From: Folkers, Greg (NIH/NIAID) [E]
Sent: Mon, 29 Mar 2021 21:21:41 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Auchincloss, Hugh (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Embry, Alan (NIH/NIAID) [E]
Subject: FW: WHO report
Attachments: 20210328- Full report COVID-19 Origins Report - embargoed[3].pdf, Annexes_20210328[1].pdf

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From: Hall, Bill (HHS/ASPA) (b) (6)
Sent: Monday, March 29, 2021 5:14 PM
To: Folkers, Greg (NIH/NIAID) [E] (b) (6); Billet, Courtney (NIH/NIAID) [E] (b) (6)
Subject: WHO report

Greg/Courtney,

Attached is the final WHO report and the annexes. Wanted to make sure Tony has it to review.

Bill

William Hall
Deputy Assistant Secretary for Public Affairs (Public Health)
Office of the Assistant Secretary for Public Affairs
U.S. Department of Health & Human Services
Washington, DC`
Direct: (b) (6)
Mobile: (b) (6)
Email: (b) (6)
www.hhs.gov

From: Myles, Renate (NIH/OD) [E]
Sent: Fri, 14 May 2021 17:15:04 +0000
To: Collins, Francis (NIH/OD) [E];Fauci, Anthony (NIH/NIAID) [E];Embry, Alan (NIH/NIAID) [E]
Cc: Hallett, Adrienne (NIH/OD) [E]
Subject: RE: Rebuttal to Ebright?

Here's the full article in case folks aren't able to access it:

NEWS
NR PLUS SCIENCE & TECH

Biosafety Expert Explains Why Fauci's NIH 'Gain-of-Function' Testimony Was 'Demonstrably False'

By ISAAC SCHORR
May 13, 2021 10:07 AM

Dr. Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, prior to a hearing at the Capitol in Washington, D.C., May 11, 2021 *(Greg Nash/Reuters)*

Dr. Richard Ebright, a professor of chemistry and chemical biology at Rutgers University and biosafety expert, is contesting NIH director Dr. Anthony Fauci's testimony before the Senate Health, Education, Labor, and Pensions Committee on Tuesday.

Dr. Fauci's claim — made during an exchange with Senator Rand Paul — that "the NIH [National Institutes of Health] has not ever and does not now fund gain of function research in the Wuhan Institute of Virology [WIV]" is "demonstrably false," according to Ebright.

At least some of the NIH-funded research conducted at the WIV "unequivocally" qualifies as gain-of-function, Ebright told NATIONAL REVIEW.

A research article written by WIV scientists, "Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus", for example, qualifies as gain-of-function and was clearly a product of NIH-funding.

Ebright insists that the research can be classified as gain-of-function under a number of different definitions, including those found in two pieces of Department of Health and Human Services guidance on the subject.

The first details the Obama administration's 2014 decision to halt domestic gain-of-function research, which it defines as that which "may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route."
The second — drafted in 2017 as Fauci was pushing to renew government funding for gain-of-function research — provides a definition of what are

called “enhanced potential pandemic pathogen (PPP)” or those pathogens “resulting from the enhancement of the transmissibility and/or virulence of a pathogen.”

Ebright claims that the work being conducted at the WIV, using NIH funds originally granted to Peter Daszak of EcoHealth Alliance, “epitomizes” gain-of-function research under the definition HHS provided in its guidance, and is the exact kind of research that led the Obama administration to conclude that gain-of-function was too dangerous to continue domestically.

‘The Wuhan lab used NIH funding to construct novel chimeric SARS-related coronaviruses able to infect human cells and laboratory animals,” he said. “This is high-risk research that creates new potential pandemic pathogens (i.e., potential pandemic pathogens that exist only in a lab, not in nature). This research matches — indeed epitomizes — the definition of ‘gain of function research of concern’ for which federal funding was ‘paused’ in 2014-2017.”

“Chimeric” coronaviruses refers to those that have been altered and enhanced by man, in this case in such a way as to make them more transmissible and dangerous to humans.

The paper drafted by WIV scientists clearly states that the underlying research was funded by, among other entities, the National Institutes of Health. The NIH’s own database of grantees lists this research and confirms that over $660,000 was spent supporting it.

Fauci appears to have been, at best, mistaken while sparring with Senator Paul on Tuesday. At worst, he was playing tenuous word games meant to deceive.

From: Collins, Francis (NIH/OD) [E] (b) (6)
Sent: Friday, May 14, 2021 12:33 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b) (6) Embry, Alan (NIH/NIAID) [E] (b) (6)
Cc: Hallett, Adrienne (NIH/OD) [E] (b) (6) Myles, Renate (NIH/OD) [E] (b) (6)
Subject: Rebuttal to Ebright?

Hi Tony and Alan,

(b) (5)

https://www.nationalreview.com/news/biosafety-expert-explains-why-faucis-nih-gain-of-function-testimony-was-demonstrably-false/

https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006698#abstract0

FC

From: (b) (6)
Sent: Sat, 26 Jun 2021 06:37:58 -0400
To: Auchincloss, Hugh (NIH/NIAID) [E]
Subject: Fwd: Re - A Coronavirus Epidemic Hit 20,000 Years Ago, New Study Finds - The New York Times
Attachments: PIIS0960982221007946.pdf

Sent from my iPhone

Begin forwarded message:

From: Steve Anderson (b) (6)
Date: June 26, 2021 at 2:27:53 AM EDT
To: "Fauci, Anthony (NIH/NIAID) [E]" (b) (6), "Collins, Francis (NIH/OD) [E]" (b) (6), "Mulach, Barbara (NIH/NIAID) [E]" (b) (6) Professor William Schief at the Scripps Research Institute (b) (6)
Cc: "Prime Minister NZ. Jacinda Ardern" (b) (6), "Professor Dame Juliet A. Gerrard. Prime Ministers Chief Advisor" (b) (6) "Professor Ian Town. Chief Science Advisor at the Ministry of" (b) (6), "Professor Peter K Dearden. Director of Genomics Aotearoa. Laboratory for" (b) (6) Professor Miguel Quiñones-Mateu (b) (6) "Professor Michael Baker. Department of Public Baker. Department Of Public" (b) (6), "Professor Nick Wilson. Dept Public Health" (b) (6), "Dr Kevin Esvelt. MIT. Daisy chain gene drive. Crispr/cas9" (b) (6), "Professor Pieter Cullis. Biochemistry and Molecular Biology" (b) (6) "Adjunct Professor of Epidemiology Wayne Koff. CEO Human Vaccines Project" (b) (6)
Subject: Re - A Coronavirus Epidemic Hit 20,000 Years Ago, New Study Finds - The New York Times

Hi Anthony. Interesting!

"Scientists looking for drugs to fight the new coronavirus might want to scrutinize the 42 genes that evolved in response to the ancient epidemic, Dr. Souilmi said. “It’s actually pointing us to molecular knobs to adjust the immune response to the virus,” he said.

Dr. Anders agreed, saying that the genes identified in the new study should get special attention as targets for drugs. “You know that they’re important,” she said. “That’s the nice thing about evolution.”"

https://www.nytimes.com/2021/06/24/science/ancient-coronavirus-epidemic.html#click=https://t.co/wh9bW08fYC

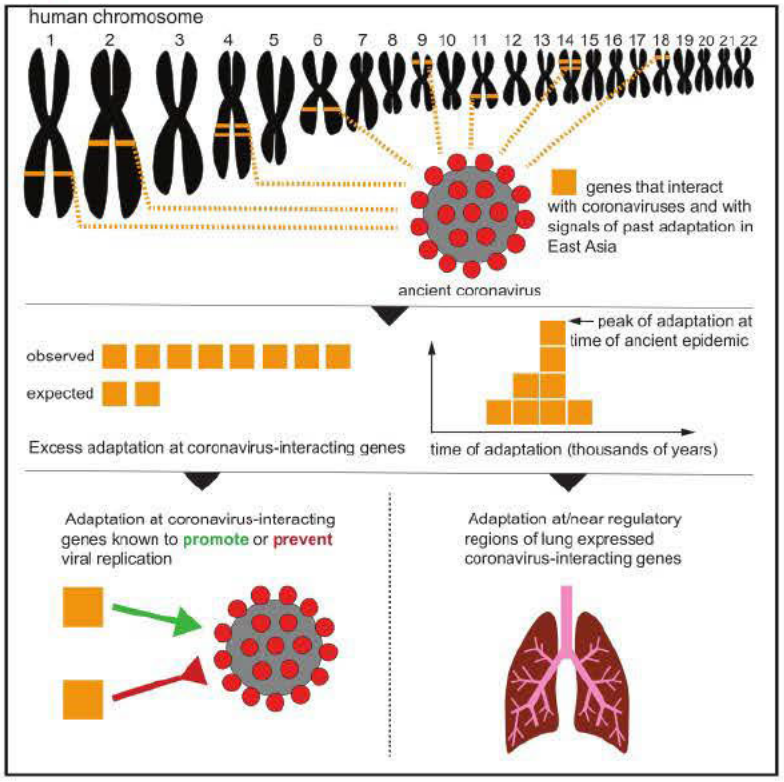
Regards Steve

Article

Current Biology

An ancient viral epidemic involving host coronavirus interacting genes more than 20,000 years ago in East Asia

Graphical abstract



Highlights

- Ancient viral epidemics can be identified through adaptation in host genomes
- Genomes in East Asia bear the signature of an ~25,000-year-old viral epidemic
- Functional analysis supports an ancient corona- or related virus epidemic

Authors

Yassine Souilmi, M. Elise Lauterbur, Ray Tobler, ..., Nevan J. Krogan, Kirill Alexandrov, David Enard

Correspondence

(b) (6)

In brief

Souilmi et al. find that strong genetic adaptation occurred in human East Asian populations, at multiple genes that interact with coronaviruses, including SARS-CoV-2. The adaptation started 25,000 years ago, and functional analysis of the adapting genes supports the occurrence of a corona- or related virus epidemic around that time in East Asia.

Souilmi et al., 2021, Current Biology *31*, 1–11
August 23, 2021
https://doi.org/10.1016/j.cub.2021.05.067

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Current Biology

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Article

An ancient viral epidemic involving host coronavirus interacting genes more than 20,000 years ago in East Asia

Yassine Souilmi,[1,2] M. Elise Lauterbur,[3] Ray Tobler,[1] Christian D. Huber,[1] Angad S. Johar,[1] Shayli Varasteh Moradi,[4] Wayne A. Johnston,[4] Nevan J. Krogan,[5,6,7,8,9] Kirill Alexandrov,[4,*] and David Enard[3,10,*]

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https://doi.org/10.1016/j.cub.2021.05.067

SUMMARY

The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has emphasized the vulnerability of human populations to novel viral pressures, despite the vast array of epidemiological and biomedical tools now available. Notably, modern human genomes contain evolutionary information tracing back tens of thousands of years, which may help identify the viruses that have impacted our ancestors—pointing to which viruses have future pandemic potential. Here, we apply evolutionary analyses to human genomic datasets to recover selection events involving tens of human genes that interact with coronaviruses, including SARS-CoV-2, that likely started more than 20,000 years ago. These adaptive events were limited to the population ancestral to East Asian populations. Multiple lines of functional evidence support an ancient viral selective pressure, and East Asia is the geographical origin of several modern coronavirus epidemics. An arms race with an ancient coronavirus, or with a different virus that happened to use similar interactions as coronaviruses with human hosts, may thus have taken place in ancestral East Asian populations. By learning more about our ancient viral foes, our study highlights the promise of evolutionary information to better predict the pandemics of the future. Importantly, adaptation to ancient viral epidemics in specific human populations does not necessarily imply any difference in genetic susceptibility between different human populations, and the current evidence points toward an overwhelming impact of socioeconomic factors in the case of coronavirus disease 2019 (COVID-19).

INTRODUCTION

Coronaviruses have been behind three major zoonotic outbreaks.[1] The first outbreak, known as SARS-CoV (severe acute respiratory syndrome coronavirus), originated in China in 2002 and infected more than 8,000 and killed more than 800 people.[2] Four years later, MERS-CoV (Middle East respiratory syndrome coronavirus) affected >2,400 and killed over 850 people (https://www.who.int). The most recent outbreak began in late 2019 when SARS-CoV-2 emerged in China, triggering an ongoing pandemic (coronavirus disease 2019 [COVID-19]).[3]

The research on SARS-CoV-2 epidemiology has revealed that socioeconomic (e.g., access to healthcare, testing, and exposure at work), demographic, and personal health factors all play a major role in SARS-CoV-2 epidemiology.[4–6] Additionally, several genetic loci that mediate SARS-CoV-2 susceptibility and severity have been found in contemporary European populations,[7–10] one of which contains a genetic variant that increases SARS-CoV-2 susceptibility that likely increased in frequency in the ancestors of modern Europeans after interbreeding with Neanderthals.[11]

Throughout the evolutionary history of our species, positive natural selection has frequently targeted proteins that physically interact with viruses—e.g., those involved in immunity or used by viruses to hijack the host cellular machinery.[12–14] In the millions of years of human evolution, selection has led to the fixation of gene variants encoding virus-interacting proteins (VIPs) (Data S1A) at three times the rate observed for other classes of genes.[13,15] Strong selection on VIPs has continued in human populations during the past 50,000 years, as evidenced by VIP

genes being enriched for adaptive introgressed Neanderthal variants and also selective sweep signals (i.e., selection that drives a beneficial variant to substantial frequencies in a population), particularly around VIPs that interact with RNA viruses (Data S1B), a viral class that includes the coronaviruses.[16,17]

The accumulated evidence suggests that ancient RNA virus epidemics have occurred frequently during human evolution; however, we currently do not know whether selection has made a substantial contribution to the evolution of human genes that interact more specifically with coronaviruses.

Accordingly, here, we investigate whether ancient coronavirus epidemics have driven past adaptation in modern human populations, by examining whether selection signals are enriched within a set of 420 VIPs that interact with coronaviruses (denoted CoV-VIPs; Data S1C) across 26 human populations from the 1000 Genomes Project.[18] These CoV-VIPs comprise 332 SARS-CoV-2 VIPs identified by high-throughput mass spectrometry (Data S1D),[19] and an additional 88 proteins that were manually curated from coronaviruses literature (e.g., SARS-CoV-1, MERS, HCoV-NL63, etc.; Data S1C)[16] and are part of a larger set of 5,291 VIPs (STAR Methods; Data S1A) from multiple viruses.[16] Our focus on VIPs is motivated by evidence indicating that these protein interactions are the central mechanism that viruses use to hijack the host cellular machinery.[16,19] Accordingly, VIPs are much more likely to have functional impacts on viruses than other proteins (STAR Methods). An alternative that we cannot exclude however is that a different type of virus that happens to use similar VIPs as coronaviruses might have driven adaptation signals at CoV-VIPs.

Our analyses find a strong enrichment in sweep signals at CoV-VIPs across multiple East Asian populations, which is absent from other populations. This suggests that an ancient coronavirus epidemic (or another virus using similar VIPs) drove an adaptive response in the ancestors of East Asians. Further, by leveraging ancestral recombination graph approaches,[20,21] we find that 42 CoV-VIPs may have come under selection around 900 generations (~25,000 years) ago and exhibit a coordinated adaptive response. We further show that the CoV-VIP genes are enriched for anti- and proviral effects and variants that affect COVID-19 etiology in the modern British population (https://grasp.nhlbi.nih.gov/Covid19GWASResults.aspx).[22,23] We further show that the inferred underlying causal mutations are situated near to regulatory variants active in lungs and other tissues impacted by COVID-19. These independent lines of evidence support an ancient coronavirus (or a similarly interacting virus) epidemic that emerged in the ancestors of contemporary East Asian populations.

RESULTS

Signatures of adaptation to an ancient epidemic

Viruses have exerted strong selective pressures on modern humans.[15,17] Accordingly, we use two statistical tests that are sensitive to such genetic signatures (i.e., selective sweeps)—nSL[24] and iHS[25]—while being insensitive to background selection.[26,27]

After scanning each of the 26 populations for selection signals, we apply an enrichment test that was previously used to detect enriched selection signals in RNA VIPs in human populations.[17] Briefly, for each population and selection statistic, we rank all genes based on the average selection statistic score observed in genomic windows ranging from 50 kb to 2 Mb (STAR Methods). Different window sizes are used because smaller windows tend to be more sensitive to weaker sweeps, whereas larger windows tend to be more sensitive to stronger sweeps (STAR Methods).[17] After ranking the gene scores, we estimate an enrichment curve (Figure 1) for gene sets ranging from the top 10 to 10,000 ranked loci (STAR Methods). The significance of the whole enrichment curve is then calculated using a genome block-randomization approach that accounts for the genomic clustering of neighboring CoV-VIPs and provides an unbiased false-positive risk (FPR) for the whole enrichment curve[28] by re-running the entire enrichment analysis pipeline on block-randomized genomes (STAR Methods).[17] For our control gene set, we use protein-coding genes situated at least 500 kb from CoV-VIPs to avoid overlapping the same sweep signals. Additionally, genes in the control sets are chosen to have similar characteristics as the CoV-VIPs (e.g., similar recombination, density of coding sequences, etc.; see STAR Methods for the complete list of factors) to ensure that any detected enrichment is virus specific rather than due to a confounding factor.[17] Finally, we also exclude the possibility that functions other than viral interactions might explain our results by running a Gene Ontology analysis (STAR Methods; Data S1E and S1F; Figures S1A and S1B).[29]

Applying this approach to each of the 26 populations from the 1000 Genomes Project dataset, we find a strong enrichment of sweep signals in CoV-VIPs that is specific to the five East Asian populations (whole enrichment curve for nSL and iHS combined FPR = 2.10^{-4}; Figures 1 and S2A–S2N; STAR Methods). No enrichment is observed for populations from other continents, including in neighboring South Asia (whole enrichment curve for nSL and iHS combined FPR > 0.05 in all cases; Figures 1 and S2F–S2I). Further, no enrichment is detected for VIP sets for 17 other viruses in East Asian populations (whole enrichment curve for nSL and iHS separately or combined; $p > 0.05$ in all cases; Figures S3 and S4). Taken together, these results suggest that coronaviruses (or a virus interacting similarly with hosts) have driven ancient epidemics in East Asia. This enrichment is unlikely to have been caused by any other virus represented in our set of 5,291 VIPs (Data S1A), but we still cannot exclude that a currently unknown type of virus that happened to use similar VIPs as coronaviruses could have been involved instead. The enrichment is most substantial for the top-ranked gene sets ranging between the top 10 and top 1,000 loci (Figure 1; whole enrichment curve FPR = 3.10^{-6} for nSL, FPR = 4.10^{-3} for iHS, and FPR = 6.10^{-5} for iHS and nSL combined) and is particularly strong for the top 200 loci in large windows (1 Mb) where a 4-fold enrichment is observed for both nSL and iHS statistics (pertaining to between 10 and 13 selected CoV-VIPs among the top 200 ranked genes; Data S1G). This suggests strong selection at multiple CoV-VIPs. That the selected haplotype structures are detected by both the iHS and nSL statistics suggests that they are unlikely to have occurred prior to 30,000 years ago, as both statistics have little power before this time point.[30]

An ancient epidemic in the ancestors of East Asians starting more than 20,000 years ago

To further test the existence of an ancient viral epidemic in East Asia, we use a recent ancestral recombination graph (ARG)-based

Please cite this article in press as: Souilmi et al., An ancient viral epidemic involving host coronavirus interacting genes more than 20,000 years ago in East Asia, Current Biology (2021), https://doi.org/10.1016/j.cub.2021.05.067

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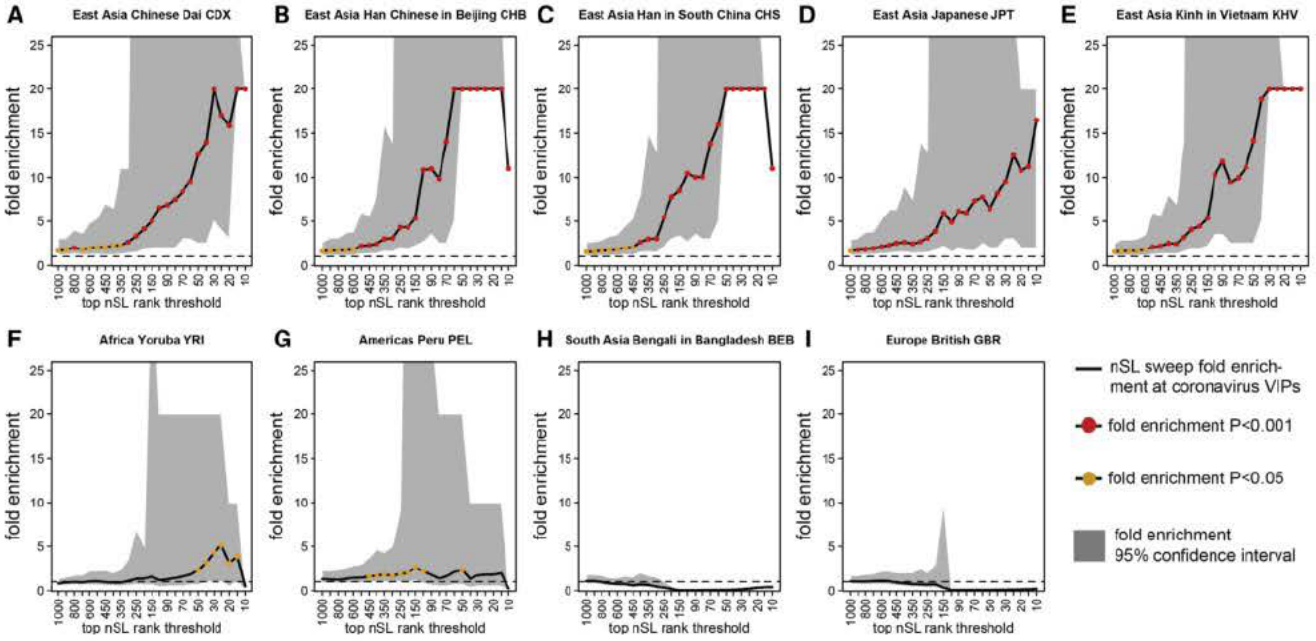

Figure 1. Coronavirus VIPs nSL ranks enrichment

(A)–(E) are East Asian populations, and (F)–(I) are populations from other continents. The y axis represents the bootstrap test (STAR Methods) relative fold enrichment of the number of genes in putative sweeps at CoV-VIPs, divided by the number of genes in putative sweeps at control genes matched for multiple confounding factors. The x axis represents the top rank threshold to designate putative sweeps. Black full line, average fold enrichment over 5,000 bootstrap test control sets. Fold enrichments greater than 20 are represented at 20. Gray area, 95% confidence interval of the fold enrichment over 5,000 bootstrap test control sets. The rank thresholds where the confidence interval lower or higher fold enrichment has a denominator of zero are not represented (for example, graph B, top 10 rank threshold). Lower confidence interval fold enrichments higher than 20 are represented at 20 (for example, graph B, top 30 rank threshold). Red dots, bootstrap test fold enrichment $p < 0.001$. Orange dots, bootstrap test fold enrichment $p < 0.05$. Note that the bootstrap test p values are not the same as the whole curve enrichment false positive risk (FPR) estimated using block-randomized genomes on top of the bootstrap test (STAR Methods). Related to STAR Methods and Figures S2–S4.

method, Relate,[20] to infer the timing and trajectories of selected loci for the CoV-VIPs. If the selective pressure responsible for the multiple independent selection events at CoV-VIPs was sudden, as expected from a new epidemic, these selection events should have started independently around the same time. By estimating ARGs at variants distributed across the entire genome, Relate can reconstruct coalescent events across time and detect genomic regions impacted by positive selection. To approximate the start time of selection, Relate estimates the first historical time point that a putatively selected variant had an observable frequency unlikely to be equal to zero (STAR Methods). We use this approximation as the likely starting time of selection (STAR Methods). Additionally, we use the iSAFE software[31]—which enables the localization of selected variants—along with a curated set of regulatory variants (expression quantitative trait loci [eQTLs]) from the GTEx Project[32] to help identify the likely causal mutations in the selected CoV-VIP genes. There is good evidence that most adaptive mutations in the human genome are regulatory mutations.[26,33–35] Accordingly, we find that iSAFE peaks are significantly closer to GTEx v8 eQTLs proximal to CoV-VIP genes than expected by chance (iSAFE proximity test; $p < 10^{-9}$; STAR Methods). Therefore, for each CoV-VIP gene, we choose a variant with the lowest Relate p value ($<10^{-3}$; STAR Methods) that is situated at or close to a GTEx eQTL associated with the focal gene to estimate the likely starting time of selection for that gene (STAR Methods; Figure S5A).

