abstracts Session 3: Vaccines Developments**	
11/19/22	8:00	9:00	Banquet Hall		**Oral3**	**Oral3**	**Co-Chairs: Neelika Malavige**	
11/19/22	8:00	9:00	Banquet Hall		**Oral3**	**Oral3**	**Co-Chair: Cherry Kang**	

11/19/22	8:00	9:00	Banquet Hall		~~**Oral3.1**~~	~~**Oral3**~~	~~CUBAN VACCINES ABDALA AND MAMBISA AGAINST COVID-19~~	~~Dr. Gerardo Guillen~~
11/19/22	8:00	9:00	Banquet Hall		~~**Oral3.2**~~	~~**Oral3**~~	~~HEPATITIS B NUCLEOCAPSID PARTICLE AS IMMUNOPOTENTIATOR OF INNATE AND ADAPTIVE IMMUNITY FOR NASAL VACCINES CIGB 2020 AND MAMBISA AGAINST COVID-19~~	~~Dr. Gerardo Guillen~~
11/19/22	8:00	9:00	Banquet Hall		**Oral3.3**	**Oral3**	GLOBAL AND REGIONAL BURDEN OF ATTRIBUTABLE AND ASSOCIATED BACTERIAL ANTIMICROBIAL RESISTANCE AVERTABLE BY VACCINATION: MODELLING STUDY	Ms. Chaelin Kim
11/19/22	8:00	9:00	Banquet Hall		**Oral3.4**	**Oral3**	NUCLEOSIDE-MODIFIED MRNA VACCINES PROTECT IFNAR -/- MICE AGAINST CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS INFECTION	Prof. Ali Mirazimi
11/19/22	8:00	9:00	Banquet Hall		**Oral3.5**	**Oral3**	BRIDGING THE IMMUNOGENICITY OF A TETRAVALENT DENGUE VACCINE CANDIDATE (TAK-003) FROM CHILDREN AND ADOLESCENTS TO ADULTS	Dr. Carolina Halim
11/19/22	8:00	9:00	Banquet Hall		**Oral3.6**	**Oral3**	IMMUNOGENICITY OF A TETANUS TOXOID CONJUGATED QUADRIVALENT MENINGOCOCCAL VACCINE (MENACYW-TT) IN MENINGOCOCCAL VACCINE-NAÏVE TODDLERS, CHILDREN, ADOLESCENTS AND ADULTS USING AN RSBA ASSAY	Dr. Danaya Chansinghakul
11/19/22	9:15	10:00	CH2		**P3**	**P3**	**PLENARY III**	
11/19/22	9:15	10:00	CH2		**P3**	**P3**	**MONKEYPOX**	
11/19/22	9:15	10:00	CH2		**P3.1**	**P3**	**Chair: Paul Tambyah**	
11/19/22	9:15	10:00	CH2		**P3.2**	**P3**		Ifedayo Adetifa
11/19/22	9:15	10:00	CH2		**P3.3**	**P3**		William Welfare
							PARALLEL SYMPOSIA - Morning	
11/19/22	10:30	12:00	CH3		**S10**	**S10**	**Hepatitis C Elimination – Global Opportunities**	
11/19/22	10:30	12:00	CH3		**S10.1**	**S10**	**Chair: Ruksana Raihan**	
11/19/22	10:30	12:00	CH3		**S10.2**	**S10**	Malaysia's Hepatitis C Elimination Journey – Global Implications and Opportunities	
11/19/22	10:30	12:00	CH3		**S10.3**	**S10**	Egypt's Experience in the Elimination of Hepatitis C	Mohamed El-Kassas
11/19/22	10:30	12:00	CH3		**S10.4**	**S10**	Innovation and integration of HCV diagnostics to facilitate linkage to care	Sem XiaoHui
11/19/22	10:30	12:00	CH1		**S11**	**S11**	**AMR Challenges in the Post COVID-19 Era**	
11/19/22	10:30	12:00	CH1		**S11.1**	**S11**	**Co-Chair: Prof Margaret IP**	
11/19/22	10:30	12:00	CH1		**S11.2**	**S11**	Global burden of bacterial antimicrobial resistance	Jason Roberts
11/19/22	10:30	12:00	CH1		**S11.3**	**S11**	Patient-centered surveillance of drug-resistant infections	Priscilla Rupali
11/19/22	10:30	12:00	CH1		**S11.4**	**S11**	Learning from COVID-19 to tackle antibiotic resistance	Nicholas Feasey
11/19/22	10:30	12:00	Banquet Hall		**S12**	**S12**	**Global Genomic Infectious Disease Surveillance and Response**	
11/19/22	10:30	12:00	Banquet Hall		**S12.1**	**S12**	**Co-chair: tbd**	
11/19/22	10:30	12:00	Banquet Hall		**S12.2**	**S12**	Spotting unseen outbreaks using WGS	Kalisvar Marimuthu
11/19/22	10:30	12:00	Banquet Hall		**S12.3**	**S12**	TB genomics for public health	Cynthia Chee
11/19/22	10:30	12:00	Banquet Hall		**S12.4**	**S12**	Towards building capacity and accelerating genomic surveillance: Lessons from Banglades	Senjuti Saha

11/19/22	10:30	12:00	Room 304 / 305		**Oral4**	**Oral4**	**Oral Abstracts Session 4 - Antimicrobial Resistance**	
11/19/22	10:30	12:00	Room 304 / 305		**Oral4**	**Oral4**	**Co-Chair: Ariza Adnan**	
11/19/22	10:30	12:00	Room 304 / 305		**Oral4**	**Oral4**	**Co-Chair: Oluchi Mbamalu**	
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.1**	**Oral4**	EXTENT OF ANTIMICROBIAL RESISTANCE (AMR) IN AN ECOSYSTEM WITH ORGANIZED LIVESTOCK FARMING IN SRI LANKA.	Dr. Basnayake Mudiyanselage Yasodha Isani Bas
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.2**	**Oral4**	IN-VITRO ACTIVITY OF CEFIDEROCOL AGAINST CARBAPENEM-RESISTANT GRAM-NEGATIVE BACILLI: FIRST STUDY FROM INDIA	Dr. Akansha Didwania
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.3**	**Oral4**	THE CONTEXT OF ANTIBIOTIC USE IN BROILER POULTRY FARMS IN BANGLADESH: A QUALITATIVE EXPLORATION	Mr. S M Murshid Hasan
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.4**	**Oral4**	PREVALANCE OF LINEZOLID-RESISTANT VANCOMYCIN-RESISTANT ENTEROCOCCUS SPECIES (LRVRE) IN CLINICAL ISOLATES FROM TERTIARY CARE HOSPITAL OF NORTH INDIA – A REAL THREAT	Dr. Shakti Jain
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.5**	**Oral4**	PRE-CLINICAL DEVELOPMENT OF HUMAN MONOCLONAL ANTIBODIES TARGETING NOVEL, CELL WALL PROTEINS IN DRUG RESISTANT FUNGAL PATHOGENS	Dr. Soumya Palliyil
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.6**	**Oral4**	EMERGENCE OF RESISTANCE TO FLUOROQUINOLONES AND THIRD-GENERATION CEPHALOSPORINS IN SALMONELLA TYPHI IN LAHORE, PAKISTAN	Dr. Farhan Rasheed
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.7**	**Oral4**	LARGE RETROSPECTIVE WGS STUDY DESCRIBES GENOMIC EPIDEMIOLOGY OF S. AUREUS IN INDIA AND REVEALS TWO NOVEL MULTI-DRUG RESISTANT SUB-LINEAGES OF S. AUREUS CLONAL COMPLEX 22	Mr. Varun Shamanna
11/19/22	10:30	12:00	Room 304 / 305		**Oral4.8**	**Oral4**	INTRAVENOUS DOXYCYCLINE OR AZITHROMYCIN OR A COMBINATION OF THE TWO FOR TREATMENT OF SEVERE SCRUB TYPHUS: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL	Prof. George Varghese
11/19/22	10:30	12:00	Room 302 / 303		**S13**	**S13**	**The global rise of sexually transmitted infections**	
11/19/22	10:30	12:00	Room 302 / 303		**S13.1**	**S13**	**chair: Yasmin A Malik**	
11/19/22	10:30	12:00	Room 302 / 303		**S13.2**	**S13**	STI Diagnostics and the Multi-verse	Gabriel Yan
11/19/22	10:30	12:00	Room 302 / 303		**S13.3**	**S13**	Resurgence of syphilis	David Lewis
11/19/22	10:30	12:00	Room 302 / 303		**S13.4**	**S13**	Ongoing public efforts to develop new treatments for gonorrhea	Fernando Pascual
11/19/22	10:30	12:00	Theatre		**PD2**	**PD2**	**Policy Discussion - Pandemic Centers**	
11/19/22	10:30	12:00	Theatre		**PD2.1**	**PD2**	Moderator: **Mary Horgan**	
11/19/22	10:30	12:00	Theatre		**PD2.2**	**PD2**		Philip AbdelMalik
11/19/22	10:30	12:00	Theatre		**PD2.3**	**PD2**		Ifedayo Adetifa
11/19/22	10:30	12:00	Theatre					
							WORKSHOP	
11/19/22	12:30	13:45	Room 302 / 303		**W5**	**W5**	**Bring your manuscript - discuss it with the Editors**	
11/19/22	12:30	13:45	Room 302 / 303		**W5.1**	**W5**	IJID Editors	Shui Shan Lee
11/19/22	12:30	13:45	Room 302 / 303		**W5.2**	**W5**	IJID One Health	Lucille Blumberg
11/19/22	12:30	13:45	Room 302 / 303		**W5.4**	**W5**	Lancet Digital Health	Rupa Sarkar

							PARALLEL SYMPOSIA - Afternoon	
11/19/22	14:00	15:30	CH2		**S14**	**S14**	**Innovation in Infectious Diseases - Adoption and Implementation**	
11/19/22	14:00	15:30	CH2		**S14**	**S14**	Organized by Adeeba and colleagues	
11/19/22	14:00	15:30	CH2		**S14.1**	**S14**	Chair: Adeeba Kamarulzaman	
11/19/22	14:00	15:30	CH2		**S14.2**	**S14**	Digitisation & Health - Reflections from the COVID Pandemic	Helmi Zakariah
11/19/22	14:00	15:30	CH2		**S14.3**	**S14**	Overview on mRNA vaccines beyond COVID-19	Kiat Ruxrungtham
11/19/22	14:00	15:30	CH2		**S14.4**	**S14**	Rapid Diagnostics	Travis Schlappi
11/19/22	14:00	15:30	CH3		**S15**	**S15**	**Panel Discussion: Antimicrobials and Vaccines - Equitable Global Access**	
11/19/22	14:00	15:30	CH3		**S15.1**	**S15**	**Moderator: Alison Holmes**	
11/19/22	14:00	15:30	CH3		**S15.2**	**S15**	**Moderator: Esmita Charani**	
11/19/22	14:00	15:30	CH3		**S15.3**	**S15**	Equitable Access	Manica Balasegaram
11/19/22	14:00	15:30	CH3		**S15.4**	**S15**	Global vaccine equity	Cherry Kang
11/19/22	14:00	15:30	CH3		**S15.5**	**S15**	Global access to antimicrobials and the emergence of AMR in the COVID-19 era	Ghassan Matar
11/19/22	14:00	15:30	Banquet Hall		**S16**	**S16**	**Fungal resistance, diagnostics and therapy updates**	
11/19/22	14:00	15:30	Banquet Hall		**S16.1**	**S16**	**Co-Chairs: Leong Chee Loon**	
11/19/22	14:00	15:30	Banquet Hall		**S16.2**	**S16**	TBD	Louis Chai
11/19/22	14:00	15:30	Banquet Hall		**S16.3**	**S16**	Chronic histoplasmosis	Anna Rozaliyani
11/19/22	14:00	15:30	Banquet Hall		**S16.4**	**S16**	Burden of serious human fungal infections in Malaysia	Rukumani Devi Velayuthan
11/19/22	14:00	15:30	Room 304 / 305		**Oral5**	**Oral5**	**Oral Abstracts Session 5- Emerging & Re-emerging Infectious Diseases**	
11/19/22	14:00	15:30	Room 304 / 305		**Oral5**	**Oral5**	**Co-Chair: Nurul Azmawati Mohamed**	
11/19/22	14:00	15:30	Room 304 / 305		**Oral5**	**Oral5**	**Co-Chair: Yasmin A. Malik**	
11/19/22	14:00	15:30	Room 304 / 305		**Oral5.1**	**Oral5**	EMERGING VIRUSES ARE AN UNDERESTIMATED CAUSE OF UNDIAGNOSED FEBRILE ILLNESS IN UGANDA	Dr. Shirin Ashraf
11/19/22	14:00	15:30	Room 304 / 305		**Oral5.2**	**Oral5**	PHYLOGENETIC ANALYSIS OF DENGUE VIRUS TYPE 2 IN RECURRING OUTBREAKS IN MALAYSIA	Ms. Zur-Raiha Hamim
11/19/22	14:00	15:30	Room 304 / 305		**Oral5.3**	**Oral5**	NEUTROPHIL-LYMPHOCYTE RATIO IN GUILLAIN-BARRÉ SYNDROME: A PROGNOSTIC MARKER TO PREDICT MECHANICAL VENTILATION IN EARLY STAGE OF THE DISEASE	Ms. Israt Jahan
11/19/22	14:00	15:30	Room 304 / 305		**Oral5.4**	**Oral5**	THE COST OF DENGUE SHOCK AND SEPTIC SHOCK IN VIETNAM	Dr. Angela McBride
11/19/22	14:00	15:30	Room 304 / 305		**Oral5.5**	**Oral5**	CROSS-LINEAGE PROTECTION BETWEEN CHIKV PRIMARY INFECTION AND MAYV SECONDARY EXPOSURE IN MICE	Mr. Rafael Rahal Guaragna Machado
11/19/22	14:00	15:30	Room 302 / 303		**S17**	**S17**	**Rabies - Key Areas of Focus for Successful Elimination**	
11/19/22	14:00	15:30	Room 302 / 303		**S17.1**	**S17**	**Co-chair: Pablo Beldomenico (ProMED Moderator)**	

11/19/22	14:00	15:30	Room 302 / 303		**S17.2**	**S17**	Do we Need Oral Bait Vaccination to Eliminate Dog Mediated Human Rabies?	Gowri Yale
11/19/22	14:00	15:30	Room 302 / 303		**S17.3**	**S17**	Mass canine vaccination: a proven One Health context for the elimination of human rabies caused by dogs	Umme Ruman Siddiqi
11/19/22	14:00	15:30	Room 302 / 303		**S17.4**	**S17**	Feasibility and Effectiveness Studies of Oral Rabies V	Kansuda Leelahapongsathon
11/19/22	16:00	17:00	CH2		**P4**	**P4**	**PLENARY IV**	
11/19/22	16:00	17:00	CH2		**P4**	**P4**	**Malaria**	
11/19/22	16:00	17:00	CH2		**P4.1**	**P4**	**Chair: Zamberi Sekawi**	
11/19/22	16:00	17:00	CH2		**P4.2**	**P4**	The Threat of Multidrug Resistant Falciparum Malaria	Chanaki Amaratunga
11/19/22	16:00	17:00	CH2		**P4.3**	**P4**	Getting back on track: the tools and strategies needed to achieve malaria elimination and eradication	Azra Ghani
11/19/22	16:00	17:00	CH2		**P4.4**	**P4**	Plasmodium knowlesi - Malaria at the One Health interface	Mun Yik Fong
11/19/22	17:15	18:45	Room 302 / 303		**Oral6**	**Oral6**	**Oral Abstracts Session 6 - Parasitology and Parasitic Infections**	
11/19/22	17:15	18:45	Room 302 / 303		**Oral6**	**Oral6**	**Co-Chair: Syafinaz Amin Nordin**	
11/19/22	17:15	18:45	Room 302 / 303		**Oral6**	**Oral6**	**Co-Chair: Chanaki Amaratunga**	
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.1**	**Oral6**	EFFECT OF BI-ANNUAL COMMUNITY-DIRECTED DISTRIBUTION WITH IVERMECTIN (CDTI) AND A COMMUNITY-BASED AWARENESS PROGRAMME ON THE INCIDENCE OF ONCHOCERCIASIS-ASSOCIATED EPILEPSY IN MAHENGE, TANZANIA.	Mr. Luis-Jorge Amaral
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.2**	**Oral6**	ASSOCIATION OF ONCHOCERCIASIS NODULE PREVALENCE IN MOTHERS AND THE DEVELOPMENT OF EPILEPSY IN THEIR CHILDREN IN MAHENGE, AN ONCHOCERCIASIS-ENDEMIC AREA OF TANZANIA: A CASE-CONTROL STUDY	Mr. Luis-Jorge Amaral
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.3**	**Oral6**	EVALUATION OF A COMMUNITY-BASED PREVENTION PROGRAMME TO DECREASE ONCHOCERCIASIS-ASSOCIATED EPILEPSY IN ONCHOCERCIASIS ENDEMIC VILLAGES IN SOUTH SUDAN	Mr. Luis-Jorge Amaral
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.4**	**Oral6**	A MOLECULAR AND IMMUNOLOGICAL SIGNATURE OF IL-10 PRODUCING CD4+ T CELLS IN VISCERAL LEISHMANIASIS	Dr. RAJIV KUMAR
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.5**	**Oral6**	PREVALENCE OF ASYMPTOMATIC LEISHMANIA INFECTION IN PEOPLE LIVING WITH HIV AND PROGRESSION TO SYMPTOMATIC VISCERAL LEISHMANIASIS IN BIHAR, INDIA	Mr. Raman Mahajan Mahajan
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.6**	**Oral6**	LEISHMANIA DONOVANI AND WUCHERERIA BANCROFTI CO-INFECTION IN AN ASYMPTOMATIC POPULATION OF VISCERAL LEISHMANIASIS	Dr. Abhishek Kumar Singh
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.7**	**Oral6**	MONITORING OF LEISHMANIA TRANSMISSION IN THE PERI-ELIMINATION PHASE: THE POTENTIAL OF SEROLOGICAL SURVEYS	Dr. Om Prakash Singh
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.8**	**Oral6**	VISCERAL LEISHMANIASIS -HIV COINFECTED PATIENTS ARE HIGHLY INFECTIOUS TO SAND FLIES IN ENDEMIC AREA OF BIHAR, INDIA	Dr. Om Prakash Singh
11/19/22	17:15	18:45	Room 302 / 303		**Oral6.9**	**Oral6**	NOVEL METHODS FOR RAPID IDENTIFICATION OF BACTERIAL PATHOGENS	Mr. Daniel Klaus Buhl
SUNDAY - NOV 20, 2022								

11/20/22							**MEET-THE-EXPERT sessions**	
11/20/22	8:00	9:00	Banquet Hall		**M7**	**M7**	**Travel Medicine Updates**	
11/20/22	8:00	9:00	Banquet Hall		**M7.1**	**M7**		Lin Hwei Chen
11/20/22	8:00	9:00	Banquet Hall		**M7.2**	**M7**		Priscilla Rupali
11/20/22	8:00	9:00	Room 302 / 303		**M8**	**M8**	**Management of Infective endocarditis**	
11/20/22	8:00	9:00	Room 302 / 303		**M8.1**	**M8**		K.M. John Chan
11/20/22	8:00	9:00	Room 304 / 305		**M9**	**M9**	**Early childhood diarrheal disease: Causes, consequences, and control strategies**	
11/20/22	8:00	9:00	Room 304 / 305		**M9.1**	**M9**		Sitara SR Ajjampur
11/20/22	8:00	9:00	Room 304 / 305		**M9.2**	**M9**		Gagandeep Cherry Kang
11/20/22	8:00	9:00	CH3		**Oral7**	**Oral7**	**Oral Abstracts Session 7 - COVID**	
11/20/22	8:00	9:00	CH3		**Oral7**	**Oral7**	**Co-Chair: Tatiana Pinto**	
11/20/22	8:00	9:00	CH3		**Oral7**	**Oral7**	**Co-Chair: Nurul Azmawati Mohamed**	
11/20/22	8:00	9:00	CH3		**Oral7.1**	**Oral7**	CLOSTRIDIUM DIFFICILLAE INFECTION IN POST-COVID PATIENTS	Dr. Silvia Cveková
11/20/22	8:00	9:00	CH3		**Oral7.2**	**Oral7**	MODELLING THE IMPACT OF COVID-19 AND ROUTINE MENACWY VACCINATION ON MENINGOCOCCAL CARRIAGE AND DISEASE IN THE UK.	Ms. Liza Hadley
11/20/22	8:00	9:00	CH3		**Oral7.3**	**Oral7**	BURDEN AND SEVERITY OF COVID-19 IN CHILDREN HOSPITALISED OVER FIVE COVID-19 WAVES IN SOWETO, SOUTH AFRICA	Prof. David Paul Moore
11/20/22	8:00	9:00	CH3		**Oral7.4**	**Oral7**	EFFECT OF HYBRID IMMUNITY, SCHOOL REOPENING, AND THE OMICRON VARIANT ON TRAJECTORY OF COVID-19 EPIDEMIC IN INDIA: A MODELLING STUDY	Ms. Farhina Mozaffer
~~**11/20/22**~~	~~8:00~~	~~9:00~~	~~CH3~~		~~**Oral7.5**~~	~~**Oral7**~~	~~HIGH RATE OF MULTIDRUG RESISTANT BACTERIAL INFECTIONS IN CRITICALLY ILL COVID-19 PATIENTS ADMITTED AT THE PEAK OF PANDEMIC IN A NATIONAL REFERRAL HOSPITAL, KENYA~~	~~Ms. Jeniffer Munyiva Mutua~~
11/20/22	8:00	9:00	CH3		**Oral7.6**	**Oral7**	A RAPID POINT-OF-CARE DIPSTICK ASSAY FOR DIFFERENTIATION OF SARS-COV-2 VARIANTS IN COVID-19 PATIENTS	Dr. DEEPJYOTI PAUL
11/20/22	8:00	9:00	CH3		**Oral7.7**	**Oral7**	COVID-19 ASSOCIATED HEPATITIS IN CHILDREN (CAH-C) DURING THE RISE OF DELTA VARIANT IN INDIA: A NEW COVID-19 COMPLICATION OR A SUPERINFECTION.	Dr. SUMIT KUMAR RAWAT
11/20/22	9:15	10:00	CH2		**P5**	**P5**	**PLENARY V**	
11/20/22	9:15	10:00	CH2		**P5.1**	**P5**	**Chair: Sally Roberts**	
11/20/22	9:15	10:00	CH2		**P5.2**	**P5**	Pancoronavirus vaccines	Linfa Wang
							PARALLEL SYMPOSIA - Morning	
11/20/22	10:30	12:00	Banquet Hall		**S18**	**S18**	**Personalized/integrated approaches across ID therapy and prevention**	
11/20/22	10:30	12:00	Banquet Hall		**S18.1**	**S18**	**Co-chairs: Alison Holmes**	
11/20/22	10:30	12:00	Banquet Hall		**S18.2**	**S18**	Translating genomics into practice	KK Tee

11/20/22	10:30	12:00	Banquet Hall		**S18.3**	**S18**	Personalized/Integrated Approaches Across Infectious Dise	Jesus Rodriguez Manzano
11/20/22	10:30	12:00	Banquet Hall		**S18.4**	**S18**	Alternatives to Antibiotics - Microbiome modulation and others	Sunny Wong
11/20/22	10:30	12:00	CH1		**S19**	**S19**	**Developing Vaccine Confidence**	
11/20/22	10:30	12:00	CH1		**S19.1**	**S19**	**Co-chairs: Zamberi Sekawi**	
11/20/22	10:30	12:00	CH1		**S19.2**	**S19**	How to build confidence in vaccines	Hannelie Meyer
11/20/22	10:30	12:00	CH1		**S19.3**	**S19**	Childhood vaccines and the pandemic	Lulu Bravo
11/20/22	10:30	12:00	CH1		**S19.4**	**S19**	Social Media, Misinformation, and Health Literacy	Enrique Castro-Sanchez
11/20/22	10:30	12:00	Room 304 / 305		**Oral8**	**Oral8**	**Oral Abstracts Session 8: IPC/Healthcare Associated Infections & Critical Care**	
11/20/22	10:30	12:00	Room 304 / 305		**Oral8**	**Oral8**	**Co-Chair: Nuntra Suwantarat**	
11/20/22	10:30	12:00	Room 304 / 305		**Oral8**	**Oral8**	**Co-Chair: Ariza Adnan**	
11/20/22	10:30	12:00	Room 304 / 305		**Oral8.1**	**Oral8**	MIXED-SPECIES BIOFILMS AND RESISTANCE TO HEAVY METALS AND DISINFECTANTS: IMPLICATIONS FOR UPEC AND UTIS	Prof. Paul Brown
11/20/22	10:30	12:00	Room 304 / 305		**Oral8.2**	**Oral8**	SPATIAL-TEMPORAL DETERMINANTS OF MDRO TRANSMISSION DYNAMICS: IMPLICATIONS FOR INFECTION CONTROL	Mr. Ashleigh Myall
11/20/22	10:30	12:00	Room 304 / 305		**Oral8.3**	**Oral8**	SCREENING PREGNANT WOMEN FOR ASYMPTOMATIC BACTERIURIA USING MULTIREAGENT URINE DIPSTICKS AT PRIMARY HEALTH CENTRES: IMPLEMENTATION EXPERIENCE FROM INDIA	Dr. Tapas Nair
11/20/22	10:30	12:00	Room 304 / 305		**Oral8.4**	**Oral8**	IN-HOSPITAL MORTALITY OF HEART FAILURE PATIENTS ASSOCIATED WITH COMMUNITY-ACQUIRED SEPSIS AND HOSPITAL-ACQUIRED SEPSIS	Dr. Restuti Hidayani Saragih
11/20/22	10:30	12:00	Room 304 / 305		**Oral8.5**	**Oral8**	CARBAPENEM RESISTANT ENTEROBACTERALES COLONIZATION & RISK OF INFECTION IN ICU PATIENTS IN A TERTIARY CARE CENTRE	Dr. Asfia Sultan
11/20/22	10:30	12:00	Room 304 / 305		**Oral8.6**	**Oral8**	MAPPING TEAM DYNAMICS AND TRAFFIC IN THE OPERATING THEATRE: IDENTIFICATION OF ROLES AND STRESSORS IN INFECTION RELATED PRACTICE AND ITS COMMUNICATION WITH SURGICAL TEAMS	Ms. Surya Surendran
11/20/22	10:30	12:00	Room 302 / 303		**S20**	**S20**	**Current updates in tuberculosis**	
11/20/22	10:30	12:00	Room 302 / 303		**S20.1**	**S20**	**Co-Chair: Syafinaz Amin Nordin**	
11/20/22	10:30	12:00	Room 302 / 303		**S20.2**	**S20**	MDRTB treatment update	Sean Wasserman
11/20/22	10:30	12:00	Room 302 / 303		**S20.3**	**S20**	Targeting Tuberculosis and Tissue destruction	Catherine Ong
11/20/22	10:30	12:00	Room 302 / 303		**S20.4**	**S20**	Advances in shortening treatment for drug-susceptible TB	Nick Paton
11/20/22	10:30	12:00	Room 302 / 303		**S20.5**	**S20**	Updates on HIV and Tuberculosis co-infection	Jorge Gonzalez Mendoza
11/20/22	10:30	12:00	Theatre		**PD3**	**PD3**	**Policy Discussion - Advancing Clinical Trials**	
11/20/22	10:30	12:00	Theatre		**PD3.1**	**PD3**	Moderator: Petrik Periyasamy	
11/20/22	10:30	12:00	Theatre		**PD3.2**	**PD3**		Adebola Olayinka
11/20/22	10:30	12:00	Theatre		**PD3.3**	**PD3**		Evelyne Kestelyn

							PARALLEL SYMPOSIA - Afternoon	
11/20/22	14:00	15:30	CH2		**S21**	**S21**	**Neglected Infectious Diseases**	
11/20/22	14:00	15:30	CH2		**S21.1**	**S21**	**Chair: Lau Yee Ling**	
11/20/22	14:00	15:30	CH2		**S21.2**	**S21**	The role of animal reservoirs in spreading human leptospirosis in Southeast Asia	Zamberi Sekawi
11/20/22	14:00	15:30	CH2		**S21.3**	**S21**	Sarcocystis: From snakes to humans	Kum Thong Wong
11/20/22	14:00	15:30	CH2		**S21.4**	**S21**	Helminth infections	Yvonne Lim
11/20/22	14:00	15:30	CH2		**S21.5**	**S21**	Chikungunya Surveillance Updates	Jaime Torres
11/20/22	14:00	15:30	CH3		**S22**	**S22**	**Hospital-Acquired Infections - How Much Can We Prevent**	
11/20/22	14:00	15:30	CH3		**S22.1**	**S22**	**Co-Chair: Zakuan Zainy Deris**	
11/20/22	14:00	15:30	CH3		**S22.2**	**S22**	**Co-Chair: Hamimah Hassan**	
11/20/22	14:00	15:30	CH3		**S22.3**	**S22**	HAI's - How much can we prevent and how to manage expectations	Gonzalo Bearman
11/20/22	14:00	15:30	CH3		**S22.4**	**S22**	Screening strategies in different resource settings	Nuntra Suwantarat
11/20/22	14:00	15:30	CH3		**S22.5**	**S22**	The Integration of IPC and Stewardship: Key Strategies	Anucha Apisarnthanarak
11/20/22	14:00	15:30	Room 304 / 305		**Oral9**	**Oral9**	**Oral Abstracts Session 9 - Hot Topics**	
11/20/22	14:00	15:30	Room 304 / 305		**Oral9**	**Oral9**	Co-Chairs: Paul Tambyah, Esmita Charani	
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.1**	**Oral9**	STRUCTURAL AND BIOCHEMICAL ELUCIDIATION OF MOSQUITO HEAT SHOCK PROTEIN 70	Dr. Soumyananda Chakraborti
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.2**	**Oral9**	INVESTIGATION OF 3-BENZOYLBENZOFURANS AND THEIR METHYLATED AND PYRAZOLE DERIVATIVES FOR POTENTIAL INHIBITION OF HIV-1 REPLICATION	Ms. Sinothile Sementha Khuzwayo
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.3**	**Oral9**	AN ESTERASE-LIKE PROTEIN (ELP) CONFERRED MALATHION AND DELTAMETHRIN RESISTANCE IN THE INDIAN FIELD POPULATION OF ANOPHELES STEPHENSI	Mr. Jatin Kumar
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.4**	**Oral9**	SYPHILIS REINFECTION IN NEW SOUTH WALES, 2014-2021	Ms. Justine Marshall
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.5**	**Oral9**	DEVELOPMENT OF CONJUGATED SECONDARY ANTIBODIES FOR WILDLIFE DISEASE SURVEILLANCE	Dr. Sunday Ochonu Ochai
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.6**	**Oral9**	THE USE OF IMAGERY IN GLOBAL HEALTH: AN ANALYSIS OF INFECTIOUS DISEASE DOCUMENTS AND A FRAMEWORK FOR ETHICAL STANDARDS	Mr. Sameed Shariq
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.7**	**Oral9**	ROLE OF ILCS IN THE PATHOPHYSIOLOGY OF ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)	Prof. Rupesh Kumar Srivastava
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.8**	**Oral9**	TRACKING ROSS RIVER VIRUS HOST DIVERSITY USING MOSQUITOES AS 'FLYING SYRINGES'	Ms. Carla J S P Vieira
11/20/22	14:00	15:30	Room 304 / 305		**Oral9.9**	**Oral9**	STUDY OF VARIOUS METHODS FOR DETECTING HLA-B* 57:01 ALLELE IN PLHIV IN EASTERN UP, INDIA.	Ms. TULIKA KUMARI RAI
11/20/22	14:00	15:30	Room 302 / 303		**S23**	**S23**	**Pediatric infectious diseases - Challenges and Opportunities**	
11/20/22	14:00	15:30	Room 302 / 303		**S23.1**	**S23**	**Chair: Lulu Bravo**	
11/20/22	14:00	15:30	Room 302 / 303		**S23.2**	**S23**	Re-emergence of measles	Lulu Bravo
11/20/22	14:00	15:30	Room 302 / 303		**S23.3**	**S23**	A Multiyear Journey Towards Reduced Water NICU	Low Jia Ming
11/20/22	14:00	15:30	Room 302 / 303		**S23.4**	**S23**	Enhancing the prevention of mother-to-child transmission of Hepatitis B	Tan Soek Siam

11/20/22	14:00	15:30	Room 302 / 303		**S23.5**	**S23**	The continuing crisis of neonatal sepsis: lessons from 19 years of surveillance in Bangladesh	Senjuti Saha
11/20/22	15:45	16:30	CH2		**P6**	**P6**	**PLENARY VI**	
11/20/22	15:45	16:30	CH2		**P6**	**P6**	**Dengue**	
11/20/22	15:45	16:30	CH2		**P6.1**	**P6**	**Chair: Alison Holmes**	
11/20/22	15:45	16:30	CH2		**P6.2**	**P6**		Evelyne Kestelyn
11/20/22	15:45	16:30	CH2		**P6.3**	**P6**		
11/20/22	16:30	16:45	CH2		**Close**	**Close**	Closing remarks: Alison Holmes	

From: Christopher Widmer on behalf of Christopher Widmer <cwidmer@isid.org>

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Cc: Sarah Claire Loeb; Aisha Abubakar; Elkin Bermudez

Subject: ISID Congress 2022 - Upload your Presentation

Date: Thursday, November 17, 2022 8:25:52 AM

Attachments: Technical Time Table_2022_11_17.xlsx - Scientific Sessions.pdf

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Greeting Speakers. If you are able, please send your presentations to the following email address with your last name and session number(s) (or any acceptable format) to the following email address spr@klccconventioncentre.com

Attached is a list of all sessions with the dates and times and locations for your reference.

You can also upload your presentations at the **Speaker Ready Room** on level 3 of the KLCC. You can ask for directions to it at the Registration counter on the same level 3. Thank you. - Christopher

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Christopher Widmer

Senior Manager of Operations

International Society for Infectious Diseases (ISID)

Cooperative Biological Research
Final Biannual Scientific Report

PROJECT INFORMATION	
Project Number:	PO: 18-0507 Project Number: 042959
Project Title:	Surveillance for emerging infectious disease pathogens at the animal-human interfaces in Thailand, in coordination with PREDICT USAID project and the bat serology study
Award Effective Date:	01 June 2018
Award End Date:	01 December 2019
Contractor:	**CHULALONGKORN UNIVERSITY** 254 Phayathai Road, Pathumwan, Bangkok 10330 Thailand
Report #:	**FINAL REPORT**
REPORT DATE:	01 December 2021
Prepared by:	Dr. Supaporn Wacharapluesadee Dr. Eric Liang (Serology data analysis)
Organization:	Faculty of Medicine, Chulalongkorn University King Chulalongkorn Memorial Hospital, Thai Red cross Society

Contents

Overall Project Summary

In this final report, 1,173 specimens from bats were collected from 5 sites (Ratchaburi (n=280), Chachoengsao (n=252), Chonburi (n=172), Bangkok (n=64), and Chanthaburi (n=405) provinces) from June 2019 through to September 2020. Samples from rodents (n=90) and macaques (n=100) were collected from Ratchaburi province from August to September 2020. All samples were tested for three viral families, namely Paramyxoviruses, Coronaviruses, and Filoviruses using the molecular technique. Seven Coronavirus species were identified, including Sarbecovirus, the SARS-CoV-2 related virus, from horseshoe bats. Nipah virus RNA was tested from pooled bat urine of Lyle flying foxes collected in May 2018. Additionally, a serology study was conducted using multiplex microsphere immunoassay (MMIA) to test antibodies against 16 viruses in 1,002 *P. lylei* bat plasma from Chonburi in 2012, 2016, 2017, and 2018; 128 archived human serum from Chonburi in 2018; and new bat plasma specimens (n=1,0360) collected in 2019 and 2020.

Background and Justification

Bats play a critical role in the transmission of zoonotic diseases, primarily viral zoonoses associated with high case-fatality rates, including Nipah virus (NiV), severe acute respiratory syndrome (SARS)-like coronavirus (CoV) including SAR-CoV-2, and Middle East Respiratory Syndrome (MERS)-like CoV infections. Ratchaburi, Chonburi, and Loei provinces in Thailand are hotspots for emerging zoonotic viruses. MERS-like CoV has previously been found in bat guano fertilizer in Ratchaburi; NiV has been previously identified in Lyle's flying fox roosting in the village in Chonburi since 2002; however, no outbreak has been reported. This research aims to understand better the role of bats and interfaces from these areas in harbouring and transmitting emerging infectious diseases (EIDs), including known and novel EID viral pathogens. This is also a disease surveillance research of wildlife-domestic animal-human interfaces, in coordination with PREDICT USAID project. Bats and animals (rodents and macaques) were sampled around the bat cave area to test NiV, MEES-like-, SARS-like CoVs, and filovirus. In addition to animal surveillance, this study also includes human subjects. Thus, the overall scope is to detect and characterize NiV and MERS like-CoVs in potentially high-risk communities and respiratory pathogens.

Project Objectives and Hypotheses

Objective/Task 0: Detect and characterize new and known epidemic and pandemic viruses in wildlife (bats, rodents, and macaques) and high-risk communities; identify animal reservoirs and amplification hosts for zoonotic viruses.

Objective/Task 1: Study bat serology for its immune response against Nipah virus, MERS-CoV, and other bat-borne viruses.

Objective/Task 2: Enhance biosecurity and serological diagnostic capabilities in Thailand

SCIENTIFIC REPORT

Overview of Scientific Achievements

1. Viral zoonotic molecular study

1.1 Sampling (oral, feces or rectal swab, blood and/or urine)

1,173 bat samples were collected from 6 sites in 5 provinces (Table 1):

100 samples from Ratchaburi in June 2019
54 samples from Chachoengsao in July 2019
60 samples from Chonburi in July 2019
80 samples from Ratchaburi in August 2019
64 samples from Bangkok in September 2019
112 samples from Chonburi in October 2019
206 samples from Chanthaburi in November 2019
199 samples from Chanthaburi in June 2020
100 samples from Chachoengsao in July 2020
98 samples from Chachoengsao in July 2020
100 samples from Ratchaburi in September 2020

90 Rodent samples were collected from 1 site

90 samples from Ratchaburi in August 2020

100 Macaque samples were collected from 1 site

100 samples from Ratchaburi in September 2020

1.2 Results: Molecular testing for viral detection

1.2.1 Nipah Virus & Paramyxovirus Family

- **197 pooled bat urine samples from Chonburi in November 2017, February 2018 and May 2018** were tested for Nipah virus using Nipah specific primers PCR.

  5/197 (2.54%) samples tested positive for Nipah virus. The nucleotide sequences of nucleocapsid protein gene showed 99.21 to 99.47 % identity to Nipah virus isolated from Bangladesh patients.

- **975 bat rectal swabs from 5 sites collected in 2019 and 2020** were tested for paramyxoviruses using PCR.

  21/975 (2.15%) samples tested positive for paramyxoviruses (Table 1). Three positive specimens were from *Hiposideros larvatus* bat from Chantaburi (n=2, and Chachoengsao (n=1), from phylogenetic analysis (Figure 2) they belong to bat Paramyxovirus found from Hipposideros in Myanmar and Thailand.

While the other 18 samples were from *Cherephon plicatus* (n=2), *Eonycteris spelaea* (n=12), *Rousettus leschenaulti* (n=2) and *Rousettus* sp (n=2), they belong to different lineage to Hipposideros virus but shared similarity to bat Paramyxovirus from Rwanda, Congo, China and Indonesia. However, there is no report of a threat to humans or other animals from the bat paramyxovirus found in this study.

- **90 rodent rectal swabs from the year 2020** were tested for paramyxoviruses using PCR.
  - No sample tested positive for paramyxoviruses.
- **100 macaque rectal swabs from the year 2020** were tested for paramyxoviruses using PCR.
  - No sample tested positive for paramyxoviruses.

1.2.2 Coronaviruses

A. Coronaviruses Quan Protocol[1]

- **1,173 bat rectal swabs from 2019 and 2020** were tested for coronaviruses using PCR.
  - 192/1,173 (16.36%) samples tested positive for coronaviruses (Table 1).
- **90 rodent rectal swabs from 2020** were tested for coronaviruses using PCR.
  - 3/90 (3.33%) samples tested positive for coronaviruses (Table 1).
- **100 macaque rectal swabs from 2020** were tested for coronaviruses using PCR.
  - No sample tested positive for coronaviruses.

B. Coronaviruses Watanabe Protocol[2]

- **1,075 bat rectal swabs from 2019 and 2020** were tested for coronaviruses using PCR.
  - 226/1,075 (21.02%) samples tested positive for coronaviruses (Table 1).
- **90 rodent rectal swabs from 2020** were tested for coronaviruses using PCR.
  - 3/90 (3.33%) samples tested positive for coronaviruses (Table 1).
- **100 macaque rectal swabs from 2020** were tested for coronaviruses using PCR.
  - No sample tested positive for coronavirus

1.2.3 Filoviruses

975 bat rectal swabs from 2019 and 2020 were tested for filoviruses using PCR.

- No sample tested positive for filovirus

90 rodent rectal swabs from 2020 were tested for filoviruses using PCR.

- No sample tested positive for filovirus

[1] PREDICT protocol modified from Quan PL, et al. Identification of a severe acute respiratory syndrome coronavirus-like virus in a leaf-nosed bat in Nigeria. MBio. 2010 Oct 29;1(4).
[2] PREDICT protocol modified from Watanabe T, et al. Development of a dose-response model for SARS coronavirus. Risk Analysis: An International Journal. 2010 Jul;30(7):1129-38.

100 macaque rectal swabs from 2020 were tested for filoviruses using PCR.

- No sample tested positive for filovirus

1.3 Whole-genome sequencing (WGS)

Five Sarbecovirus PCR positive specimens were further characterized for the whole-genome sequence. WGS was performed using enrichment library preparation (Respiratory Viral Oligos Panel, RVOP) and an Illumina MiSeq 3000 sequencer, according to the manufacturer instructions.using the RVOP enrichment library preparation protocol (Illuminar, USA). The complete genome sequence was a success in one specimen; No. RacCS203. All five genome sequences were submitted and can be accessed via NCBI GenBank; accession number MW251308 (complete genome; RacCS203), MW251310-12 (partial genome; RacCS224, RacCS253, RacCS264, and RacCS271).

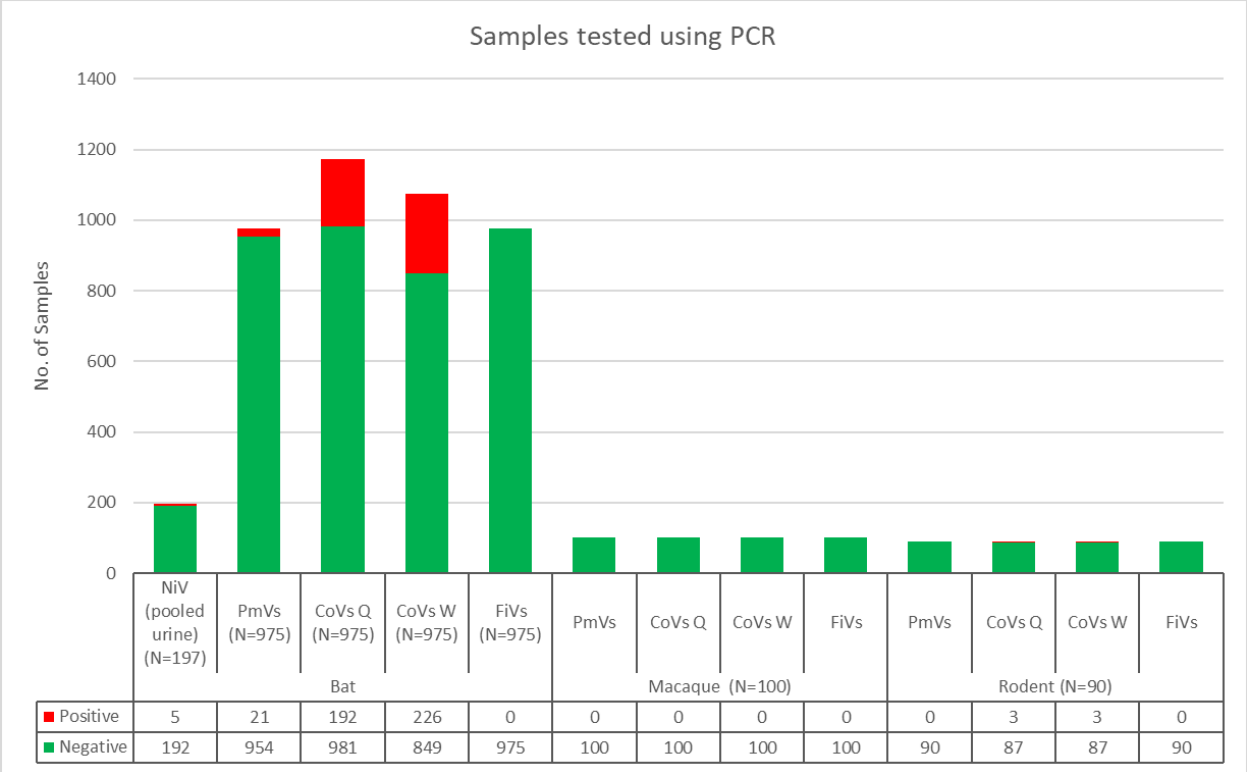

Figure 1: Summary of samples tested using PCR

Table 1. PCR results of Coronavirus (CoV) (2 protocols) and Paramyxovirus (PmV) from rectal swab specimens of bats, rodents, and macaques.

Event Name	Collected Date	Animal ID	No. tested	No. of Positive sample-viral group		
				CoV (Q protocol)	CoV (W protocol)	PmV
Bat						
Ratchaburi-Wat Khao Chong Pran	2019Jun14	B19043-142	100	All Neg	All Neg	1
Chachoengsao-1 (Wat Khao Tham Raet)	2019Jul23	B19143-196	54	5-Hibecovirus 9-unclassified AlphaCoV	3- unclassified AlphaCoV	1
Chonburi-Wat Khao Cha-ang	2019Jul24	B19197-256	60	1-Nobecovirus (HKU9) 1-Nobecovirus (GCCDC1) 2-Minunacovirus 1-unclassified Decavirus	5-Nobecovirus (HKU9) 4-Nobecovirus (GCCDC1)	2
Ratchaburi-Wat Khao Chong Pran	2019Aug16	B19257-336	80	27-unclassified AlphaCoV	20-unclassified AlphaCoV	All Neg
Bangkok-Kasetsart university	2019Sep14	B19337-389 B19391-401	64	2-Nobecovirus (HKU9) 1-Pedacovirus [99.3% identity to Porcine epidemic diarrhea virus (PEDV), GenBank accession no. MN314264]	3-Nobecovirus (HKU9)	All Neg
Chonburi-Wat Khao Cha-ang	2019Oct17	B19402-513	112	10-Nobecovirus (GCCDC1) 1-Minunacovirus	30-Nobecovirus (GCCDC1)	1
Chantaburi-Khao Soi Dao	2019Nov08	B19514-719	206	3-Nobecovirus (HKU9) 10-Nobecovirus (GCCDC1) 5-unclassified Decacovirus	53 32-Nobecovirus (HKU9) 21-Nobecovirus (GCCDC1)	6
Chantaburi-Khao Soi Dao	2020Jun11	B20001-004 B20006-200	199	82 30-Nobecovirus (HKU9) 18-Nobecovirus (GCCDC1) 1-Hibecovirus 26-unclassified Decacovirus 6-unclassified AlphaCoV	102 68-Nobecovirus (HKU9) 29-Nobecovirus (GCCDC1) 5- unclassified AlphaCoV	9

Event Name	Collected Date	Animal ID	No. tested	No. of Positive sample-viral group		
				CoV (Q protocol)	**CoV (W protocol)**	**PmV**
Chachoengsao-2 (Ang Rue Nai)	2020Jun19	B201-300	100	**13-Sarbecovirus**	Negative	notdone
Chachoengsao-2 (Ang Rue Nai)	2020Jul	B543-640	98	**14-Sarbecovirus**	Not done	notdone
Ratchaburi-Wat Khao Chong Pran	2020Sep12	B20838-937	100	4-unclassified AlphaCoV 2-Hibecovirus	1-Nobecovirus (HKU9) 2-Nobecovirus (GCCDC1) 3-unclassified AlphaCoV	1
TOTAL			1,173	192	226	21
Macaque						
Ratchaburi-Wat Tham Nam	2020Sep01	P20001-100	100	All Negative	All Negative	All Negative
Rodent						
Ratchaburi-Wat Khao Chong Pran	2020Aug29	R20001-090	90	3-Embecovirus	3-Embecovirus	All Negative

1.4 Discussion

Specimens from the bat (13 species), rodent, and macaque were collected during 2019 and 2020 and tested for three viral families to detect EID bat-borne viruses. Three viral families included coronavirus, paramyxovirus and filovirus.