Using this approach, we observe 42 CoV-VIPs (Data S1H; Figure S5A) with selection starting times clustered around 870 generations ago (~200 generations wide, potentially due to noise in our estimates; Figure 2). While this amounts to about four times more selected CoV-VIP genes than were detected using either nSL or iHS (both detected around ten CoV-VIPs among the top 200 ranked genes; Data S1G), this is not unexpected, as Relate has more power to detect selection events than nSL and iHS when the beneficial allele is at intermediate frequencies (typically <60%; Figure 3; see Enard and Petrov,[17] Ferrer-Admetlla et al.,[24] and Voight et al.[25]). The tight clustering of starting times forms a highly significant peak (peak significance test $p = 2.3.10^{-4}$; Figure 2) when comparing the observed clustering of CoV-VIPs start times with the distribution of inferred start times for randomly sampled sets of genes (STAR Methods). Further, this significance test is not biased by the fact that CoV-VIPs are enriched for sweeps, as the test remains highly significant ($p = 1.10^{-4}$) when using random control sets with comparable high-scoring nSL statistics (STAR Methods). Thus, the tight temporal clustering of selection events is a specific feature of the CoV-VIPs, rather than a confounding aspect of any gene set similarly enriched for sweeps.

Consequently, our results are consistent with the emergence of a viral epidemic ~900 generations, or ~25,000 years (28 years per generation),[36] ago that drove a burst of strong positive selection in East Asia. Selection events starting 900 generations ago

Please cite this article in press as: Souilmi et al., An ancient viral epidemic involving host coronavirus interacting genes more than 20,000 years ago in East Asia, Current Biology (2021), https://doi.org/10.1016/j.cub.2021.05.067

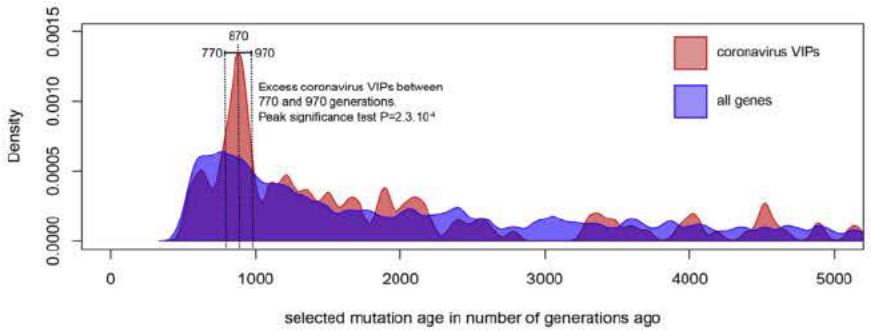

Figure 2. Timing of selection at CoV-VIPs

The figure shows the distribution of selection start times at CoV-VIPs (pink distribution) compared to the distribution of selection start times at all loci in the genome (blue distribution). Details on how the two distributions are compared by the peak significance test, and how the selection start times are estimated with Relate, are provided in STAR Methods. Related to STAR Methods and Figure S1.

clearly predate the estimated split of different East Asian populations included in the 1000 Genomes Project from their shared ancestral population.[18]

Although selective pressures other than a coronavirus or another unknown type of virus with similar host interactions might also contribute to these patterns, we note that the signal is restricted specifically at CoV-VIPs and none of 17 other viruses that we tested exhibit the same temporal clustering (peak significance test $p > 0.05$ in all cases; STAR Methods). Further, this test remained highly significant when retesting the clustering of CoV-VIPs using only RNA VIPs as the control set ($p = 4.10^{-4}$; Data S1B). Importantly, the estimate of an ancient viral epidemic starting ~25,000 years ago in East Asia is remarkably congruent with the 23,000 years estimate for the emergence of sarbecoviruses (the viral family of SARS-CoV-2).[37]

Strong selection drove coordinated changes in multiple CoV-VIP genes over 20,000 years

To learn more about the start and duration of selection acting in East Asia, we use CLUES[21] to infer allele frequency trajectories and selection coefficients for the inferred beneficial mutations proximal to the 42 CoV-VIP genes with selection starting 900 generations ago according to Relate (Figure 3). We anticipate that selection was probably strongest when the naive host

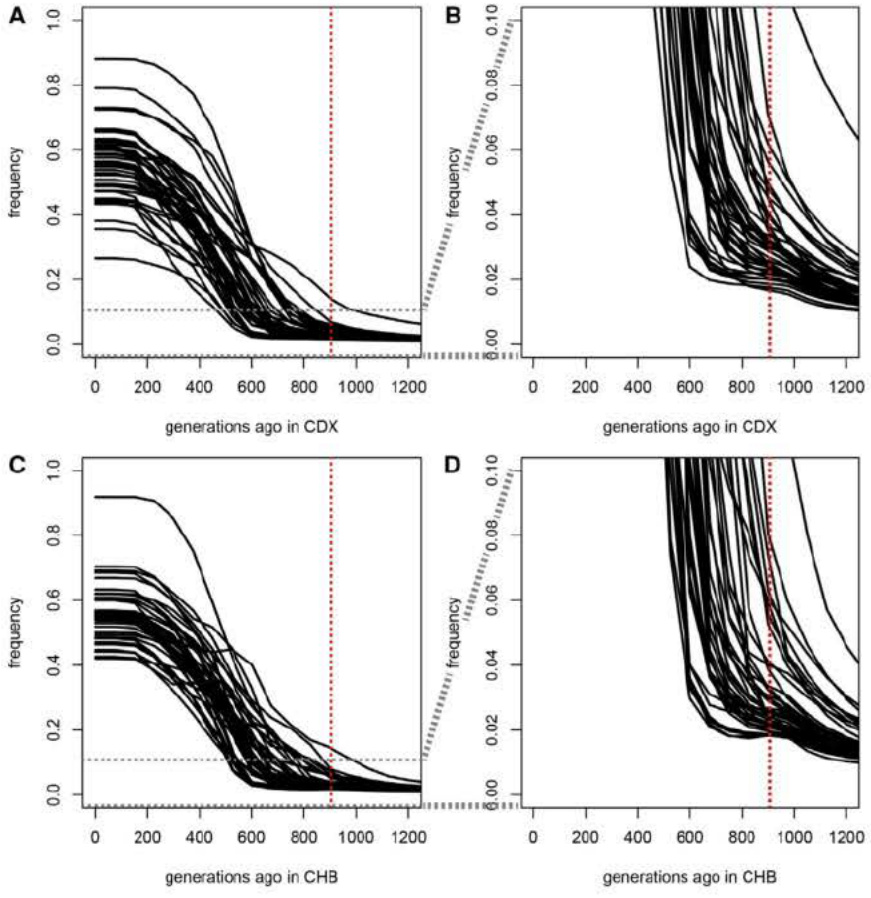

Figure 3. Selected CoV-VIPs allele frequency trajectories over time estimated by CLUES in East Asia

Each frequency trajectory is for one of the 42 Relate selected mutations at CoV-VIPs within the peak around 900 generations ago (STAR Methods).

(A) Frequency trajectories in the Chinese Dai CDX 1000 Genomes population.

(B) Same but zoomed in from frequencies 0%–10%.

(C) Frequency trajectories in the Han Chinese from Beijing CHB 1000 Genomes population.

(D) Same but zoomed in from frequencies 0%–10%.

Related to STAR Methods.

Please cite this article in press as: Souilmi et al., An ancient viral epidemic involving host coronavirus interacting genes more than 20,000 years ago in East Asia, Current Biology (2021), https://doi.org/10.1016/j.cub.2021.05.067

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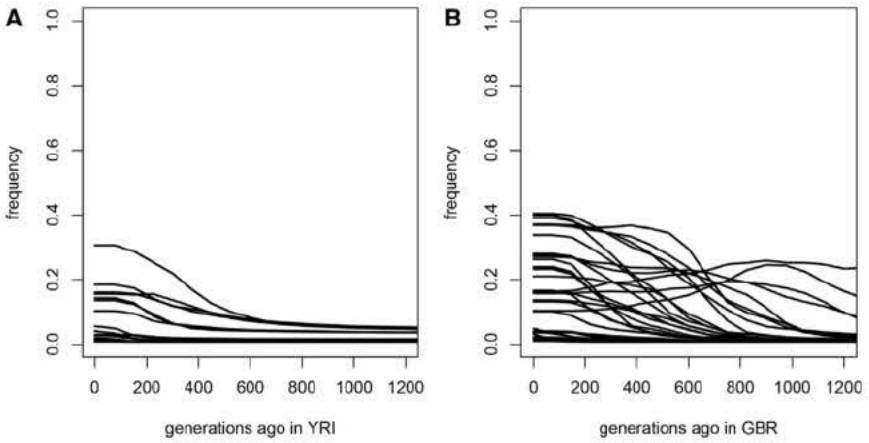

Figure 4. Selected CoV-VIPs allele frequency trajectories over time estimated by CLUES in Africa (Yoruba) and Europe (British)

Same as Figure 3.

(A) Yoruba population. The graph includes 17 frequency trajectories, the 25 other alleles selected in East Asia being absent in the Yoruba sample (but not Africa overall; see Data S1I).

(B) British population. The graph includes 35 frequency trajectories, the other seven alleles selected in East Asia being absent in the British sample.

Related to STAR Methods.

population was first infected, before gradually waning as the host population adapted to the viral pressure.[38] Similarly, a decrease in the virulence of the virus over time, a phenomenon that has been reported during long-term bouts of host-virus coevolution,[39] would also result in the gradual decrement of selection over time. Hence, for each of the 42 CoV-VIPs predicted to have come under selection ~900 generations ago, we use CLUES to estimate the selection coefficient in two successive time intervals (between 1,000 and 500 generations ago and from 500 generations ago to the present), predicting that selection would be stronger in the oldest interval. We note that a 500 generations interval was reported as the approximate time span that CLUES provides reliable estimates for humans.[21] Following the protocol of Stern et al.,[40] we base our estimates on two of the five East Asian populations (i.e., Dai and Beijing Han Chinese; Figures 3A and 3B and 3C and 3D, respectively).

CLUES infers more complex frequency trajectories than an abrupt jump in frequency 900 generations ago. Instead, the estimated trajectories (Figures 3A–3D) suggest that 900 generations ago is the approximate time when the bulk of the selected variants reached a frequency of a few percent or more and when there is an acceleration in the frequency increase (Figures 3B and 3D). Note that this does not contradict the strong peak of selection times starting around 900 generations ago found by Relate, as this is the time when Relate estimates frequencies clearly distinguishable from zero (STAR Methods). This might correspond to the transition between the establishment and exponential phases of the sweeps and might imply that the selective pressure could be older than 900 generations. Although the slow starts of frequency increases make it hard to pinpoint when selection started exactly, the vast majority of the selected alleles appear to have reached 5% or higher frequencies by 600 generations, thus making it highly unlikely that the selection would have started later. Frequency trajectories estimated in the Yoruba African population (Figure 4A) or the British European population (Figure 4B) also show very low initial frequencies. The selected variants in East Asia are found nowadays at very low frequencies, especially in Africa (Data S1I).

The selected mutations are estimated to have continually increased in frequency in East Asia until ~200 generations (~5,000 years) ago (Figures 3A and 3C). Accordingly, CLUES estimates high selection coefficients between 1,000 and 500 generations ago (Dai average s = 0.034; Beijing Han average s = 0.042; Figures 5A and 5B) but much weaker selection coefficients from 500 generations ago to the present (Dai average s = 0.002; Beijing Han average s = 0.003; Figures 5A and 5B). These patterns are consistent with the appearance of a strong selective pressure that triggered a coordinated adaptive response across multiple independent loci, which waned through time as the host population adapted to the viral pressure and/or as the virus became less virulent.

Validation of direct physical interactions between selected COV-VIPs and SARS-CoV-2 proteins

To further validate that an ancient viral epidemic was responsible for the observed selection signals, next we test whether the 35 out of 42 selected CoV-VIPs that interact with SARS-CoV-2 (as opposed to other coronaviruses in our dataset) are indeed CoV-VIPs and directly interact with SARS CoV-2 viral proteins. While these interactions were originally identified by high-throughput mass spectrometry,[19] high-throughput mass spectrometry can sometimes identify indirect interactions in a larger protein complex or false positives altogether.[41] We co-express the candidate CoV-VIPs:SARS CoV-2 protein pairs in a cell-free protein expression system and test their interactions using an AlphaLISA protein:protein interaction assay (STAR Methods). This approach (Figure S6A) was previously used for rapid analysis of intra-viral PPI network of Zika virus.[42] The assay is expected to detect ~70% of protein interactions with human proteins (30% false negative rate; STAR Methods). Out of 35 selected SARS-CoV-2 CoV-VIPs, 33 interacting protein pairs can be tested with the assay (STAR Methods). Figure 6 highlights the results for six of the 33 CoV-VIPs, while Figure S6 presents the results for the remaining CoV-VIPs. Among the 33 interactions tested, we confirm 24 or 73%, the expected confirmation rate (taking the false negative rate into account) if 100% or close to 100% of the selected CoV-VIPs are indeed CoV-VIPs (Figures 6A–6C and S6B; Data S1J). This very high validation rate further strengthens the evidence for an ancient viral epidemic in East Asia.

Selected CoV-VIPs are enriched for antiviral and proviral factors

To further clarify that a viral epidemic caused the strong burst of selection, and not another ecological pressure acting on the

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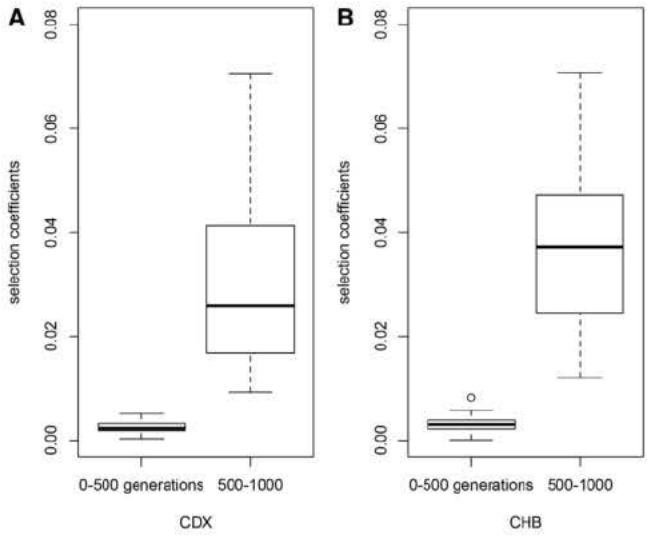

Figure 5. Coronavirus selected VIPs selection coefficients estimated by CLUES

This figure shows classic R boxplots of selected coefficients at the 42 Relate selected mutations within the peak around 900 generations ago (STAR Methods).

(A) Selection coefficients in the Chinese Dai CDX 1000 Genomes population. (B) Selection coefficients in the Han Chinese from Beijing CHB 1000 Genomes population. Left: average selection coefficients between 0 and 500 generations ago are shown. Right: average selection coefficients between 500 and 1,000 generations ago are shown.

Related to STAR Methods.

same set of genes, we test whether the 42 selected CoV-VIPs are enriched for genes with antiviral or proviral effects relative to other CoV-VIPs (i.e., loci that are known to have a detrimental or beneficial effect on the virus, respectively). Because the relevant literature for coronaviruses is currently limited, we extend our set of anti- and proviral loci to include loci reported for diverse viruses with high confidence from the general virology literature (STAR Methods; Data S1K and S1L). We find that 21 (50%) of the 42 CoV-VIPs that came under selection ~900 generations ago have high-confidence anti- or proviral effects (versus 29% for all 420 CoV-VIPs), a significant inflation in such effects (hypergeometric test $p = 6.10^{-4}$) that further supports our claim that the underlying selective pressure was most likely a viral epidemic.

Selected mutations lie near regulatory variants active in SARS-CoV-2-affected tissues

Coronavirus infections in humans are known to have pathological consequences for specific tissues; therefore, we investigate whether the genes selected in East Asia are also enriched for regulatory functions in similar tissues. In light of our finding that many putative causal mutations in CoV-VIPs are proximal to eQTLs, we investigate whether selected mutations are situated closer to eQTLs for a given tissue than expected by chance, as this would indicate that the tissue was negatively impacted by the virus (prompting the adaptive response). Note that the GTEx eQTLs we use are not specific to a single tissue and are often shared between tissues. However, each tissue still has its own specific combination of eQTLs. Briefly, we estimate a proximity-based metric that quantifies the distance between the location of the causal mutation estimated by iSAFE and the tissue-specific eQTLs for the 42 loci with selection starting ~900 generations ago and compare this to the same distances observed among randomly sampled sets of CoV-VIPs (Figure 7; STAR Methods).

We find that GTEx lung eQTLs lie closer to predicted causal mutations among the 42 putative selected loci than for any other tissue ($p = 3.10^{-5}$; Figure 7). Several additional tissues known to be negatively affected by coronavirus—blood and arteries,[43,44] adipose tissue,[45] and the digestive tract[46]—also exhibit closer proximities between putative causal loci and eQTLs than expected by chance (Figure 7). Interestingly, the spleen shows no tendency for eQTLs to lie closer to selected loci than expected around 900 generations ago compared to other evolutionary times, perhaps because the spleen is replete with multiple immune cell types that might be more prone to regular adaptation to diverse pathogens over time.[47] Note that tissues with more eQTLs tend to have more significant p values. For example, skeletal muscle has a lower proximity ratio than stomach but also a lower p value due to higher statistical power (more eQTLs). However, we find no correlation (Pearson's correlation test $p = 0.6$) between the total number of GTEx v8 eQTLs[32] for a given tissue and the proximity ratio for each tissue. Thus, different proximity ratios between tissues do not just reflect a statistical power bias. We further show that iSAFE locates adaptation particularly closer to more lung-specific eQTLs compared to other tissues (Figure S7; STAR Methods). Our results indicate that the tissues impacted in the inferred viral epidemic in East Asia match those affected by SARS-CoV-2.

Coronavirus VIPs are enriched for SARS-CoV-2 susceptibility and COVID-19 severity loci

Our results indicate that many of the selected CoV-VIPs now sit at intermediate frequencies in modern East Asian populations. We anticipate that these segregating loci should make a measurable contribution to the inter-individual variation in SARS-CoV-2 susceptibility and COVID-19 severity among contemporary populations in East Asia. While a genome-wide association study (GWAS) scan has yet to be reported for a large East Asian cohort, two GWASs were recently released that used sizable British cohorts to investigate SARS-CoV-2 susceptibility (1,454 cases and 7,032 controls; henceforth called the susceptibility GWAS) and severity (325 cases [deaths] versus 1,129 positive controls; henceforth called the severity GWAS; data from the UK Biobank;[22,23] https://grasp.nhlbi.nih.gov/Covid19GWASResults.aspx). Because we use a non-East Asian population, we only ask, as a functional validation of a viral pressure, whether there is an overlap between the selected loci in East Asia and stronger COVID-19 GWAS hits in the UK Biobank. We do not look at all at the directionality or the size of effects. It is indeed unclear that those would be transposable between populations, given that here we provide evidence that different pathogens may have influenced evolution in different human populations. This also means that we make no claim at all here about any decrease or increase of virus susceptibility in any given human population compared to others. Furthermore, we use the UK-Biobank cohort instead of the complete COVID-19 Host Genetics Initiative meta-GWAS data (https://

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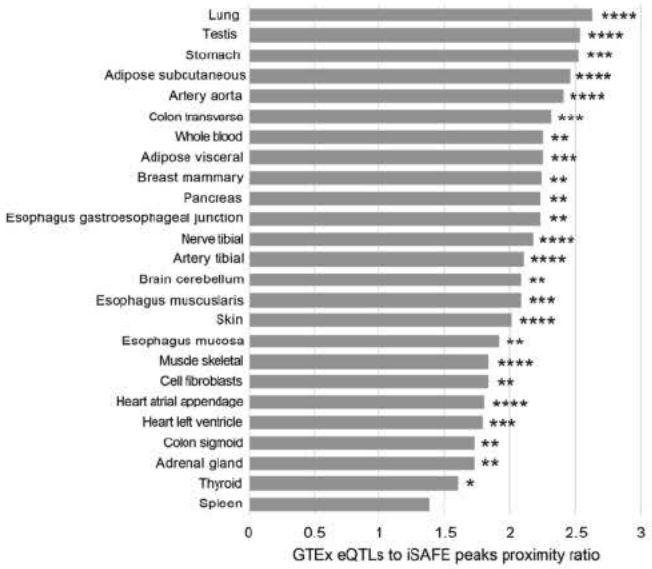

Figure 6. Validation of selected CoV-VIPs/SARS-CoV-2 protein interactions using cell-free expressed proteins

(A) A representative image of SDS-PAGE gel loaded with *in vitro* translation reactions co-expressing human VIPs/SARS-CoV-2 proteins in *Leishmania tarentolae* (LTE) system. Human proteins were tagged with EGFP at N terminus, and the viral proteins were tagged with mCherry at C terminus. The protein bands were visualized by fluorescence scanning; viral proteins: M,ORF9c, ORF10, and NSP5; human proteins: ACADM, C20orf4, PMPCA, NDFIP2, PPT1, and ARF6.

(B) A plot of representative signals of AlphaLISA interaction assay for VIP/viral protein pairs shown in (A). Zika virus self-dimerizing C-protein tagged with Cherry and EGFP was used as positive interaction control. As the negative control, we used FKBP-rapamycin-binding (FRB) domain.

(C) Graphic summary of the VIPs/SARS-CoV-2 interaction analysis: the confirmed interactions are shown with green circle, whereas interactions that could not be conformed using this assay are depicted as red diamond.

Related to STAR Methods and Figure S6.

www.covid19hg.org/),[7,8] to avoid population stratification to the best extent possible (a legitimate concern with a trait clearly affected by socioeconomic factors).

While we are unable to precisely identify the causal variants for the selected CoV-VIP genes observed in the ancestors of East Asians—nor would these variants necessarily occur as outliers in a GWAS conducted on the British population—we note that it is possible that other variants in the same CoV-VIP genes may also produce variation in SARS-CoV-2 susceptibility and COVID-19 severity among modern British individuals.

By contrasting variants in CoV-VIPs against those in random sets of genes, we find that variants in CoV-VIPs have significantly lower p values for both the susceptibility GWAS and severity GWAS than expected (simple permutation test $p < 10^{-9}$ for both GWAS tests; STAR Methods). More importantly, the 42 CoV-VIPs with selection starting ~900 generations ago have even lower GWAS p values compared to other CoV-VIPs ($p = 0.0015$ for susceptibility GWAS and $p = 0.023$ for severity; STAR Methods). This result indicates that the selected genes inferred in our study might contribute to individual variation in COVID-19 etiology in modern human populations in the UK, providing further evidence that a coronavirus or another virus with similar host interactions may have been the selection pressure behind the adaptive response we observe in East Asia. Notably, the strongest GWAS hits identified by the COVID-19 Host Genetics Initiative (listed at https://www.covid19hg.org/publications/) do not overlap with the 42 CoV-VIPs selected in East Asia. The lack of overlap is however not surprising and a result of the design of our analysis (STAR Methods).

Selected CoV-VIP genes include multiple known drug targets

Our analyses suggest that the 42 CoV-VIPs identified as putative targets of an ancient coronavirus (or another virus using similar host interactions) epidemic might play a functional role in SARS-CoV-2 etiology in modern human populations. We find that four of these genes (*SMAD3*, *IMPDH2*, *PPIB*, and *GPX1*) are targets of eleven drugs currently used or investigated in clinical trials to mitigate COVID-19 symptoms (STAR Methods). While this number is not higher than expected when compared to other CoV-VIPs (hypergeometric test $p > 0.05$), we note that most of the 42 genes identified here have yet to be the focus of trials. In addition to the four selected CoV-VIP genes targeted by coronavirus-specific drugs, five additional selected CoV-VIPs are targeted by multiple drugs to treat a variety of non-coronavirus pathologies (Data S1). An additional six of the 42 selected CoV-VIPs have been identified by Finan et al.[48] as part of the "druggable genome" (Data S1M).

DISCUSSION

We identified a set of 42 CoV-VIPs exhibiting a coordinated adaptive response that likely emerged more than 20,000 years ago (Figure 2). This pattern was unique to East Asian populations (as classified by the 1000 Genomes Project). We show that this selection pressure produced a strong response across the 42 CoV-VIP genes that gradually waned and resulted in the selected loci plateauing at intermediate frequencies. Further, we demonstrate that this adaptive response is likely the outcome of a viral epidemic, as attested by the clustering of putatively selected loci around variants that regulate tissues known to exhibit COVID-19-related pathologies, and the enrichment of variants associated with SARS-CoV-2 susceptibility and severity, as well as anti- and proviral functions, among the 42 CoV-VIP genes selected starting around 900 generations ago.

An important limitation is that some of our analyses rely upon comparative datasets that were generated in contemporary human populations that have different ancestries than the East Asian populations where the selected CoV-VIP genes were detected. In particular, both of the eQTL and GWAS datasets come from large studies that are focused on contemporary populations from Europe and none of the five European populations in our study exhibit the selection signals observed in East Asia. More direct confirmation of the causal role of 42 CoV-VIP genes in COVID-19 etiology will require the appropriate GWAS to be conducted in East Asian populations. The detection of genetic associations among the 42 CoV-VIPs in a GWAS on contemporary East Asians would provide further evidence that one or more coronaviruses, or another virus using similar interactions, comprised the selection pressure that drove the observed adaptive response. Moreover, a high-powered GWAS in East Asian populations would be

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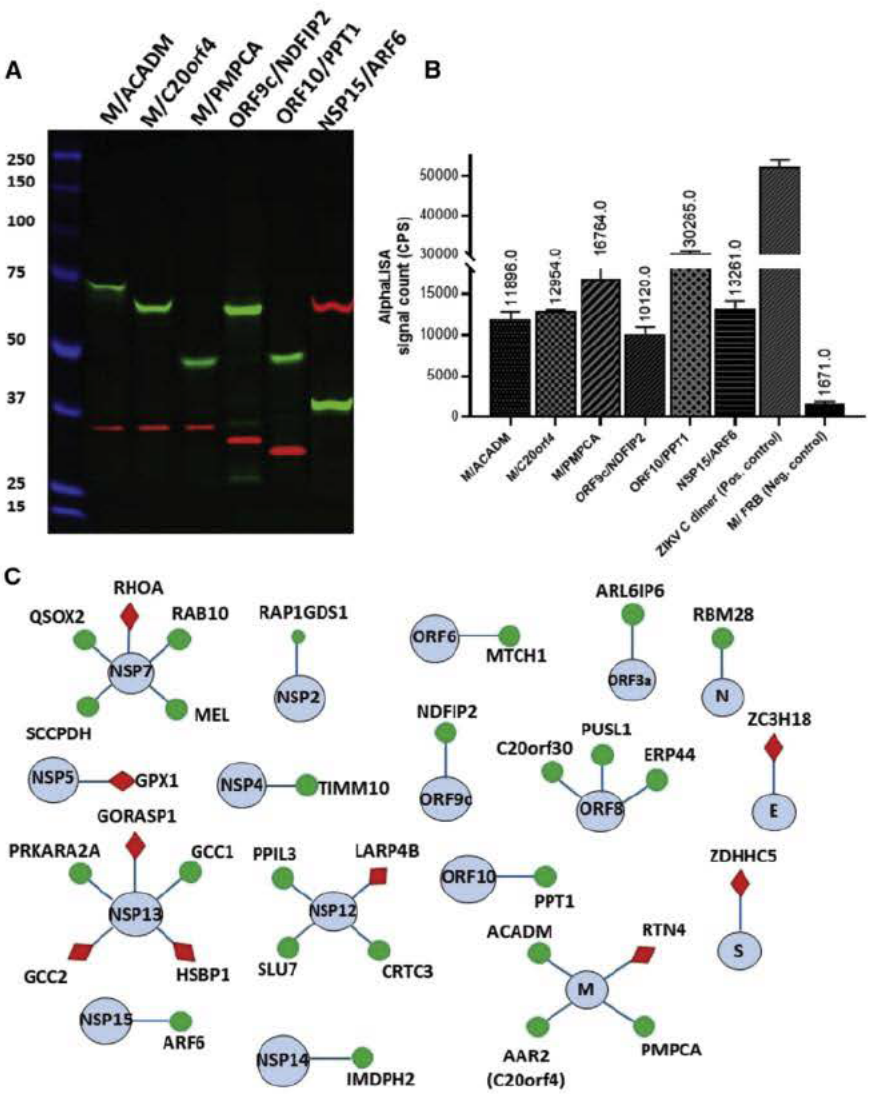

Figure 7. Proximity of selection signals to GTEx eQTLs at the 42 selected CoV-VIPs compared to random CoV-VIPs

The histogram shows how close selection signals localized by iSAFE peaks are to the GTEx eQTLs from 25 different tissues, at peak-VIPs compared to randomly chosen CoV-VIPs (STAR Methods). How close iSAFE peaks are to GTEx eQTLs compared to random CoV-VIPs is estimated through a proximity ratio. The proximity ratio is described in the STAR Methods. It quantifies how much closer iSAFE peaks are to eQTLs of a specific GTEx tissue, compared to random expectations that take the number and structure of iSAFE peaks as well as the number and structure of GTEx eQTLs into account (STAR Methods). ****Proximity ratio test $p < 0.0001$. ***Proximity ratio test $p < 0.001$. **$p < 0.01$. *$p < 0.05$. Note that lower proximity ratios can be associated with smaller p values for tissues with more eQTLs (due to decreased null variance; for example, skeletal muscle versus pancreas). Related to STAR Methods and Figure S5.

required to identify the loci that currently impact individual variation in COVID-19 etiology in East Asian individuals. Because of these limitations, and because it would be extremely difficult to control for all the other factors that differ across the world (including socioeconomic factors), our results do not represent evidence for any difference in either increased or decreased genetic susceptibility in any human population.

Insights into ancient viral epidemics from modern human genomes

A particularly salient feature of the adaptive response observed for the 42 CoV-VIPs is that selection appears to be acting continuously over an ~20,000 years period. The profile of selection in the host East Asian populations is consistent with a new viral pressure that ancestral populations had never experienced previously but that subsequently remained present for a very long period of time. As this manuscript was in the final stages of preparation, the first host-virus interactomes were published for SARS-CoV-1 and MERS-CoV,[49] which exhibit an extensive overlap with the SARS-CoV-2 interactome used in the present study.[19] This suggests that coronaviruses share a broad set of host proteins that they interact with, which should apply to ancient coronaviruses. These patterns are consistent with one or more coronaviruses driving selection that produced the signals reported here. Still, we cannot exclude that another currently unknown type of viruses might have been responsible, which used the same interactions as coronaviruses with human proteins.

Further validation of the historical trajectories of the causal mutations at selected genes is still needed, including more finely resolved temporal and geographic patterns that could be derived from ancient DNA sampled from across East Asia; however, the requisite ancient samples are currently lacking. Nonetheless, we note the geographic origin of several modern outbreaks of coronaviruses in East Asia point to East Asia being a likely location where these ancient populations came into contact with the virus. Our results suggest that East Asia might have also been a natural range for coronavirus reservoir species during the last 25,000 years.[50]

Applied evolutionary medicine: Using evolutionary information to combat COVID-19

The net result of the ancient selection patterns on the CoV-VIPs in ancient human populations is the creation of genetic differences among individuals now living in East Asia and between East Asians and populations distributed across the rest of the world. As we demonstrate in this study, this evolutionary genetic information can be exploited by statistical analyses to identify loci potentially involved in the epidemiology of modern diseases—COVID-19 in the present case. Such evolutionary

information may ultimately assist in the development of future drugs and therapies by complementing information obtained from more traditional epidemiological and biomedical research. While such studies provide information on a specific gene, the evolutionary approach adopted here leverages evolutionary information in modern genomes to identify candidate genomic regions of interest. This is similar to the information provided by GWAS—i.e., lists of variants or genes that are potentially associated with a particular trait or disease—though we note that the information provided by evolutionary analyses comes with an added understanding about the historical processes that created the underlying population genetic patterns.

The current limitation shared by population genomic approaches, such as GWAS and the evolutionary analyses presented here, is that they identify statistical associations rather than causal links. Further evidence of causal relationships between the CoV-VIPs and COVID-19 etiology could be obtained by examining which viral proteins the selected CoV-VIPs interact with, thus establishing the specific viral functions that are affected.

The ultimate confirmation of causality requires functional validation. It remains to be established whether the genes we have identified in this study might help drug-repurposing efforts and provide a basis for future drug and therapeutic development.

By leveraging the evolutionary information contained in publicly available human genomic datasets, we were able to infer ancient viral epidemics impacting the ancestors of contemporary East Asian populations. Importantly, our evolutionary genomics analyses have identified several new candidate genes that might provide novel drug targets (Data S1). More broadly, our findings highlight the utility of thinking about the possible contribution of evolutionary genomic approaches into standard medical research protocols. Indeed, by revealing the identity of our ancient pathogenic foes, evolutionary genomic methods may ultimately improve our ability to predict—and thus prevent—the epidemics of the future.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.05.067.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments, Y.S., R.T., K.A., and D.E.; performed the experiments, Y.S., M.E.L., R.T., S.V.M., W.A.J., and D.E.; interpreted the results, Y.S., M.E.L., R.T., C.D.H., A.S.J., S.V.M., W.A.J., K.A., and D.E.; wrote the manuscript, Y.S., R.T., S.V.M., and D.E.; contributed resources/reagents, N.J.K., K.A., and D.E.

DECLARATION OF INTERESTS

The Krogan Laboratory has received research support from Vir Biotechnology and F. Hoffmann-La Roche. N.J.K. has consulting agreements with the Icahn School of Medicine at Mount Sinai, New York, Maze Therapeutics, and Interline Therapeutics; is a shareholder of Tenaya Therapeutics; and has received stocks from Maze Therapeutics and Interline Therapeutics. The other authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
1000 Genomes Project – Phase 3	Auton et al.[18]	ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/
VIPs	this manuscript	Data S1
Relate-estimated coalescence rates, allele ages and selection P values for the 1000GP	Speidel et al.[20]	https://zenodo.org/record/3234689
GTEx expression	GTEx Consortium[32]	https://gtexportal.org/home/datasets
Protein-protein interactions (IntAct)	Luisi et al.[51]	https://www.ebi.ac.uk/intact
The density of conserved segments (PhastCons)	Siepel et al.[52]	http://hgdownload.cse.ucsc.edu/goldenPath/hg19/phastCons46way/
The density of regulatory elements	N/A	http://hgdownload.soe.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeRegDnaseClustered
The recombination rate	Hinch et al.[33]	https://www.well.ox.ac.uk/~anjali/AAmap/
Software and algorithms		
selscan (compute nSL).	Szpiech and Hernandez[54]	https://github.com/szpiech/selscan
hapbin (compute his)	Maclean et al.[55]	https://github.com/evotools/hapbin
Gene Set Enrichment Pipeline	Enard and Petrov[17]	https://github.com/DavidPierreEnard/Gene_Set_Enrichment_Pipeline
Relate	Speidel et al.[20]	https://myersgroup.github.io/relate/
CLUES	Stern et al.[21]	https://github.com/35ajstern/clues
iSAFE	Akbari et al.[31]	https://github.com/alek0991/iSAFE
Reagents		
NucleoBond Xtra Midi kit for transfection-grade plasmid DNA	Machery-Nagel SCIENTIFIX PTY LTD, AUS	catalog #740410.5
Anti-GFP AlphaLISA Acceptor bead	Perkin Elmer	catalog #AL133M
Streptavidin Alphascreen Donor bead	Perkin Elmer	catalog #6760002
OptiPlate-384, White Opaque 384-well Microplate	Perkin Elmer	catalog #6007290
Proxy-Plate-384, White shallow 384-well Microplate	Perkin Elmer	catalog #6008280
Bolt 4 to 12%, Bis-Tris, 1.0 mm Mini Protein Gel, 12-well	Thermofisher scientific	catalog #NW04122BOX
NuPAGE sample buffer (4x)	Life Technologies	catalog #NP0007
Prestained Protein Ladder, All blue standard	Biorad	catalog #1610373
NuPAGE MOPS SDS Running Buffer (20X)	Thermofisher scientific	catalog #NP0001

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, David Enard denard@email.arizona.edu.

Materials availability

This study did not generate new unique reagents. The list of reagents used is provided in the Key resources table.

Data and code availability

The pipeline required to reproduce the analysis, as well as a complete list of VIPs for diverse viruses, are available at https://github.com/DavidPierreEnard/Gene_Set_Enrichment_Pipeline

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All the sources of bioinformatic data used in the analysis are provided in the Key resources table.

METHOD DETAILS

Terminology

For convenience, the 42 CoV-VIPs that we infer to have started coming under selection around 900 generations ago are called peak-VIPs in the STAR Methods.

Coronavirus VIPs

We used a dataset of 5,291 VIPs (Data S1A). Of these, 1,920 of these VIPs are high confidence VIPs identified by low-throughput molecular methods, while the remaining VIPs were identified by diverse high-throughput mass-spectrometry studies. Using VIPs to find the genomic footprints of an ancient epidemic is justified by the fact that VIPs do not just interact with viruses. These interactions are in fact functionally consequential for viruses. The 420 CoV-VIPs are part of a much larger set of VIPs found to interact to date with more than 20 different viruses that infect humans[16] (Data S1A). In total, there are currently 5,291 VIPs (Data S1A). Of these, 1,920 high confidence VIPs were annotated manually by curating the virology literature and correspond to VIPs that were identified by low-throughput molecular methods.[16] These VIPs were often identified by virologists who hypothesized that the interaction existed in the first place based on previous virology knowledge. The other 3,371 VIPs identified by multiple high-throughput mass spectrometry experiments, such as the one conducted to identify the 332 SARS-CoV-2 VIPs.[19]

To confirm that VIPs are indeed functionally important for viruses beyond just interacting physically, and represent a viable way of detecting specific viral selective pressures that trigger host adaptation, we verify that VIPs have antiviral or proviral effects on the viral replication cycle on which positive selection can act. More specifically, we need to confirm that VIPs have much more frequent proviral or antiviral effects compared to non-VIPs. To test this, we are currently manually annotating all protein-coding genes in the human genome that were involved in published low-throughput expression perturbation experiments to assess their effects on viruses, and manually curable in PubMed. Such expression perturbation experiments typically include RNAi knock-down experiments or overexpression experiments. These experiments are useful to annotate proviral or antiviral effects. Indeed, decreasing the expression of an antiviral VIP should be beneficial to viral replication, while increasing the expression of an antiviral VIP should be detrimental to the virus. Conversely, decreasing the expression of a proviral VIP should be detrimental to viral replication, while increasing the expression of a proviral VIP should be beneficial. We consider only low-throughput expression perturbation experiments, where the expression of only one candidate gene is perturbed. This excludes high throughput genome-wide RNAi screens known for their high false positive and high false negative rates. Using these criteria, we have so far found that 855, or 66% of 1,300 already annotated low-throughput VIPs have a known antiviral or proviral effect. Of the 2,627 high-throughput VIPs that we already annotated, 426 or 16% have a known antiviral or proviral effect. Of the 3,913 non-VIPs that we already annotated, 171 or 4% have a known antiviral of proviral effect. Although we have not annotated all human protein-coding genes yet, the large numbers already annotated imply that these proportions are very likely to be close to the final proportions when all genes are annotated.

Thus, approximately two-thirds of low-throughput VIPs have known antiviral or proviral effects that were revealed by expression perturbation experiments such as gene knock-down or overexpression. The 16% proportion of high throughput VIPs known to have a clear antiviral or proviral effect is much lower than the two-thirds of low-throughput VIPs with antiviral or proviral effects, but it is important to consider that high-throughput VIPs have not been investigated anywhere near as much as the low-throughput ones. In contrast, only 4% of non-VIPs with no known viral interaction have published antiviral or proviral effects. Both low-throughput and high-throughput VIPs are thus far more often functionally consequential for viruses compared to non-VIPs (simple permutation test $p < 10^{-16}$ in both cases). Note that because they will dilute the signal rather than create it, a certain amount of random, false-positive high-throughput interactions are expected to be conservative when trying to detect ancient epidemics.

Focusing specifically on the 420 CoV-VIPs, we find that 121 or 28.9% of them already have published antiviral or proviral effects (Data S1K). Of the 332 SARS-CoV-2 VIPs, 83 or 25% of them have antiviral or proviral effects (Data S1K), often independently confirmed in multiple viruses. The SARS-CoV-2 VIPs are thus more than six times more likely to have antiviral or proviral effects than non-VIPs, which supports the high quality of the mass spectrometry screen conducted by Gordon et al.,[19] as confirmed by our own validations of interactions (Figure 6). Note that it is unrealistic to expect much higher percentages at SARS-CoV-2 VIPs, given that coronaviruses are only starting to be more thoroughly investigated, and have been much less investigated than other viruses such as HIV or IAV (Influenza Virus).

Validation of selected SARS-CoV-2 CoV-VIPs

We co-express the selected SARS-CoV-2 CoV-VIPs:SARS-CoV-2 protein pairs in *Leishmania tarentolae* (LTE) cell-free protein expression system and test their interactions using AlphaLISA protein: protein interaction assay. This approach (Figure S6A) was previously used for rapid analysis of intra-viral PPI network of ZIKA virus.[42] Two of the 35 selected SARS-CoV-2 CoV-VIPs, UBAP2 and FBN2, are missing from the analysis because they are not available in the DNASU plasmid repository (see below). All proteins were tagged with either EGFP or Cherry fluorescent proteins and with the exception of GCC2 and RTN4 could be detected on SDS-PAGE upon cell-free co-expression (Figures 6A and S6B). These two host proteins have large molecular weights (Data S1) that make proper

protein folding challenging, which likely explain detection failure. When the *in vitro* translation reactions were subjected to AlphaLISA interaction analysis, out of 33 interacting protein pairs 24 were positively confirmed by our assay (Figures 6A–6C and S6B). Of the two negative results for GCC2 and RTN4, only GCC2 is represented in lane 23 of Figure S6B for comparison with the positive results. The 73% (24/33) validation rate is likely to be an underestimation of the actual true interactions in this experimental set due to the limitations of the expression system and also the details of biochemistry of the individual proteins. The obtained results probably contain a significant number of false negatives due to two factors. First, we have demonstrated that LTE cell-free system can produce approximately 70% of human proteins in full length, folded and monodispersed form.[56] Therefore, it is likely that at least some of the human proteins have not been expressed in functional form. Furthermore, post-translational modifications and functional states of proteins may modulate their interactions with viral ORFs. For example one of these proteins in this set, RhoA, is post translationally prenylated and is carboxyl methylated *in vivo*.[57,58] Due to the lack of isoprenoid pyrophosphate precursors this modification is likely to be absent in its LTE produced version. Furthermore, its nucleotide bound form (GDP versus GTP) modulates its interaction with many RhoA binding proteins[59] and may not be optimal in the current experimental set up. Moreover, protein-focused assay optimization is likely to reveal additional positive interactions in this set.

For gene sequences and generation of Cell-free expression vectors, the DNA sequences of SARS-CoV-2 were sourced from the isolate of 2019-nCoV/USA-WA1/2020, (accession number MN985325) and based on the published annotation of the genome sequence of SARS-CoV-2.[19] The viral genes were synthesized and inserted into pCellFree_G06 gateway destination vector (available in Addgene, Plasmid # 67140; https://www.addgene.org/67140/ by Gene Universal. The human gene plasmids were generated by DNASU plasmid repository (Arizona State University, US). The genes were cloned into pmCell-free_KA1 gateway destination vector (available in Addgene, Plasmid #145369; https://www.addgene.org/145369/). The synthesized plasmids DNA were amplified and isolated by NucleoBond Xtra Midi kit.

For the Cell-free co-expression of CoV-VIPs and SARS-CoV-2 protein pairs, the protein pairs were co-expressed in the LTE cell-free expression system. The *Leishmania tarentolae* translation competent extract and the feeding solution for protein expression were prepared as previously described.[60] The DNA templates for N-terminal-GFP (8-12 nM) and C-terminal-Cherry (10-15 nM) tagged proteins were added concomitantly to the LTE reaction mixture and the samples were incubated for 5h at 25°C for expression. The expression of proteins was performed using 384-well Proxiplate in 10 μL volume. The Protein expression was detected by measuring GFP and Cherry fluorescence using Tecan Spark multimode microplate reader (Tecan Australia Pty). In addition, for analysis of co-translated eGFP and mCherry fused proteins, the LTE reactions were mixed with 1:1 v/v of 2x NuPAGE sample buffer and loaded on a Bolt 4%–12% Bis-Tris protein gel. The proteins were detected by scanning the gel using ChemiDoc MP System (Bio-Rad, Australia).

AlphaLISA assays were performed in Optiplate-384 plus plates using Anti-GFP AlphaLISA Acceptor and Streptavidin Donor beads. Alpha beads were prepared according to the protocol provided by the manufacturer (https://www.perkinelmer.com/Content/TDLotSheet/AS112D_AS112_2587358.pdf). Briefly, the acceptor and donor beads stocks (5 mg/mL) were diluted to 100 μg/mL (5x) in AlphaLISA assay buffer (Buffer A: 25 mM HEPES, 50 mM NaCl, 0.1% BSA and 0.01% Nonidet P-40; pH:7.5). The biotinylated mCherry nanobody diluted in buffer A (final concentration of 4 nM) was added into microplate wells followed by the addition of lysate containing putative interacting proteins and 5 μL of the acceptor beads (5x). The samples were incubated for 30 min at room temperature. Subsequently, 5 μL of donor beads (5x) were added to samples under low light conditions and incubated for 30 minutes at room temperature. For all experiments, samples were prepared in triplicate and the assay was repeated two times. The AlphaLISA signal was detected with Tecan Spark multimode microplate reader using the following settings: Mode: AlphaLISA, Excitation time: 130 ms, Integration time: 300 ms.

Genomes and sweeps summary statistics

To detect signatures of adaptation in various human populations, we used the 1000 Genomes Project phase 3 dataset[18] which provides chromosome level phased data for 26 distinct human populations representing all major continental groups. To measure nSL separately in each of the 26 populations, we use the selscan software available at https://github.com/szpiech/selscan.[54] To measure iHS, we use the hapbin software available at https://github.com/evotools/hapbin.[55]

Ranking of sweep signals at protein-coding genes and varying window sizes

To detect sweep enrichments at CoV-VIPs, we first order, separately in each of the 26 1000 Genomes populations, human Ensembl[61] (version 83) protein-coding genes according to the intensity of the sweep signals at each gene. As a proxy for the intensity of these signals, we use the average of either iHS or nSL across all the SNPs with iHS or nSL values within a window of fixed size, centered at the genomic center of genes, halfway between the most upstream transcription start site and the most downstream transcription end site. We then rank the genes according to the average iHS or nSL (more precisely their absolute values) in these windows. We get six rankings for six different fixed window sizes: 50kb, 100kb, 200kb, 500kb, 1,000kb and 2,000kb. We do this to account for the variable size of sweeps of different strengths. We then estimate the sweep enrichment at CoV-VIPs compared to controls over all these different window sizes considered together, or at specific sizes, as described below and in Enard & Petrov.[17] Note that we use up to 2,000kb windows to measure the average nSL or iHS, while we use control genes that are at least 500kb away from CoV-VIPs. This means that a fraction of control windows can overlap CoV-VIP windows. This makes our comparison conservative by reducing the visible excess of sweep signals at CoV-VIPs compared to control genes, since a proportion of the controls now also reflect the enrichment at CoV-VIPs, albeit not to the same extent as windows actually centered on CoV-VIPs.

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Estimating the whole ranking curve enrichment at CoV-VIPs and its statistical significance

To estimate a sweep enrichment in a set of genes, a typical approach is to use the outlier approach to select, for example, the top 1% of genes with the most extreme signals. Here we use a previously described approach to estimate a sweep enrichment while relaxing the requirement to identify a single top set of genes. Instead of, for example, only estimating an enrichment in the top 100 genes with the strongest sweep signals, we estimate the enrichment over a wide range of top X genes, where X is allowed to vary from the top 10,000 to the top 10 with many intermediate values (10000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2500, 2000,1500, 1000, 900, 800, 700, 600, 500, 450, 400, 350, 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10). This creates an enrichment curve as in Figure 1. Figure 1 shows the estimated relative fold enrichments at CoV-VIPs compared to controls, from the top 1,000 to the top 10 nSL. The statistical significance of the whole enrichment curve can then be estimated by using block-randomized genomes, as described in Enard & Petrov.[17] All the methodological details on how we use block-randomized genomes to estimate the sensitivity and False Positive Risk of the pipeline are described in reference,[17] and the readers can refer to that reference for further details. In brief, block-randomized genomes make it possible to generate a large number of random whole enrichment curves while maintaining the same level of clustering of genes in the same candidate sweeps as in the real genome, which effectively controls for gene clustering. Comparing the real whole enrichment curve to the random ones then makes it possible to estimate an unbiased false-positive risk[28] (also known as False Discovery Rate in the context of multiple testing) for the observed whole enrichment curve at CoV-VIPs. A single false positive risk can be estimated for not just one curve but by summing over multiple curves combined, thus making it possible to estimate a single false positive risk over any arbitrary numbers of rank thresholds, window sizes, summary statistics, and populations. For instance, we estimate the false-positive enrichment risk of $p = 2.10^{-4}$ at CoV-VIPs for rank threshold from the top 10,000 to top 10, over six window sizes, for the five East Asian populations in the 1000 Genomes data, and for both nSL and iHS, all considered together at once. This makes our approach more versatile and sensitive to selection signals ranging from a few very strong sweeps, to many, more moderately polygenic hitchhiking signals. The entire pipeline to estimate false-positive risks with block-randomized genomes is available at https://github.com/DavidPierreEnard/Gene_Set_Enrichment_Pipeline. Note that the false positive risk estimates fully take into account the extra variance expected from shrinking the pool of potential control genes by requiring control genes that match CoV-ViPs for multiple confounding factors.[17]

Building sets of controls matching for confounding factors

To estimate a sweep enrichment at CoV-VIPs, we compare CoV-VIPs with random control sets of genes selected far enough (> 500kb) from CoV-VIPs that they are unlikely to overlap the same large sweeps. We do not compare CoV-VIPs with completely random sets of control genes. Instead, we use a previously described bootstrap test to build random control sets of genes that match CoV-VIPs for a number of potential confounding factors that might explain a sweep enrichment, rather than interactions with viruses. The bootstrap test has been described in detail,[17] and is available at https://github.com/DavidPierreEnard/Gene_Set_Enrichment_Pipeline.