No tested virus was found from macaque rectal swab specimens. Three positive murine coronaviruses were from rodent rectal specimens. However, to our knowledge, there is no report of any threat from this virus to humans.

Coronavirus was found in bat from all studied sites. At Ratchaburi-Wat Khao Chong Pran site where specimens from *Cherephon plicatus* bats were collected three times, CoV was not found in June 2019, but unidentified AlphaCoV was detected in August 2019, and unclassified AlphaCoV, Hibecovirus, and Nobevirus were detected in September 2020 (Table 1).

Sabecovirus (SARS-CoV-2 related virus) was detected from horseshoe bat (*Rhinolophus accuminatus*) from Chachoengsao province (**Figure S4a)**. The small polymerase gene fragment (290 base pairs) showed 96% similarity to human SAR-CoV-2, but its whole genome (RacCS203) showed 91% identity (**Figure S4b)**. The ability of the virus to enter the human cell using ACE-2 receptor was performed at Professor Linfa's laboratory, Duke-NUS, Singapore. The RacCS203 virus could not bind to the human ACE-2 receptor (Wacharapluesadee, et al., 2021). However, Sarbecovirus did not identify in the other 12 bat species from this study.

Nobecovirus was primarily found in fruit bat species; *Cynopterus sphinx*, *Eonycteris spelaea*, *Rousettus leschenaultia*, *Rousettus amplexicaudatus* and *Rousettus* sp (Table 2, supplement figures). In contrast, unclassified decavirus was detected in insect-eating and fruit bats species; *Eonycteris spelaea*, *Hipposideros armiger*, *Hipposideros larvatus*, *Rousettus leschenaultia*, *Rousettus amplexicaudatus* and *Rousettus* sp. In addition, unclassified AlphaCoV was identified in *Cherephon plicatus*, and *Hipposideros larvatus*. Minunacovirus was detected only from *Miniopterus magnate*. Finally, Pedacovirus was found in *Myotis horsfieldii* and showed 99% identity to the virus from porcine (Porcine epidemic diarrhea virus).

Nobecovirus is the most abundant found from this study. It is the subgenus of viruses in the genus Betacoronavirus, previously known as group 2d coronaviruses (HKU9 strain). It originates in (fruit) bat but not other animal species as far as our knowledge. There is no evidence that it can cause disease to humans or other animals.

Two PCR protocols for the detection of CoV were performed in this study. The Watanabe protocol could detect Nobevirus better than Quan PCR protocol. Whereas Sarbecovirus and Hebecovirus (Betacoronavirus) could be identified by Quan but not Watanabe protocol. Combining both protocols for CoV detection in bat gives better sensitivity and avoids the false-negative result.

Wacharapluesadee S, Tan CW, Maneeorn P, Duengkae P, Zhu F, Joyjinda Y, Kaewpom T, Chia WN, Ampoot W, Lim BL, Worachotsueptrakun K, Chen VC, Sirichan N, Ruchisrisarod C, Rodpan A, Noradechanon K, Phaichana T, Jantarat N, Thongnumchaima B, Tu C, Crameri G, Stokes MM, Hemachudha T, Wang LF. Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. Nat Commun. 2021 Feb 9;12(1):972. doi: 10.1038/s41467-021-21240-1. Erratum in: Nat Commun. 2021 Feb 25;12(1):1430. PMID: 33563978; PMCID: PMC7873279.

Table 2 Coronaviruses (sub-genus) found from 13 bat species in the study

Bat species	Location	No. tested	unclassified AlphaCoV	unclassified Decacovirus	Minuna covirus	Peda covirus	Hibe covirus	Nobe covirus	Sarbe covirus	Total	%
Cherephon plicatus	Ratchaburi	280	32	nf	nf	nf	2	3	nf	37	13.21
Cynopterus sphinx	Choburi	3	nf	nf	nf	nf	nf	2	nf	2	66.67
Cynopterus sphinx	BKK	63	nf	nf	nf	nf	nf	3	nf	3	4.76
Eonycteris spelaea	Chantaburi	170	nf	1	nf	nf	nf	67	nf	68	40.00
Eonycteris spelaea	Chonburi	143	nf	nf	nf	nf	nf	37	nf	37	25.87
Hipposideros armiger	Chantaburi	17	nf	1	nf	nf	nf	nf	nf	1	5.88
Hipposideros armiger	Chachoengsao-1	9	nf	nf	nf	nf	1	nf	nf	1	11.11
Hipposideros armiger	Chonburi	7	nf	nf	nf	nf	nf	nf	nf	0	0.00
Hipposideros larvatus	Chantaburi	20	4	1	nf	nf	1	1	nf	7	35.00
Hipposideros larvatus	Chachoengsao-1	44	9	nf	nf	nf	4	nf	nf	13	29.55
Hipposideros lekaguli	Chonburi	7	nf	nf	nf	nf	nf	nf	nf	0	0.00
Miniopterus magnate	Chonburi	5	nf	nf	3	nf	nf	nf	nf	3	60.00
Myotis horsfieldii	BKK	1	nf	nf	nf	1	nf	nf	nf	1	100.00
Rhinolophus accuminatus	Chachoengsao -2	198	nf	nf	nf	nf	nf	nf	**27**	27	13.64
Rhinolophus shameli	Chonburi	2	nf	nf	nf	nf	nf	nf	nf	0	0.00
Rousettus amplexicaudatus	Chantaburi	70	nf	4	nf	nf	nf	20	nf	24	34.29
Rousettus leschenaultia	Chantaburi	37	nf	1	nf	nf	nf	8	nf	9	24.32
Rousettus leschenaultia	Chonburi	4	nf	1	nf	nf	nf	1	nf	2	50.00
Rousettus sp	Chantaburi	91	nf	25	nf	nf	nf	37	nf	62	67.39
Taphozous melanopogon	Chachoengsao	1	nf	nf	nf	nf	nf	nf	nf	0	0.00
Taphozous melanopogon	Chonburi	1	nf	nf	nf	nf	nf	nf	nf	0	0.00
TOTAL	297	1173	45	34	3	1	8	179	27	297	25.30

2. Viral zoonotic serological study

2.1 Specimens: plasma separated from collected blood samples

2.1.1 Archived specimens (n=1,130)

- 358 bats' plasma samples were collected from Chonburi in January 2012 through to January 2013
- 104 bats' plasma samples were collected from Chonburi in November 2016
- 302 bats'plasma samples were collected from Chonburi in February 2017 through to November 2017
- 238 bats'plasma samples were collected from Chonburi in February 2018 through to May 2018
- 128 humans' plasma samples were collected from Chonburi in May 2018

2.1.2 New specimens (n=1,036)

- 540 **bats'** plasma samples were collected from Ratchaburi, Chachoengsao, Chanthaburi and Bangkok in June 2019 through to November 2019
- 298 **bats'** plasma samples were collected from Chanthaburi and Ratchaburi in June 2019 through to November 2019
- 88 **rodents'** plasma samples were collected from Ratchaburi in August 2020
- 100 **macaques'** plasma samples were collected from Ratchaburi in September 2020

2.2 Results: Viral detection using MMIA Serology testing

Human and bat sera samples were screened in a multiplex microsphere-based immunoassay (MMIA). 16 viruses and two additional human ACE-2-using bat SARS-related CoVs (Table 3) were prepared and provided by the Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD, USA.

We detected 23.4% (234/1002) of flying foxes had NiV-specific IgG, and that henipavirus RBP (NiV/GhV)-reactive IgG were detected in 43.9% of flying foxes (Figure 2 and Table 4). Suprsingly, we detected GhV RBP-reactive IgG in several flying fox serum samples. Comparaively, no other bat species had detectable henipavirus RBP-binding IgG.

Flying foxes also had 15.1% (151/1002) seroprevalence for any fiovirids (Table 4). The highest preferential reactivity was observed against Bundibugyo virus GP (Figure 3). Cross-reaction among the ebolaviruses GPs were observed, in addition several flying fox serum samples possessed IgG that reacted with Lloviu virus and marburgviruses. Rousette bats were positive for Mengla virus IgG, but no other filovirids. Additionally, *Chaerephon spp.* sera had some IgG reactivity across ebolavirus GPs but reacitivty was low.

Futhermore, flying foxes had evidence of IgG anitbodies that bound to bat SARS-related CoVs, Rs4784 and Rs4231, spike proteins.

One human and one non-human primate serum sample possessed IgG that were most reactive with Ebola virus GP, however, reactivity was low relative to the upper limits of detection.

2.3 Discussion

The serology data supports the well-characterized host-virus relationship between Lyle's flyig foxes and NiV. NiV seroprevalance of 23 – 44% is consistent with HeV in Australian flying foxes and NiV in Bangladesh sampled Indian flying foxes. Using this confirmed natural reservoir of NiV and a robust sample size of sera, we were able to apply LCA to define threshold cutoffs that were broadly applicable to the multiplex serology assay. We found minimal evidence of henipavirus, filovirid, and betacoronavirus infection outside of Lyle's flying foxes. The negative serology data is further supportive that there may be virus host-restrictions and that ecological or behavorial barriers exist that limit enzootic transmission among bat species.

The specific detection of Mengla virus reactive IgG in rousette bats is consistent with rousette bats being a natural host of Megnla virus and dianloviruses across South and Southeast Asia. However, the IgG levels to Mengla virus were low, suggesting that other dianloviruses may be circulating in rousette bats located with Thailand and distinct from Mengla virus, though retaining conserved antigenic similarities. Serology data indicates that *Pteropus, Chaerephon,* and *Rousettus spp* should be the focus of continued serological and nucleic acid detection for novel Asiatic filovirids.

We observed no evidence of subclinical human exposure Nipah virus or other henipavirses.

Lastly, Lyle's flying foxes were reactive with *Sarbecovirus* spike proteins (14%), whereas, no other bats had evidence of prior infection. Human SARS-CoV-2 research has indicaed that cross-reactions exist between SARS-CoV-2 (*Sarbecovirus*) and other distantly related betacoronaviruses such as HCoV-OC43 *(Embecovirus*). Thus, in the absence of other coronavirus spike proteins we are limited in our interpretation of coronavirus serology in flying foxes. Horseshoe bats are the known source and host of SARS-CoVs and bat SARS-related CoVs (*Sarbecoviruses*). It is more likely that flying foxes are host of antigenically-related betacoronaviruses as opposed to CoVs in the *Sarbecovirus* lineage.

Table 3. List of virus antigen used in this study.

Virus species	Abbreviation	Soluble Glycoprotein	Bead No.
Ebolaviruses			
Ebola virus	EBOV	$sGP_{(1,2)}$	34
Bundibugyo virus	BDBV	$sGP_{(1,2)}$	64
Bombali virus	BOMV	$sGP_{(1,2)}$	55
Tai forest virus	TAFV	$sGP_{(1,2)}$	57
Sudan virus	SUDV	$sGP_{(1,2)}$	77
Reston virus (monkey isolate)	RESTVm	$sGP_{(1,2)}$	85
Reston virus (pig isolate)	RESTVp	$sGP_{(1,2)}$	72
Marburgviruses			
Marburg virus	MARV	$sGP_{(1,2)}$	37
Ravn virus	RAVV	$sGP_{(1,2)}$	62
Cuevavirus			
Lloviu virus	LLOV	$sGP_{(1,2)}$	66
Dianloviruses			
Měnglà virus	MLAV	$sGP_{(1,2)}$	22
Henipaviruses			
Hendra virus	HeV	sG	43
Nipah virus (Malaysia strain)	NiV	sG	46
Cedar virus	CedV	sG	53
Mojiang virus	MojV	sG	29
Ghana virus	GhV	sG	35

Table 4. Multiplex microsphere-based immunoassay (MMIA) results against three viral families.

		Filovirus			Henipavirus			Coronavirus
Genus	n	Positive	Single Positive	Multiple Positive	Positive	Single Positive	Multiple Positive	Positive
Pteropus	1002	15.07% (151/1002)	7.39% (74/1002)	7.68% (77/1002)	43.91% (440/1002)	23.35% (234/1002)	20.56% (206/1002)	13.17% (132/1002)
Hipposideros	103	0% (0/103)	-	-	0% (0/103)	-	-	0% (0/103)
Rhinolophus	7	0% (0/7)	-	-	0% (0/7)	-	-	0% (0/7)
Rousettus	190	2.63% (5/190)	2.63% (5/190)	-	0% (0/190)	-	-	0% (0/190)
Charephon	175	6.29% (11/175)	5.14% (9/175)	1.29% (2/175)	0.57% (1/175)	0% (0/175)	0.57% (1/175)	0% (0/175)
Cynopterus	64	0% (0/64)	-	-	0% (0/64)	-	-	0% (0/64)
Eonycteris	195	0% (0/195)	-	-	0% (0/195)	-	-	0% (0/195)
Miniopterus	4	0% (0/4)	-	-	0% (0/4)	-	-	0% (0/4)
Myotis	5	0% (0/5)	-	-	0% (0/5)	-	-	0% (0/5)
Tazophus	2	0% (0/2)	-	-	0% (0/2)	-	-	0% (0/2)
Unspeciated	100	4% (4/100)	3% (3/100)	1% (1/100)	0% (0/100)	-	-	0% (0/100)
Human	128	2.34% (3/128)	1.56% (2/128)	0.78% (1/128)	0.78% (1/128)	-	0.78% (1/128)	0% (0/128)
Macaque	100	1% (1/100)	1% (1/100)	-	0% (0/100)	-	-	0% (0/100)
Rodent	88	0% (0/88)	-	-	0% (0/88)	-	-	0% (0/88)

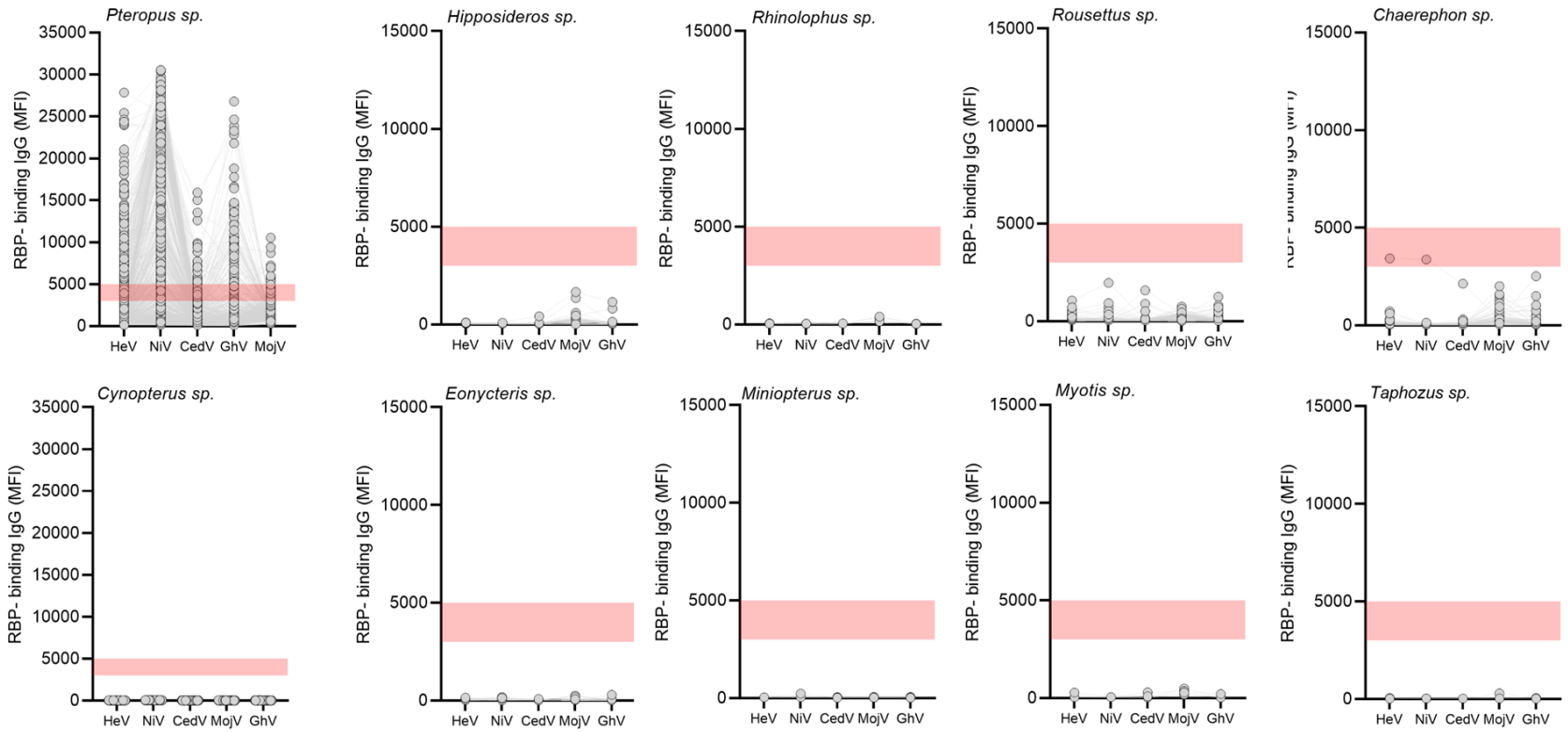

Figure 2 Multiplex microsphere-based immunoassay (MMIA) results of 10 bat genus against five antigen beads; HeV, Hendra virus; NiV, Nipah virus; CedV, Cedar virus; MojV, Mojiang virus; GhV, Ghana virus

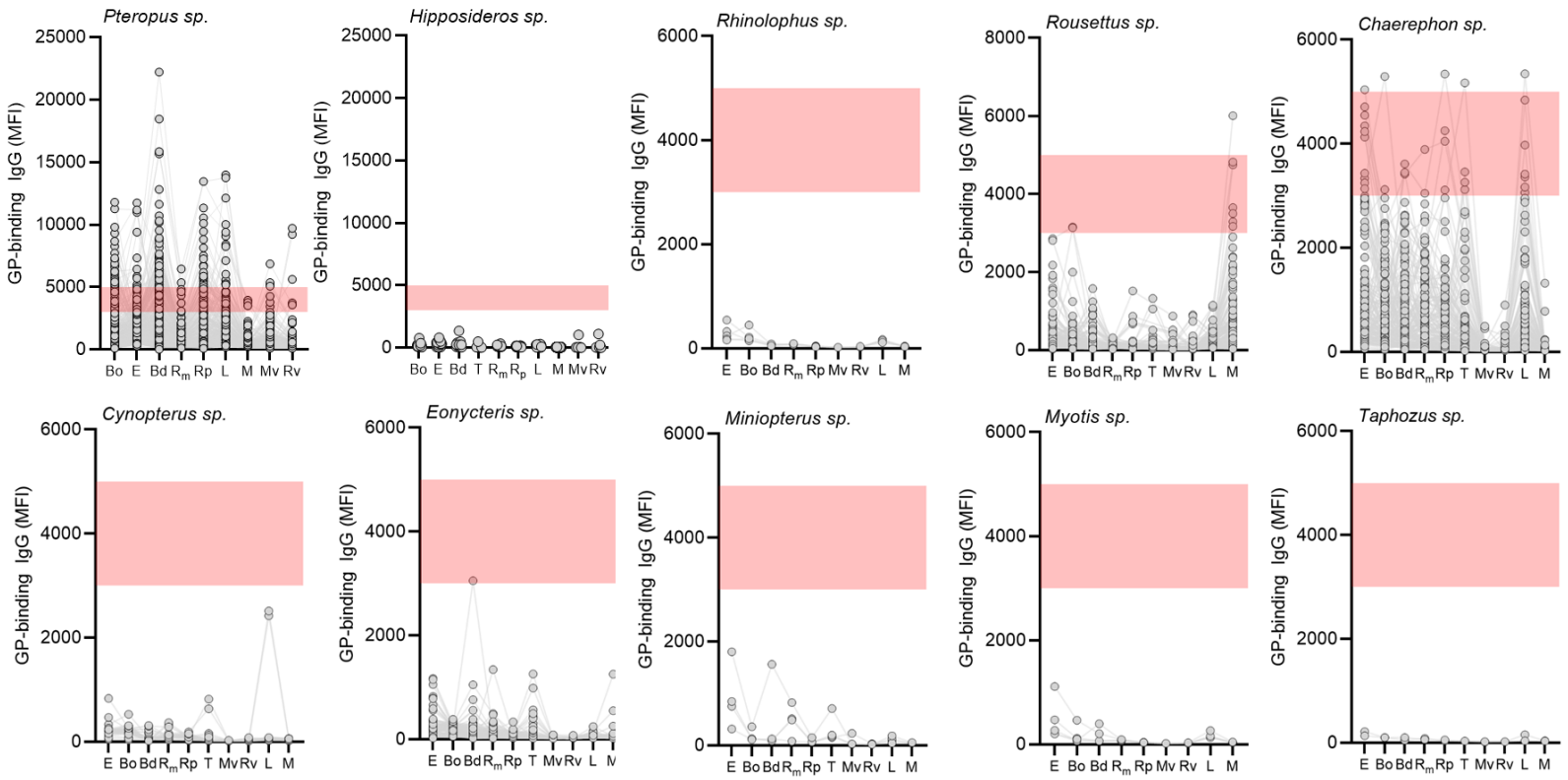

Figure 3 Multiplex microsphere-based immunoassay (MMIA) results of 10 bat genus against ten antigen beads; Bo, Bombali virus; E, Ebola virus; Bd, Bundibugyo virus; T, Tai Forest virus; Rm, Reston virus; Rp, Reston virus; L, Lloviu virus; M, Mengla virus; Mv, Marburg virus; Rv, Ravn virus

Methods

Bat capture and sample collection

Bats were captured each time using mist net for flying foxes or butterfly net for small bats. Captured animals were removed immediately and put into cotton bag individually. Bats were not euthanized, and they were released after measurements were taken and samples were collected. Bats were identified morphometrically, and species, sex, reproductive status, FA length and body mass were determined. Rectal swab was collected from each individual bat and immediately put into Lysis buffer. The samples were transported to laboratory on ice within 48 hours and stored at -80^{o}C until further analysis.

Bat Pooled urine sample collection

Bat urine samples were collected using a plastic sheet. Plastic sheets were laid at 26 spots under the trees where the urine and faeces of fruit bats were expected to be deposited as indicated by the presence of previous droppings. Each sheet was 1.5 x 1.5 meters. Sterile cotton swabs were used to soak up the urine on the plastic sheet. These were immersed immediately into 9 mL of Lysis buffer. Two cotton swabs were pooled in each Lysis buffer tube. The tubes were kept cold by placing them in a cooled box and transported back to the laboratory within 24 hours.

Bats' packed red blood cells and serum collection

Non-heparinized capillary tubes were used in blood collection from brachial vein. Bats were bled with caution to maintain a ratio no greater than 10 µL of collected blood to 1 g of bat body weight (equivalent to 1% of bodyweight). The capillary tubes were kept vertically for 30 minutes. Then the capillary tubes were centrifuged at 1,000xg for 5 minutes. Sterile pipette tips were used to separate packed red blood cells from serum in each sample. The packed red blood cells were placed in 500 µL VTM, and the serum was stored in 0.5 mL sterile tubes.

Both the packed red blood cells in VTM and the serum sample were kept in -80˚C freezer until further analyses.

Rodent capture and sample collection

Free ranging rodents were captured through pit traps and box traps. Captured rodents were removed immediately and put into cotton bags individually. Rodents were not euthanized, and were released after measurements were taken and samples were collected. Sampling included venipuncture; fecal, urine & external parasite collection; skin scrape of skin lesions; oropharyngeal, urogenital & rectal swabs; hair clipping; physical measurements (weight, height), photos, and dentition examination. Blood was drawn from the orbital vein and collected into a vial. This was only performed on anesthetized rodents. Femoral, ventral tail vein or jugular venipuncture were used for larger rodents (e.g. grass cutters). In all rodents, blood volumes of no more than 1% of body weight were drawn (example 0.5 ml blood from a 50 g rodent).