We include 11 different potential confounding factors in the bootstrap test:

- average GTEx expression in 53 GTEx V8 tissues.
- GTEx expression in lymphocytes.
- GTEx expression in testis.
- the number of protein-protein interactions from the Intact database, curated by Luisi et al.[51]
- the Ensembl (v83) coding sequence density in a 50kb window centered on each gene.
- the density of conserved segments identified by PhastCons.[52] (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/phastCons46way/).
- the density of regulatory elements, estimated by the density of Encode DNase I V3 Clusters (http://hgdownload.soe.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeRegDnaseClustered/) in a 50kb window centered on each gene.
- the recombination rate in a 200kb window centered on each gene.[53]
- the GC content in a 50kb window centered on each gene.
- the number of bacteria each gene interacts with, according to the Intact database (as of June 2019; https://www.ebi.ac.uk/intact/).
- the proportion of genes that are immune genes according to Gene Ontology annotations GO:0006952 (defense response), GO:0006955 (immune response), and GO:0002376 (immune system process) as of May 2020.

Two other factors commonly controlled for in selection analyses are gene length and SNP density. In our controls, gene length is accounted for by the functional density controls such as the density of coding, conserved, and regulatory elements. Gene length could be an issue if longer genes mean higher densities of functional elements more likely to adapt. But we match functional densities, and thus gene length is not an issue. SNP density could be problematic, because the values of haplotype-based summary statistics such as iHS or nSL can be sensitive to the local SNP density. To test the potential impact of SNP density, we add the number of SNPs in East Asia in 50kb windows centered on genes, and the number of SNPs in larger, 500kb windows centered on genes, to the 11 confounding factors already included in the matching process. We find that adding SNP density to the other confounding factors affects the observed sweep enrichment at CoV-VIPs in East Asia very weakly (top 1,000 rank thresholds, 1Mb and 2Mb windows, nSL+iHS: $FPR = 6.10^{-5}$; compare Figures S2O–S2S to Figure 1).

We further show that the strong sweep excess at CoV-VIPs is also visible when not controlling for confounding factors at all (Figures S2J–S2N; iHS+nSL FPR < 10^{-5}). This confirms that the control genes selected by the bootstrap test when matching confounding factors are not unusual with respect to their sweep prevalence, as also shown by the FPR analysis.

Host intrinsic functions do not explain the pattern and timing of adaptation at CoV-VIPs

An important limitation to consider when inferring ancient epidemics is that VIPs do not just interact with viruses, but are also involved in multiple hosts intrinsic functions. These host functions could in theory explain the enrichment and timing of adaptation at CoV-VIPs, rather than interactions with a coronavirus or related virus. This would happen as a result of specific host functions being enriched at CoV-VIPs, and also intrinsically enriched in adaptive signals independently of any interaction with viruses. Host functions not enriched at CoV-VIPs are not expected to generate an enrichment in adaptation at CoV-VIPs, because the lack of enrichment means that they are present in similar or smaller proportions in the rest of the genome.

Thus, if host functions enriched at CoV-VIPs, rather than viral interactions, explain adaptation at CoV-VIPs, we expect that i) genes with these host functions should be enriched in sweep signals even when they don't interact with coronaviruses and ii) genes with these host functions should have started adapting around 900 generations ago, to also explain the timing of adaptation at CoV-VIPs, even when they do not interact with coronaviruses.

To estimate the role of host intrinsic functions, we use the functional annotations from the Gene Ontology (GO) for GO biological processes, GO molecular functions, and GO cellular localizations. In total, there are 106 GO annotations that are enriched at CoV-VIPs compared to the matched controls already used to assess the sweep enrichment ($p < 0.001$ based on 10,000 matched control sets). Of these 106 GO annotations, only 20 have a more than two-fold enrichment among CoV-VIPs (and 50 genes or more among non-CoV-VIPs; Data S1E) and are thus more likely to contribute to the strong sweep enrichment at CoV-VIPs. We first test if these 20 GO annotations are enriched in sweeps independently of any interaction with coronaviruses. To do this, we use the same bootstrap test used to compare CoV-VIPs with matched controls, but this time we compare genes with the GO annotations, with control genes far enough (> 500kb) from any other gene with these annotations. To make sure that a significant enrichment would have nothing to do with coronaviruses, we exclude from this comparison any gene closer than 500kb to any CoV-VIP. In total, there are 1723 genes with at least one of the 20 highly enriched GO annotations, and 3701 far enough potential control genes. Using exactly the same iHS and nSL enrichment curves used to detect a sweep enrichment at CoV-VIPs (STAR Methods), we do not find any significant enrichment at the 1723 genes compared to matched controls (whole enrichment curves for nSL and iHS combined, $p = 0.15$). Furthermore, we do not find any significant enrichment in strong sweeps signals within the nSL or iHS top 1000 ($p = 0.77$), as we do at CoV-VIPs (Figure 1). When considered individually rather than all together, only four of the 20 functions have a significant sweep enrichment ($p < 0.05$; Data S1E).

To test whether these four functions explain the sweep enrichment at CoV-VIPs, we test this sweep enrichment at CoV-VIPs again, but this time excluding all genes included in the four previous GO annotations. The sweep enrichment at the remaining CoV-VIPs (91% of them) is the same as when testing all CoV-VIPs (the sum of differences between observed and expected numbers over all the nSL sweep rank thresholds, over all window sizes, and over all five East Asian populations is 14,620 for 352 CoV-VIPs included in the test, versus 15,848 for 385 CoV-VIPs included when not excluding GO functions, in other words almost perfectly proportional to the number of genes included in the test), thus showing that these four host functions do not explain the sweep enrichment at CoV-VIPs. Moreover, further excluding all genes with the 20 GO annotations over-represented more than twofold at CoV-VIPs, we find that the remaining CoV-VIPs (58% of them) have a stronger sweep enrichment than when considering all CoV-VIPs (the sum of differences between observed and expected numbers is 10,843 for 222 genes included in this test, proportionally more than the 15,848 sum of differences for 385 CoV-VIPs when not excluding GO functions). Excluding the genes with any of 106 over-represented GO annotations at CoV-VIPs, we also find that the remaining CoV-VIPs (16% of them) have a stronger sweep enrichment than when considering all CoV-VIPs (sum of differences 4,575 for 62 CoV-VIPs). Host intrinsic functions, as annotated by GO, thus cannot explain the sweep enrichment at CoV-VIPs.

Nevertheless, we further test which GO annotations enriched at CoV-VIPs have a significant peak of Relate times around 900 generations ago, as we did before for CoV-VIPs. To do this, we consider all GO annotations enriched at COV-VIPs, but this time compared to completely random controls, rather than compared to control sets matched for confounding factors as before. Indeed, we previously tested the significance of the peak around 900 generations ago at CoV-VIPs compared to completely random controls (STAR Methods), and here we do the same for a fair comparison. Compared to fully random controls, CoV-VIPs are significantly enriched in 316 GO annotations ($p < 0.001$). Of these 316 GO annotations, 238 are enriched more than two-fold, many more than the 20 GO annotations enriched more than two-fold when using controls matched for confounding factors. This shows that controlling for the confounding factors that we take into account (STAR Methods) effectively controls for many other correlated host intrinsic functions. A total of 39 GO annotations are enriched more than four fold at CoV-VIPs when compared to fully random controls. When considered all together, all the 1,134 genes in the genome other than CoV-VIPs, but with at least one of these 39 highly enriched GO annotations, do not have a significant peak of Relate times (peak significance test $p = 0.18$). When considered individually, only 16 of all the initial 316 over-represented GO annotations have a significant peak between 770 and 970 generations ago (Data S1F; peak significance test $p < 0.05$). When removing all CoV-VIPs with at least one of these 16 GO annotations (31% of them), the magnitude of the peak around 900 generations ago at the remaining CoV-VIPs compared to all CoV-VIPs is not affected (Figures S1A and S1B).

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Taken together, these results make it very unlikely that host intrinsic functions explain the patterns and timing of adaptation observed at CoV-VIPs, and make a causal role of coronavirus-like viruses more plausible. Below, we provide further, virus-focused functional evidence, further supporting this.

Estimating adaptation start times at specific genes with Relate

As times of emergence of adaptive mutations, we use the publicly available estimates from Relate (https://myersgroup.github.io/relate/). Relate estimates mutation emergence times while controlling for fluctuations of population size over time, based on the coalescence rates it reconstructs after inferring ancestral recombination graphs at the scale of the whole genome.[20] Relate provides two times of emergence of mutations, one low estimate (less generations ago), and one high estimate (more generations ago). The low time estimate corresponds to the time when Relate estimates an elevated probability that the frequency of the mutation is different from zero (95% confidence interval, most recent time estimate). The high time estimate corresponds to the time when Relate estimates that the probability is not too small that the frequency of the mutation is different from zero. For our purpose of estimating when selection started, the low time estimate is the best suited, because it provides an estimate of when the frequency of a selected mutation was already high enough to distinguish from zero, for those mutations where selection started from a very low frequency. For cases where selection started with standing genetic variants that were already distinguishable from zero, the Relate low time estimates for the emergence of mutations do not provide a good proxy for when selection actually started. Thus, if we were able to estimate when selection started for standing genetic variants, we might be able to observe an even stronger peak than the one we see when just relying on those variants where selection started from low frequencies.

Using the low Relate time estimates is also justified due to the fact that the sweep establishment phase can take very variable amounts of time before the start of the sweep exponential phase. During the establishment phase, selected alleles are still mostly governed by drift which makes pinpointing the actual starting time of selection difficult. In this context, the low Relate time estimates provide an estimate of the time when the selected alleles were no longer at very low frequencies not statistically different from zero, and closer to entering the exponential phase, which provides a more certain time estimate for when selection started for certain.

An important step is then to choose at each CoV-VIP locus, and all the other control loci, which Relate mutation to use to get a single time estimate for each locus. Note that here we make an assumption that each locus has experienced only one single adaptive event. Given our finding that iSAFE peaks at CoV-VIPs are much closer to GTEx V8 eQTLs than expected by chance, it is likely that the selected adaptive mutations are regulatory mutations at, or close to annotated eQTLs for a specific gene. They are not necessarily exactly located at eQTLs, because current eQTLs annotations may still be incomplete, and in our case we use eQTLs identified in GTEx V8 using mostly European individuals, even though we analyze selection signals in East Asian populations. Because of these limitations, we use the Relate estimated time at the mutation where Relate estimates the lowest positive selection p value within 50kb windows centered on eQTLs. We also only consider variants with a minor allele frequency greater than 20%, given the signals detected by iHS and nSL that only have some power to detect incomplete sweeps above 20% frequencies.[24,25] This also excludes a potential risk of confounding by low frequency neutral or weakly deleterious variants, that can show selection-like patterns when their only way to escape removal early on is through a chance, rapid frequency increase that can look like selection. The Relate selection test is based on faster than expected coalescence rates given the population size at any given time, and its results are publicly available at https://myersgroup.github.io/relate/. Note that the mutation with the lowest Relate p value does not always overlap with an iSAFE peak (Figure S5A), which is not entirely surprising if the haplotype signals exploited by both Relate and iSAFE partly deteriorated due to recombination since the time selection at CoV-VIPs was strong (Figures 3 and 5). Both of these methods are indeed designed to locate the selected variant right after, or during, active selection.

Because we work with five different East Asian populations, we more specifically select the variant with the lowest Relate selection test p value on average across all the five East Asian populations. Then, we also use the corresponding average low Relate mutation time estimate across the five East Asian populations. We do not attempt to estimate the selection time and p value by considering all 1000 Genomes East Asian individuals tested together by Relate, because then the Relate selection test is at a greater risk of being confounded by population structure. Finally, we only consider CoV-VIPs and other control genes with an average Relate selection test p value lower than 10^{-3}, to make sure that we indeed use estimated times at selected variants.

The peak significance test

To test if the peak of Relate time estimates around 900 generations ago at CoV-VIPs (Figure 2) is expected simply by chance or not, we designed a peak significance test. The test compares the peak at CoV-VIPs, with the top peaks obtained when repeatedly randomly sampling sets of genes. We first identify the most prominent peak at CoV-VIPs by visual inspection of the pink distribution of Relate times for CoV-VIPs compared to the blue distribution of Relate times for all protein-coding genes with an estimated Relate time (Figure 2). To build these distributions, top Relate selected mutations shared between multiple neighboring genes (CoV-VIPs or controls) are counted only once, to avoid a confounding effect of gene clustering (152 selected variants at CoV-VIPs, 1771 selected variants for all protein coding genes). The peak around 900 generations ago (870 generations more exactly) spans approximately 200 generations, where the pink distribution is clearly above the blue one. We then use a 200 generations-wide window, sliding every generation from 0 to 6,000 generations to verify the peak more rigorously. Sliding one generation after another, each time we count the difference between the number of Relate selected variants at CoV-VIPs that fall in the sliding 200 generations window, and the number of Relate selected variants at all other genes that are not CoV-VIPs, weighted by the percentage of variants found at CoV-VIPs, to correct for the different size of the two sets of variants. Using this sliding window approach, the top of the peak is found at 870

generations, with a difference of 19.5 additional Relate selected variants between 770 and 970 (870 plus or minus 100) at CoV-VIPs compared to the null expectation.

We then repeat the sliding of a 200 generations window to identify the maximum peak and measure the same difference, but this time for random sets of Relate selected variants of the same size (152 selected variants out of the 1,771 selected variants). To estimate p values, we then compare the actual observed difference with the distribution of differences generated with one million random samples.

As mentioned in the Results, one potential issue is that we run the peak significance test after we already know that CoV-VIPs are enriched for iHS and nSL top sweeps, and especially enriched for nSL top sweeps. This enrichment may skew the null expectation for the distribution of Relate times at CoV-VIPs. In other words, there is a risk that any set of genes with the same sweep enrichment might exhibit the same peak as CoV-VIP. As a result, comparing CoV-VIPs with randomly chosen non-CoV-VIPs may not be appropriate. To test this, we repeat the peak significance test, but this time comparing the peak at CoV-VIPs with the peaks at random sets of non-CoV-VIPs that we build to have the same distribution of nSL ranks as CoV-VIPs. To do this, we define nSL bins between ranks 1 and the highest rank with a rank step of 100 between each bin, and we count how many Relate selected variants fall in each bin (each gene has one nSL rank and one Relate selected variant). To build the random set, we then fill each of the 100 bins with the same number of random non-CoV-VIPs, as long as their nSL rank falls within that bin. We use the average nSL rank over the five East Asian populations, and the lower population-averaged rank of either 1 Mb or 2Mb window sizes (where we observe the strongest enrichment at CoV-VIPs, see Results). The results of the peak significance test are unchanged when using the matching nSL distribution (peak significance test $p = 1.10\text{-}4$ versus $p = 2.3.10\text{-}4$ without matching nSL distribution).

In further agreement with the fact that the sweep enrichment does not confound the peak significance test, the peak at CoV-VIPs stands out more when repeating the peak significance test using a smaller nSL top rank limit (Figure S1C). In this case, we compare sets of CoV-VIPs and sets of controls both enriched in stronger sweep signals. Thus, if stronger sweep signals at CoV-VIPs biased the peak significance test, we would expect the peak to fade away when comparing only CoV-VIPs and controls both with stronger nSL signals. Conversely, we observe that half of the CoV-VIPs with the weaker nSL signals (population-averaged nSL rank higher than 7,200 for both 1Mb and 2Mb windows) do not show a significant peak (peak significance test $p = 0.53$).

The iSAFE peaks/eQTL proximity test

Adaptation in the human genome was likely mostly regulatory adaptation through gene expression changes.[26,33–35] To test if positive selection at CoV-VIPs involved regulatory changes, we ask whether the signals of adaptation around CoV-VIPs are localized closer than expected by chance to GTEx eQTLs that affect the expression of CoV-VIPs in present human populations. We use proximity instead of exact colocalization because we do not expect selection signals in East Asia to colocalize perfectly with eQTLs identified mostly from European tissue samples. The genomic regions at or close to CoV-VIP GTEx eQTLs are likely enriched for CoV-VIP regulatory elements, and therefore the most likely place to find CoV-VIP-related adaptations in the genome. To localize where adaptation occurred, we use the iSAFE method that was specifically designed for this purpose.[31] iSAFE scans the genome and estimates a score that increases together with proximity to the actual selected mutation. The higher the score, the higher the odds that the scored variant is itself the selected one, or close to the selected one. An important caveat is that iSAFE is designed to localize where selection happened right after it happened, or as selection is still ongoing. In our case, we have evidence that selection was strong at CoV-VIPs only more than 500 generations (~14,000 years) ago, and then much weaker more recently (Figure 5). This could be an issue, because we expect that recombination events that occurred after the strong selection might have deteriorated the iSAFE signal that relies on haplotype structure. This is because recombination mixes together the haplotypes that hitchhiked with the selected mutation, with those that did not. In line with this, we often do not observe simple, clean iSAFE score peaks, but instead, iSAFE score plateaus and more rugged peaks (Figure S5A). For this reason, we designed an approach to not only identify the top of simple iSAFE peaks, but also more rugged peaks or plateaus. First, to measure iSAFE scores, we combine all the haplotypes from the five East Asian populations together as input, since we found that the selection signal at CoV-VIPs is common to all these populations (iSAFE parameters:–IgnoreGaps–MaxRegionSize 250000000–window 300–step 100–MaxFreq 0.95–MaxRank 15). We then use a 500kb window sliding every 10kb to identify the highest local iSAFE value in the 500kb window (Figure S5B). Once we have the highest local iSAFE value and coordinate, we define a broader iSAFE peak as the region both upstream and downstream where the iSAFE values are still within 80% of the maximum value (Figure S5B). This way, we can better annotate iSAFE plateaus and rugged peaks, and take into account the fact that they can span more than just a narrow local maximum (Figure S5A).

Once the local iSAFE peaks are identified, we can ask how close GTEx eQTLs are to these peaks compared to random expectations. We first measure the distance of each CoV-VIP GTEx eQTL to the closest iSAFE peak. To avoid redundancy, we merge eQTLs closer than 1kb to each other into one test eQTL at the closest, lower multiple of 1,000 genomic coordinates (for example 3,230 and 3,950 would both become 3,000). We then measure the average of the log of the distance between all CoV-VIPs and their closest iSAFE peak. We use the log (base 10) of the distance, because it matters if the eQTL/iSAFE peak distance is 100 bases instead of 200kb, but it does not really matter if the distance is 200kb or 600kb, because the iSAFE peak at 200kb is likely not more related to the eQTL than the peak at 600kb. Once we have the average of log-distances, we compare it to its random expected distribution. To get this random distribution, we measure the log-distance between each CoV-VIP eQTL and the iSAFE peaks, but after shifting the iSAFE scores left or right by a random value between 1Mb and 2.5Mb (Figure S5B; less, or no shift at all if this falls within telomeres or centromeres). We shift by at least 1Mb to make sure that we do not rebuild the original overlap of iSAFE peaks with eQTLs again and again (some iSAFE peaks, or more precisely rugged peaks and plateaus can be wide and include several hundred kilobases; see

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Figure S5A). The random shifting effectively breaks the relationship between eQTLs and iSAFE peaks, while maintaining the same overall eQTL and peak structure (and thus variance for the test). The random log-distance distribution then provides an overall random average log-distance to compare the observed average long-distance with, as well as estimate a p value.

Then, to more specifically ask if lung eQTLs at CoV-VIPs or the eQTLs of other specific tissues are closer to iSAFE peaks than expected by chance, we can do the same but only using the eQTLs of that specific tissue. The analysis represented in Figure 7 is however more complicated than just testing if CoV-VIP eQTLs for a specific tissue are closer to iSAFE peaks than expected by chance by randomly sliding iSAFE values. Instead, what we ask is whether the 42 peak-VIPs have eQTLs for a given tissue that are even closer to iSAFE peaks than the eQTLs of all CoV-VIPs in general. To test this, for example with lung eQTLs, we first estimate how close lung eQTLs are to iSAFE peaks at peak-VIPs, compared to random expectations, by measuring the difference between the observed and the average random log-distance, just as described before. We then count the number of peak-VIPs with lung eQTLs (19 out of 25 peak-VIPs with GTEx eQTLs), and we randomly select the same number of any CoV-VIP (which may randomly include peak-VIPs) as long as the random set of CoV-VIPs has the same number of lung eQTLs (plus or minus 10%) as the set of peak VIPs with lung eQTLs (the same gene can have multiple eQTLs for one tissue). We make sure that the tested and the random sets have similar numbers of genes and eQTLs so that the test has the appropriate null variance. We then measure the difference between the observed log-distance, and the randomly expected average log-distance for the random set of CoV-VIPs, exactly the same way we did before for the actual set of peak-VIPs. We then measure the ratio of the observed difference in log-distance between peak-VIPs and the random expectation after many random shiftings (1,000), divided by the average of the same difference measured over many random sets of CoV-VIPs. The final ratio tells us how much closer lung eQTLs are to iSAFE peaks at peak-VIPs compared to CoV-VIPs in general, and still takes the specific eQTLs and iSAFE peak structures at each locus into account, since we compare differences in log-distances expected while preserving the same eQTL and iSAFE peak structure (see above the description of the random coordinate shifting). One important last detail about the test is that because we already found that the 50% of loci with the lowest nSL signals do not show a peak of selection at CoV-VIPs around 900 generations ago (see Results), we do not use these loci in this test since any iSAFE peak there is much more likely to represent random noise, not actual selection locations, and thus likely to dilute genuine signals. Using this test, we find that lung and other tissues' eQTLs at peak-VIPs are much closer to iSAFE peaks than they are at CoV-VIPs in general. This test thus specifically tells that adaptation happened closer to lung eQTLs, specifically around 900 generations ago compared to other evolutionary times. By estimating the same ratio for 24 other tissues with at least 10 peak-VIPs with the specific tested tissue eQTLs, we can finally rank each tissue for its more pronounced involvement in adaptation ~900 generations ago, as done in Figure 7. It is particularly interesting in this respect that the tissue with least evidence for being more involved in adaptation at that time more than other evolutionary times is spleen. Spleen indeed likely represents a good negative control as a tissue strongly enriched in immune cell types and likely to have evolved adaptively for most of evolution.

A possible limitation is that eQTLs tend to be shared between many tissues, and only a minority of eQTLs are tissue-specific. This means that in our analysis, specific tissues may stand out only because they share their eQTLs with other tissues that were the primary targets of selection. In order to better identify which specific tissues may have been the strongest targets of selection, we consider again the 42 CoV-VIPs selected 900 generations ago, but this time we ask how much closer than expected by chance their eQTLs are to the location of selection (estimated by iSAFE), as a function of increasing eQTL tissue specificity. We define the tissue specificity of a given eQTL for tissue A as the total number of tissues where GTEx found the eQTL (tissue A + other tissues). We find that for most tissues, eQTLs that are increasingly more specific to these tissues, also tend to be found more and more randomly located compared to the location of selection (Figure S7). Out of 25 tissues, lung is the only one with a clear pattern of more lung-specific eQTLs being closer to the location of selection compared to random expectations (Figure S7, red curve).

UK Biobank GWAS analysis

To compare the UK Biobank GWAS p values at different loci, we assigned one p value for each gene, either CoV-VIPs, peak-VIPs or other genes, even though each gene locus can have many variants with associated GWAS p values. To assign just one single GWAS p value to each gene, we selected the variant with the lowest p value at or very close (< 1kb) to GTEx eQTLs for a specific gene, in line with the fact that GWAS hits tend to overlap eQTLs ,[62] and to remain consistent with the rest of our manuscript. We then compared the average p value between different sets of genes using classic permutations (one billion iterations).