Macaques capture and sample collection

Free ranging and captive macaques will be chemically restrained by darting with anesthetic or through manual chemical injection, and handled only for the duration of sampling, thorough physical examination (PE), PIT tagging or other marking, and morphometrics. Macaque will be captured using net cages (made of rope) or metal traps placed on flat ground in a secure area or on a pallet constructed on a tree. Trapped animals will be transferred to a transfer cage with a sliding door and covered. Sampling procedures for non-human primates will include venipuncture; fecal, urine, milk (if a lactating female), and external parasite collection; oral, nasal, urogenital and anal swabs, plucked hair and milk if/when available. Blood samples from macaques will primarily be collected from the forearm veins cephalic, radial, median, and ulnar veins sampling will include non-invasive specimen collection of oral swabs from specially designed dental ropes and opportunistic collection of fresh feces and urine.

Human serum collection

Blood samples from healthy humans in hotspots were collected. After blood collection in red top vacutainers, the tubes were incubated at room temperature to let blood clot for 30 mins. To remove clots, blood was centrifuged at 1,000xg for 5 min. Following centrifugation, the separated serum was immediately transferred into 1.5 mL sterile tubes. Serum samples were immediately frozen in aliquots of 100 L at 80°C.

PCR assays

1. MERS-like CoV PCR

Hemi-nested Reverse Transcription PCR (RT-PCR) was performed using broadly reactive consensus PCR primers for CoV, targeting the RNA-dependent RNA polymerase (RdRp) gene. A total of 5µl of extracted nucleic acid was added to 50µl of reaction mixture of OneStep RT-PCR kit (QIAGEN, Hilden, Germany), per manufacturer's instructions, and reacted with each forward primer and reverse primer[3]. Hemi-nested PCR amplifications were performed using 2µl of first amplification product and 48µl of reaction mixture containing 1.0 unit of Platinum Taq DNA polymerase in 2.5mM MgCl2, 400µM dNTPs, 0.6µM of second forward primer and 0.6µM of the same reverse primer as the first round of RT-PCR. Amplification product of 282 bp was visualized using 2% agarose gel electrophoresis. All positive PCR products were further sequenced for confirmation and strain characterization.

[3] Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, Kreher P, Lattwein E, Eschbach-Bludau M, Nitsche A, Bleicker T. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Eurosurveillance. 2012 Dec 6;17(49):20334.

2. NiV nested RT-PCR

NiV nucleoprotein (N)-specific primers were used for first-round and nested PCR[4]. The PCR product was sized by gel electrophoresis in 2% agarose (NiV-PCR product is 227-bp). All PCR positive samples were re-amplified with heminested PCR[5]. The heminested primer pairs were NP1F/NP2R and NP1R/NP2F, which resulted in 342 bp and 283 bp PCR products, respectively. PCR products were purified and sequenced using direct sequencing.

3. Paramyxovirus Nested RT-PCR

Polymerase (*pol*)-specific primers were used for first-round and nested PCR[6]. The PCR product was sized by gel electrophoresis in 1.5% agarose (*pol* -PCR product is 561-bp). PCR products were purified and sequenced using direct sequencing.

4. Filoviruses Nested RT-PCR

Protocol for Filovirus detection was modified[7]. RNA polymerase L (L)-specific primers were used for first-round and nested PCR. The PCR product was sized by gel electrophoresis in 1.5% agarose (L-PCR product is <630-bp). PCR products were purified and sequenced using direct sequencing.

5. Coronavirus Nested RT-PCR

Alphacoronaviruses

RNA-dependent RNA polymerase (RdRp) gene specific primers were used for first-round and nested PCR[8]. The PCR product was sized by gel electrophoresis in 1.5% agarose (RdRp-PCR product is 434-bp). PCR products were purified and sequenced using direct sequencing.

Betacoronaviruses; severe acute respiratory syndrome coronaviruses (SARS-CoVs)

RNA-dependent RNA polymerase (RdRp) gene specific primers were used for first-round and nested PCR (Quan P, *et al.*, 2010). The PCR product was sized by gel electrophoresis in 2% agarose (RdRp-PCR product is 328-bp). PCR products were purified and sequenced using direct sequencing.

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[4] Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhome L, Rollin P, Stockton P, Rupprecht CE, Ksiazek TG, Hemachudha T. Bat Nipah virus, Thailand. Emerging infectious diseases. 2005 Dec;11(12):1949.

[5] Wacharapluesadee S, Hemachudha T. Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats. Journal of virological methods. 2007 Apr 1;141(1):97-101.

[6] Tong S, Chern SW, Li Y, Pallansch MA, Anderson LJ. Sensitive and broadly reactive reverse transcription-PCR assays to detect novel paramyxoviruses. Journal of clinical microbiology. 2008 Aug 1;46(8):2652-8.

[7] Zhai J, Palacios G, Towner JS, Jabado O, Kapoor V, Venter M, Grolla A, Briese T, Paweska J, Swanepoel R, Feldmann H. Rapid molecular strategy for filovirus detection and characterization. Journal of clinical microbiology. 2007 Jan 1;45(1):224-6.

[8] Watanabe S, Masangkay JS, Nagata N, Morikawa S, Mizutani T, Fukushi S, Alviola P, Omatsu T, Ueda N, Iha K, Taniguchi S. Bat coronaviruses and experimental infection of bats, the Philippines. Emerging infectious diseases. 2010 Aug;16(8):1217.

6. Sequencing

The positive PCR products were gel purified using the NucleoSpin® Gel and PCR Clean-up kit and sequenced directly using an automated ABI PRISM 377 DNA sequencer. Sequences were cleaned using the Bio-edit program and aligned with reference sequences collected from GenBank.

Genome characterization by next generation sequencing (NGS)

Whole genome sequencing (WGS) using NGS technology was performed on five nucleic acid specimens with relatively strong PCR positive signals. WGS was performed using enrichment library preparation (Respiratory Viral Oligos Panel, RVOP) and an Illumina MiSeq 3000 sequencer, according to the manufacturer instructions.

Genome data analysis (collaborated with Prof. Linfa's team)

Raw reads were first imported into Geneious Prime (version 2020.2.3) for downstream analysis and trimmed of adaptors with BBDuk (version 38.84). De novo assembly was conducted with clean reads by SPAdes (version 3.13.0, http://cab.spbu.ru/software/spades/) in Metagenome mode. The longest contig for each sample was then blasted against SARS-CoV-2 reference genome (MN908947) to evaluate the completeness of genome. The name RacCS203 was assigned to the best contig (29,853 nt). Each sample was then individually mapped to the reference RacCS203 genome using Geneious assembler. Coverage map, low coverage and Variant/SNP was further analyzed in Geneious. Annotation of RacCS203 was done by comparing and transferring the annotation of human SARS-CoV-2 and other related CoVs (RaTG13, BJ01, GX-P4L, SL-ZXC21, SL-ZC45 and RmYN02) after nucleotide sequence alignment done by MAFFT in Geneious Prime software. Individual gene alignment was generated by Geneious alignment and used to plot the phylogeny tree by the maximum-likelihood method with the general-time-reversible (GTR) model and 1,000 bootstrap replicates in PHYML 3.0 software. Similarity plot was generated by SimPlot (version 3.5.1). The accession number of the genome sequences used in the phylogeny analysis are tabulated in Supplementary Table 1.

Serology assay

Virus Glycoprotein Antigen-Base Multiplex Serology Assay

Human and bat sera samples were screened in a multiplex microsphere-based immunoassay (MMIA). Envelope attachment glycoporteins from henipaviruses (RBP, receptor-binidng protein), filovirids (GP), and bat SARS-related CoVs (spike) were expressed in native-like quaternary conformations then coupled to magnetic microspheres. The antigen-coupled microspheres representing 16 viruses and two additional human ACE-2-using bat SARS-related CoVs (Table 3) were prepared and provided by the Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD, USA. Bat and human samples were diluted at 1:500 in PBS and incubated with antigen-coupled microspheres. After sera incubation with antigen-coupled microspheres, samples were washed, incubated with biotinylated-Protein A and biotinylated Protein G (1:1 ratio) (Thermo Fisher Scientific, Waltham, MA, USA), washed and then finally incubated with streptavidin-phycoerythrin (PE) (Bio-Rad, Hercules, CA, USA). Antigen-antibody complexes were screend on a Bio-Rad Bio-Plex 200 HTF multiplexing system (Bio-Rad), and IgG levels were measured as median fluorescence intensities (MFI).

Analysis method

In the absence of a true control group the assay cutoff for antigen-positive IgG was generated using the assay results for NiV serology in Lyle's flying foxes (*Pteropus lylei*), a confirmed NiV reservoir and a well-studied host-virus relationship. A latent cluster analysis (LCA) was preformed on 1,002 serum and plasma samples using R-Studio. This LCA generated four distinct clusters of IgG data with three threshold cutoffs; clusters between 0-300 MFI, 301-3,357 MFI, 3,358-30,549 MFI, and above 30,550 MFI, represent naïve, cross-reactive exposures/IgG decay, recent antigen-specific exposure, and saturating positives, respectively. IgG values above 3,357 MFI were regarded as likely positive. To standardize this cutoff across antigens, and to account for the a range of cerntainity/uncerntanity between clusters and variance, we established an indeterminate range of 3,000 – 5,000 MFI. Thus, samples with IgG levels < 3,000 MFI were negative, those > 5,000 MFI were positive and those falling in between are indeterminate. Indetermine may relate to cross-reactive IgG responses with the antigens included in the serology panels, or decay of antigen-specific IgG responses to levels that are below what we would consider positive of circulating antibodies.

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Supplement data

Phylogenetic tree analysis of Paramyxovirus, Coronavirus (Quan and Watanabe protocols).

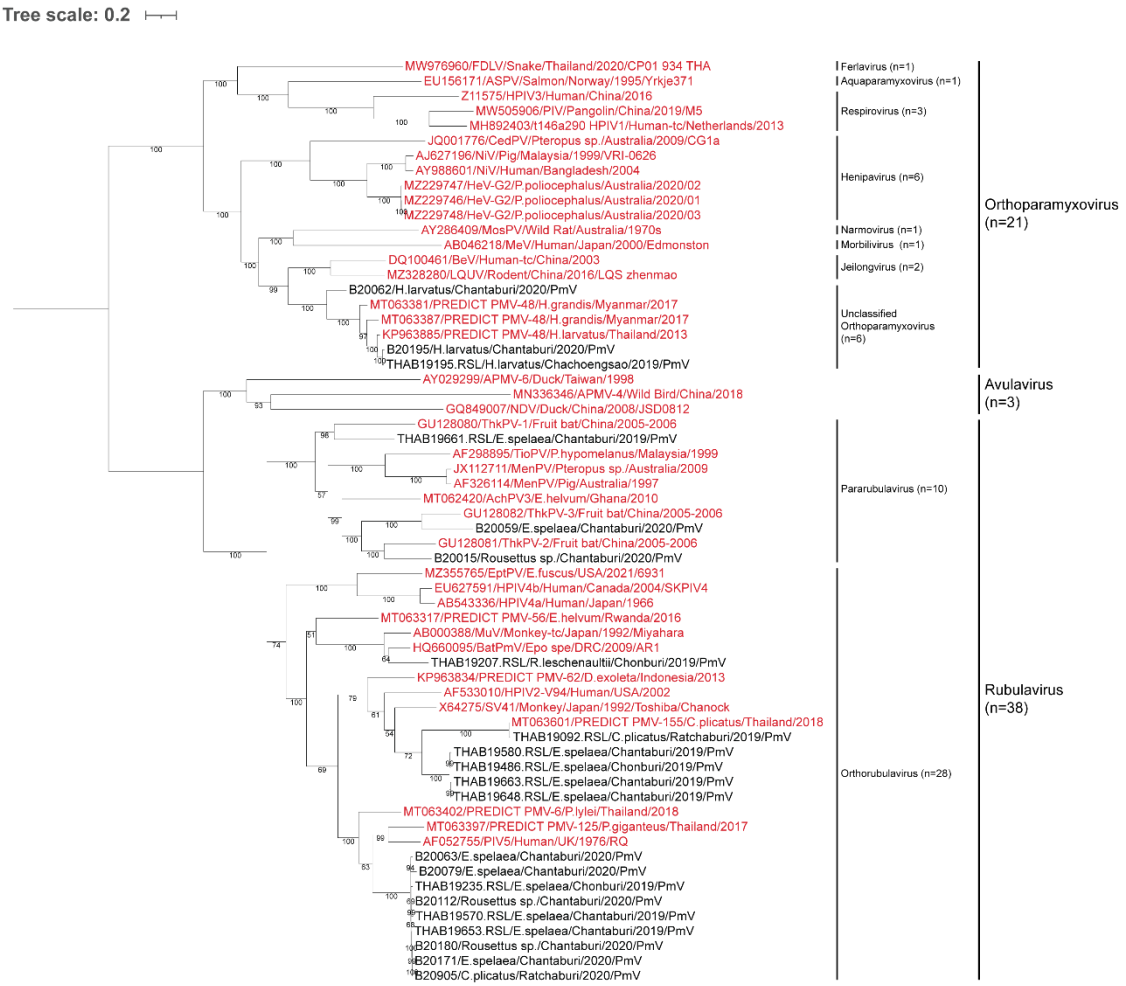

Figure S1. Phylogenetic tree generated using 530 nucleotides of the polymerase gene sequences of **Paramyxovirus (PmV)** *Hiposideros larvatus (n=3), Cherephon plicatus* (n=2), *Eonycteris spelaea* (n=12), *Rousettus leschenaulti* (n=1), and *Rousettus* sp (n=3) from this study (21 specimens, black) and reference strains of PmV (red). The location of the sampled bat in this study was indicated in the sequence name. The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected GTR+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application.

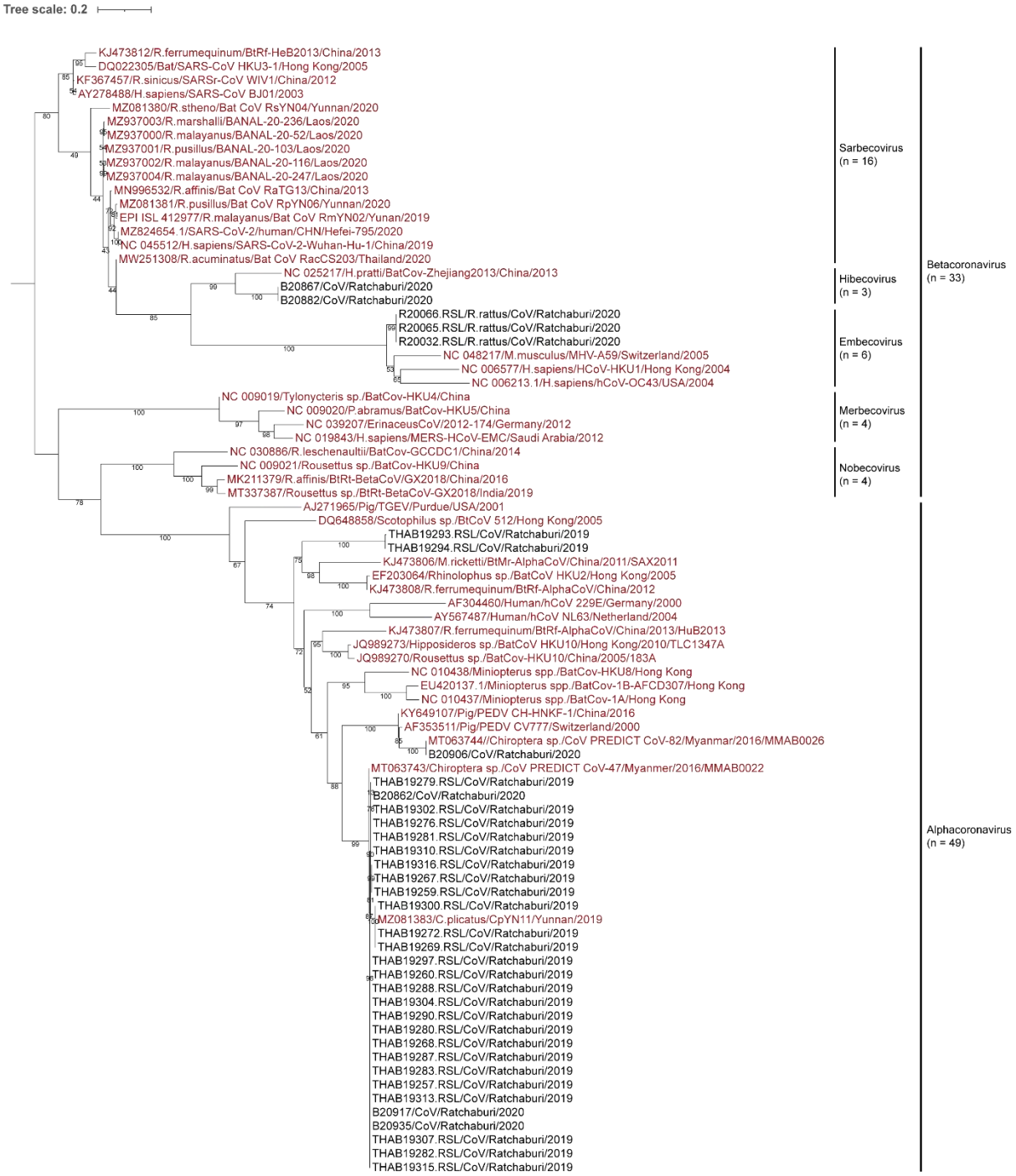

Figure S2. Phylogenetic tree generated using 286 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from the bat (*Cherephon plicatus,* n=33) and rodent(Rodentia Rattus, n=3) from **Ratchaburi** province in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected TIM2+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Quan et al.

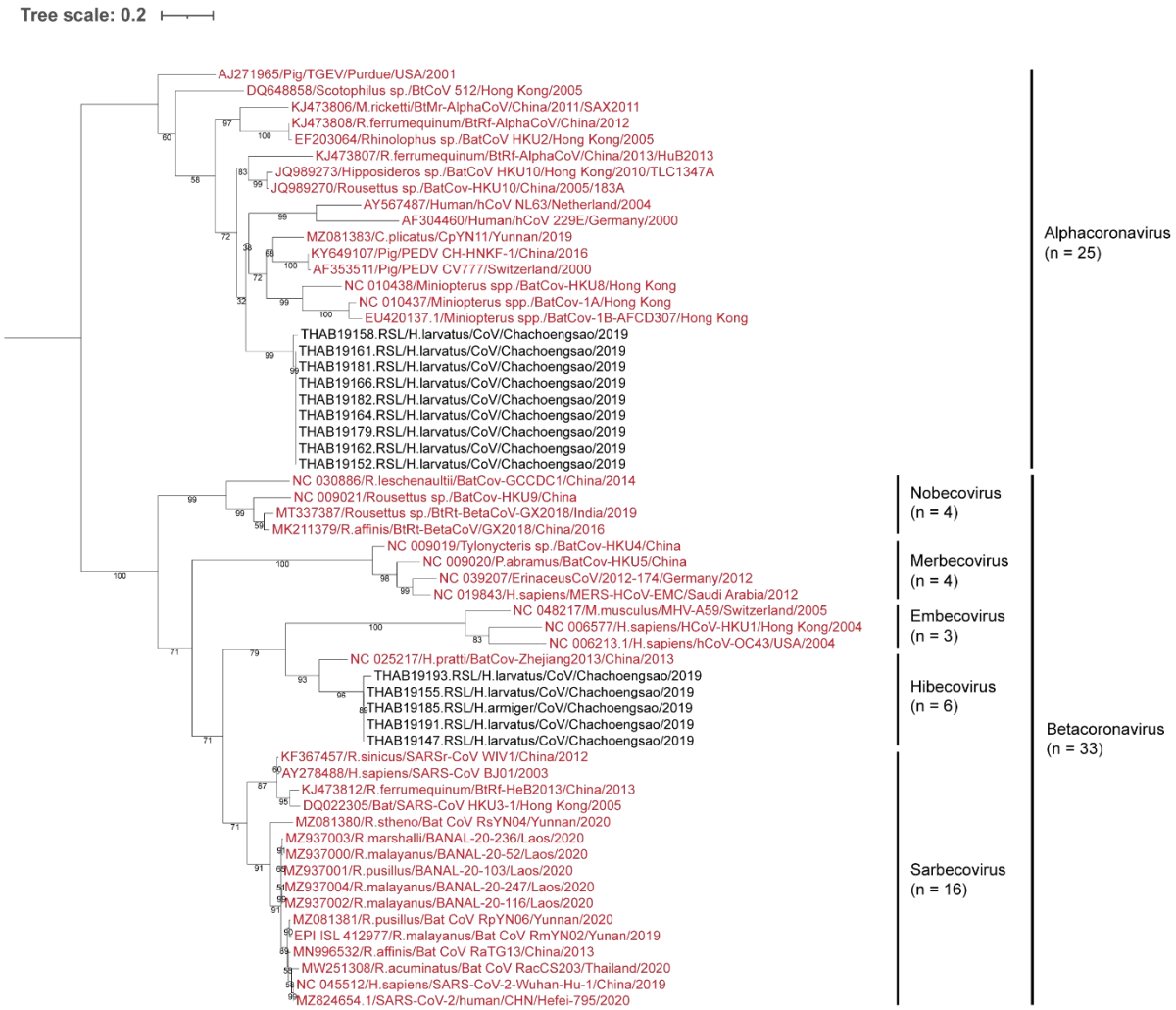

Figure S3. Phylogenetic tree generated using 252 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Hiposideros larvatus* bat (n=13) from Chachoengsao province (Wat Khao Tham Raet) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected TIM2+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Quan et al.

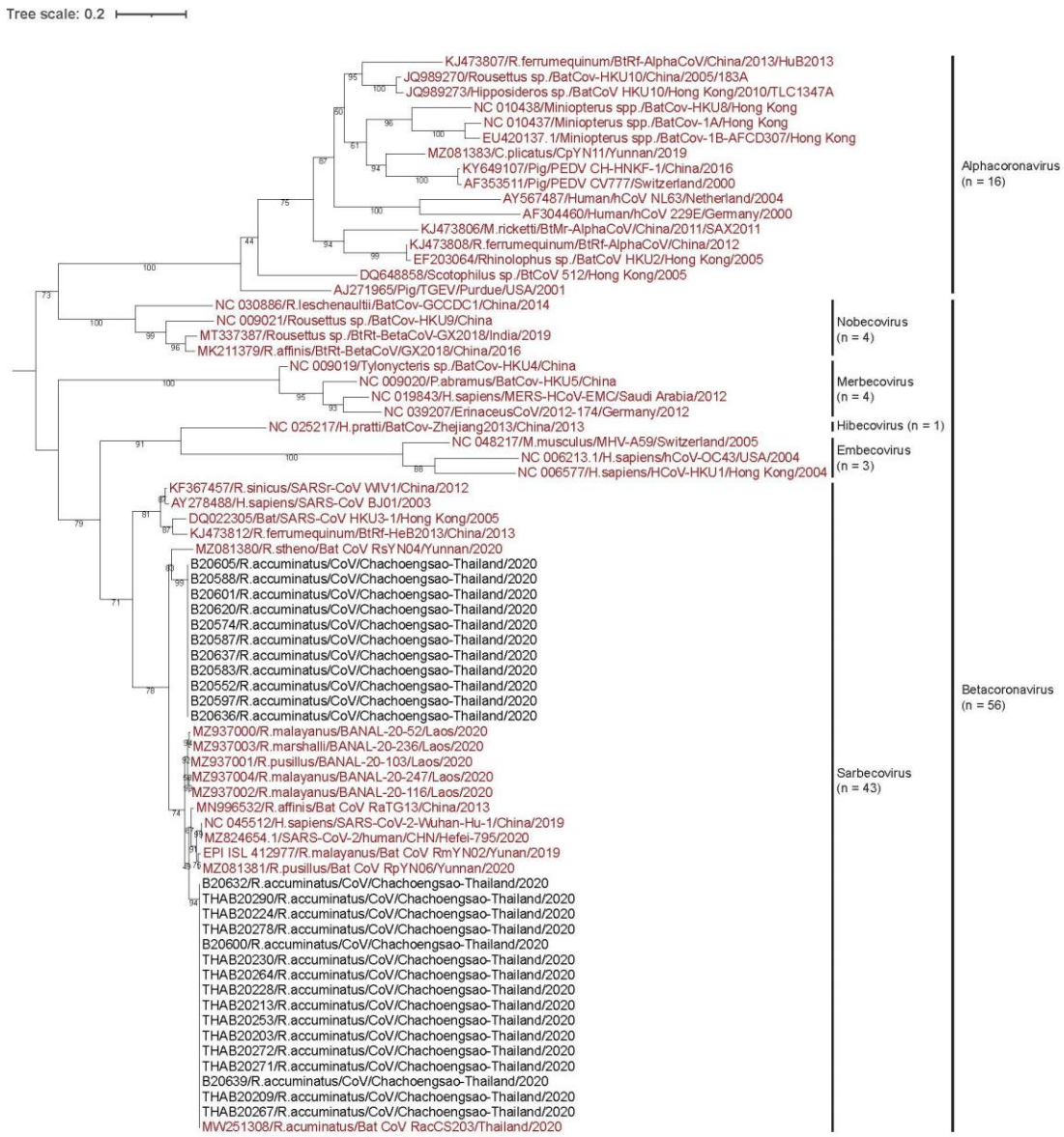

Figure S4a. Phylogenetic tree generated using 290 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Rhinolophus accuminatus* bat (n=17) from Chachoengsao province (Khao Ang Rue Nai) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected TIM2+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Quan et al.