We note that the top-ranking loci identified by the COVID-19 Host Genetics Initiative (*IFNAR2*, *OAS*, *RAVER1*, *DPP9*, *LZTFL1*, etc.) are broadly acting immune factors. These factors do not interact with viral proteins, and are instead involved in immune signaling cascades that are not specific to a given virus. We therefore do not expect an overlap with the more coronavirus-specific CoV-VIPs that we use here. We also note that we do not necessarily expect the strongest GWAS hits in Europe to be strong hits in other populations. This is particularly true when the investigated trait is the response to a pathogen, given that we show in this manuscript that the evolution of this response was probably population-specific. In addition, although adaptation implies a functional genetic effect, a genetic effect does not necessarily mean it has adaptive potential. Finally, the list of the very top GWAS hits might be sensitive to population stratification, and still change depending on how much population stratification is controlled for. The average strength of the GWAS hits over many Cov-VIPs that we focus on is likely to be less sensitive to these issues. The lack of overlap with the strongest COVID-19 Host Genetics Initiative hits is therefore not very surprising. It also does not take away the fact that we found an enrichment in stronger GWAS hits on average at CoV-VIPs and especially at selected CoV-VIPs.

Drug targets identification

We queried the databases DGIdb,[63] and PanDrugs[64] for drugs targeting CoV-VIPs and peak-VIPs. For hits from PanDrugs we limited the results to only genes that are in direct interaction with the designated drug. Drugs targeting peak-VIPs are presented in Data S1M. In addition, we present a list of peak-VIPs that are not currently drug targets, but have been previously identified in Finan et al.[48] as viable drug targets (druggable genome).

QUANTIFICATION AND STATISTICAL ANALYSIS

The Method details provide in-depth descriptions of the quantifications and statistical analyses used in this manuscript.

From: Fauci, Anthony (NIH/NIAID) [E]
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Cc: Awwad, David (NIH/NIAID) [C];Arms, Erin (NIH/NIAID) [E]
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RESEARCH ARTICLE

Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files. The complete genome sequences of the 11 bat SARS-related coronaviruses newly identified in this study have been deposited in the GenBank database and assigned accession numbers KY417142 to KY417152, respectively.

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Abstract

A large number of SARS-related coronaviruses (SARSr-CoV) have been detected in horseshoe bats since 2005 in different areas of China. However, these bat SARSr-CoVs show sequence differences from SARS coronavirus (SARS-CoV) in different genes (S, ORF8, ORF3, *etc*) and are considered unlikely to represent the direct progenitor of SARS-CoV. Herein, we report the findings of our 5-year surveillance of SARSr-CoVs in a cave inhabited by multiple species of horseshoe bats in Yunnan Province, China. The full-length genomes of 11 newly discovered SARSr-CoV strains, together with our previous findings, reveals that the SARSr-CoVs circulating in this single location are highly diverse in the S gene, ORF3 and ORF8. Importantly, strains with high genetic similarity to SARS-CoV in the hypervariable N-terminal domain (NTD) and receptor-binding domain (RBD) of the S1 gene, the ORF3 and ORF8 region, respectively, were all discovered in this cave. In addition, we report the first discovery of bat SARSr-CoVs highly similar to human SARS-CoV in ORF3b and in the split ORF8a and 8b. Moreover, SARSr-CoV strains from this cave were more closely related to SARS-CoV in the non-structural protein genes ORF1a and 1b compared with those detected elsewhere. Recombination analysis shows evidence of frequent recombination events within the S gene and around the ORF8 between these SARSr-CoVs. We hypothesize that the direct progenitor of SARS-CoV may have originated after sequential recombination events between the precursors of these SARSr-CoVs. Cell entry studies demonstrated that three newly identified SARSr-CoVs with different S protein sequences are all able to use human ACE2 as the receptor, further exhibiting the close relationship between strains in this cave and SARS-CoV. This work provides new insights into the origin and evolution of SARS-CoV and highlights the necessity of preparedness for future emergence of SARS-like diseases.

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Author summary

Increasing evidence has been gathered to support the bat origin of SARS coronavirus (SARS-CoV) in the past decade. However, none of the currently known bat SARSr-CoVs is thought to be the direct ancestor of SARS-CoV. Herein, we report the identification of a diverse group of bat SARSr-CoVs in a single cave in Yunnan, China. Importantly, all of the building blocks of SARS-CoV genome, including the highly variable S gene, ORF8 and ORF3, could be found in the genomes of different SARSr-CoV strains from this single location. Based on the analysis of full-length genome sequences of the newly identified bat SARSr-CoVs, we speculate that the direct ancestor of SARS-CoV may have arisen from sequential recombination events between the precursors of these bat SARSr-CoVs prior to spillover to an intermediate host. In addition, we found bat SARSr-CoV strains with different S proteins that can all use the receptor of SARS-CoV in humans (ACE2) for cell entry, suggesting diverse SARSr-CoVs capable of direct transmission to humans are circulating in bats in this cave. Our current study therefore offers a clearer picture on the evolutionary origin of SARS-CoV and highlights the risk of future emergence of SARS-like diseases.

Introduction

Severe Acute Respiratory Syndrome (SARS) is a severe emerging viral disease with high fatality characterized by fever, headache and severe respiratory symptoms including cough, dyspnea and pneumonia [1]. Due to its high transmissibility among humans, after its first emergence in southern China in late 2002, it rapidly led to a global pandemic in 2003 and was marked as one of the most significant public health threats in the 21st century [2,3]. The causative agent, SARS coronavirus (SARS-CoV), has been previously assigned to group 2b CoV and is now a member of the lineage B of genus *Betacoronavirus* in the family *Coronaviridae* [4]. It shares similar genome organization with other coronaviruses, but exhibits a unique genomic structure which includes a number of specific accessory genes, including ORF3a, 3b, ORF6, ORF7a, 7b, ORF8a, 8b and 9b [5,6].

Masked palm civets (*Paguma larvata*) were initially hypothesized to be the animal origin of SARS-CoV [7,8]. However, since a large number of genetically diverse SARS-related coronaviruses (SARSr-CoV) have been detected in multiple species of horseshoe bats (genus *Rhinolophus*) from different areas of China and Europe in the aftermath of SARS, it is prevailingly considered that SARS-CoV originated in horseshoe bats with civets acting as the intermediate amplifying and transmitting host [9–16]. Recently we have reported four novel SARSr-CoVs from Chinese horseshoe bats that shared much higher genomic sequence similarity to the epidemic strains, particularly in their S gene, of which two strains (termed WIV1 and WIV16) have been successfully cultured *in vitro* [17,18]. These newly identified SARSr-CoVs have been demonstrated to use the same cellular receptor (angiotensin converting enzyme-2 [ACE-2]) as SARS-CoV does and replicate efficiently in primary human airway cells [17–19].

Despite the cumulative evidence for the emergence of SARS-CoV from bats, all bat SARSr-CoVs described so far are clearly distinct from SARS-CoV in the S gene and/or one or more accessory genes such as ORF3 and ORF8, suggesting they are likely not the direct ancestor of SARS-CoV. Thus a critical gap remains in our understanding of how and where SARS-CoV originated from bat reservoirs. Previously, we reported a number of bat SARSr-CoVs with diverse S protein sequences from a single cave in Yunnan Province, including the four strains

mentioned above most closely related to SARS-CoV [17,18]. Here we report the latest results of our 5-year longitudinal surveillance of bat SARSr-CoVs in this single location and systematic evolutionary analysis using full-length genome sequences of 15 SARSr-CoV strains (11 novel ones and 4 from previous studies). Efficiency of human ACE2 usage and the functions of accessory genes ORF8 and 8a were also evaluated for some of the newly identified strains.

Results

Continued circulation of diverse SARSr-CoVs in bats from a single location

We have carried out a five-year longitudinal surveillance (April 2011 to October 2015) on SARSr-CoVs in bats from a single habitat in proximity to Kunming city, Yunnan province, China, which was mainly inhabited by horseshoe bats. A total of 602 alimentary specimens (anal swabs or feces) were collected and tested for the presence of CoVs by a Pan-CoV RT-PCR targeting the 440-nt RdRp fragment that is conserved among all known α- and β-CoVs [20]. In total, 84 samples tested positive for CoVs. Sequencing of the PCR amplicons revealed the presence of SARSr-CoVs in the majority (64/84) of the CoV-positive samples (Table 1). Host species identification by amplification of either *Cytb* or *ND1* gene suggested that most (57/64) of the SARSr-CoV positive samples were from *Rhinolophus sinicus*, while the remaining 7 samples were from *Rhinolophus ferrumequinum*, *Rhinolophus affinis* and from *Aselliscus stoliczkanus* which belongs to the family *Hipposideridae*.

Based on the preliminary analysis of the partial RdRp sequences, all of the 64 bat SARSr-CoV sequences showed high similarity among themselves and with other reported bat SARSr-CoVs and SARS-CoVs from humans and civets. To understand the genetic diversity of these bat SARSr-CoVs, the most variable region of the SARSr-CoV S gene, corresponding to the receptor-binding domain (RBD) of SARS-CoV, were amplified and sequenced. Due to low viral load in some samples, RBD sequences were successfully amplified only from 49 samples. These RBD sequences displayed high genetic diversity and could be divided into two large clades, both of which included multiple genotypes. Clade 1 strains shared an identical size and higher amino acid (aa) sequence identity with SARS-CoV RBD, while clade 2 had a shorter size than SARS-CoV S due to two deletions (5 and 12–13 aa, respectively) (S1 Fig). Co-infections by two strains of different clades were detected in two samples, Rs3262 and Rs4087 (S1 Fig).

Table 1. Summary of SARSr-CoV detection in bats from a single habitat in Kunming, Yunnan.

Sampling time	Sample type	Sample Numbers			SARSr-CoV + bat species (No.)
		Total	CoV +	SARSr-CoV +	
April, 2011	anal swab	14	1	1	*R. sinicus* (1)
October, 2011	anal swab	8	3	3	*R. sinicus* (3)
May, 2012	anal swab & feces	54	9	4	*R. sinicus* (4)
September, 2012	feces	39	20	19	*R. sinicus* (16)
					R. ferrumequinum (3)
April, 2013	feces	52	21	16	*R. sinicus* (16)
July, 2013	anal swab & feces	115	9	8	*R. sinicus* (8)
May, 2014	feces	131	8	4	*A. stoliczkamus* (3)
					R. affinis (1)
October, 2014	anal swab	19	4	4	*R. sinicus* (4)
May, 2015	feces	145	3	0	
October, 2015	anal swab	25	6	5	*R. sinicus* (5)
Total		***602***	***84***	***64***	***R (61) A (3)***

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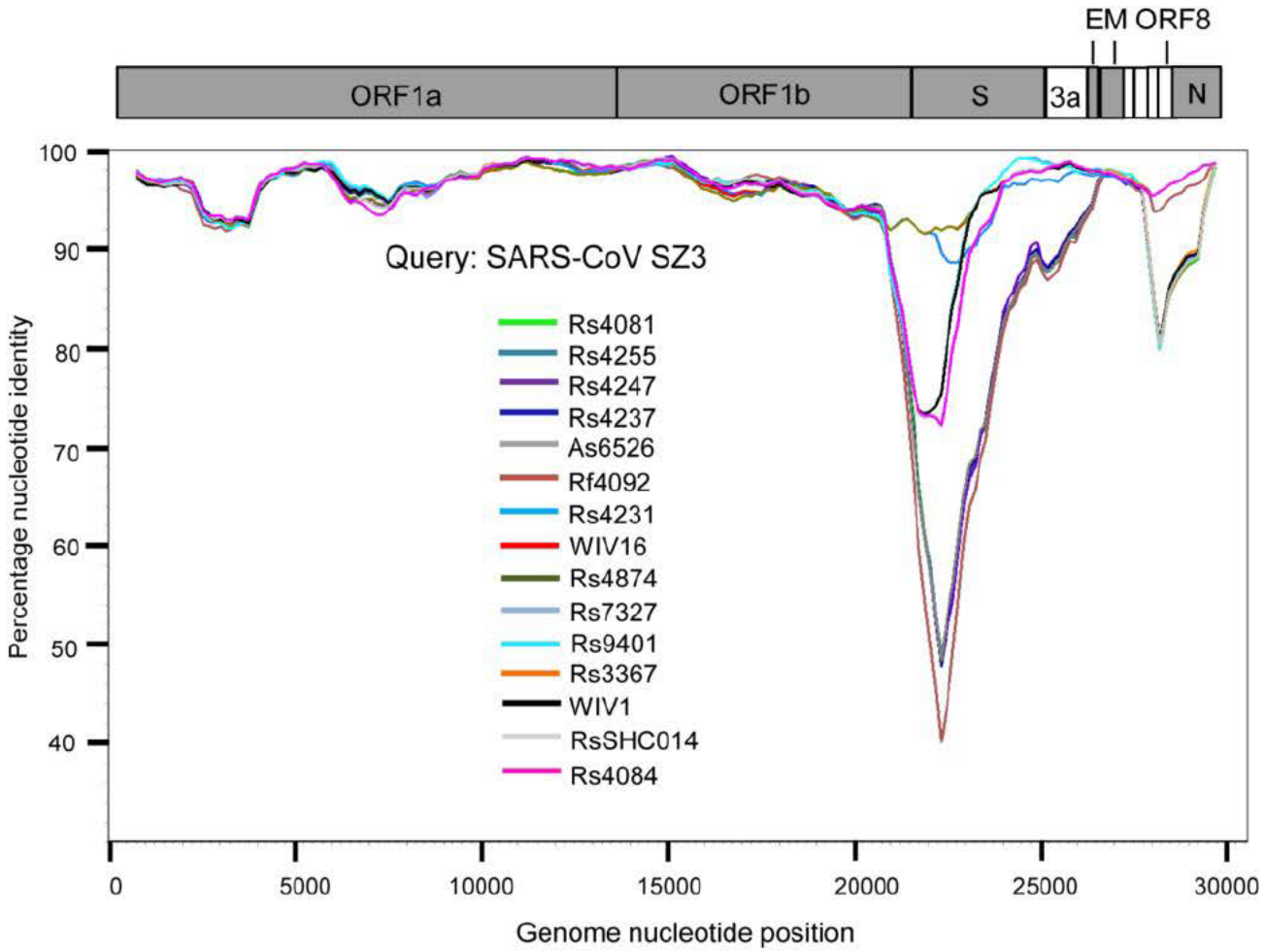

Fig 1. Similarity plot based on the full-length genome sequence of civet SARS CoV SZ3. Full-length genome sequences of all SARSr-CoV detected in bats from the cave investigated in this study were used as reference sequences. The analysis was performed with the Kimura model, a window size of 1500 base pairs and a step size of 150 base pairs.

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Genomic characterization of the novel SARSr-CoVs

Based on the diversity of RBD sequences, 11 novel SARSr-CoV strains named by abbreviation of bat species and sample ID (Rs4081, Rs4084, Rs4231, Rs4237, Rs4247, Rs4255, Rs4874, Rs7327, Rs9401, Rf4092 and As6526) were selected for full-length genomic sequencing based on sample abundance, genotype of RBD as well as sampling time. For each RBD genotype and each time of sampling, at least one representative strain was selected. The genome size of these novel SARSr-CoVs ranged from 29694 to 30291 nucleotides (nt). This gave a total of 15 full-length genomes of bat SARSr-CoVs from this single location (13 from *R.sinicus*, and one each from *R. ferrumequinum* and *A. stoliczkanus*), including our previously reported strains, Rs3367, RsSHC014, WIV1 and WIV16 [17,18]. The genomes of all 15 SARSr-CoVs circulating in this single cave shared 92.0% to 99.9% nt sequence identity. The overall nt sequence identity between these SARSr-CoVs and human and civet SARS-CoVs is 93.2% to 96%, significantly higher than that observed for bat SARSr-CoVs reported from other locations in China (88–93%) [9,10,12,14,21,22]. The genome sequence similarity among the 15 SARSr-CoVs and SARS-CoV SZ3 strain was examined by Simplot analysis (Fig 1). The 15 SARSr-CoVs are

highly conserved and share a uniformly high sequence similarity to SARS-CoV in the non-structural gene ORF1a (96.6% to 97.1% nt sequence identity, 98.0% to 98.3% aa sequence identity) and ORF1b (96.1% to 96.6% nt sequence identity, 99.0% to 99.4% aa sequence identity). In contrast, a considerable genetic diversity is shown in the S gene (corresponding to SZ3 genome position 21477 to 25244) and ORF8 (corresponding to SZ3 genome position 27764 to 28132) (Fig 1).

The 11 novel SARSr-CoVs identified from this single location generally shared similar genome organization with SARS-CoV and other bat SARSr-CoVs. In our previous study, we identified an additional ORF termed ORFx present between ORF6 and ORF7 in strain WIV1 and WIV16 [18,23]. In this study, ORFx was also found in the genomes of Rs7327 and Rs4874. Compared with that of WIV1 and WIV16, the length of ORFx in Rs7327 and Rs4874 was extended to 510 nt due to a deletion of 2 nt in a poly-T sequence that resulted in a shift of reading frame (Fig 2 and S2 Fig).

Co-circulation of different bat SARSr-CoVs with S, ORF8 and ORF3 sequences similar to those in SARS-CoV at a single location

The primary difference between SARS-CoV and most bat SARSr-CoVs is located in S gene. The S protein is functionally divided into two subunits, denoted S1 and S2, which is responsible for receptor binding and cellular membrane fusion, respectively. S1 consists of two domains, the N-terminal domain (NTD) and C-terminal domain (CTD) which is also known as the RBD in SARS-CoV [24]. SARS-CoV and bat SARSr-CoVs share high sequence identity in the S2 region in contrast to the S1 region. Among the 15 SARSr-CoVs identified from bats in the surveyed cave, six strains with deletions in their RBD regions (Rs4081, Rs4237, Rs4247, Rs4255, Rf4092 and As6526) showed 78.2% to 80.2% aa sequence identity to SARS-CoV in the S protein, while the other nine strains without deletions were much more closely related to SARS-CoV, with 90.0% (Rs4084) to 97.2% (Rs4874) aa sequence identity. These nine SARSr-CoVs can be further divided into four genotypes according to their S1 sequences (Fig 2): RsSHC014/Rs4084 showed more genetic differences from SARS-CoV in both NTD and RBD regions; The RBD sequences of SARSr-CoV Rs7327, Rs9401 and previously reported WIV1/Rs3367 closely resembled that of SARS-CoV. However, they were distinct from SARS-CoV but similar to RsSHC014 in NTD. In contrast, we found a novel SARSr-CoV, termed Rs4231, which shared highly similar NTD, but not RBD sequence with SARS-CoV (Figs 2 and 3). Its S protein showed 94.6% to 95% aa sequence identity to those of human and civet SARS-CoVs (S1 Table). Strains with both NTD and RBD highly homologous to those of SARS-CoV were also present in this cave. In addition to WIV16 which we described previously [18], Rs4874 was also found to have the S protein closest to SARS-CoV S (> 97% aa sequence identity) of all the bat SARSr-CoVs reported to date (Figs 2 and 3). In addition to the SARSr-CoVs subjected to full-length genome sequencing, we also obtained the RBD and NTD sequences from other samples collected in this cave. The sequences with high identity to SARS-CoV RBD were amplified from 10 more *R. sinicus* samples. SARSr-CoVs with this genotype of RBD were detected in different seasons throughout the five years. Strains containing the NTD similar to SARS-CoV were only found in 2013 (S2 Table).

ORF8 is another highly variable gene among different SARS-CoV and SARSr-CoV strains [25,26]. We aligned the ORF8 nt sequences of the representative SARSr-CoVs discovered in this surveillance with those of other SARSr-CoVs and SARS-CoVs (Fig 4). Though WIV16, WIV1, Rs4231 and RsSHC014 were genetically closer to SARS-CoV in S gene, they contained a single 366-nt ORF8 without the 29-nt deletion present in most human SARS-CoVs and showed only 47.1% to 51.0% nt sequence identity to human and civet SARS-CoVs. However,

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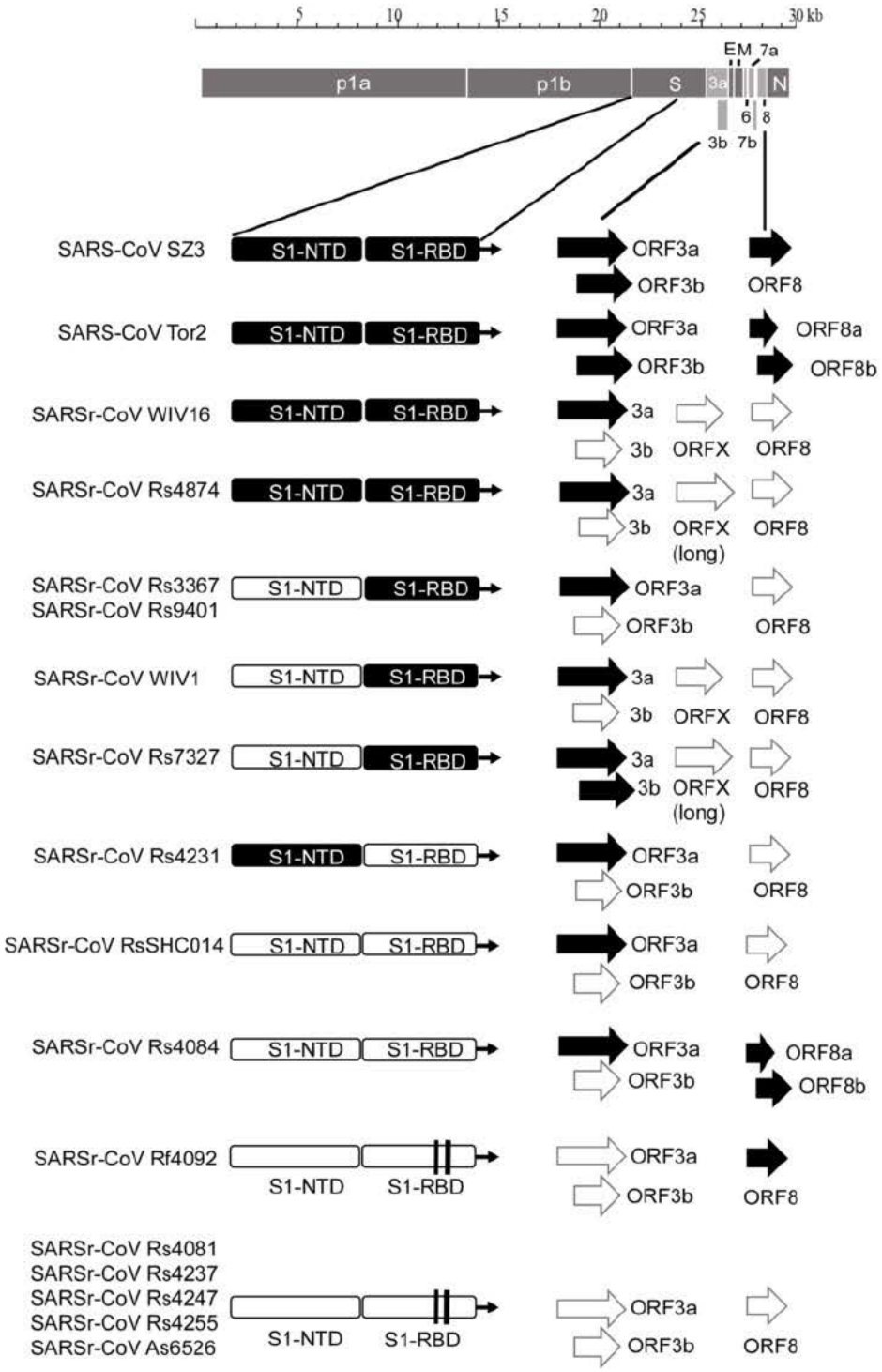

Fig 2. Schematic diagram illustrating the genomic regions or ORFs with most variation between different SARS-CoV and SARSr-CoV isolates. Coding regions of the N-terminal domain (NTD) and receptor-binding domain (RBD) of the spike protein, ORF3a/b and ORF8 (8a/b) in bat SARSr-CoV genomes highly similar to those in SARS CoV genome are indicated with black boxes or arrows while the hollow boxes or arrows represent corresponding regions with less sequence similarity to those of SARS-CoV. The deletions in the RBD of some SARSr-CoVs are indicated by two vertical lines.

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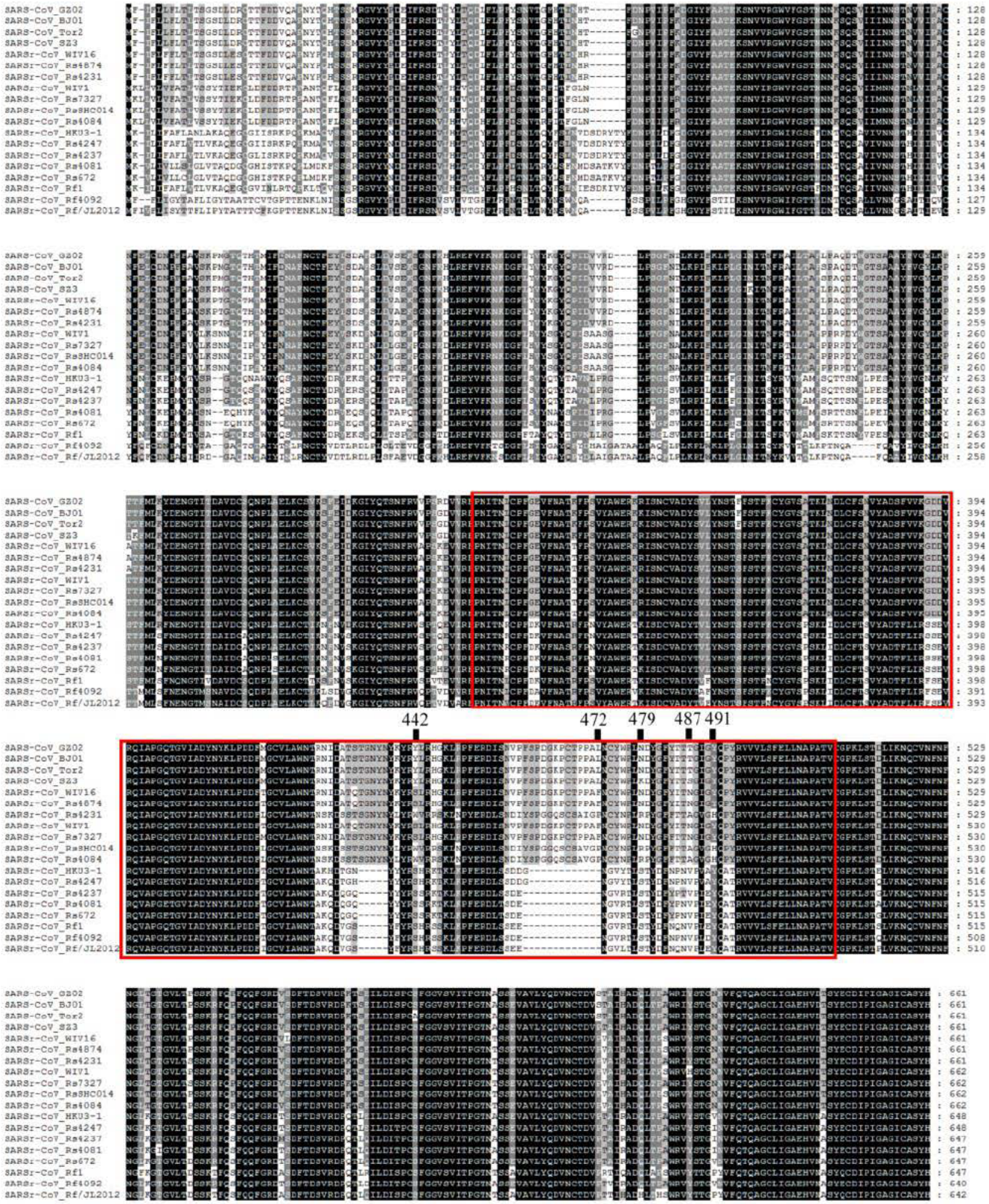

Fig 3. Amino acid sequence comparison of the S1 subunit (corresponding to aa 1–660 of the spike protein of SARS-CoV). The receptor-binding domain (aa 318–510) of SARS-CoV and the homologous region of bat SARSr-CoVs are indicated by the red box. The key aa

residues involved in the interaction with human ACE2 are numbered on top of the aligned sequences. SARS-CoV GZ02, BJ01 and Tor2 were isolated from patients in the early, middle and late phase, respectively, of the SARS outbreak in 2003. SARS-CoV SZ3 was identified from civets in 2003. SARSr-CoV Rs 672 and YN2013 were identified from *R. sinicus* collected in Guizhou and Yunnan Province, respectively. SARSr-CoV Rf1 and JL2012 were identified from *R. ferrumequinum* collected in Hubei and Jilin Province, respectively. WIV1, WIV16, RsSHC014, Rs4081, Rs4084, Rs4231, Rs4237, Rs4247, Rs7327 and Rs4874 were identified from *R.sinicus*, and Rf4092 from *R. ferrumequinum* in the cave surveyed in this study.

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the ORF8 of strain Rf4092 from *R. ferrumequinum* exhibited high similarity to that of civet SARS-CoV. It possessed a single long ORF8 of the same length (369 nt) as that of civet SARS--CoV strain SZ3, with only 10 nt mutations and 3 aa mutations detected (Fig 4). Similar ORF8 sequences were also amplified from other 7 samples collected in the cave during 2011 to 2013, from both *R. ferrumequinum* and *R. sinicus* (S2 Table). The ORF8 of Rs4084 was highly similar to Rf4092's but was split into two overlapping ORFs, ORF8a and ORF8b, due to a short 5-nt deletion (Figs 2 and 4). The position of start codons and stop codons of the two ORFs were consistent with those in most human SARS-CoV strains. Excluding the 8-aa insertion, Rs4084 and SARS-CoV strain BJ01 displayed identical aa sequence of ORF8a, and only three different

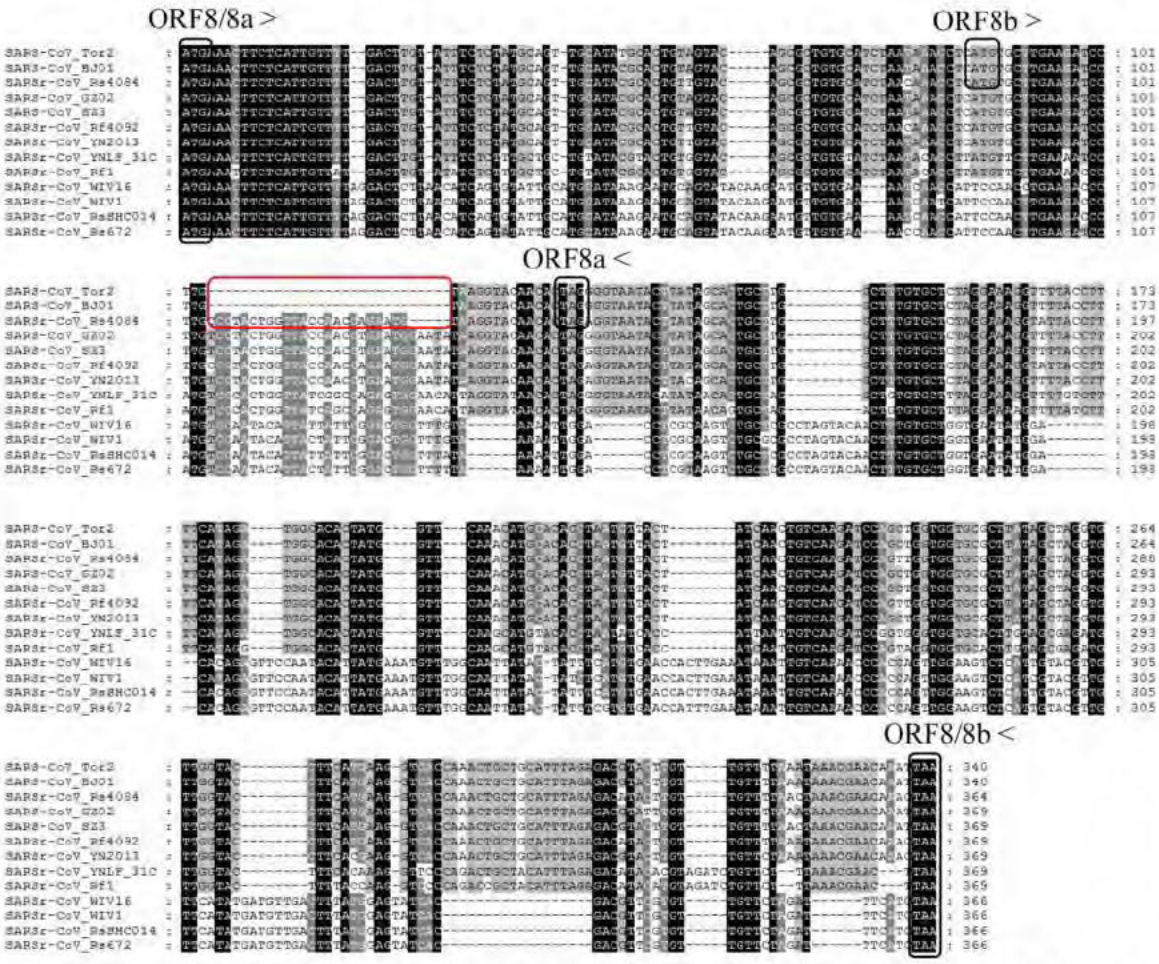

Fig 4. Alignment of nucleotide sequences of ORF8 or ORF8a/8b. The start codons and stop codons of ORF8, 8a and 8b are marked with black boxes and the forward and reverse arrows, respectively. The deletion responsible for the split ORF8a and 8b in human SARS-CoV BJ01, Tor2 and bat SARSr-CoV Rs4084 is marked with red boxes. See the legend for Fig 3 for the origin of various sequences used in this alignment.

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aa residues were observed between their ORF8b (Fig 4). To our knowledge, Rs4084 was the first bat SARSr-CoV reported that resembled the late human SARS-CoVs in both ORF8 gene organization and sequence.

Another key difference between SARS-CoV and bat SARSr-CoV genomes is the ORF3 coding region [10,17,21]. We analyzed the ORF3a sequences amplified from 42 samples and found that most of the SARSr-CoVs closely related to SARS-CoV in the S gene shared higher ORF3a sequence similarity (96.4% to 98.9% aa identity) with SARS-CoV (S3 Fig and S2 Table). The ORF3b of SARS CoV, sharing a large part of its coding sequence with the ORF3a, encodes a 154-aa protein [27], but it is truncated to different extents at the C-terminal in previously described bat SARSr-CoVs including WIV1 and WIV16 (S4 Fig). In the current study, we identified a non-truncated ORF3b for the first time (Rs7327), which maintained the nuclear localization signal at its C-terminal. Moreover, it shared 98.1% aa sequence identity with SARS-CoV strain Tor2 with only three aa substitutions (S4 Fig). Thus, Rs7327 is the bat SARSr-CoV most similar to SARS-CoV in the ORF3 region known to date.

Recombination analysis

The full-length genome sequences of all 15 SARSr-CoVs from the surveyed cave were screened for evidence of potential recombination events. Both similarity plot and bootscan analyses revealed frequent recombination events among these SARSr-CoV strains. It was suggested that WIV16, the closest progenitor of human SARS-CoV known to date [18], was likely to be a recombinant strain from three SARSr-CoVs harbored by bats in the same cave, namely WIV1, Rs4231 and Rs4081, with strong P value ($<10^{-30}$). Breakpoints were identified at genome positions nt 18391, 22615 and 28160 (Fig 5A). In the genomic region between nt 22615 and 28160, which contained the region encoding the RBD and the S2 subunit of the S protein, WIV16 was highly similar to WIV1, sharing 99% sequence identity. In contrast, in the region between nt 18391 and 22615, which covered a part of ORF1b and the region encoding the NTD of the S gene, WIV16 showed substantially closer relationship to Rs4231. Meanwhile, the ORF1ab sequences upstream from nt 18391 of WIV16 displayed the highest genetic similarity (99.8% nt sequence identity) to that of Rs4081.

Evidence of recombination event was also detected in the genome of the novel SARSr-CoV Rs4084, which had a unique genome organization with split ORF8a and 8b. The previously reported strain RsSHC014 and the newly identified strain Rf4092 were suggested to be the major and minor parent of Rs4084, respectively (P value $< 10^{-80}$). The breakpoint was located at nt 26796 (S5 Fig). In the region downstream of the breakpoint including ORF8, Rs4084 showed closet genetic relationship with Rf4092, sharing 98.9% nt sequence identity, while it shared the highest nt sequence identity (99.4%) with RsSHC014 in the majority of its genome upstream from the breakpoint.

When civet SARS-CoV SZ3 was used as the query sequence in similarity plot and bootscan analysis, evidence for recombination events was also detected (Fig 5B). In the region between the two breakpoints at the genome positions nt 21161 and nt 27766, including the S gene, closer genetic relationship between SZ3 and WIV16 was observed. However, from position nt 27766 towards the 3' end of its genome, a notably close genetic relationship was observed between SZ3 and Rf4092 instead. Throughout the non-structural gene, moreover, SZ3 shared a similarly high sequence identity with WIV16 and Rf4092. It indicates that civet SARS-CoV was likely to be the descendent from a recombinant of the precursors of WIV16 and Rf4092, or that the SARSr-CoVs found in this cave, like WIV16 or Rf4092, may have been the descendants of the SARS-CoV lineage.

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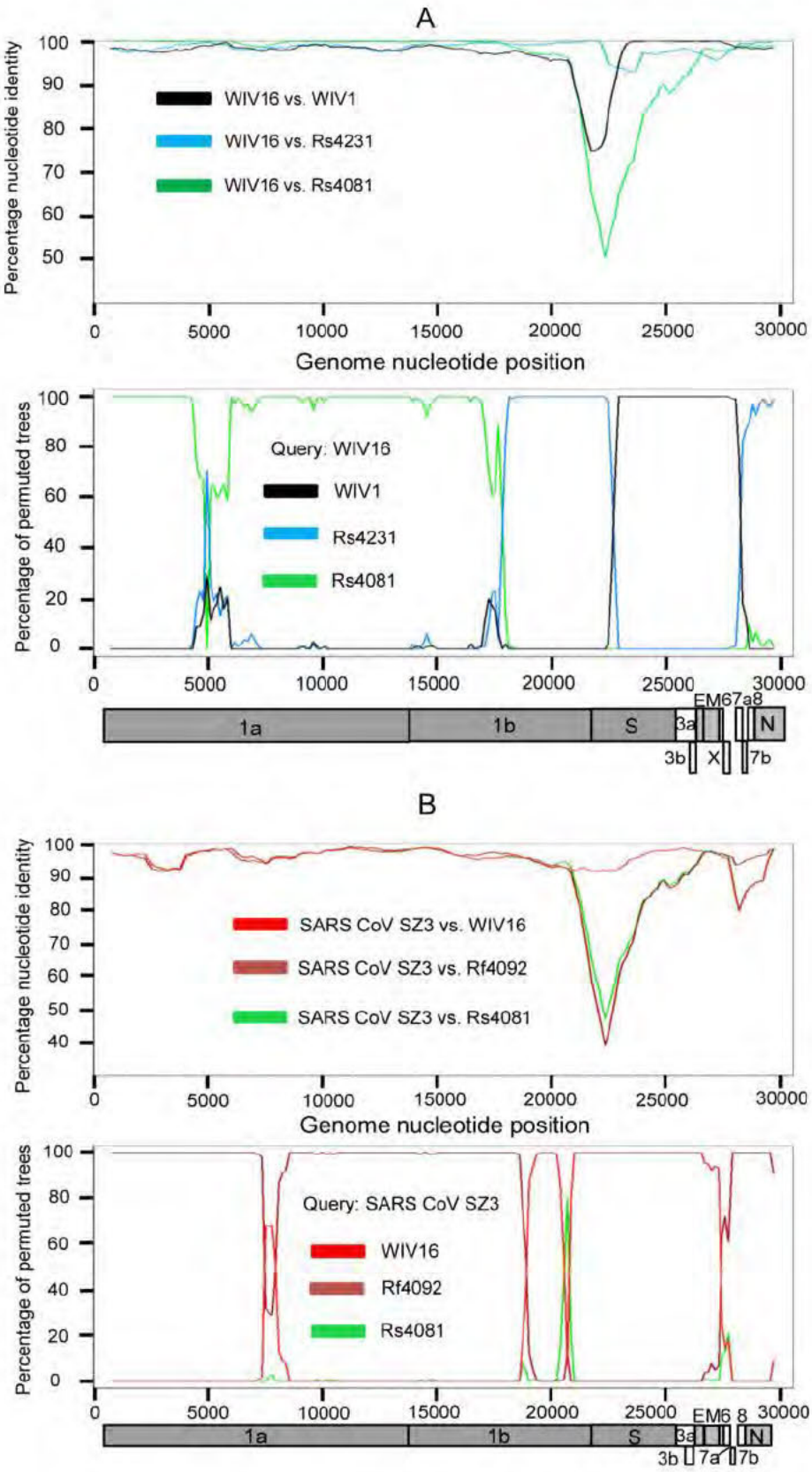
A
Percentage nucleotide identity
WIV16 vs. WIV1
WIV16 vs. Rs4231
WIV16 vs. Rs4081
Genome nucleotide position
Percentage of permuted trees
Query: WIV16
WIV1
Rs4231
Rs4081
1a
1b
S
3a
EM
6
7a
8
N
3b
X
7b
B
SARS CoV SZ3 vs. WIV16
SARS CoV SZ3 vs. Rf4092
SARS CoV SZ3 vs. Rs4081
Query: SARS CoV SZ3
WIV16
Rf4092
Rs4081

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Fig 5. Detection of potential recombination events by similarity plot and boot scan analysis. (A) Full-length genome sequence of SARSr-CoV WIV16 was used as query sequence and WIV1, Rs4231 and Rs4081 as reference sequences. (B) Full-length genome sequence of SARS-CoV SZ3 was used as query sequence and SARSr-CoV WIV16, Rf4092 and Rs4081 as reference sequences. All analyses were performed with a Kimura model, a window size of 1500 base pairs, and a step size of 150 base pairs. The gene map of query genome sequences are used to position breakpoints.

https://doi.org/10.1371/journal.ppat.1006698.g005

Phylogenetic analysis

Phylogenetic trees were constructed using the nt sequences of nonstructural protein gene ORF1a and ORF1b. Unlike the high genetic diversity in the S gene, nearly all SARSr-CoVs from the bat cave we surveyed were closely clustered, and showed closer phylogenetic relationship to SARS-CoV than the majority of currently known bat SARSr-CoVs discovered from other locations, except YNLF_31C and 34C which were recently reported in greater horseshoe bats from another location in Yunnan [22] (Fig 6). The phylogeny of SARSr-CoVs in ORF1a and ORF1b appeared to be associated with their geographical distribution rather than with host species. Regardless of different host bat species, SARS-CoV and SARSr-CoVs detected in bats from southwestern China (Yunnan, Guizhou and Guangxi province) formed one clade, in which SARSr-CoV strains showing closer relationship to SARS-CoV were all from Yunnan. SARSr-CoVs detected in southeastern, central and northern provinces, such as Hong Kong, Hubei and Shaanxi, formed the other clade which was phylogenetically distant to human and civet SARS-CoVs (Fig 6 and S6 Fig).

Rescue of bat SARSr-CoVs and virus infectivity experiments

In the current study, we successfully cultured an additional novel SARSr-CoV Rs4874 from a single fecal sample using an optimized protocol and Vero E6 cells [17]. Its S protein shared 99.9% aa sequence identity with that of previously isolated WIV16 and it was identical to WIV16 in RBD. Using the reverse genetics technique we previously developed for WIV1 [23], we constructed a group of infectious bacterial artificial chromosome (BAC) clones with the backbone of WIV1 and variants of S genes from 8 different bat SARSr-CoVs. Only the infectious clones for Rs4231 and Rs7327 led to cytopathic effects in Vero E6 cells after transfection (S7 Fig). The other six strains with deletions in the RBD region, Rf4075, Rs4081, Rs4085, Rs4235, As6526 and Rp3 (S1 Fig) failed to be rescued, as no cytopathic effects was observed and viral replication cannot be detected by immunofluorescence assay in Vero E6 cells (S7 Fig). In contrast, when Vero E6 cells were respectively infected with the two successfully rescued chimeric SARSr-CoVs, WIV1-Rs4231S and WIV1-Rs7327S, and the newly isolated Rs4874, efficient virus replication was detected in all infections (Fig 7). To assess whether the three novel SARSr-CoVs can use human ACE2 as a cellular entry receptor, we conducted virus infectivity studies using HeLa cells with or without the expression of human ACE2. All viruses replicated efficiently in the human ACE2-expressing cells. The results were further confirmed by quantification of viral RNA using real-time RT-PCR (Fig 8).

Activation of activating transcription factor 6 (ATF6) by the ORF8 proteins of different bat SARSr-CoVs

The induction of the ATF6-dependent transcription by the ORF8s of SARS-CoV and bat SARSr-CoVs were investigated using a luciferase reporter, 5×ATF6-GL3. In HeLa cells transiently transfected with the expression plasmids of the ORF8s of bat SARSr-CoV Rf1, Rf4092 and WIV1, the relative luciferase activities of the 5×ATF6-GL3 reporter was enhanced by 5.56 to 9.26 folds compared with cells transfected with the pCAGGS empty vector, while it was

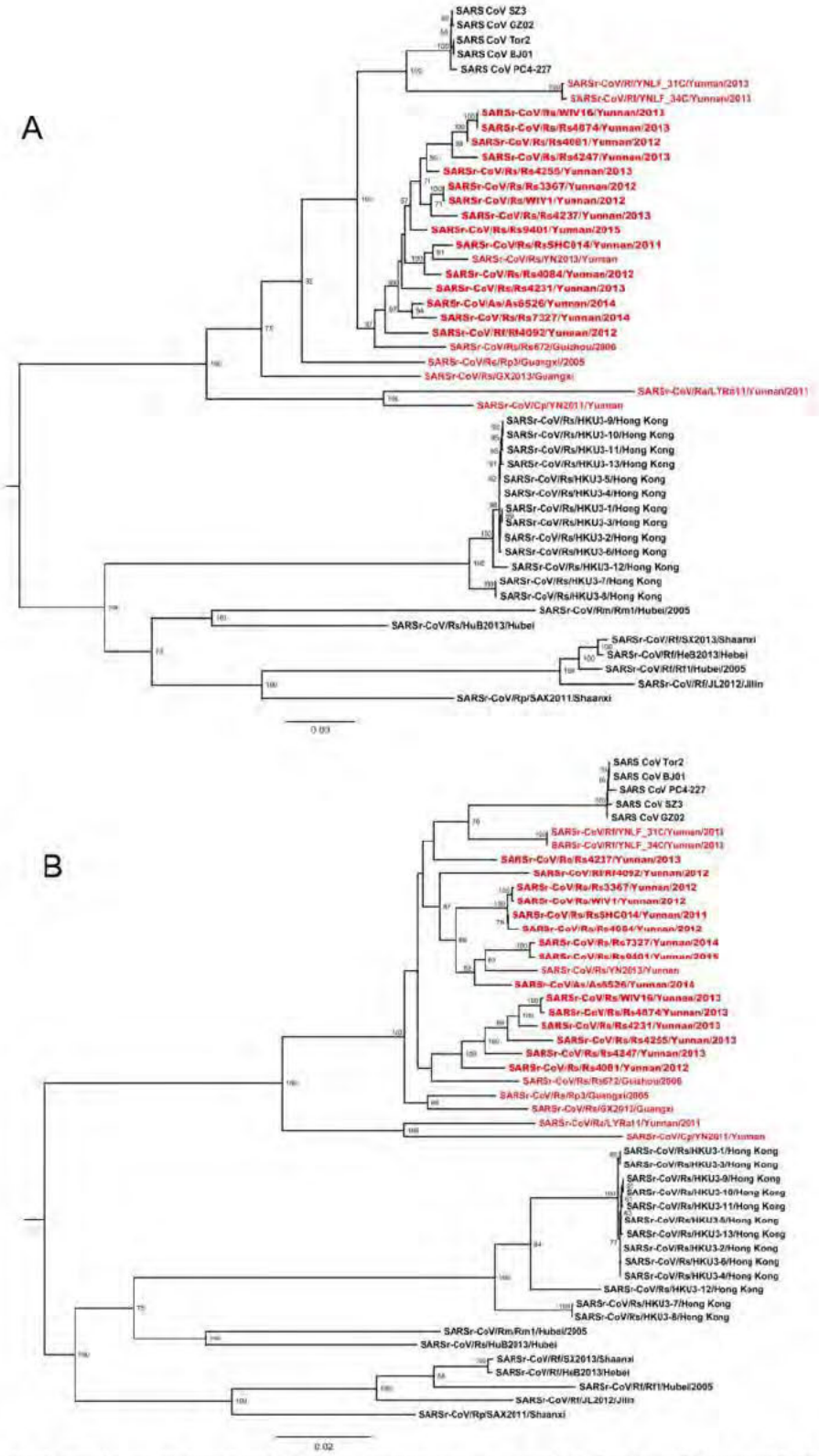

Fig 6. Phylogenetic trees based on nucleotide sequences of ORF1a (A) and ORF1b (B). The trees were constructed by the maximum likelihood method using the LG model with bootstrap values determined by 1000 replicates. Only bootstraps > 50% are shown. The scale bars represent 0.03 (A) and 0.02 (B) substitutions per

nucleotide position. Rs, *Rhinolophus sinicus*; Rf, *Rhinolophus ferremequinum*; Rm, *Rhinolophus macrotis*; Ra, *Rhinolophus affinis*; Rp, *Rhinolophus pusillus*; As, *Aselliscus stoliczkanus*; Cp, *Chaerephon plicata*. SARSr-CoVs detected in bats from the single cave surveyed in this study are in bold. Sequences detected in southwestern China are indicated in red.