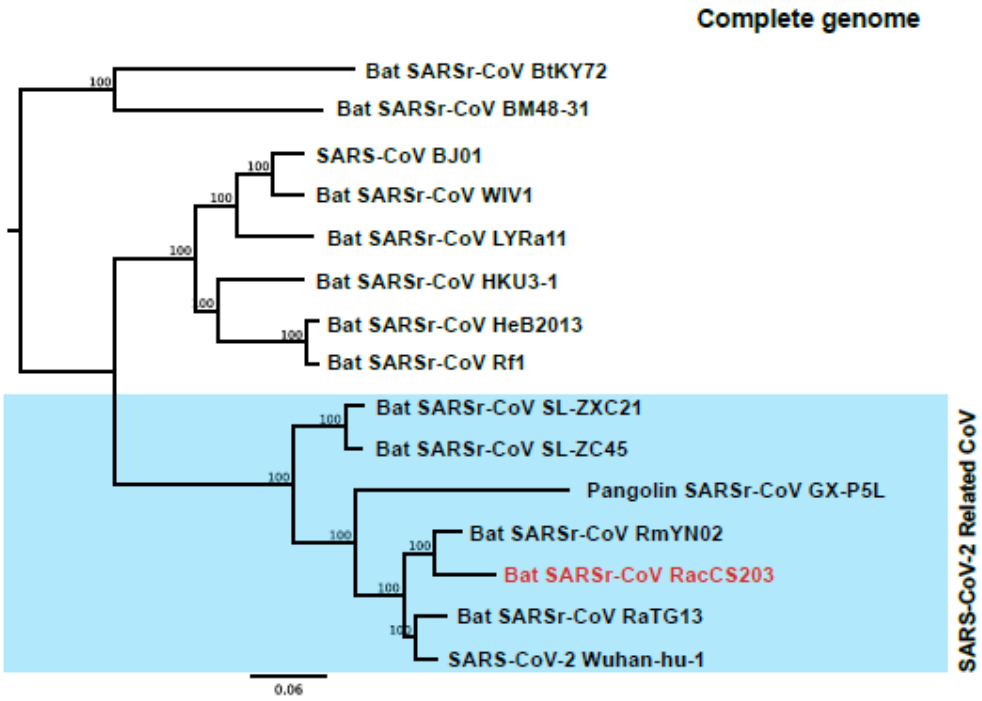

Figure S4b. Phylogenetic tree based on whole-genome sequences of SARS-related CoV from *Rhinolophus accuminatus* (RacCS203) from Khao Ang Rue Nai National park, Chachoengsao province.

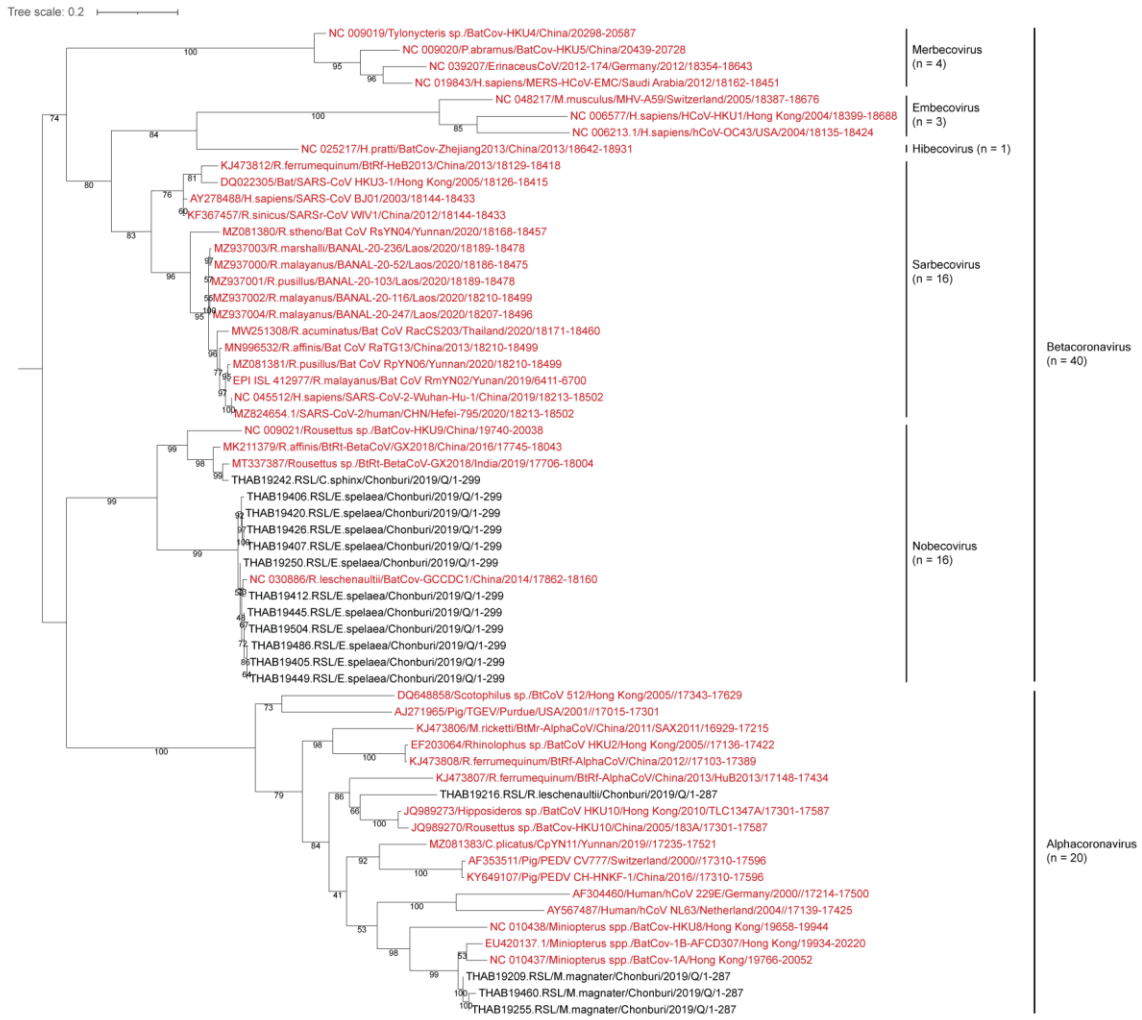

Figure S5. Phylogenetic tree generated using 287 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Miniopterus magnater* (n=3), *Rousettus leschenaultii* (n=1), *Cynopterus sphinx* (n=1), *Eonycteris spelaea* (n=11) from Chonburi province (Wat Khao Cha-Ang) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected TIM2+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Quan et al.

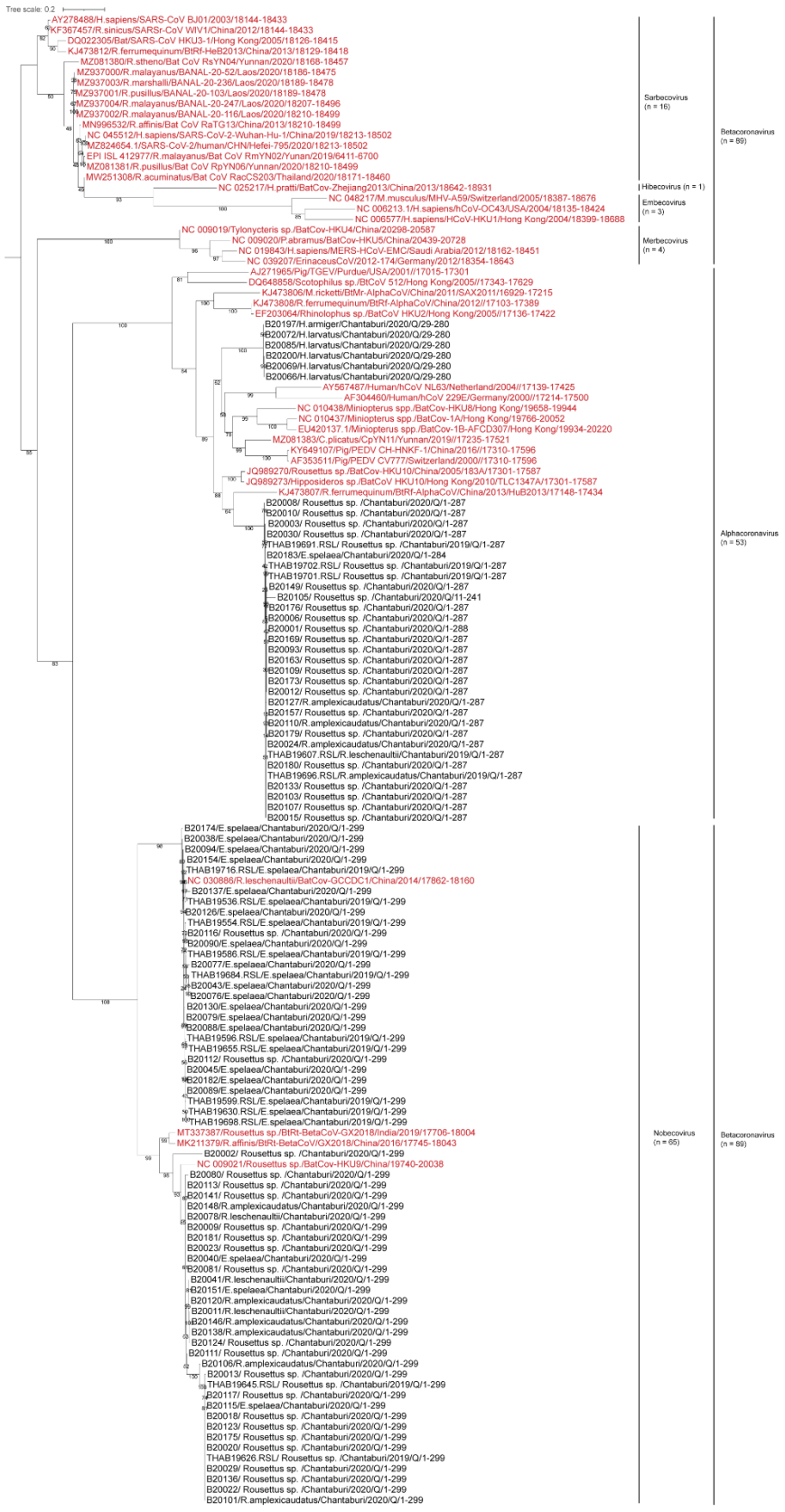

Figure S6. Phylogenetic tree generated using 142 nucleotides of the polymerase gene sequences of Coronavirus (CoV) *Hipposideros larvatus* (n=5), *Hipposideros armiger* (n=1), *Rousettus* sp. (n=48), *Eonycteris spelaea* (n=30), *Rousettus amplexicaudatus* (n=10), and *Rousettus leschenaultii* (n=4) from Chantaburi province (Khao Soi Dao) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected TIM2+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Quan et al.

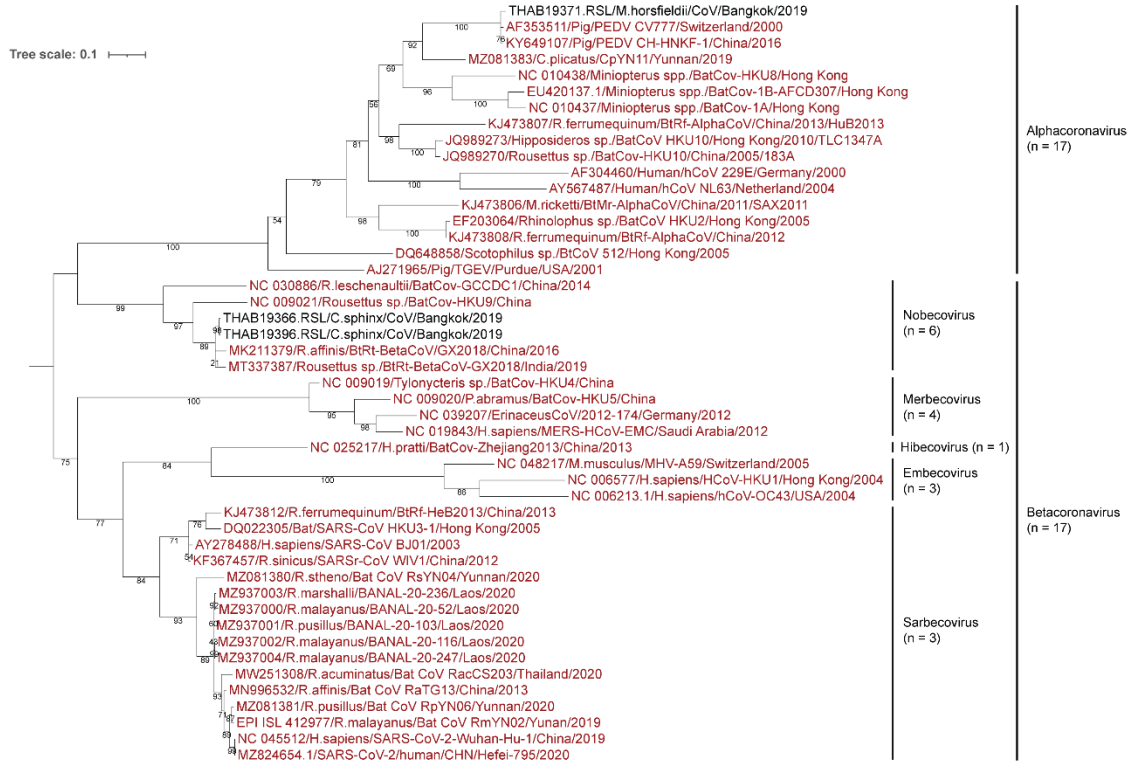

Figure S7. Phylogenetic tree generated using 287 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Myotis horsfieldii* (n=1) and *Cynopterus sphinx* (n=2) from Bangkok (Kasetsart University) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected TIM2+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Quan et al.

Figure S8. Phylogenetic tree generated using 387 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from the bat (*Cherephon plicatus,*n=26) and rodent (*Rodentia Rattus*, n=3) from Ratchaburi in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected GTR+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Watanabe et al.

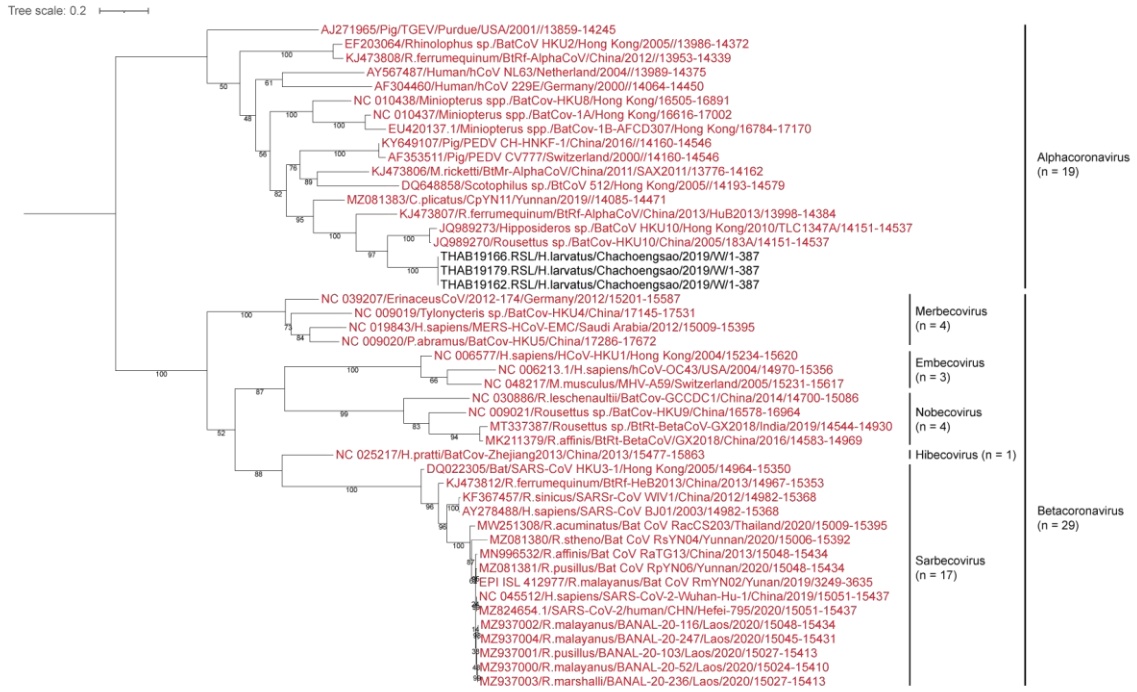

Figure S9. Phylogenetic tree generated using 387 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Hiposideros larvatus* bat (n=3) from Chachongsao (Wat Khao Tham Raet) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected GTR+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Watanabe et al.

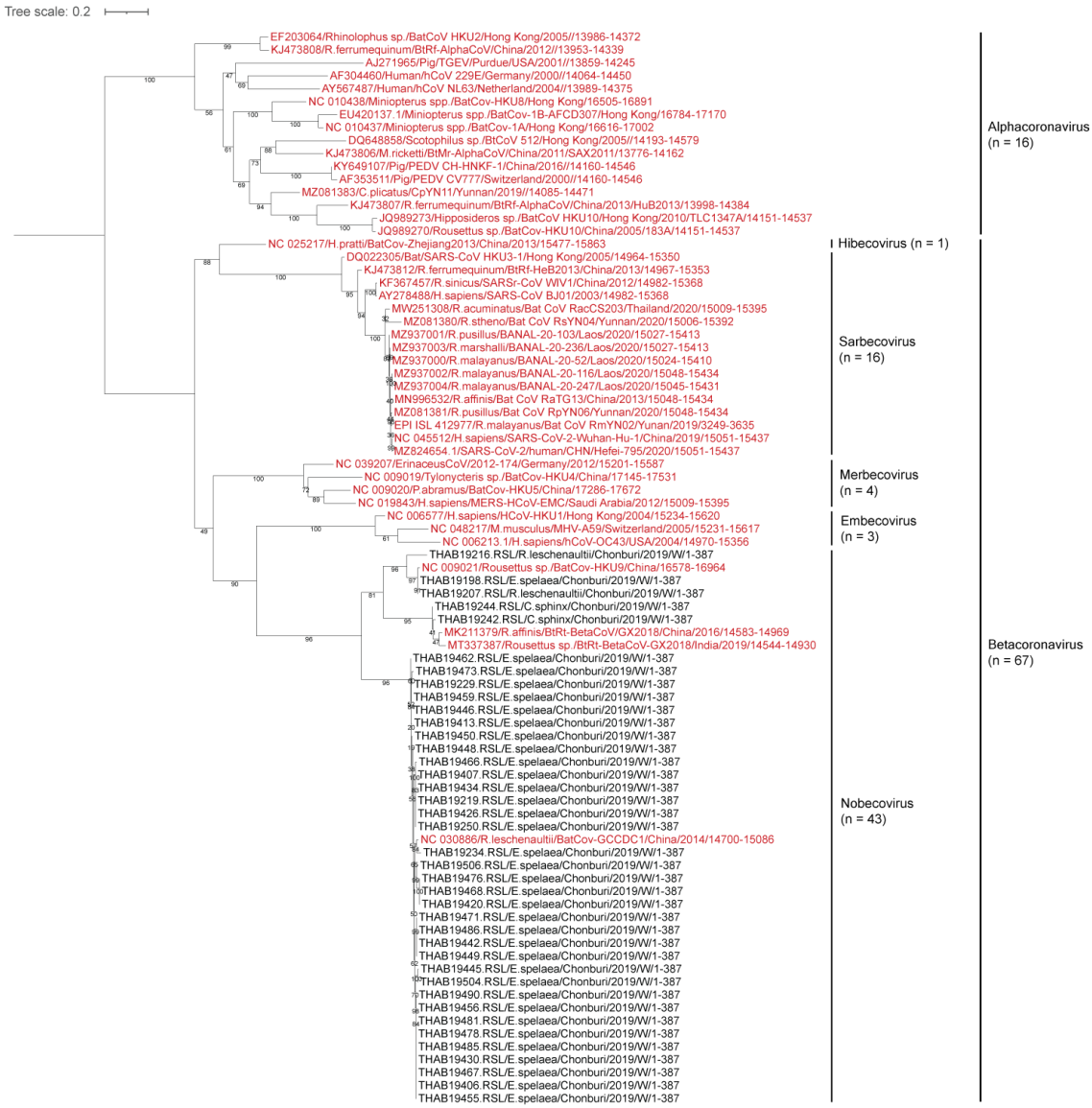

Figure S10. Phylogenetic tree generated using 387 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Rousettus leschenaultii* (n=2), *Eonycteris spelaea* (n=35), and *Cynopterus sphinx* (n=2) from Chonburi (Wat Khao Cha-ang) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected GTR+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Watanabe et al.

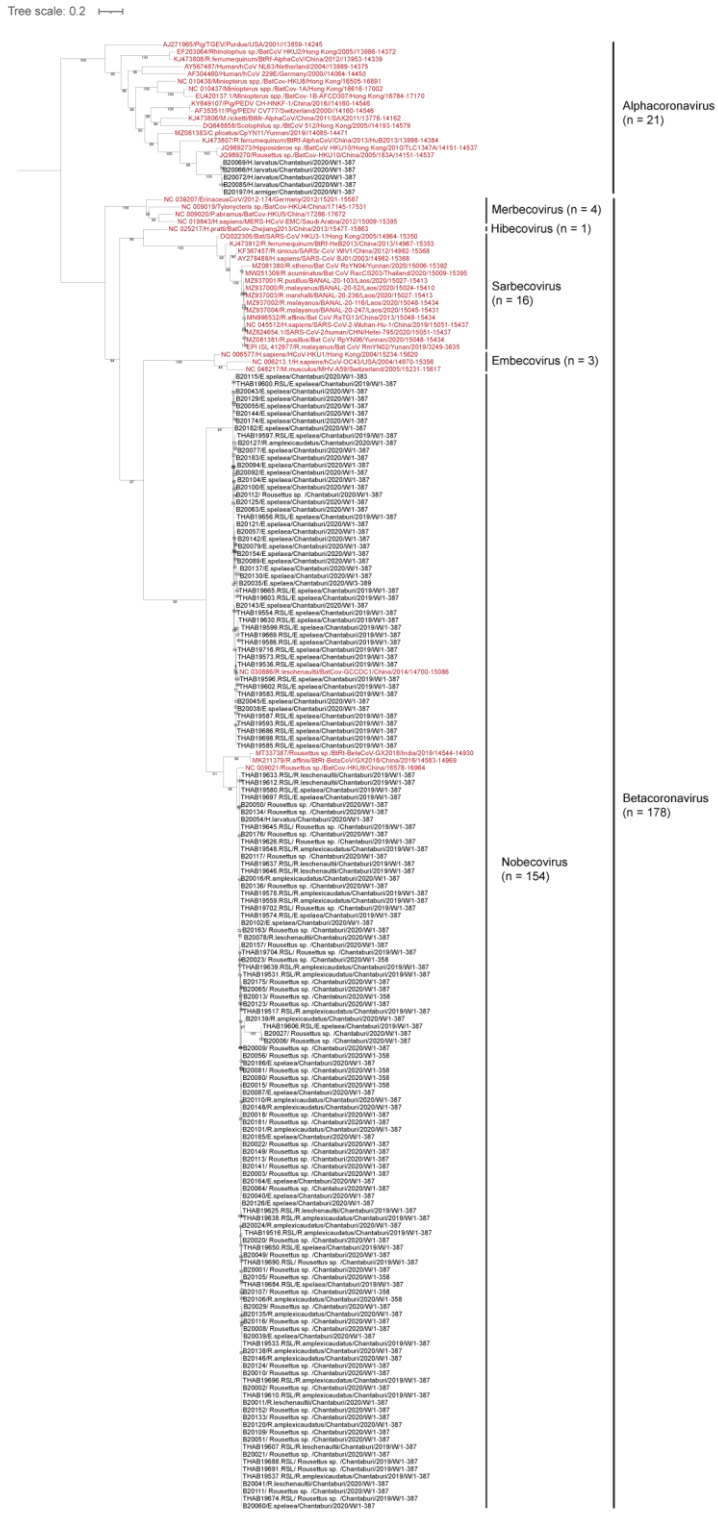

Figure S11. Phylogenetic tree generated using 387 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Hipposideros larvatus* (n=5), *Hipposideros armige*r (n=1), *Eonycteris spelaea* (n=63), *Rousettus* sp. (n=53), *Rousettus amplexicaudatus* (n=24), and *Rousettus leschenaultii* (n=9) from Chantaburi (Khao Soi Dao) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected GTR+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Watanabe et al.