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increased by 4.42 fold by the SARS-CoV GZ02 ORF8. As a control, the treatment with tunicamyxin (TM) stimulated the transcription by about 11 folds (Fig 9A). The results suggests that various ORF8 proteins of bat SARSr-CoVs can activate ATF6, and those of some strains have a stronger effect than the SARS-CoV ORF8.

Induction of apoptosis by the ORF8a of the newly identified bat SARSr-CoV

We conducted transient transfection to examine whether the ORF8a of SARSr-CoV Rs4084 triggered apoptosis. As shown in Fig 9B, 11.76% and 9.40% of the 293T cells transfected with the SARSr-CoV Rs4084-ORF8a and SARS-CoV Tor2-ORF8a expression plasmid underwent apoptosis, respectively. In contrast, transfection with the empty vector resulted in apoptosis in only 2.79% of the cells. The results indicate that Rs4084 ORF8a has an apoptosis induction activity similar to that of SARS-CoV [28].

Discussion

Genetically diverse SARSr-CoVs have been detected in various horseshoe bat species across a wide geographic range in China in the past decade [9–12,14,29]. However, most bat SARSr-CoVs show considerable genetic distance to SARS-CoV, particularly in the highly variable S1, ORF8 and ORF3 regions [10,25]. Recently, several novel SARSr-CoVs have been described to be more closely related to SARS-CoV, either in the S gene or in ORF8. The S proteins of RsSHC014, Rs3367, WIV1 and WIV16, which were reported in our previous studies, shared 90% to 97% aa sequence identities to those of human/civet SARS-CoVs [17,18]. Another strain from *Rhinolophus affinis* in Yunnan termed LYRa11 showed 90% aa sequence identity to SARS-CoV in the S gene [13]. In addition, two studies have described 4 novel SARSr-CoVs (YNLF_31C/34C and GX2013/YN2013) which possessed a full-length ORF8 with substantially higher similarity to that of SARS-CoV [22,30]. These findings provide strong genetic evidence for the bat origin of SARS-CoV with regard to the S gene or ORF8. However, all of these SARSr-CoVs were distinct from SARS-CoV in at least one other gene, suggesting that none of them was the immediate progenitor of SARS-CoV. Moreover, these SARSr-CoVs were discovered in bat populations from physically distinct locations. The site of origin of the true progenitor of SARS-CoV and the evolutionary origin of SARS-CoV have until now remained elusive. In the current study, we have identified a bat habitat potentially important for SARSr-CoV evolution where a series of recombination events have likely occurred among different SARSr-CoV strains, which provides new insights into the origin of SARS-CoV.

SARS first emerged in Guangdong province in late 2002 [7]. However, SARSr-CoVs discovered in bats from neighboring areas of Guangdong to date have shown phylogenetic disparity from SARS-CoV especially in the S gene [9,10,14], suggesting SARS-CoV may have originated from another region. Our analysis of the phylogeny of SARS-CoVs and all known bat SARSr-CoVs using the nt sequence of their non-structural ORF1a and ORF1b genes, which constitute the majority of the genome, shows that SARSr-CoV evolution is strongly correlated with their geographical origin, but not host species. It is noteworthy that SARSr-CoVs detected in Yunnan are more closely related to SARS-CoV than strains from other regions in China. This finding implies that Yunnan, or southwestern China, is more likely to be the geographical source

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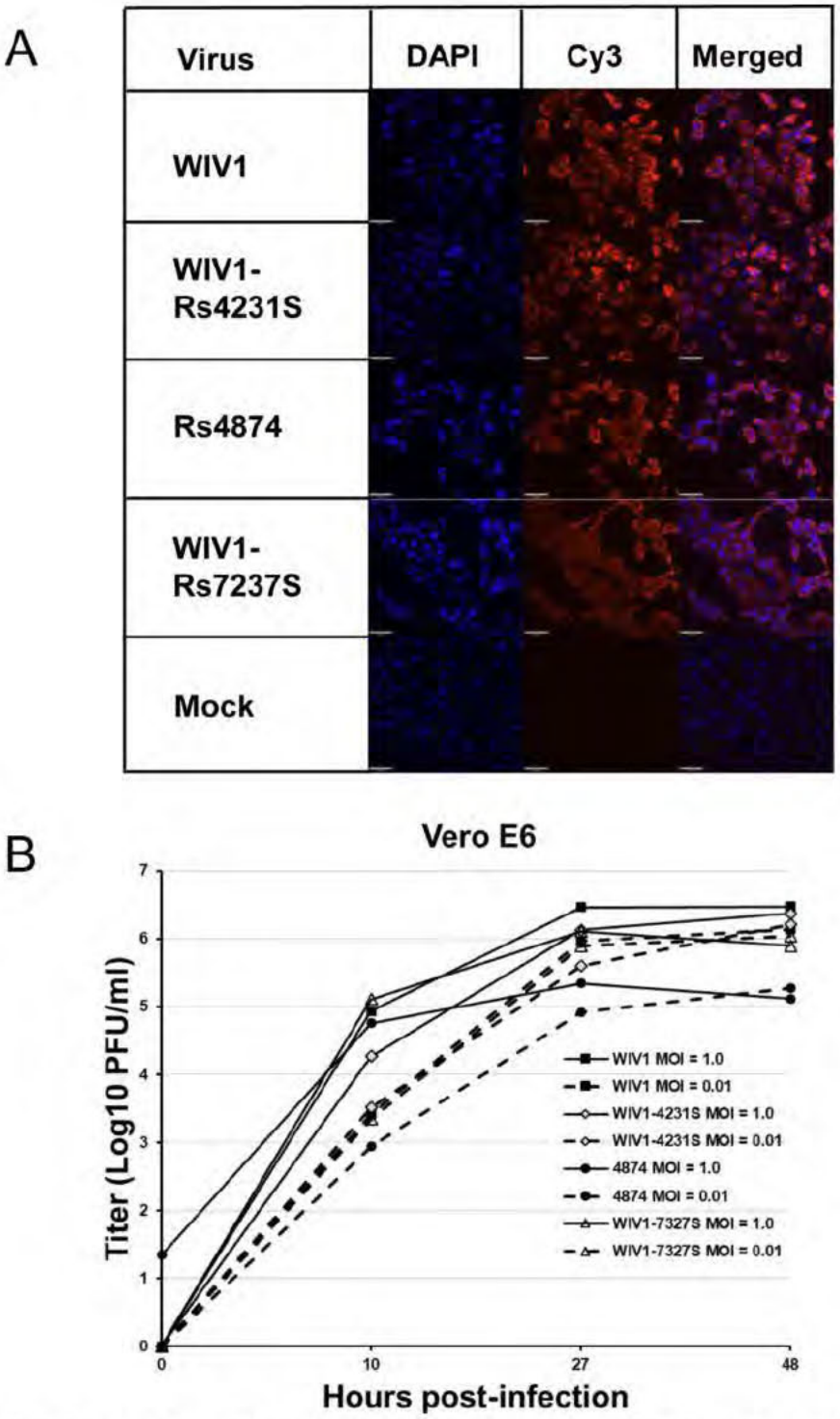

Fig 7. Infection of Vero E6 cells by bat SARSr-CoV WIV1, Rs4874, WIV1-Rs4231S and WIV1-Rs7327S. (A) The successful infection was confirmed by immunofluorescent antibody staining using rabbit antibody against the SARSr-CoV Rp3 nucleocapsid protein. The columns (from left to right) show staining of nuclei (blue), virus replication (red), and both nuclei and virus replication (merged double-stain images). (B) The growth curves in Vero E6 cells with a MOI of 1.0 and 0.01.

https://doi.org/10.1371/journal.ppat.1006698.g007

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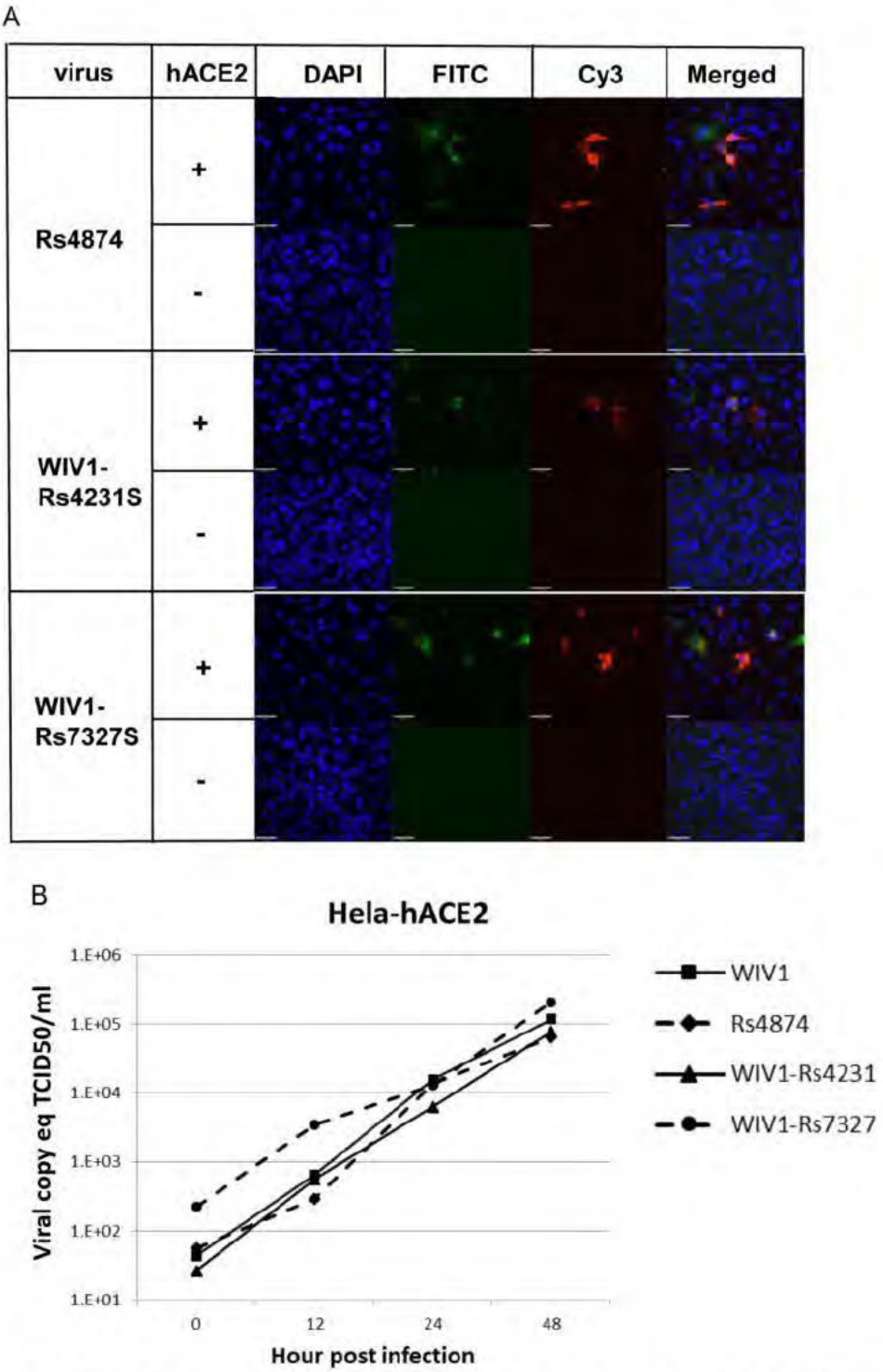

Fig 8. Analysis of receptor usage by immunofluorescence assay (A) and real-time PCR (B). Virus infectivity of Rs4874, WIV1-Rs4231S and WIV1-Rs7327S was determined in HeLa cells with and without the expression of human ACE2. ACE2 expression was detected with goat anti-human ACE2 antibody followed by fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat IgG. Virus replication was detected with rabbit antibody against the SARSr-CoV Rp3 nucleocapsid protein followed by cyanine 3 (Cy3)-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI (49,6-diamidino-2-phenylindole). The columns (from left to right) show staining of nuclei (blue), ACE2 expression (green), virus replication (red) and the merged triple-stained images, respectively.

https://doi.org/10.1371/journal.ppat.1006698.g008

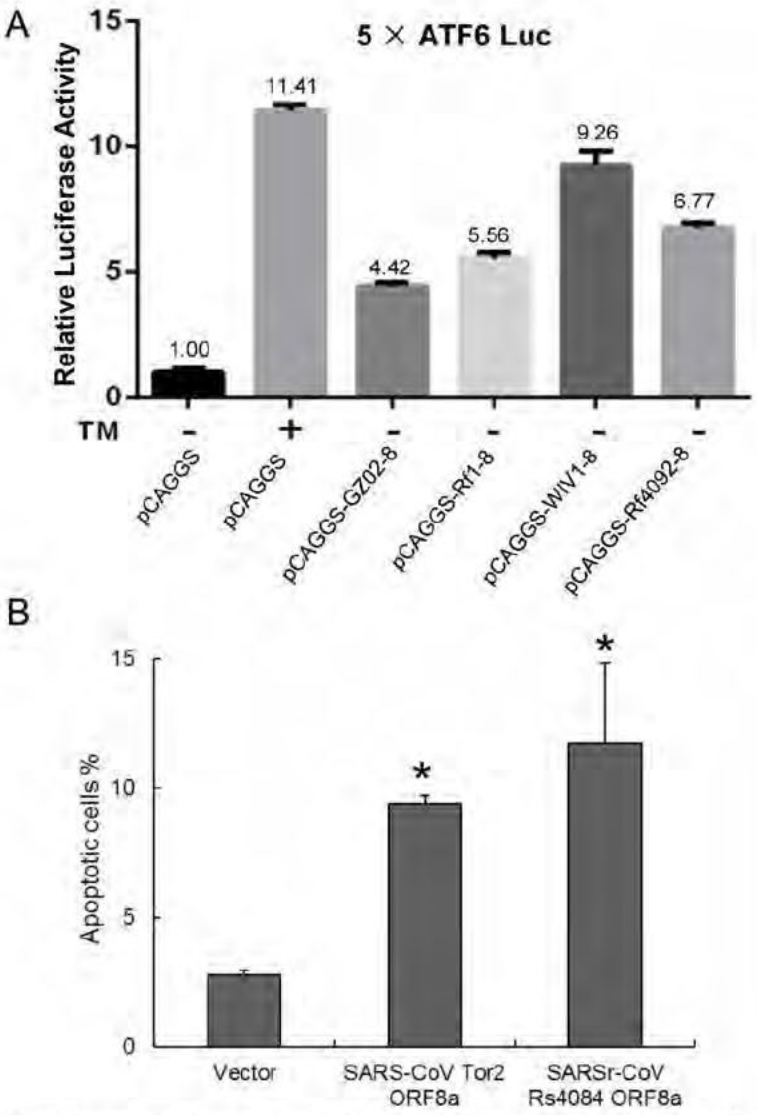

Fig 9. Functional characterization of diverse ORF8 and ORF8a proteins of bat SARSr-CoVs. (A) The ORF8 proteins of SARS-CoV and bat SARSr-CoVs induces the ATF6-dependent transcriptional activity. HeLa cells were transiently transfected with the pcAGGS expression plasmids of the ORF8 of SARS-CoV GZ02, bat SARSr-CoV Rf1, WIV1 and Rf4092 and the reporter plasmid 5×ATF6-GL3 for 40h. Control cells were co-transfected with the reporter plasmid and the empty pCAGGS vector for 24h, and treated with or without TM (2μg/ml) for an additional 16h. The cell lysates were harvested for dual luciferase assay and data are shown as the average values from triplicate wells. (B) The ORF8a proteins of SARS-CoV and bat SARSr-CoV triggered apoptosis. 293T cells were transfected with the expression plasmids of the ORF8a of SARS-CoV Tor2 and bat SARSr-CoV Rs4084 and a pcAGGS vector control for 24h. Apoptosis was analyzed by flow cytometry after annexin V staining and the percentage of apoptotic cells were calculated. Data are shown as the average values from triplicate cells. Error bars indicate SDs. * $P<0.05$.

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of SARS-CoV than other regions in China, but data from more extensive surveillance are yet needed to support this inference.

In our longitudinal surveillance of SARSr-CoVs in a single cave in Yunnan where we discovered Rs3367, RsSHC014, WIV1 and WIV16, the CoV prevalence in fecal samples varied among different sampling time. Generally, a higher prevalence was observed in autumn (September and October) than in spring and early summer (April and May). This may be due to

the establishment of a susceptible subpopulation of newborn bats which had not developed their own immunity after the parturition period [31]. Another factor may be the changes in the composition of bat species in the cave at different sampling dates. For example, in September 2012 when the CoV prevalence reached 51.3%, the majority of samples were from *R. sinicus*, but in May 2015 when only 3 out of the 145 samples tested positive, *Aselliscus stoliczkanus* was the predominant bat species in the cave. We failed to amplify the RBD sequences from 15 of the 64 SARSr-CoV positive samples. Most of these samples had comparatively low viral concentration ($< 10^7$ copies/g) (S8 Fig), as revealed by our previous quantitative studies [32]. The unsuccessful amplification of RBD in some samples with high viral concentration was probably because of the more divergent sequences in this region of these SARSr-CoV genomes.

In this cave, we have now obtained full-length genome sequences of additional 11 novel SARSr-CoVs from bats. Our findings suggest the co-circulation of different bat SARSr-CoVs highly similar to SARS-CoV in the most variable S1 (NTD and RBD), ORF8 and ORF3 regions, respectively, in this single location. In the ORF1a, ORF1b, E, M and N genes, the SARSr-CoVs circulating in this cave also shared > 98% aa sequence identities with human/civet SARS-CoVs. Thus, all of the building blocks of the SARS-CoV genome were present in SARSr-CoVs from this single location in Yunnan during our sampling period. Furthermore, strains closely related to different representative bat SARSr-CoVs from other provinces (e.g. Rs672, HKU3 and Rf1) in the RBD region were also detected there. Therefore, this cave could be regarded as a rich gene pool of bat SARSr-CoVs, wherein concurrent circulation of a high diversity of SARSr-CoV strains has led to an unusually diverse assemblage of SARSr-CoVs.

During our 5-year surveillance in this single cave, we first reported Rs3367 and WIV1 in 2013, with RBD sequence closely resembling that of SARS-CoV [17]. More recently, we discovered WIV16 which had an RBD almost identical to WIV1's but shared much higher similarity with SARS-CoV than WIV1 in the NTD region of S1, making it the closest SARSr-CoV to the epidemic strains identified to date [18]. In this study, we found a novel strain Rs4231 from the same location sharing almost identical NTD sequence with WIV16 but distinct from it in the RBD, with evidence of a recombination event. Our recombination analysis indicated that a recombination event may have taken place at the junction between the coding region of NTD and RBD in the Rs4231 and WIV1 genomes and resulted in WIV16. Recombination at this genomic position also happened among other SARSr-CoVs relatively distant to SARS-CoV found in this location (e.g. Rs4081 and Rs4247, S5 Fig). The frequent recombination at this hotspot in the S gene increased the genetic diversity of SARSr-CoVs harbored in these bat populations and might have been responsible for the generation of the S gene of the direct progenitor strain of SARS-CoV.

The genomes of SARS-CoVs from patients during the early epidemic phase and civet SARS-CoVs all contained a single full-length ORF8 [3,7]. We have found that a number of bat SARSr-CoVs from this cave possessed a complete ORF8 highly similar to that of early human/civet SARS-CoV (>97% nt sequence identity), represented by strain Rf4092 (S3C Fig). This provided further evidence for the source of human SARS-CoV ORF8 in bats [22,30]. In contrast, the ORF8 was split into overlapping ORF8a and ORF8b in most human SARS-CoV strains from later-phase patients due to the acquisition of a 29-nt deletion [8,26]. In this study, we have discovered for the first time a bat SARSr-CoV with ORF8a and ORF8b highly similar to the later-phase human SARS-CoVs, though the split of ORF8 in the bat SARSr-CoV and that in human SARS-CoV were two independent events. Our recombination analysis suggests that this strain, Rs4084, likely acquired its ORF8 from Rf4092 through recombination, followed by the development of the 5-nt deletion which led to the splitting. It suggests that ORF8 region in bat SARSr-CoV genomes is prone to deletions as in human SARS-CoV [3,25]. Finally, the recombination analysis suggests that an ancestral strain of SARS-CoV SZ3 would have been generated if the recombination around ORF8 had occurred between the lineages that led to WIV16 and Rf4092.

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Taken together, the evidence of recombination events among SARSr-CoVs harbored by bats in this single location suggests that the direct progenitor of SARS-CoV may have originated as a result of a series of recombination within the S gene and around ORF8. This could have been followed by the spillover from bats to civets and people either in the region, or during movement of infected animals through the wildlife trade. However, given the paucity of data on animal trade prior to the SARS outbreak, the likely high geographical sampling bias in bat surveillance for SARSr-CoVs in southern China, and the possibility that other caves harbor similar bat species assemblages and a rich diversity of SARSr-CoVs, a definite conclusion about the geographical origin of SARS-CoV cannot be drawn at this point.

R. sinicus are regarded as the primary natural host of SARS-CoV, as all SARSr-CoVs highly homologous to SARS-CoV in the S gene were predominantly found in this species. However, it is noted that two SARSr-CoVs previously reported from *R. ferrumequinum* showed the closest phylogenetic position to SARS-CoV in the ORF1a/1b trees. These strains were discovered in another location in Yunnan 80 km from the cave surveyed in the current study [22]. This information also supports the speculation that SARS-CoV may have originated from this region. Nonetheless, since the correlation between the host species and the phylogeny of SARSr-CoV ORF1ab seems limited, more SARSr-CoV sequences need to be obtained from different *Rhinolophus* bat species in both locations in Yunnan, and from other locations in southern China. In particular, it will be important to assess whether *R. ferrumequinum* played a more important role in the evolution of SARS-CoV ORF1ab.

The cave we studied is located approximately 60 km from the city of Kunming. Beside a number of rhinolophid and hipposiderid species from which SARSr-CoVs have been detected, other bats like myotis were also present there. The temperature in the cave is around 22–25˚C and the humidity around 85%-90%. The physical nature of the cave is not unique, but it does appear to host a particularly dense population of bats in the reproductive season. Similar caves co-inhabited by bat populations of different species are not rare in other areas in Yunnan. We propose that efforts to study the ecology, host species diversity, and viral strain populations of these caves may provide critical information on what drives SARSr-CoV evolution.

Our previous studies demonstrated the capacity of both WIV1 and WIV16 to use ACE2 orthologs for cell entry and to efficiently replicate in human cells [17,18]. In this study, we confirmed the use of human ACE2 as receptor of two novel SARSr-CoVs by using chimeric viruses with the WIV1 backbone replaced with the S gene of the newly identified SARSr-CoVs. Rs7327's S protein varied from that of WIV1 and WIV16 at three aa residues in the receptor-binding motif, including one contact residue (aa 484) with human ACE2. This difference did not seem to affect its entry and replication efficiency in human ACE2-expressing cells. A previous study using the SARS-CoV infectious clone showed that the RsSHC014 S protein could efficiently utilize human ACE2 [33], despite being distinct from SARS-CoV and WIV1 in the RBD (S1 Fig). We examined the infectivity of Rs4231, which shared similar RBD sequence with RsSHC014 but had a distinct NTD sequence, and found the chimeric virus WIV1-Rs4231S also readily replicated in HeLa cells expressing human ACE2 molecule. The novel live SARSr-CoV we isolated in the current study (Rs4874) has an S gene almost identical to that of WIV16. As expected, it is also capable of utilizing human ACE2. These results indicate that diverse variants of SARSr-CoV S protein without deletions in their RBD are able to use human ACE2. In contrast, our previous study revealed that the S protein of a *R. sinicus* SARSr-CoV with deletions (Rp3) failed to use human, civet and bat ACE2 for cell entry [34]. In this study, in addition to Rs4231 and Rs7327, we also constructed infectious clones with the S gene of Rs4081, Rf4075, Rs4085, Rs4235 and As6526, which all contained the deletions in their RBD. These 7 strains, plus Rs4874 and the previously studied WIV1 and RsSHC014, could represent all types of S variants of SARSr-CoVs in this location (S3A Fig). However, none of the strains

with deletions in the RBD could be rescued from Vero E6 cells. Therefore, the two distinct clades of SARSr-CoV S gene may represent the usage of different receptors in their bat hosts.