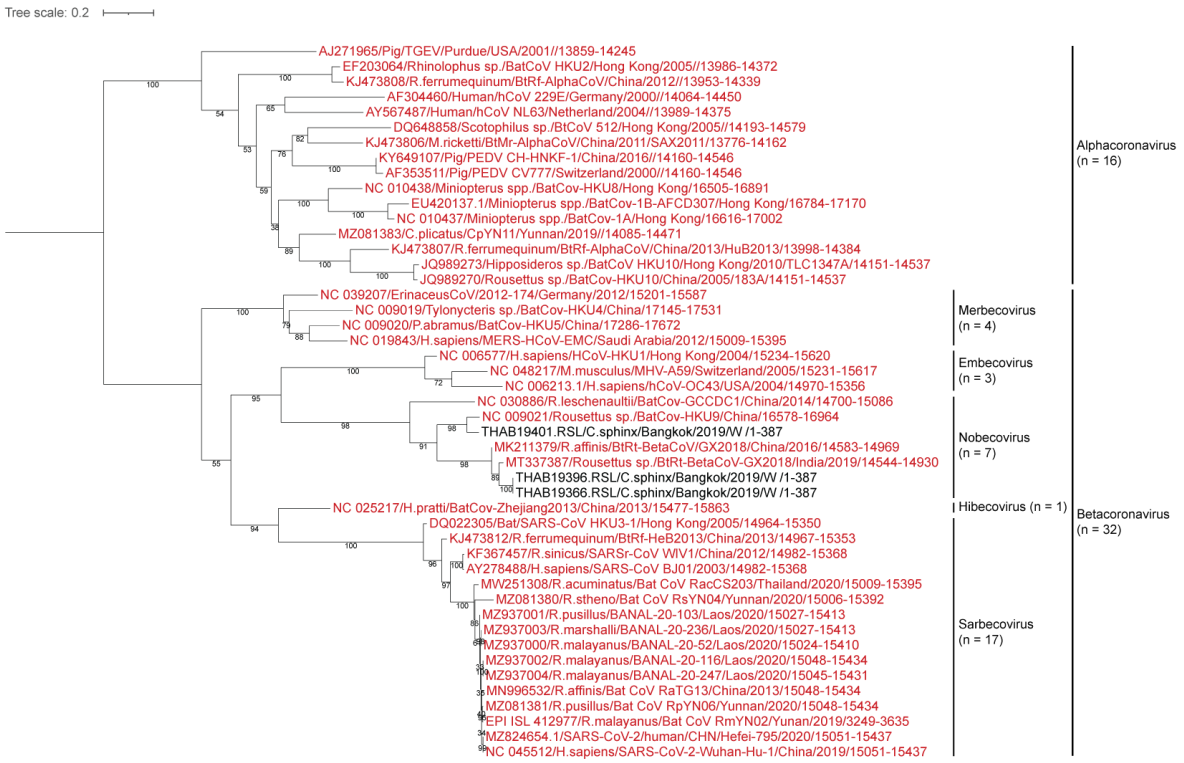

Figure S12. Phylogenetic tree generated using 387 nucleotides of the polymerase gene sequences of Coronavirus (CoV) from *Cynopterus sphinx* (n=3) from Bangkok (Kasetsart University) in this study (black) and reference strains of CoV (red). The maximum-likelihood method, with 1000 replicates bootstrap test, and ModelFinder automatically selected GTR+F+I+G4 substitution model during running IQ-TREE version 2.1.4 was used for analysis. The tree was visualized with the iTOL web application. PCR protocol was modified from Watanabe et al.

From: Supaporn Wacharapluesadee on behalf of Supaporn Wacharapluesadee <spwa@hotmail.com>
To: Plum, Gary R.; Stokes, Martha M CIV (US); Scott Vitarelli
Cc: Nareeporn Unisearch; Opass ID; Eric D Laing; Supaporn Wacharapluesadee
Subject: Final Report Project no. 042959: Deliverables for Bat Serology Study (CHULA)
Date: Wednesday, December 1, 2021 5:56:32 AM
Attachments: Final Scientific Report_Chulalongkorn_Proj 042959.pdf

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Dear Dr. Martha,

I've attached the Final Biannual Scientific Report of project number 042959 (PO: 18-0507, BLACK & VEATCH), project title "Surveillance for emerging infectious disease pathogens at the animal-human interfaces in Thailand, in coordination with PREDICT USAID project and the bat serology study" .

For your kind consideration and approval for the final (8^{th}) payment.

I apologize for the delay in sending the final report.

Best,
Supaporn and team

Supaporn Wacharapluesadee, PhD

King Chulalongkorn Memorial Hospital
Rama4 road, Patumwan, Bangkok, Thailand 10330
Skype ID: supapornwa

From: Simmi Ghai <siriporn.ghai@gmail.com>
Sent: Sunday, September 15, 2019 4:33 PM
To: Plum, Gary R. <PlumGR@bv.com>; Stokes, Martha M CIV (US) <martha.m.stokes.civ@mail.mil>
Cc: P'Chu <spwa@hotmail.com>; joe_scswu@yahoo.com <joe_scswu@yahoo.com>; Tippawan Pissawong <tpw029@gmail.com>
Subject: Re: Deliverables for Bat Serology Study (CHULA)

Dear Marty, Gary,

Please see the attached 6th deliverable, project status update report.

Best Regards,
Simmi

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Siriporn (Simmi) Ghai (MNeuroSci)
Neuroscientist / International Research Coordinator

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Tel: +66870778121 // Fax: +662-652-3122

On Wed, 10 Jul 2019 at 14:30, Plum, Gary R. <PlumGR@bv.com> wrote:

> Great news Simmi, thank you for the update.
>
> **Gary R. Plum**
> Program Director
> Federal Business
>
> Black & Veatch Special Projects Corp.
> Insular Life Corporate Center, Tower 1, 6th Floor, Insular Life Drive,
> Filinvest Corporate City, Alabang, Muntinlupa, Metro Manila, Philippines
> **D** +1 913-458-3402 **O** +63 2-358-3564 **M** +63 917-511-3624
> **E** PlumGR@BV.com
>
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>
> **From:** Simmi Ghai <siriporn.ghai@gmail.com>
> **Sent:** Wednesday, July 10, 2019 2:10 PM
> **To:** Plum, Gary R. <PlumGR@bv.com>
> **Cc:** Stokes, Martha M CIV (US) <martha.m.stokes.civ@mail.mil>; P'Chu <spwa@hotmail.com>; joe_scswu@yahoo.com; Tippawan Pissawong <tpw029@gmail.com>
> **Subject:** Re: Deliverables for Bat Serology Study (CHULA)
>
> Dear Gary,
>
> We confirm receipt of payment. Please see the attached.
>
> Best Regards,
> Simmi
>
> --
> Siriporn (Simmi) Ghai (MNeuroSci)
> Neuroscientist / International Research Coordinator
>
> Thai Red Cross Emerging Infectious Diseases - Health Science Centre
> WHO Collaborating Centre for Research and Training on Viral Zoonoses
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On Sun, 23 Jun 2019 at 05:45, Plum, Gary R. <PlumGR@bv.com> wrote:

> Thanks Simmi! I will get payment processed as soon as possible.
>
> **Gary R. Plum**
> Program Director
> Federal Business
>
> Black & Veatch Special Projects Corp.
> Insular Life Corporate Center, Tower 1, 6th Floor, Insular Life Drive,
> Filinvest Corporate City, Alabang, Muntinlupa, Metro Manila, Philippines
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> **E** PlumGR@BV.com
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>
> **From:** Simmi Ghai <siriporn.ghai@gmail.com>
> **Sent:** Friday, June 21, 2019 3:21 PM
> **To:** Plum, Gary R. <PlumGR@bv.com>
> **Cc:** Stokes, Martha M CIV (US) <martha.m.stokes.civ@mail.mil>; P'Chu <spwa@hotmail.com>; joe_scswu@yahoo.com; Tippawan Pissawong <tpw029@gmail.com>
> **Subject:** Re: Deliverables for Bat Serology Study (CHULA)
>
> Dear Gary,
>
> Please see the signed invoice attached.
>
> Best Regards,
> Simmi
>
> --
> Siriporn (Simmi) Ghai (MNeuroSci)
> Neuroscientist / International Research Coordinator
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> Thai Red Cross Emerging Infectious Diseases - Health Science Centre
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On Thu, 20 Jun 2019 at 12:22, Plum, Gary R. <PlumGR@bv.com> wrote:

> Hello Simmi
>
> I am very sorry to say that I just found this submittal along with the earlier submittal of CHULA's Project Status Update Report in my quarantined messages.
>
> Please find the attached invoice for CHULA's review and signature for these two submittals. As soon as you can return the signed invoice I will get the payment processed.
>
> Thank You
>
> **Gary R. Plum**
> Program Director
> Federal Business
>
> Black & Veatch Special Projects Corp.
> Insular Life Corporate Center, Tower 1, 6th Floor, Insular Life Drive,
> Filinvest Corporate City, Alabang, Muntinlupa, Metro Manila, Philippines
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> **E** PlumGR@BV.com
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>
> **From:** Simmi Ghai <siriporn.ghai@gmail.com>
> **Sent:** Wednesday, June 12, 2019 9:12 PM
> **To:** Plum, Gary R. <PlumGR@bv.com>
> **Cc:** Stokes, Martha M CIV (US) <martha.m.stokes.civ@mail.mil>; P'Chu <spwa@hotmail.com>; joe_scswu@yahoo.com; Tippawan Pissawong <tpw029@gmail.com>
> **Subject:** Re: Deliverables for Bat Serology Study (CHULA)
>
> Dear Gary,
>
> Please see the attached 5th deliverable, 2nd Biannual Scientific report.
>
> Best Regards,
> Simmi
>
> --
> Siriporn (Simmi) Ghai (MNeuroSci)
> Neuroscientist / International Research Coordinator
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> Thai Red Cross Emerging Infectious Diseases - Health Science Centre
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On Wed, 20 Mar 2019 at 11:22, Plum, Gary R. <PlumGR@bv.com> wrote:

> Thanks Simmi!
>
> Sent from my iPhone
>
> On 20 Mar 2019, at 11:37 AM, Simmi Ghai <siriporn.ghai@gmail.com> wrote:
>
> > Dear Marty, Gary,
> >
> > Please see the attached 4th deliverable, project status update report.
> >
> > Please let us know if you have any questions/comments. Thanks!
> >
> > Best Regards,
> > Simmi
> >
> > --
> > Siriporn (Simmi) Ghai (MNeuroSci)
> > Neuroscientist / International Research Coordinator
> >
> > Thai Red Cross Emerging Infectious Diseases - Health Science Centre
> > WHO Collaborating Centre for Research and Training on Viral Zoonoses
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> >
> > On Tue, 1 Jan 2019 at 00:48, Simmi Ghai <siriporn.ghai@gmail.com> wrote:
> >
> > > Dear Gary, Marty,
> > >
> > > Please see the attached deliverables 2 and 3, status update and biannual scientific reports.
> > >
> > > Please let us know if you have any questions/comments. Thanks!
> > >
> > > Best Regards,
> > > Simmi
> > >
> > > --
> > > Siriporn (Simmi) Ghai (MNeuroSci)
> > > Neuroscientist / International Research Coordinator

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On Tue, 2 Oct 2018 at 19:08, Plum, Gary R. <PlumGR@bv.com> wrote:

> Thank you Simmi,
>
> Receipt acknowledges.
>
> v/r
>
> **Gary R. Plum**
> Program Director
> Federal Business
>
> Black & Veatch Special Projects Corp.
> Insular Life Corporate Center, Tower 1, 6th Floor, Insular Life Drive,
> Filinvest Corporate City, Alabang, Muntinlupa, Metro Manila, Philippines
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> **E** PlumGR@BV.com
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> **Building a World of Difference.®**
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>
> **From:** Simmi Ghai [mailto:siriporn.ghai@gmail.com]
> **Sent:** Monday, October 01, 2018 7:49 AM
> **To:** Plum, Gary R. <PlumGR@bv.com>; Stokes, Martha M CIV (US) <martha.m.stokes.civ@mail.mil>; christopher.r.lewis16.civ@mail.mil
> **Cc:** P'Chu <spwa@hotmail.com>; joe_scswu@yahoo.com
> **Subject:** Deliverables for Bat Serology Study (CHULA)
>
> Dear Gary, Martha, Chris,
>
> Please see the attached project plan and schedule for the serology study (1st deliverable).
>
> Please let us know if you have any questions or comments. Thank you!

Best Regards,
Simmi

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Siriporn (Simmi) Ghai (MNeuroSci)
Neuroscientist / International Research Coordinator

Thai Red Cross Emerging Infectious Diseases - Health Science Centre
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SPECIFIC AIMS

Preventing spillovers of bat-borne viruses into intermediate hosts is critical to stopping pandemics. Human outbreaks of Hendra virus, Nipah virus, and multiple coronaviruses likely resulted from transmission from bats through intermediate hosts [1–6]. Spillovers into intermediate hosts represent important opportunities for bat-borne viruses, where they can adapt and become more infectious to humans. **Until and unless we better understand and prevent spillovers of bat-borne viruses into intermediate hosts, we will be severely limited in our ability to stop pandemics.**

Henipaviruses (including Nipah and Hendra viruses) **have characteristics that suggest they could pose a meaningful pandemic threat**: human infections are highly fatal [7], there are currently no commercially available therapies or vaccines, and they can spread person-to-person through the respiratory route [7]. Henipaviruses are not currently transmitted efficiently between people [7], but this could change, posing a threat for a henipavirus pandemic. **Nearly half of all reported human henipavirus infections were associated with contact with sick domesticated animals** [7–10]. The largest human Nipah outbreak ever identified, in Malaysia and Singapore, resulted from spillover from flying foxes (genus *Pteropus*) into pigs, with subsequent transmission to humans [11,12]. In this outbreak, **Nipah adapted to transmit well among pigs, with a reproductive number >1** [1]. Horses have been important intermediate hosts for a purported Nipah outbreak in the Philippines [9] and for Hendra virus in Australia [13].

In Bangladesh, antibodies against henipaviruses have been found in several potential intermediate host species [14], **including pigs, cows, goats, dogs, and cats,** although the specific henipaviruses infecting these animals remains unknown. There is some suggestion that animals may be infected through contact with bat-bitten fruits or contaminated tree sap [14], though **the spillover pathways into domesticated animals remain unclear, particularly for carnivores.** Each spillover into a potential intermediate host poses a risk for virus adaptation to a new host and **it is prudent to understand the frequency and transmission pathways for these spillovers.**

There is evidence that Nipah virus is transmitted to humans in Bangladesh through contact with sick intermediate hosts. During two Nipah outbreaks, human cases were more likely to have had contact with sick cows than controls [15]. In another outbreak, a child reported exposure to goats who had died from neurological illness [16]. Despite this potential risk, **detected human henipavirus cases in Bangladesh have rarely been associated with intermediate hosts** [17]. **The most likely explanation for this is systematic biases in human surveillance systems** because they are 1) optimized to identify humans infected through date palm sap consumption and 2) specific to Nipah virus, to the exclusion of other henipaviruses. More **targeted studies of henipavirus transmission at the domesticated animal-human interface are needed to uncover the true risk to humans.**

This multidisciplinary study, integrating epidemiology, ecology, and anthropology, will identify spillover pathways for henipaviruses into domesticated animals in Bangladesh and the risk they pose to human health. We will investigate henipavirus transmission at the bat-domesticated animal-human interface in Faridpur District, the most intense site of human henipavirus spillover in Bangladesh [18].

Specific Aim 1: Identify drivers of henipavirus spillovers into domesticated animals. Spillovers into humans vary substantially from year to year [18]. We hypothesize that there is also substantial temporal variation in domesticated animal spillovers driven by infection dynamics in bats, weather patterns, and domesticated animal contact with bats. We will build statistical models to identify the relative contribution of each of these factors.

Specific Aim 2: Describe which henipaviruses are being transmitted from bats to domesticated animals. We will sample sick animals in Faridpur and will sequence any detected henipaviruses. We hypothesize that domesticated animal species will be infected by Nipah virus, as well as other henipaviruses.

Specific Aim 3: Determine the risk of henipavirus transmission from domesticated animals to humans. We hypothesize that undetected henipavirus spillovers in humans are occurring through contact with sick domesticated animals. We will identify human henipavirus infections following contact with domesticated animals and identify the types of contact associated with transmission.

In this study, **we will identify henipaviruses spillovers into humans through intermediate hosts, including how and when transmission occurs.** Based on this understanding, we can update human public health surveillance systems and public health prevention strategies to reduce pandemic risk of henipavirus.

RESEARCH STRATEGY – SIGNIFICANCE

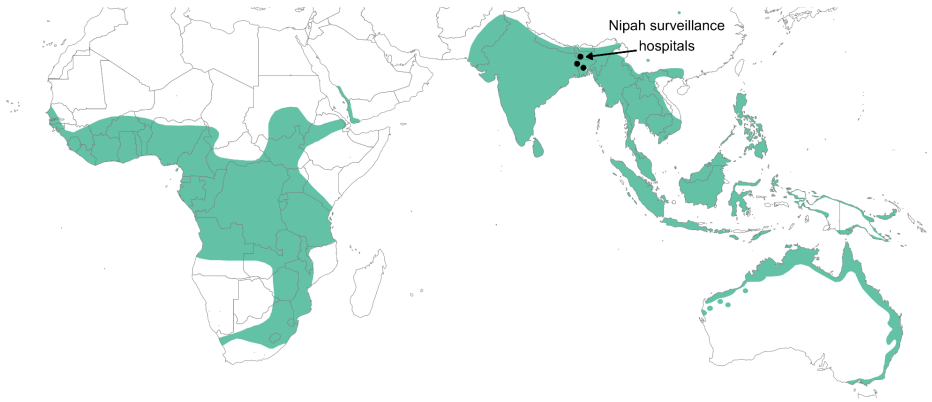
The current reactionary model for emerging infectious disease response is inadequate for preventing pandemics. In the last 50 years, the world witnessed the emergence or re-emergence of HIV, Nipah, SARS-CoV, SARS-CoV-2, H1N1 influenza, Zika, and Ebola with surprise and scarce preparation. **While research on these pathogens post-emergence eventually revealed important aspects of their ecology and evolution, this knowledge has not been successfully distilled into meaningful lessons about the nature and frequency of spillover events** or the necessary policy and infrastructure to prevent such events. If we could detect and study spillover events prior to larger outbreaks – **specifically when, where, and how they occur** – then we can work to prevent them.

To provide a model for this preemptive approach to spillovers, we must identify an appropriate pathogen. The high-profile pathogens listed above are all **RNA viruses**. The proportion of RNA viruses that can infect both animals and humans (zoonotic viruses) is substantially higher than in DNA viruses [19,20]. **RNA viruses have a wider host range on average than DNA viruses** [21] and zoonotic potential increases with host breadth [19]. The increased host breadth of RNA viruses is facilitated by **high genomic mutation rates** [22], which support within-host adaptation following cross-species transmission [23], and the **usage of evolutionary conserved binding sites** to enter host cells [24–27]. Another common feature of emerging zoonotic viruses is the involvement of **intermediate hosts** in the initial spillover event, as demonstrated by the role of farmed civets in the emergence of SARS [4,28], camels in outbreaks of MERS [6,29,30], and horses in the case of Hendra virus [3,31]. These intermediate hosts, often domesticated animals, may have **more frequent contact with humans** than the wild reservoir hosts and therefore increase the likelihood of spillover into humans [32]. Additionally, **genomic adaptation of viruses taking place within intermediate hosts** that are more phylogenetically related to humans than the reservoir host could **select for mutations that favor onward transmission** to humans [23,33,34].

Henipaviruses, single-stranded RNA viruses (family *Paramyxoviridae*), are a **highly appropriate model** for studying the nature of spillovers of zoonotic viruses. Henipaviruses, including Nipah and Hendra viruses, have caused outbreaks of human illness with high case fatality in Australia, Malaysia, Singapore, Bangladesh, India, and the Philippines [7–9,13,31,35,36]. Henipaviruses bind to the ephrin-B2 [37,38] and ephrin-B3 [39] receptors using the attachment glycoprotein (G) to enter host cells. The **conserved nature of these receptors allows henipaviruses to infect cells of a wide diversity of species** beyond their reservoir hosts, pteropodid fruit bats [40–48], including human, horse, pig, cat, dog, and mouse [49]. Pigs and cats are also susceptible to experimental infection with Nipah virus [50,51]. The broad host range of henipaviruses has facilitated human outbreaks through initial spillover from bats into domesticated animals in three cases: the multiple outbreaks of Hendra virus in horses and humans in Australia since 1994 [13,31], the 1998-1999 outbreaks in Malaysia and Singapore began in farmers and abattoir workers in close contact with infected pigs [2,11,52] and a 2014 outbreak in the Philippines occurred from contact between humans and horses [9]. While the conserved host receptors allow henipaviruses to infect human cells, observed outbreaks with person-to-person transmission have not been sustained for more than five generations [7]. However, the **elevated mutation rate of henipaviruses as RNA viruses** [22] and their geographic niche in a densely populated and interconnected region of the world (**Figure 1**) highlight the need for a deeper understanding of spillover pathways. **Each new spillover event from bats into a new host represents an opportunity for a new henipavirus strain to emerge with greater transmissibility in humans and thus greater pandemic potential** [53].

Figure 1.

Range of pteropodid fruit bat hosts with henipavirus sequences.



It is clear that **monitoring human cases alone is insufficient to understand the nature and frequency of henipavirus spillover events**. Further support comes from the case of Bangladesh. Bangladesh is the only country with systematic surveillance of human henipavirus infections, specifically Nipah virus [54], so the human spillover events outside of Bangladesh have exclusively been

detected as part of outbreaks. Since Bangladesh represents a small portion of the geographic range that pteropodid fruit bat hosts of henipaviruses occupy in Africa, Asia, and Australia (**Figure 1**), **many henipavirus spillovers into humans likely go undetected**. Furthermore, active surveillance for acute cases has been ongoing in Bangladesh since 2007, yet an estimated half of all human cases in the catchment area of hospitals still go undetected [55].

Looking for infections exclusively in humans is also an overly narrow approach for detecting henipavirus spillover events, especially given the importance of intermediate hosts in past outbreaks of henipaviruses in Australia, Malaysia, Singapore, and the Philippines. There is also evidence that **screening specifically for Nipah or Hendra virus may be an overly narrow approach as bats may be excreting a diversity of henipaviruses.** In Bangladesh, there is evidence of henipavirus exposure in domesticated animals living around *Pteropus* bat roosts in areas where human Nipah virus outbreaks have been identified [14]. Antibodies detected in animals in this study **cross-reacted with but did not cross-neutralize Nipah virus in cattle, goats, and pigs** [14], suggesting that Nipah virus strains or henipaviruses with antigenically-distinct receptor-binding pockets may be the source of these exposures.

The work detailed in this proposal will use **henipaviruses in Bangladesh as a model system to understand the nature and frequency of spillovers through intermediate hosts**. This work will illuminate **when and how spillovers are occurring** along the bat-domesticated animal-human pathway in Bangladesh (**Figure 2**), thereby providing the information necessary to **preempt larger outbreaks**. Findings from this study will allow us to **target interventions to prevent spillovers into intermediate hosts and mitigate the risk of viral mutations in these hosts that could lead to a human pandemic**. Furthermore, the approaches devised for this work provide a scaffold for studying other emerging zoonotic diseases to develop a **general understanding of pathogen spillover** and effective methods of prevention.

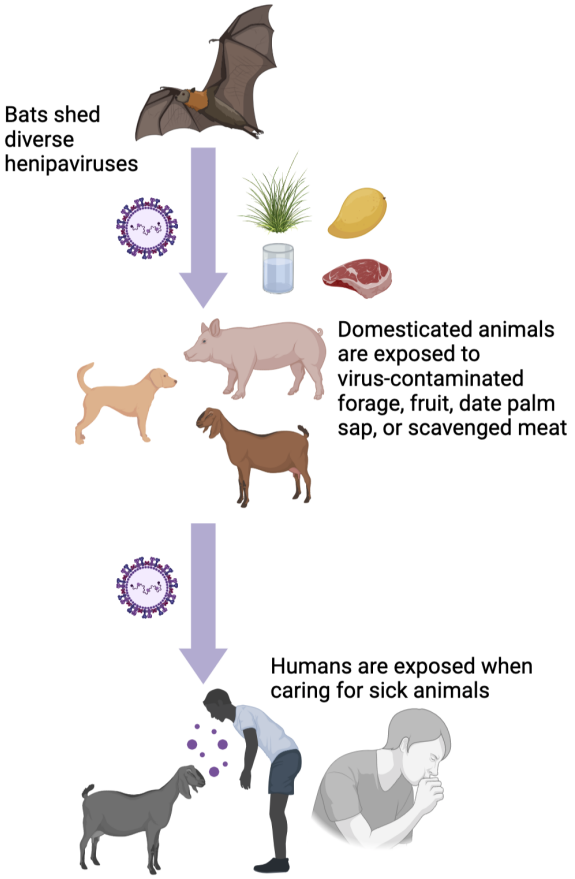


Figure 2. Henipavirus spillover from bats to humans through domesticated animal hosts.

RESEARCH STRATEGY – INNOVATION

The emergence of highly transmissible henipaviruses depends upon multiple layers of viral interactions between multiple species. Despite the risk posed by henipaviruses and the complex nature of henipavirus ecology, studies which prospectively address every layer of risk – from dynamics in the reservoir host, to spillover to intermediate hosts, to subsequent human infections – are exceedingly rare. **We are unaware of studies which have attempted to simultaneously identify and explain transmission pathways from bats to intermediate hosts and from intermediate hosts to humans.** The comprehensive study we propose is only possible with a multidisciplinary team that includes expertise across bat health, human behavior, viral ecology, henipavirus serology, and epidemiology.

Although the first detected Nipah virus spillover occurred in Malaysia, annual spillovers have been reported from Bangladesh since 2001, resulting in more reported human spillovers than any country in the world. **Given strong evidence of henipavirus infections in domesticated animals, particularly pigs** [2,14,56–58], **and the close contact humans have with sick animals in Bangladesh** [59,60], **we would expect to see frequent infections in humans resulting from contact with sick animals.** However, the majority of human Nipah spillovers *detected* in Bangladesh, primarily through hospital-based surveillance, have been associated with date palm sap consumption [16,61], where humans are infected directly from bats. At a first glance, this evidence suggests that human henipavirus infections are rarely acquired through intermediate hosts. However, **there are very good reasons to believe that current human henipavirus surveillance strategies systematically exclude human infections acquired through contact with sick animals.** The existing hospital-based surveillance for human henipavirus infections likely excludes spillover from intermediate hosts in four key ways. First, surveillance in hospitals for Nipah virus occurs only during the winter months when humans most frequently consume date palm sap, which is a well-known pathway of transmission from bats to humans [54]. In a recent analysis of zoonotic exposures among patients with severe

infections in Nipah surveillance hospital sites, most patients who had contact with a sick animal sought care outside of the winter season [62]. Therefore, **since human surveillance for Nipah is limited to winter, spillovers from contact with sick animals will be missed.** Second, current surveillance for Nipah in hospitals targets identification and testing of patients with encephalitis [54]. In Malaysia, where humans were infected from contact with sick pigs, many patients presented with atypical pneumonia [63]. **If henipavirus infections in humans are associated with respiratory illness rather than neurological illness, then spillovers through intermediate hosts may be systematically missed in Bangladeshi surveillance**. Third, hospital-based surveillance for Nipah cases assumes that most persons with infections will seek care from a hospital. Prior studies of care seeking in Bangladesh show that a minority of patients experiencing symptoms compatible with encephalitis or severe respiratory disease actually seek care at hospital [64]. **Communities that raise pigs in Bangladesh are very poor and highly marginalized** [59] **and are therefore even less likely to seek care should they become ill.** Seroprevalence surveys of domesticated animals in Bangladesh suggested that pigs are at highest risk of infection (>40% seroprevalence) [14], and could pose the greatest risk as an intermediate host, which is consistent with the large outbreak reported from Malaysia and Singapore [2,11,63,65], so excluding humans who have regular contact with pigs is a major limitation. Therefore, **hospital-based surveillance for human henipavirus infections very likely excludes any illness related to exposure to sick pigs.** Finally, human surveillance for henipavirus infections in Bangladesh is specific to Nipah virus. Serologic evidence in domesticated animals suggests the circulation of antigenically-distinct Nipah virus strains or closely-related henipaviruses [14]. If **these non-Nipah infections** in animals led to human infections, they **would be missed by Nipah-specific PCR-based diagnostics for infection**. Surveillance for human infections with henipaviruses is expensive because spillovers are identified in a minority of patients tested [54]. **If findings from this study show that current surveillance truly excludes spillovers through intermediate hosts, the evidence will be immediately useful for improving surveillance systems and designing interventions to prevent spillover.**

Past **serologic studies of bat and domesticated animal henipavirus infections** are limited because they **cannot easily distinguish between the type of henipavirus that caused the infection** [14]. Past studies of human spillovers are severely limited because they rely primarily on PCR-based diagnostics which are specific to Nipah virus, potentially missing other henipavirus spillovers [7,54]. **The serologic assays we will use in this study are uniquely designed to overcome these difficulties in identifying and distinguishing between henipavirus infections.** Through a multiplex pan-henipavirus antibody-binding assay and surrogate virus neutralization test that have been optimized for non-clinical research and biosurveillance, **we will be able to detect the serological footprints of all five known henipaviruses: Nipah virus, Hendra virus, Cedar virus, Ghana virus and Mojiang virus.** The majority of antibodies that develop after virus infections are conformation-dependent and bind to three-dimensional epitopes present in the quaternary structure of virus proteins. The henipavirus envelope attachment glycoprotein (G) interacts with the ephrin receptors, mediates cellular entry, and is the primary target of protective neutralizing antibodies. Serum samples will be simultaneously incubated with each recombinant henipavirus G_t antigen, **permitting antibody epitope and affinity competition, and improving specific antibody binding to the homotypic antigen representing the probable prior henipavirus infection**. This approach will allow us to improve specificity for Nipah virus antibody detection and investigate whether other henipaviruses are circulating that may be missed by nucleic acid detection or serology assays that only target Nipah virus.

RESEARCH STRATEGY – APPROACH

Preliminary data

We have known that **humans are infected by bat henipaviruses through contact with sick domesticated animals** since 1994, when Hendra virus was first discovered [13,66]. Hendra virus has caused multiple outbreaks of respiratory or neurological illness in horses and veterinarians and horse trainers became ill after close contact with sick animals [31]. A much larger outbreak of the related Nipah virus occurred among humans and pigs in Malaysia and Singapore in 1998-1999 [2,11,52]. It is hypothesized that this outbreak was sparked by exposure of pigs to Nipah virus via dropped fruit from bats visiting cultivated mango trees adjacent to intensive pig farms [59,60]. Sustained respiratory transmission in pigs led to an outbreak in workers on pig farms and in abattoirs, totaling 265 human cases and 105 deaths. **The outbreak ended only after one million pigs were culled, highlighting the importance of intermediate hosts** [2]. A recent henipavirus outbreak in the Philippines in 2014 involved neurological illness in 9 horses and in 17 humans who either slaughtered horses, consumed horse meat, or had close contact with an infected person [9]. There is also **evidence that outbreaks of Nipah virus in Bangladesh have involved contact with animals** [7,15]. During two outbreaks in 2001 and 2003, epidemiologic studies showed that cases were more likely to have had contact with sick cows than controls

[15]. In addition, one child with Nipah reported exposure to goats who had died from apparent neurological illness [16]. *P. medius* bats feed frequently on cultivated fruit trees in populated areas [61], dropping partially eaten fruits that are consumed by humans or fed to domesticated animals [14,61]. **Dogs, cats, and pigs may be exposed to bats or their excreta, and therefore Nipah virus, when hunting or scavenging underneath bat roosts** [8,9,59], though evidence about these exposures is limited. **We will build upon this accumulated knowledge about potential routes of henipavirus transmission from domesticated animals to humans in the design of our human contact studies.**

The **diversity and host range of henipaviruses posing a threat to animal and human health continues to expand**. Following the discovery of Hendra virus and Nipah virus, Cedar virus was discovered in Australian flying foxes [42], Ghana virus from straw-colored fruit bats [41], and Mojiang virus from rats in China [67]. The broad species tropism of **Hendra virus and Nipah virus is facilitated by virus receptor-usage of ephrin-B2 and ephrin-B3, which are highly conserved proteins across mammals** [37–39]. **Many types of domesticated animals have evidence of henipavirus infections** in parts of the world where bats carry these viruses, though past studies have not identified specifically which viruses are infecting which species. Horses, pigs, goats, dogs, and cats have been infected as part of known human henipavirus outbreaks [2,8,9,68–70] and serological surveys have identified antibodies against henipaviruses in cattle, goats, and pigs in Bangladesh [14], sheep and goats in Ghana [56], pigs in Uganda [58], and pigs and horses in Nigeria [57]. **We will focus our study of henipavirus spillover to domesticated animals on species that have been previously identified as possible intermediate hosts.**

Faridpur District is the perfect location to study spillovers of henipaviruses because we have strong evidence of infections in bats, domesticated animals, and humans. Henipavirus infection has been repeatedly detected in multiple *Pteropus medius* fruit bat roosts across Bangladesh since 2003 **[15,71–73]**. **However, the longest running henipavirus surveillance effort in bats is in Faridpur District, and we will conduct our study among animals and humans living near this roost site.** Faridpur District has reported more spillovers of Nipah virus in humans than any other district in Bangladesh, so **we know that spillover risk exists in this locale**. *Detected* human spillovers of Nipah virus in Faridpur have primarily been associated with direct bat-to-human transmission through consumption of date palm sap [17,61], though current surveillance strategies may miss many spillovers (see Innovation). **Serologic studies have also detected henipavirus infections in domesticated animals in Faridpur and the surrounding areas.** In one study, 1112 cattle, goats and pigs were tested, including 80 cattle and 80 goats from Faridpur District. Sixteen percent (N=181) of domesticated animals had cross-reactive antibodies to Nipah virus, though none neutralized Nipah [14]. **Twenty-one percent of cattle and goats enrolled in the study from Faridpur District (34/160) had cross-reactive antibodies; animals with antibodies were more than three times more likely than those without antibodies to be fed juice from fruits or dropped fruit**, presumably contaminated with bat urine or saliva [14].

The combination of ecological forces that leads to henipavirus exposure in domesticated animals in Bangladesh is unknown, but we will build upon the understanding of these dynamics from the Hendra virus system in Australia and preliminary findings from Bangladesh. Ultimately, henipavirus spillovers are likely driven by multiple factors, including bat virus dynamics, weather patterns, and contact patterns between bats and domesticated animals. In Australia, climate change, urbanization, and nutritional stress have led to increases in viral shedding and contact with horses as bats move into human habitats in search of food [3]. Preliminary data from Bangladesh suggests that the **inter-annual variation in human spillovers is strongly associated with cold winter temperatures**, but how these variables are related is unclear [74].

In Bangladesh, human henipavirus spillovers likely go undetected because surveillance is focused on severe illness and is highly specific to Nipah virus. Serological surveys in healthy or mildly ill humans have identified henipavirus exposure in Malaysia, Singapore, and Cameroon [52,65,75–77]. Hospital-based surveillance for human encephalitis looks specifically for Nipah virus infections, using PCR with specific primers and Nipah specific serologic assays [54]. In one study in Bangladesh, patients with encephalitis with no evidence of Nipah virus infection were more likely to die if they recently drank date palm sap, **suggesting that they may have been infected by another bat virus transmitted through the same route** [62]. **Recently, a novel Hendra virus variant was detected in horses in Australia** by broadening the specificity of the diagnostics being used in the surveillance system (Annand et al., in review); this virus had previously gone undetected because diagnostics were specific to previously identified Hendra variants. **Given this evidence, we will use laboratory assays that will be able to identify a broad range of henipaviruses infecting domesticated animals and humans to uncover hidden spillovers.**

In this study we will utilize a multiplex pan-henipavirus assay which has been recently developed and already used to investigate henipavirus infections across multiple species. Expression of native-like

soluble henipavirus G tetramers (sG_{tet}) has been previously described [78] and these Hendra virus (HeV) and Nipah virus (NiV) antigens have been used in ELISAs and Luminex xMAP-based multiplex serology assays for over 15 years to detect antibodies in domestic animals and wildlife [14,71,79–81]. Since the discovery of Cedar (CedV), Ghana (GhV), and Mojiang (MojV) viruses, the group at USU has expressed G from each virus and developed a pan-henipavirus sG_{tet}-based microsphere-based multiplex immunoassay (MMIA). When applied to field-collected wildlife biosurveillance, NiV-reactive IgG were detected in Indian flying foxes (*Pteropus medius*) with expected cross-reactivity to HeV G (**Figure 3**). Sera were serially diluted two-fold, and at low dilutions, cross-reactive IgG to heterotypic HeV G was observed. Specificity for NiV G increased with dilutions, demonstrating that these Indian flying foxes had the highest IgG titer specific to NiV, permitting us to strengthen our interpretation of the serological footprint of NiV.

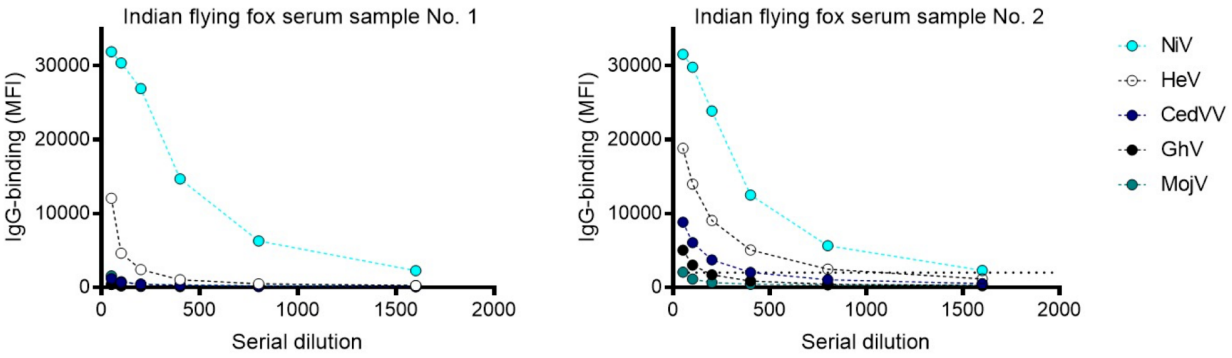


Figure 3. Screening Indian flying fox sera with sG_{tet} pan-henipavirus MMIA for evidence of exposure to Nipah virus (NiV).

The same assay was used to screen human sera collected in an acute febrile illness study in Cambodia (Laing et al., unpublished data)**. Although we detected minimal reactivity with Nipah virus G, several individuals had a distinct pattern of sera cross-reactivity with positive IgG to Ghana virus, Cedar virus, and Hendra virus** (**Figure 4**). These serological footprints to other known henipaviruses would have been missed with a single NiV antigen-based assay. As this testing was performed outside the area endemic for Ghana virus and Ghana virus is phylogenetically ancestral to Nipah virus and Hendra virus, these seropositive results are consistent with the presence of unknown henipaviruses in Southeast Asia that are ancestral and antigenically-distinct from NiV.

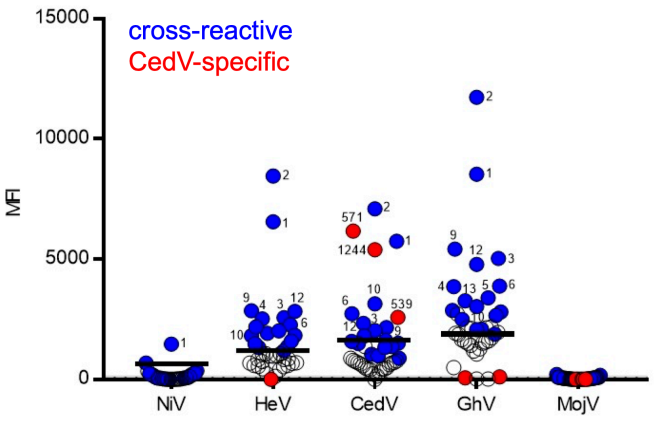


Figure 4. Serologic evidence of human exposure to unknown henipaviruses antigenically-related to GhV (N=1469 individuals). Cross-reactive sera are individually numbered.

Overall strategy

In Year 1 of the grant, we will set up a field site in Faridpur, Bangladesh to study henipavirus spillovers from bats to domesticated animals and subsequent transmission to humans (**Figure 5**). In Year 1, we will map the animal and human populations surrounding the *Pteropus* bat roost site in Faridpur and follow bats and animals through Year 4 to identify spillovers and explain their drivers (Aim 1). We will identify which viruses are spilling over through sick animal surveillance during Years 1-4 among domesticated animals living near the bat roost (Aim 2). Finally, we will conduct a cross-sectional serosurvey in humans and also identify humans with contact with sick animals to identify transmission of henipaviruses from intermediate hosts (Aim 3). Each aim will span multiple years of the grant (**Figure 6**).

Figure 5.
Project timeline.

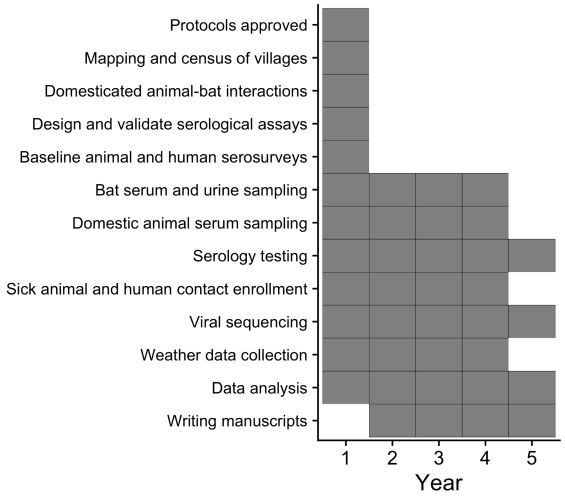
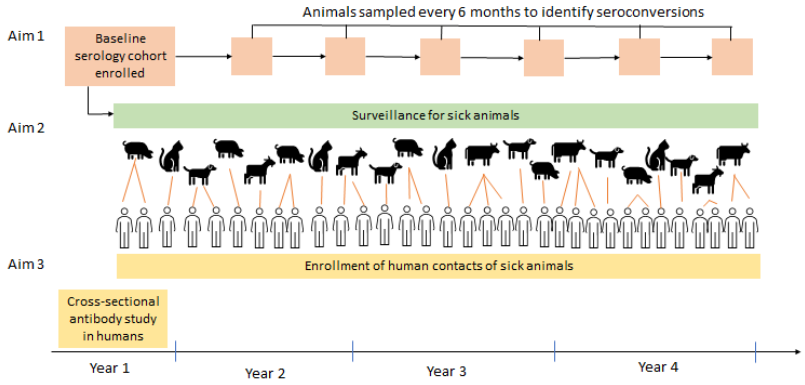


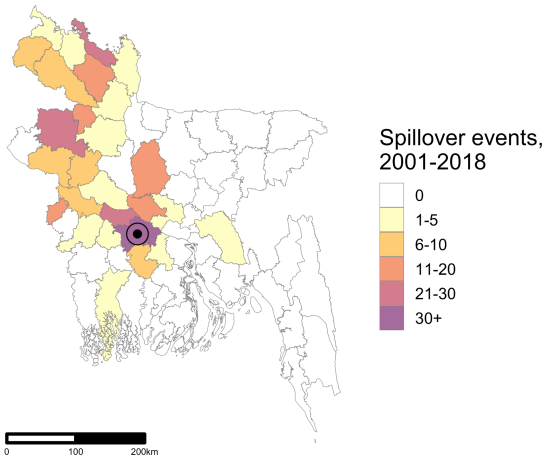
Figure 6. Domesticated animal and human sampling across Aims 1 - 3



Study Site

Research will be conducted in five villages surrounding bat roosts in Faridpur District where we have been conducting surveillance for Nipah virus shedding and seroprevalence since 2006 [71]. **Faridpur represents an optimal study site for henipavirus spillover because of the high incidence of human infections and numerous studies identifying infections in bats and intermediate hosts.** More human Nipah spillovers have been identified in Bangladesh than any other place in the world and most of these have been detected in Faridpur District (**Figure 7**). Nipah virus shedding in urine has been detected repeatedly in longitudinal surveys of bats in Faridpur during 2007-2012 [71] and since 2017 (Gurley et al., unpublished data). Cattle and goats [14] and dogs and cats (Islam et al., unpublished data) with antibodies against henipaviruses have also been reported from studies in Faridpur.

Figure 7.
Proposed study site in Faridpur.



Aim 1 Strategy

We hypothesize that temporal variation in **domesticated animal spillovers is driven by infection dynamics in bats, weather patterns, and domesticated animal contact with bats.** We will investigate bat viral dynamics and identify spillovers into domesticated animals over time in Faridpur. We will describe through surveys and observations the types and frequency of contact between bats and domesticated animals. Finally, we will build statistical models to identify the relative contribution of bat shedding, weather patterns, and animal behavior to the risk of spillover into domesticated animals.

Mapping and census of study area: Research field teams will identify the five closest villages to the focal *Pteropus medius* bat roost in Faridpur. We will first speak with local leaders to delineate the boundaries of the villages nearest the main roost and identify any additional *P. medius* bat colonies within the study site. Once boundaries are established, teams will then map the location of households and other structures, populations of domesticated animals (cattle, goats, pigs, dogs, and cats), any additional fruit bat roost sites, cultivated fruit trees (e.g, mango, lychee), date palm trees, and any other natural resources that are shared between host groups (**Figure 8**). GPS locations for each point will be recorded and then used to develop preliminary maps of each village. A census of all households in each village will then be performed, recording all of the members of the household and the number and species of animals they own. In Bangladesh, pigs are often raised in mobile herds, or in ethnic minority communities [59,82]. Therefore, we will ensure that one of the enrolled villages includes pig raisers and we will also identify the nearest mobile pig herd that makes frequent visits to this area and map out their typical foraging trajectory. Based on our previous experience, we would expect that there are 200 households and 960 domesticated animals in each village

around the Faridpur site.

Bat henipavirus seroprevalence over time: In previous studies, henipavirus seroprevalence in bat populations have correlated with detected viral shedding in bats. Over a five year study in Faridpur, five of eighteen sampling events were associated with detection of Nipah virus in a bat and **six out of eight viral detections occurred during times when seroprevalence in the roost was waning** [71]. We will measure changes in seroprevalence in the fruit bat populations living in the focal roosts in Faridpur during Years 1-4 of the study. A target of 50 bats will be captured for blood serum sampling every month (**Figure 5**) and tested for henipavirus antibodies using the same multiplex pan-henipavirus assay as the domesticated animals. These tests will be done in near-real time to detect population level changes in seroprevalence. Changes in the proportion of juvenile and adult bats in the roosts will be monitored over time to compare with any observed changes in seroprevalence. All captured bats will be marked with microchips so that any change in serostatus (conversion or reversion) can be recorded for recaptured individuals.

Describing contact between bats and domesticated animals: We will use qualitative approaches, quantitative surveys, and camera observations to identify when and how domesticated animals have contact with bats and bat secretions. First, teams will enroll members of each village for a social mapping exercise. In this exercise, **members will collaborate in focus groups to identify the known forms of interactions between domesticated animals and bats.** For example, domesticated animal-bat interactions might involve livestock grazing underneath bat roosts or popular bat feeding sites (**Figure 8A**); domesticated animals being fed bat-bitten fruits or date palm sap (**Figure 8B**); or cats and dogs scavenging dead bats or bat placenta.

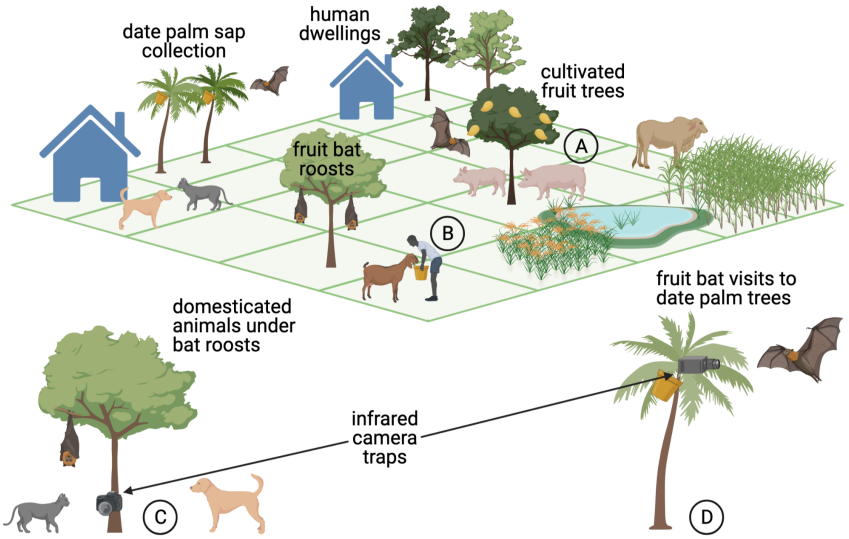


Figure 8. Mapping and recording interactions between bats, humans and domesticated animals.

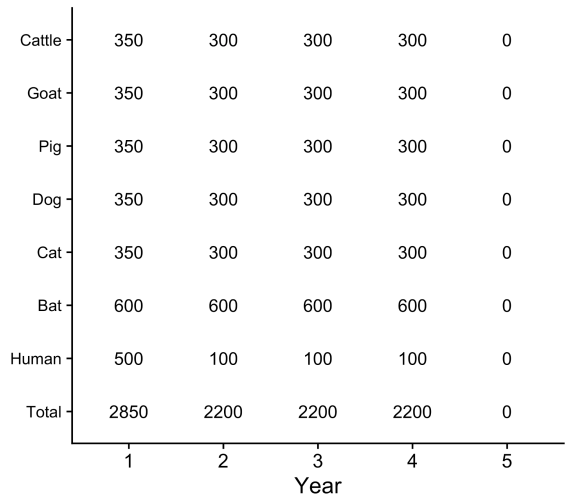
Second, **nighttime space use and food resource consumption will be described using camera traps** in Year 1. This is important because some interactions between domesticated animals and bats may not be captured through social mapping because they occur at night and are not observed by humans. We are particularly interested in observing if dogs and cats scavenge underneath bat roosts (**Figure 8C**) during the birthing season when placentas and aborted fetuses may be more commonly available underneath roosts. A motion-sensor tripped infrared camera will be mounted at the base of each bat roost tree – including the main roost site and any additional roosts identified during mapping – for three consecutive nights each month throughout Year 1 to record visitations by animals at night and any interactions they may have with bats.

We will also use camera traps to capture bat visits to date palm trees and fruit trees that are identified through social mapping as sources of food for domesticated animals, particularly cattle, goats and pigs (**Figure 8D**). Based on social mapping, we will identify which date palm trees are used for collecting fresh sap that is consumed by domesticated animals and the cultivated fruit trees that are reported to be popular feeding sites for bats. Cameras will be mounted at the top of selected trees near the collection pot or near the ripe fruit to observe bats touching or licking sap or feeding on the fruit [83]. Bat visits to date palm sap trees will be photographed for three consecutive nights in winter (between December and February) when fresh sap is collected. Bat visits to fruit trees will be photographed during the period of peak ripeness. All cameras will be set at dusk and record animal visits until cameras are retrieved at dawn. A hired guard will protect the cameras during each night they are mounted.

Third, we will use structured surveys to quantify contacts between bats and domesticated animals. For all animals enrolled in the seroprevalence cohort (see below) **owners will be asked to provide information on the specific behavior and feeding patterns for each animal.** This information will be used to identify risk factors for spillover into domesticated animal hosts.

Seroprevalence and spillover force of infection in domesticated animals: Teams will aim to enroll approximately **350 each of cattle, goats, pigs, dogs, and cats in Year 1 from the five surveyed villages around Faridpur** (**Figure 9**); 350 animals will be sampled at this step so that we will successfully identify the 300 seronegative animals required for the sample size for the seroincidence cohort (see below). We will randomly choose households listed in the census that own at least one of the target species and keep enrolling households until we reach the target sample size for each species. For some species, there may be only 350 animals total across all surveyed villages, so we will sample all animals. For animals at higher density (>350 across all villages), we will prioritize sampling juveniles and then fill in with adults from unique households (one animal per household). Assuming that virus exposure varies by household, this sampling approach decreases dependence between observations.

Figure 9.
Serum sampling schedule.



To establish a baseline seroprevalence estimate and identify seronegative animals for a follow-up cohort, teams will capture animals and take a sample of whole blood. Blood samples will be centrifuged to separate the serum and immediately preserved in liquid nitrogen in the field. We will use microchips to mark dogs, cats, and pigs. For cattle and goats, we will use physical descriptions including age, sex, body weight, color, and special markings combined with the owner's address to identify individual animals. **Serum samples will then be transferred to the icddr,b laboratory to be tested for the presence of henipavirus antibodies using the multiplex pan-henipavirus assay**. During capture, teams will record the age in months of each animal if known from the owner. In the few cases that exact age is not known, teams will classify animals into juvenile or adult age groups. The serology data will then be used to calculate baseline seroprevalence by animal species. Where age data is available, **we will calculate seroprevalence by age classes and will fit catalytic models to estimate the average annual force of infection from henipavirus in the region for each animal species** [82]. These estimates will be used as a baseline for comparison to seroincidence in the prospective study.

> *Power calculation:* Based on a survey showing henipavirus seroprevalence 1.1% in cattle, 1.2% in goats, 3.2% in dogs, and 4.7% in cats (Islam et al., unpublished data), a sample size of 350 animals is sufficient to measure a seroprevalence greater than zero in the baseline survey, with a power of 0.8 and a significance level of 0.05.

Prospective identification of spillovers in domesticated animals: Among animals screened for henipavirus antibodies, **teams will identify 300 juvenile cattle, goats, pigs, dogs, and cats that are henipavirus seronegative at baseline to follow over time to identify spillovers**. If our estimates of the number of seropositive animals at baseline are too low, and we do not identify sufficient seronegative animals in that survey for prospective follow up, we will test additional animals until we reach our targets for the prospective study. Owners of these animals will be approached to consent to enroll the animals in a study to prospectively identify henipavirus spillovers during Years 1-4. **Each animal will be bled every six months to identify henipavirus seroconversion.** Animals who are between the ages of 3-6 months will be prioritized for prospective follow-up because they are old enough so that any maternal antibodies have waned, yet young enough so that they have at least six months of life left (many livestock are consumed after they reach a year of age). If any members of the cohort seroconvert or cannot be captured due to disappearance or death, a new seronegative animal will be identified and added to the cohort. The same multiplex pan-henipavirus assay that was used in the baseline serosurvey and the bat seroprevalence study will be used (methods below). We will use serologic evidence of infection to estimate the incidence of henipavirus spillovers into domesticated animals during Years 2–4 for each animal species.

> *Power calculation:* Using the combined data on seroincidence in domesticated animals and the animal behavior data collected during interviews with owners and from household surveys, we can test the hypothesis that animals with high-risk exposures (e.g., feeding on dropped fruit or date palm sap) are more likely to seroconvert over the period of monitoring than animals without high-risk exposures. Assuming that half of all animals sampled during Years 2-4 have one or more high-risk exposures, this sample size (2250 animals per group) will be sufficient to detect a small effect size for the difference in

seroincidence between exposed animals and unexposed animals (4% vs. 1%), with a power of 0.8 and a significance level of 0.05.

Multiplex pan-henipavirus assay: The team at USU has expressed all five henipavirus G (Yan et al., submitted) as sG_{tet} and developed a pan-henipavirus multiplex serology assay [84] that can detect henipavirus-specific and cross-reactive antibodies. Field-collected serum samples will be screened at an initial dilution of 1:500 and seropositive samples will be serially diluted to determine titers and improve specificity if cross-reaction are observed. NiV IgG-positive samples will be further characterized for neutralizing antibodies using a bead-based surrogate virus neutralization test using the same Luminex xMAP-based technology as the binding assay [79].

Statistical models to identify drivers of henipavirus spillovers into domesticated animals: Henipavirus seroincidence data from domesticated animals collected in six-month intervals during Years 1-4. To test the relative influence of different factors on seroincidence in domesticated animals, **we will summarize data on henipavirus infection dynamics in bats, weather patterns, and changes in domesticated animal-bat contacts for the same six-month intervals.** Summarized bat data will include the average number of bats in the roost, the average number of adults and juveniles, the average seroprevalence, and the direction and magnitude of prevalence change over the six-month period. Weather data from the nearest station in Faridpur District will be obtained from the NOAA National Climatic Data Center or the Bangladesh Meteorological Department. Variables related to temperature, precipitation, and any adverse weather events will be summarized in six-month intervals. The contacts that domesticated animals have with bats (e.g., feeding on dropped fruit or date palm sap, grazing underneath roosts or bat feeding sites) will be summarized from information provided by owners during interviews and from household surveys. Generalized linear models will then be fit to seroincidence data to test the relative contribution of different explanatory variables.

Limitations: The greatest limitation of our approach is that we are unable to control how many spillovers occur; it is possible that we may identify too few spillovers to make strong inferences about the drivers of those spillovers. However, past serostudies in domesticated animals suggest that spillovers are common [14,56–58]. Serological methods are inherently limited as tools for virus discovery as they are indirect measurements of virus exposure and closely-related viruses share conserved epitopes that can lead to misidentification of specific versus cross-reactive antibodies. While we expect that the specificity of the serological assay to henipaviruses will be high as shown with preliminary Indian flying fox sera (**Figure 2**), even small deficits in specificity can interfere with interpreting low estimates of seroprevalence or seroincidence, since many positives may be false positives. However, since we will also know the high-risk exposures that animals have with bats, we can make inference about the serologic test results.

Aim 2 Strategy

Given the evidence from previous serologic studies of domesticated animals in Bangladesh, we hypothesize that **spillovers from bats to domesticated animal species will include Nipah virus, as well as other henipaviruses**. In this aim, we will use samples collected as part of the bat sampling and sick animal surveillance described in Aim 1 to identify which henipaviruses are spilling over from bats into domesticated animals.

Sick domesticated animal sampling: Animal owners enrolled in Aim 1 (300 individuals from each species) **will be given a number to call to report any illnesses they observe in their animals**, as well as educational materials about how to reduce risk of infection from sick animals and safe disposal of carcasses. **We will use the best available data on clinical signs of henipavirus infections and viral shedding patterns in domesticated animals to guide our surveillance and sampling efforts.** There are no published accounts of clinical illness or viral shedding among cattle and goats. However, studies of cats, dogs, and pigs provide a basis for defining relevant clinical signs and optimal biological specimen collection to identify viral shedding. Experimentally and naturally infected cats can develop severe disease characterized by fever and increase respiratory rate, and shed virus in their respiratory tract [9,50,51,85,86]. Naturally infected dogs may not show overt signs of illness, though sample sizes are small; however, evidence suggests that virus replicates in their respiratory tract [87]. Experimentally infected pigs shed Nipah virus from the nasopharynx, but show limited clinical signs [51,86,88]. However, naturally infected pigs did experience respiratory disease and mortality [2,69].

When we receive a call about a sick animal, we will dispatch a veterinarian to provide medical advice and **collect oral and nasal swabs from the sick animals**. If the animal expires before we reach the village, we will collect swabs from the carcass postmortem. These samples will be stored in viral transport medium and stored at cryogenic temperatures for molecular testing. Humans whose animals are enrolled in the sick animal surveillance will receive educational materials about how to safely care for their animals should they become ill. They will also be provided with information about the best place to seek care in their locality should they become

sick. If any humans become sick following contact with a sick animal, we will assist them with seeking care at the nearest government hospital in Faridpur town where biological samples for laboratory testing could be collected.

Bat virus shedding: During bat sampling events each month (Aim 1), field teams will also **collect urine using plastic tarps placed underneath roosts overnight and will collect individual urine samples from captured bats** [71]. These urine samples will be stored in viral transport medium and will be transported back to the icddr,b laboratory using liquid nitrogen dry shippers.

Virus testing and sequencing: Once per year, urine and swab samples will be sent under cryogenic temperatures to the Rocky Mountain Laboratories at NIH for testing. RNA will be extracted and cDNA generated using SuperScript IV reverse transcriptase (Thermo Fisher Scientific, Waltham, MA). Genus-level PCR primers have been developed and validated to target a conserved region of the RNA-dependent RNA polymerase (RdRp) gene for henipaviruses, morbilliviruses, and respiroviruses [89]. The expected PCR amplicon size is approximately 600 base pairs. Upon detection of a PCR-positive sample, amplicons will be sequenced using Sanger sequencing and identified using the NCBI Basic Local Alignment Search Tool (BLAST). Positive samples will be further analyzed by full-genome sequencing using a combination of VirCapSeq [90], long-range or unbiased deep sequencing, or a combination of different next-generation DNA/RNA sequencing approaches including the Illumina and PacBio platforms. Phylogenetic trees will be generated from RdRp and full genome sequences to examine relationships between viruses identified in domesticated animals and bats within each of the six-month sampling periods. Close genetic relatedness between viruses in bats and domesticated animals sampled in the same time period will corroborate evidence from serology that henipaviruses were actively spilling over.

Limitations: The greatest risk of this approach is that we may not identify any animals shedding virus over the course of the study. Given the previous studies on seroprevalence in domesticated animals, we are confident that we will find animals who have been infected. However, we do not understand well the clinical presentation of naturally infected animals and may, therefore, be unable to detect viral shedding because animal infections are largely asymptomatic, we have targeted the wrong clinical syndromes, or we have not collected the optimal biological species. If after the first two years of the study we have identified seropositive animals but no clinical illness has been reported by their owners, we will add regular sampling of the affected species to identify asymptomatic shedding events.

Aim 3 Strategy

In this aim, we will identify human henipavirus infections and the risk associated with contact with sick domesticated animals. In the Year 1 of the study, we **will enroll a random sample of adults living in our study area and test their serum for evidence of henipavirus infection**. Beginning in Year 2, we will ask all **humans who have had contact with sick animals identified in Aim 2** to report details of their contact with the animal and provide serum for testing.

Population-based henipavirus seroprevalence survey: A random sample of adults living in our study area will be approached to enroll in the serosurvey. Enrolled **participants will be asked to provide a 5 mL blood sample for testing using the multiplex pan-henipavirus assay.** In addition, **participants will complete a quantitative questionnaire about their typical exposures to domesticated animals**, particularly when those animals are sick. These questionnaires will be informed by our prior studies of interactions between humans and domesticated animals in Bangladesh [59,60,91]. Exposures to sick animals will focus on possible contact with animals' respiratory secretions or blood, as studies suggest that these fluids are most likely to have high viral loads in experimental studies [50,51,86,88].

> *Power calculation:* In a serosurvey of 1469 individuals with acute febrile illness in Cambodia, 2.9% had antibodies reactive to one or more henipaviruses (Laing et al., unpublished data). Assuming that seroprevalence is only 0.5% in our baseline serosurvey of healthy humans, a sample size of 400 people will be sufficient to conclude that seroprevalence is greater than zero, with a power of 0.8 and a significance level of 0.05.

Serosurveys of humans with contact with sick domesticated animals: We will identify the humans who had close contact with the sick domesticated animals identified in Aim 2 and ask them to enroll in our prospective study of human infections. Human contacts will be provided with information about where to seek care should they become ill following exposure to sick animals (see Aim 2). However, we assume that many of these infections will be mild or asymptomatic so we will use the multiplex pan-henipavirus assay to identify human

infections. At the time of exposure with the sick animal, **contacts will be asked to provide details about their exposure to the animal**. Thirty days following the last exposure to the animal, **we will follow up to collect a 5 mL blood sample for serologic testing.**

After we complete the serologic testing of sick domesticated animals identified in Aim 2, we will know which animals suffered from henipavirus infections. We will use those results to split the human contacts into two groups: **those who had contact with animals with henipavirus and those who did not.** We will compare the seroprevalence between these two groups and will use data on their animal contacts to identify risk factors for human infections.

> *Power calculation:* We hypothesize that humans having close contact with sick animals confirmed positive for henipavirus infection are more likely to be seropositive than humans without known contact with positive animals. Assuming that one sick animal will be detected per week in the study and each sick animal has on average two human contacts, a total of 100 persons will be enrolled per year and 400 over the whole study. If 15% of sick animals have evidence of henipavirus infection, the total number of humans having contact with henipavirus-infected animals (60) and humans without known contact with infected animals (340) will be sufficient to detect a moderate effect size for the difference in henipavirus seroprevalence between these groups (10% vs. 1%), with a power of 0.8 and a significance level of 0.05.

Limitations: The key limitation of our approach is a lack of prior data at this interface. We are unaware of any past studies in Bangladesh that examined exposure to henipaviruses in humans having contact with sick domesticated animals, so we have used what we believe are reasonable estimates for the number of contacts that sick animals have, the proportion of sick domesticated animals infected with henipaviruses, and the difference in seroprevalence between exposed and unexposed humans. Previous serological surveys from the 1998-1999 Nipah outbreak in Malaysia showed that 11% of persons working on pig farms with reported human encephalitis cases had Nipah virus antibodies whereas only 6% of persons working on control farms with no encephalitis cases had antibodies [52].

We could face two possible difficulties here: we may not identify enough humans to enroll, or we may identify more than we can test within our planned budget. If we cannot enroll enough sick animals to meet the necessary sample size to test our hypothesis about human seroprevalence and exposure, we will enroll additional humans into the study based on reported history of exposure to sick animals. Conversely, if there are many more sick animals than we expect or sick animals have a high number of human contacts, we will only test those persons having contact with sick animals of species identified as high-risk during the animal seroprevalence study in Year 1 of the grant.

Study Team: Collectively, our multidisciplinary team brings more than 100 years of experience in the study of henipaviruses, including surveillance, diagnostics and laboratory experiments, transmission at the human-animal interface, and development of behavioral interventions and medical countermeasures, and our investigative team benefits from a significant track record of successful henipavirus collaborations. Dr. Gurley is a member of the WHO's Nipah Virus R&D Taskforce and spent 12 years at icddr,b. She will serve as PI of this project and has worked with colleagues at icddr,b, including Drs. Islam and Rahman and Ms. Sultana, on Nipah virus research since 2004. Dr. Gurley has ongoing collaborations with all of the Key Personnel and consultants listed in this proposal. Drs. Gurley, Plowright, Luby, Munster, Laing, and Salje currently collaborate on the Preventing Emerging Pathogenic Threats (PREEMPT) project with icddr,b colleagues and this proposal will build upon these strong existing collaborations built over many years. Dr. Laing is a virologist who has been involved in the development and technology transfer of serological tools and biosurveillance of henipaviruses since 2014. He is a leader in the development of diagnostics for emerging zoonotic viruses and has considerable experience in transferring diagnostic capacity. The pan-henipavirus antigens and MMIA SOPs that will be transferred to icddr,b have been previously transferred to collaborators' labs in PERHILITAN, Kuala Lumpur, Malaysia; RML, Hamilton, MT, USA; Duke-NUS, Singapore; National Centre for Biological Sciences, Bangalore, India; University of Pretoria, RSA; Chulalongkorn University, Bangkok, Thailand; NAMRU-2 Phnom Penh detachment, Cambodia; and the Zoological Society of London, UK, where cross-laboratory validations are presently underway. Dr. Islam is a wildlife veterinarian who has led field investigations of henipavirus infections in bats and domesticated animals with other investigators on this proposal since 2009. He will serve as the primary lead for the icddr,b field work. Dr. Rahman is a virologist who leads the zoonotic infections virology laboratory at icddr,b and is currently funded to establish Luminex-based henipavirus serologic testing with Dr. Laing from USU as a part of PREEMPT. He will oversee the serologic testing at icddr,b. Ms. Sultana is an anthropologist who brings more than a decade of experience in Nipah research, including qualitative investigations into behaviors related to transmission. She will lead human behavioral components of the project, including surveys of human-animal

contact. Dr. Luby spent eight years at icddr,b where he led the Nipah virus research portfolio; he is a member of the WHO's Nipah Virus R&D Taskforce and will provide invaluable input on linking research output to practical public health action. Dr. Munster leads the Virus Ecology Unit at the NIH Rocky Mountain Laboratories and currently tests biological samples from bats in Bangladesh for henipaviruses as part of the PREEMPT project. His laboratory will identify henipaviruses in bats and sick animals. Drs. Plowright and Salje will be retained as consultants on the project for their unique expertise. Dr. Plowright is the PI of the PREEMPT project and studies the ecological drivers of henipavirus spillover. Her research on Hendra virus in Australia has been used to predict spillovers and she will advise the project on ecological drivers of transmission. Dr. Salje will build on his eight-year collaboration with the group on Nipah virus related research to lend his expertise on modeling disease transmission using serological studies for this project.

During Years 1, 3, and 5 of the project, all the Key Personnel will meet in Bangladesh to review scientific goals, progress, and plan scientific analyses and manuscripts. Dr. Gurley will visit Bangladesh twice yearly, during all years of the study, to maintain strong collaborations with the icddr,b team and closely coordinate the research. Team meetings with all scientific collaborators will occur virtually twice per year to review the findings of the study and scientific outputs. The PI will hold standing weekly meetings with icddr,b collaborators (and others, as needed) to review progress and field activities. Day-to-day communication will occur over Slack, through email, and by phone/Zoom calls; these communication tools are regularly used successfully in existing collaborations among team members.

Conclusion

Henipaviruses are emerging zoonotic viruses that pose a significant pandemic risk; in 2015, t**he World Health Organization named Nipah virus one of the most dangerous emerging zoonotic infections**, alongside SARS and others. While we know that these bat viruses are spilling over into domesticated animals and humans, our knowledge of the drivers of these spillover pathways remain lacking in sufficient detail to enable us to design prevention strategies. **We remain ignorant about henipavirus spillovers through intermediate hosts – including the specific viruses spilling over, the frequency and distribution of spillovers, and the pathways of transmission – at our own peril.** Our study aims to **uncover and explicate these spillovers** and describe the specific risks that lead to infection among domesticated animals and humans **in a part of the world with frequent and ongoing detections of human henipavirus infections.**

The knowledge gained from this study will be **immediately applicable to human and animal health programs in Bangladesh** and other countries where henipaviruses circulate in bats. Although there are many plausible pathways of transmission from bats to domesticated animals, only through increased specificity of true risk can we identify prevention strategies. Information about henipavirus risk and spillover pathways to domesticated animals can be **translated into surveillance and health messages for animal owners**. By learning about which henipaviruses infect humans, and how they are infected, we can **advise public health surveillance programs on how to optimize detection and epidemiologic investigation of cases across Bangladesh**. Our investigations about spillovers in Faridpur can also be scaled-up to other areas of Bangladesh and countries where henipaviruses circulate in bats so that we can truly begin to appreciate the scale of henipavirus spillovers in the global landscape. Finally, we believe that **this framework for uncovering spillovers through intermediate hosts will be applicable to the many other emerging bat-borne viruses where spillovers remain hidden**.

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Subject:	RO1 submitted
Date:	Tuesday, June 8, 2021 12:37:04 PM
Attachments:	specific_aims.pdf research_strategy.pdf references.pdf

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Colleagues,

I'm writing to share that our RO1 application has been submitted. The specific aims and research strategy we submitted is attached here, for your records.

Special thanks to Clif for his tireless efforts to get things together, and the really lovely figures.

All the best,
Emily