The full-length ORF8 protein of SARS-CoV is a luminal endoplasmic reticulum (ER) membrane-associated protein that induces the activation of ATF6, an ER stress-regulated transcription factor that activates the transcription of ER chaperones involved in protein folding [35]. We amplified the ORF8 genes of Rf1, Rf4092 and WIV1, which represent three different genotypes of bat SARSr-CoV ORF8 (S3C Fig), and constructed the expression plasmids. All of the three ORF8 proteins transiently expressed in HeLa cells can stimulate the ATF6-dependent transcription. Among them, the WIV1 ORF8, which is highly divergent from the SARS-CoV ORF8, exhibited the strongest activation. The results indicate that the variants of bat SARSr-CoV ORF8 proteins may play a role in modulating ER stress by activating the ATF6 pathway. In addition, the ORF8a protein of SARS-CoV from the later phase has been demonstrated to induce apoptosis [28]. In this study, we have found that the ORF8a protein of the newly identified SARSr-CoV Rs4084, which contained an 8-aa insertion compared with the SARS-CoV ORF8a, significantly triggered apoptosis in 293T cells as well.

Compared with the 154-aa ORF3b of SARS-CoV, the ORF3b proteins of all previously identified bat SARSr-CoVs were smaller in size due to the early translation termination. However, for the first time, we discovered an ORF3b without the C-terminal truncation in a bat SARSr-CoV, Rs7327, which differed from the ORF 3b of SARS-CoV GZ02 strain at only one aa residue. The SARS-CoV ORF3b antagonizes interferon function by modulating the activity of IFN regulatory factor 3 (IRF3) [27]. As previous studies suggested, the nuclear localization signal-containing C-terminal may not be required for the IFN antagonist activity of ORF3b [36]. Our previous studies also demonstrated that the ORF3b protein of a bat SARSr-CoV, termed Rm1, which was C-terminally truncated to 56 aa and shared 62% aa sequence identity with SARS-CoV, still displayed the IFN antagonist activity [37]. It is very interesting to investigate in further studies whether Rs7327's ORF3b and other versions of truncated ORF3b such as WIV1 and WIV16 also show IFN antagonism profiles.

As a whole, our findings from a 5-year longitudinal study conclusively demonstrate that all building blocks of the pandemic SARS-CoV genome are present in bat SARSr-CoVs from a single location in Yunnan. The data show that frequent recombination events have happened among those SARSr-CoVs in the same cave. While we cannot rule out the possibility that similar gene pools of SARSr-CoVs exist elsewhere, we have provided sufficient evidence to conclude that SARS-CoV most likely originated from horseshoe bats via recombination events among existing SARSr-CoVs. In addition, we have also revealed that various SARSr-CoVs capable of using human ACE2 are still circulating among bats in this region. Thus, the risk of spillover into people and emergence of a disease similar to SARS is possible. This is particularly important given that the nearest village to the bat cave we surveyed is only 1.1 km away, which indicates a potential risk of exposure to bats for the local residents. Thus, we propose that monitoring of SARSr-CoV evolution at this and other sites should continue, as well as examination of human behavioral risk for infection and serological surveys of people, to determine if spillover is already occurring at these sites and to design intervention strategies to avoid future disease emergence.

Materials and methods

Ethics statement

All sampling procedures were performed by veterinarians with approval from Animal Ethics Committee of the Wuhan Institute of Virology (WIVH05210201). The study was conducted in accordance with the Guide for the Care and Use of Wild Mammals in Research of the People's Republic of China.

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Sampling

Bat samplings were conducted ten times from April 2011 to October 2015 at different seasons in their natural habitat at a single location (cave) in Kunming, Yunnan Province, China. All members of field teams wore appropriate personal protective equipment, including N95 masks, tear-resistant gloves, disposable outerwear, and safety glasses. Bats were trapped and fecal swab samples were collected as described previously [9]. Clean plastic sheets measuring 2.0 by 2.0 m were placed under known bat roosting sites at about 18:00 h each evening for collection of fecal samples. Fresh fecal pellets were collected from sheets early in the next morning. Each sample (approximately 1 gram of fecal pellet) was collected in 1ml of viral transport medium composed of Hank's balanced salt solution at pH7.4 containing BSA (1%), amphotericin (15 μg/ml), penicillin G (100 units/ml), and streptomycin (50 μg/ml), and were stored at -80˚C until processing. Bats trapped for this study were released back into their habitat.

RNA extraction, PCR screening and sequencing

Fecal swab or pellet samples were vortexed for 1 min, and 140 μl of supernatant was collected from each sample after centrifuge at 3000 rpm under 4˚C for 1min. Viral RNA was extracted with Viral RNA Mini Kit (Qiagen) following the manufacturer's instructions. RNA was eluted in 60 μl of buffer AVE (RNase-free water with 0.04% sodium azide, Qiagen), aliquoted, and stored at -80˚C. One-step hemi-nested RT-PCR (Invitrogen) was employed to detect the presence of coronavirus sequences as described previously using a set of primers that target a 440-nt fragment in the RNA-dependent RNA polymerase gene (*RdRp*) of all known alpha- and betacoronaviruses [20]. For the first round PCR, the 25 μl reaction mix contained 12.5 μl PCR 2 × reaction mix buffer, 10 pmol of each primer, 2.5 mM $MgSO_4$, 20 U RNase inhibitor, 1 μl SuperScript III/Platinum Taq Enzyme Mix and 5 μl RNA template. The amplification was performed as follows: 50˚C for 30 min, 94˚C for 2 min, followed by 40 cycles consisting of 94˚C for 15 sec, 52˚C for 30 sec, 68˚C for 40 sec, and a final extension of 68˚C for 5 min. For the second round PCR, the 25 μl reaction mix contained 2.5 μl PCR reaction buffer, 5 pmol of each primer, 50 mM $MgCl_2$, 0.5mM dNTP, 0.1 μl Platinum Taq Enzyme (Invitrogen) and 1 μl product of the first round PCR. The amplification was performed as follows: 94˚C for 3 min followed by 35 cycles consisting of 94˚C for 30 sec, 52˚C for 30 sec, 72˚C for 40 sec, and a final extension of 72˚C for 7 min. The RBD region was amplified using the one-step nested RT-PCR method previously described [17].

PCR products were gel purified and sequenced with an ABI Prism 3730 DNA analyzer (Applied Biosystems, USA). PCR products with low concentration or generating heterogeneity in the sequencing chromatograms were cloned into pGEM-T Easy Vector (Promega) for sequencing. The positive samples in this study were termed using the abbreviated name of bat species plus the sample ID number (e.g. Rs4081). To confirm the bat species of individual sample, PCR amplification of cytochrome b (*Cytob*) or NADH dehydrogenase subunit 1 (*ND1*) gene was performed using DNA extracted from the feces or swabs [38,39].

Sequencing of full-length genomes

Full genomic sequences of 11 SARSr-CoVs were determined by One-step PCR (Invitrogen) amplification of overlapping genomic fragments with degenerate primers designed by multiple alignment of available SARS-CoV and bat SARSr- CoV sequences deposited in GenBank, and additional specific primers designed from the results of previous rounds of sequencing in this study. Primer sequences are available upon request. Sequences of the 5' and 3' genomic ends were obtained by 5' and 3' RACE (Roche), respectively. PCR products with expected size were gel-purified and subjected directly to sequencing. Each fragment was sequenced at least twice.

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The sequencing chromatogram of each product was thoroughly examined and sequence heterogeneity was not observed. For some fragments with low concentration of amplicons, the PCR products were cloned into pGEM-T Easy Vector (Promega) for sequencing. At least five independent clones were sequenced to obtain a consensus sequence. Co-presence of sequences of distinct SARSr-CoVs was not found in any of the amplicons. The sequences of overlapping genomic fragments were assembled to obtain the full-length genome sequences, with each overlapping sequence longer than 100 bp.

Evolution analysis

Full-length genome sequences of the 15 SARSr-CoVs detected from bats in the cave surveyed in this study were aligned with those of selected SARS-CoVs using MUSCLE [40]. The aligned sequences were scanned for recombination events by Recombination Detection Program (RDP) [41]. The potential recombination events suggested by strong *P* values ($<10^{-20}$) were further confirmed using similarity plot and bootscan analyses implemented in Simplot 3.5.1 [42]. Phylogenetic trees based on nucleotide sequences were constructed using the Maximum Likelihood algorithm under the LG model with bootstrap values determined by 1000 replicates in the PhyML (version 3.0) software package [43].

Virus isolation

The Vero E6 cell line was kindly provided by Australian Animal Health Laboratory, CSIRO (Geelong, Australia). Vero E6 monolayer was maintained in DMEM medium supplemented with 10% fetal calf serum (FCS). Fecal samples (in 200 μl buffer) were gradient centrifuged at 3,000–12,000 g, and the supernatant was diluted 1:10 in DMEM before being added to Vero E6 cells. After incubation at 37˚C for 1 h, the inoculum was removed and replaced with fresh DMEM medium with 2% FCS. The cells were incubated at 37˚C and checked daily for cytopathic effect. All tissue culture media were supplemented with triple antibiotics penicillin/streptomycin/amphotericin (Gibco) (penicillin 200 IU/ml, streptomycin 0.2 mg/ml, amphotericin 0.5 μg/ml). Three blind passages were carried out for each sample. After each passage, both the culture supernatant and cell pellet were examined for presence of SARSr-CoV by RT-PCR using specific primers targeting the RdRp or S gene. The viruses which caused obvious cytopathic effect and could be detected in three blind passages by RT-PCR were further confirmed by electron microscopy.

Construction of recombinant viruses

Recombinant viruses with the S gene of the novel bat SARSr-CoVs and the backbone of the infectious clone of SARSr-CoV WIV1 were constructed using the reverse genetic system described previously [23] (S9 Fig). The fragments E and F were re-amplified with primer pairs (FE, 5'-AGGGCCCACCTGGCACTGGTAAGAGTCATTTTGC-3', R-EsBsaI, 5'-ACTGGTCTCTTCGTTTAGTTATTAACTAAAATATCACTAGACACC-3') and (F-FsBsaI, 5'-TGAGGTCTCCGAACTTATGGATTTGTTTATGAG-3', RF, 5'-AGGTAGGCCTCTAGGGCAGCTAAC-3'), respectively. The products were named as fragment Es and Fs, which leave the spike gene coding region as an independent fragment. BsaI sites (5'-GGTCTCN|NNNN-3') were introduced into the 3' terminal of the Es fragment and the 5' terminal of the Fs fragment, respectively. The spike sequence of Rs4231 was amplified with the primer pair (F-Rs4231-BsmBI, 5'-AGTCGTCTCAACGAACATGTTTATTTTCTTATTCTTTCTCACTCTCAC-3' and R-Rs4231-BsmBI, 5'-TCACGTCTCAGTTCGTTTATGTGTAATGTAATTTGACACCCTTG-3'). The S gene sequence of Rs7327 was amplified with primer pair (F-Rs7327-BsaI, 5'-AGTGGTCTCAACGAACATGAAATTGTTAGTTTTAGTTTTTGCTAC-3' and R-

Rs7327-BsaI, 5'- TCAGGTCTCAGTTCGTTTATGTGTAATGTAATTTAACACCCTTG-3'). The fragment Es and Fs were both digested with BglI (NEB) and BsaI (NEB). The Rs4231 S gene was digested with BsmBI. The Rs7327 S gene was digested with BsaI. The other fragments and bacterial artificial chromosome (BAC) were prepared as described previously. Then the two prepared spike DNA fragments were separately inserted into BAC with Es, Fs and other fragments. The correct infectious BAC clones were screened. The chimeric viruses were rescued as described previously [23].

Determination of virus infectivity by immunofluorescence assay

The HeLa cell line was kindly provided by Australian Animal Health Laboratory, CSIRO (Geelong, Australia). HeLa cells expressing human ACE2 were constructed as described previously [17]. HeLa cells expressing human ACE2 and Vero E6 cells were cultured on coverslips in 24-well plates (Corning) incubated with the newly isolated or recombinant bat SARSr-CoVs at a multiplicity of infection (MOI) = 1.0 for 1h. The inoculum was removed and the cells were washed twice with PBS and supplemented with medium. Vero E6 cells without virus inoculation and HeLa cells without ACE2 were used as negative control. Twenty-four hours after infection, cells were rinsed with PBS and fixed with 4% formaldehyde in PBS (pH7.4) at 4˚C for 20 min. ACE2 expression was detected by using goat anti-human ACE2 immunoglobulin followed by FITC-labelled donkey anti-goat immunoglobulin (PTGLab). Virus replication was detected by using rabbit antibody against the nucleocapsid protein of bat SARSr-CoV Rp3 followed by Cy3-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI. Staining patterns were observed under an FV1200 confocal microscope (Olympus).

Determination of virus replication in Vero E6 cells by plaque assay

Vero E6 cells were infected with WIV1, Rs4874, WIV1-Rs4231S, and WIV1-Rs7327S at an MOI of 1.0 and 0.01. After incubation for an hour, the cells were washed with DHanks for three times and supplied with DMEM containing 2% FCS. Samples were collected at 0, 10, 27, and 48 h post infection. The viral titers were determined by plaque assay.

Determination of virus replication in HeLa cells expressing human ACE2 by quantitative RT-PCR

HeLa cells expressing human ACE2 were inoculated with WIV1, Rs4874, WIV1-Rs4231S, and WIV1-Rs7327S at an MOI of 1.0, and were incubated for 1h at 37˚C. After the inoculum was removed, the cells were supplemented with medium containing 1% FBS. Supernatants were collected at 0, 12, 24 and 48h. Virus titers were determined using quantitative RT-PCR targeting the partial N gene with a standard curve which expresses the correlation between Ct value and virus titer (shown as TCID50/ml). The standard curve was made using RNA dilutions from the purified Rs4874 virus stock (with a titer of 2.15×10^6 TCID50/ml). For qPCR, RNA was extracted from 140 μl of each supernatant with Viral RNA Mini Kit (Qiagen) following manufacturer's instructions and eluted in 60 μl AVE buffer. The PCR was performed with the TaqMan AgPath-ID One-Step RT–PCR Kit (Applied Biosystems) in a 25 μl reaction mix containing 4 μl RNA, 1 × RT–PCR enzyme mix, 1 × RT–PCR buffer, 40 pmol forward primer (5'-GTGGTGGTGACGGCA AAATG-3'), 40 pmol reverse primer (5'-AAGTGAAGCTTCTGG GCCAG-3') and 12 pmol probe (5'-FAM-AAAGAGCTCAGCCCCAGATG-BHQ1-3'). The amplification was performed as follows: 50˚C for 10 min, 95˚C for 10 min followed by 50 cycles consisting of 95˚C for 15 sec and 60˚C for 20 sec.

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Plasmids

The ORF8 genes of bat SARSr-CoV WIV1 and Rf4092 and the ORF8a gene of bat SARSr-CoV Rs4084 were amplified by PCR from the viral RNA extracted from the isolated virus or fecal samples. The ORF8 gene of SARS-CoV GZ02 and bat SARSr-CoV Rf1, and the ORF8a gene of SARS-CoV Tor2 were synthesized by Tsingke Biological Technology Co., Ltd (Wuhan, China). All genes were cloned into the pCAGGS vector constructed with a C-terminal HA tag. Expression of the proteins was confirmed by Western blotting using a mAb against the HA tag. Five tandem copies of the ATF6 consensus binding sites were synthesized and inserted into the pGL3-Basic vector to construct the luciferase reporter plasmid 5×ATF6-GL3, in which the luciferase gene is under the control of the c-*fos* minimal promoter and the ATF6 consensus binding sites.

Luciferase reporter assay

HeLa cells in 24-well plates were transfected using Lipofectamine 3000 reagent (Life Technologies) following the manufacturer's instruction. Cells per well were co-transfected with 600ng of the 5×ATF6-GL3 reporter plasmid, with 300ng of each expression plasmid of SARS-CoV and SARSr-CoV ORF8 or empty vector and 20ng of pRL-TK (Promega) which served as an internal control. The cells were incubated for 24h, and were treated with or without 2μg/ml tunicamycin for 16h. Cells were harvested and lysed. Luciferase activity was determined using a dual-luciferase assay system (Promega). The experiment was performed in triplicate wells.

Quantification of apoptotic cells

293T cells in 12-well plates were transfected using Lipofectamine 3000 reagent (Life Technologies) following the manufacturer's instruction. Cells per well were transfected with 3μg of the expression plasmid of SARS-CoV Tor2 or SARSr-CoV Rs4084 ORF8a, or the empty vector. 24h post transfection, apoptotic cells were quantified by using the Annexin V-fluorescein isothiocyanate (FITC)/PI Apoptosis Detection Kit (Yeasen Biotech, Shanghai) in accordance with the manufacturer's instruction. Apoptosis was analyzed by flow cytometry. The experiment was performed in triplicate wells.

Accession numbers

The complete genome sequences of bat SARS-related coronavirus strains As6526, Rs4081, Rs4084, Rf4092, Rs4231, Rs4237, Rs4247, Rs4255, Rs4874, Rs7327 and Rs9401 have been deposited in the GenBank database with the accession numbers from KY417142 to KY417152, respectively.

Supporting information

S1 Fig. Alignment of amino acid sequences of the receptor-binding motif (corresponding to aa 424–495 of SARS-CoV S protein). Two clades of the SARSr-CoVs identified from bats in the studied cave are indicated with vertical lines on the left.
(PPTX)

S2 Fig. Alignment of nucleotide sequences of a genomic region covering ORF6 to ORF7a. ORFX is located between ORF6 and ORF7a in the genomes of WIV1, WIV16, Rs7327 and Rs4874. The start codon and stop codon of ORFX are marked with red boxes. The deletion responsible for the long ORFX in Rs7327 and Rs4874 is marked with the blue box.
(PPTX)

S3 Fig. Phylogenetic analyses based on nucleotide sequences of the S gene (A), ORF3a (B) and ORF8 (C). The trees were constructed by the maximum likelihood method using the LG model with bootstrap values determined by 1000 replicates. Only bootstraps > 50% are shown. Rs, *Rhinolophus sinicus*; Rf, *Rhinolophus ferremequinum*; Rm, *Rhinolophus macrotis*; Ra, *Rhinolophus affinis*; Rp, *Rhinolophus pusillus*; As, *Aselliscus stoliczkanus*; Cp, *Chaerephon plicata*. SARSr-CoVs detected in bats from the single cave surveyed in this study are in bold.
(PPTX)

S4 Fig. Alignment of amino acid sequences of ORF3b protein.
(PPTX)

S5 Fig. Detection of potential recombination events by similarity plot and boot scan analysis. (A) Full-length genome sequence of SARSr-CoV Rs4084 was used as query sequence and RsSHC014, Rf4092 and Rs4081 as reference sequences. (B) Full-length genome sequence of SARSr-CoV Rs4237 was used as query sequence and SARSr-CoV Rs4247, Rs4081 and Rs3367 as reference sequences. All analyses were performed with a Kimura model, a window size of 1500 base pairs, and a step size of 150 base pairs.
(PPTX)

S6 Fig. Chinese provinces where bat SARSr-CoVs have been detected.
(PPTX)

S7 Fig. The successful or failed rescue of the chimeric SARSr-CoVs. (A) Cytopathic effects in Vero E6 cells transfected with the infectious BAC clones constructed with the backbone of WIV1 and various S genes of different bat SARSr-CoV strains. Microphotographs were taken 24 hours post transfection. (B) The culture media supernatant collected from the cells transfected with the infectious BAC clones was used to infect Vero E6 cells. Immunofluorescent assay (IFA) was performed to detect infection and viral replication. Cells were fixed 24 hours post infection, and stained using rabbit antibody against the SARSr-CoV Rp3 nucleocapsid protein and a Cy3-conjugated anti-rabbit IgG.
(PPTX)

S8 Fig. Quantification of SARSr-CoV in individual bat fecal samples. The number of genome copies of SARSr-CoV per gram of bat feces was determined by quantitative real-time PCR targeting the RdRp gene. Samples from which the SARSr-CoV RBD sequences were successfully amplified are indicated in red.
(PPTX)

S9 Fig. Spike substitution strategy. The original fragments E and F were shortened to leave spike gene as an independent fragment. The new fragments were designated as Es and Fs. BsaI or BsmBI sites were introduced into the junctions of Es/Spike and Spike/Fs. Then any spike could be substituted into the genome of SARSr-CoV WIV1 through this strategy.
(TIF)

S1 Table. Comparison of the novel bat SARSr-CoVs identified in this study with human/civet SARS-CoVs and previously described bat SARSr-CoVs.
(DOCX)

S2 Table. Distribution of SARSr-CoVs highly similar to SARS-CoV in the variable S, ORF3 and ORF8 genes in the single cave.
(DOCX)

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S1 Dataset. Full-length genome sequences of bat SARSr-CoVs newly identified in this study.
(FAS)

Acknowledgments

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 21 Jul 2021 14:47:55 +0000
To: Melissa.Healy@latimes.com
Bcc: Greg Folkers (b) (6);Billet, Courtney (NIH/NIAID) [E]
Subject: FW: Interview request: The Los Angeles Times
Attachments: Holmes_SARS-CoV-2_Origins.pdf

Melissa:

I understand that you want to speak with me about yesterday's hearing and other issues (see below). I would be happy to chat with you. In case you have not seen this preprint paper from Holmes et al, I am attaching it. In addition, I will provide a link to the NIH official statement on Gain of Function research → https://www.nih.gov/news-events/gain-function-research-involving-potential-pandemic-pathogens

We can discuss these if you wish during our conversation. I will call you later.

Best regards,

Tony

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From: Conrad, Patricia (NIH/NIAID) [E] (b) (6)
Sent: Tuesday, July 20, 2021 4:59 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b) (6)
Subject: Fwd: Interview request: The Los Angeles Times

Sent from my iPhone

Begin forwarded message:

From: "Deatrick, Elizabeth (NIH/NIAID) [E]" (b) (6)
Date: July 20, 2021 at 4:53:46 PM EDT
To: "Conrad, Patricia (NIH/NIAID) [E]" (b) (6)
Cc: NIAID COGCORE <COGCORE@mail.nih.gov>, NIAID Media Inquiries <mediainquiries@niaid.nih.gov>, NIAID FOG <fog@niaid.nih.gov>
Subject: Interview request: The Los Angeles Times

Melissa Healy
The Los Angeles Times
Melissa.Healy@latimes.com
240-643-8919
Seeking: Comment from Dr. Fauci on EcoHealth, origins of SARS-CoV-2, etc.
Deadline: unknown; likely soon. Will update when available

Hi Patty,

This writer from the LA Times would like to write about today's hearing, and has questions about "the confabulation of two possibilities under investigation: 1) that the virus, collected by scientists from animals in the wild, may have escaped from the lab via an unplanned breach; and 2) the charge that scientists there genetically manipulated it before it escaped (or was released)." She is seeking additional comment from Dr. Fauci about his statement that it is "molecularly impossible" that U.S.-funded research could been involved in the origins of SARS-CoV-2. She would also like him to state his current opinion on the "origin of the SARS-CoV-2 virus and the possibility that it may have been under the purview at some point of WIV scientists working under an NIH-research grant to EcoHealth."

Would Dr. Fauci be interested in speaking with her?

Best,
Elizabeth Deatrick
Technical Writer-Editor
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
(b) (6)

Please note that I am not a spokesperson for NIAID and should not be quoted as such.

The Origins of SARS-CoV-2: A Critical Review

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Since the first reports of a novel SARS-like coronavirus in December 2019 in Wuhan, China, there has been intense interest in understanding how SARS-CoV-2 emerged in the human population. Recent debate has coalesced around two competing ideas: a "laboratory escape" scenario and zoonotic emergence. Here, we critically review the current scientific evidence that may help clarify the origin of SARS-CoV-2.

Evidence supporting a zoonotic origin of SARS-CoV-2

Coronaviruses have long been known to present pandemic risks. SARS-CoV-2 is the ninth documented coronavirus that infects humans and the seventh identified in the last 20 years[1,2]. All previous human coronaviruses have zoonotic origins, as have the vast majority of human viruses. The emergence of SARS-CoV-2 bears several signatures of these prior zoonotic events. It displays clear similarities to SARS-CoV that spilled over into humans in Foshan, Guangdong province, China in November 2002, and again in Guangzhou, Guangdong province in 2003[3]. Both these SARS-CoV emergence events were associated with markets selling live animals and involved species, particularly civets and raccoon dogs[4], that were also sold live in Wuhan markets in 2019[5] and are known to be susceptible to SARS-CoV-2 infection[6]. Animal traders working in 2003, without a SARS diagnosis, were documented to have high levels of IgG to SARS-CoV (13% overall and >50% for traders specializing in civets[7]). Subsequent serological surveys found ~3% positivity rates to SARS-CoV related (SARSr-CoV) viruses in residents of Yunnan province living close to bat caves[8], demonstrating regular exposure in rural locations. The closest known relatives to both SARS-CoV and SARS-CoV-2 are viruses from bats in Yunnan, although animals from this province have been preferentially sampled. For both SARS-CoV and SARS-CoV-2, there is a considerable geographic gap between Yunnan and the location of the first human cases, highlighting the difficulty in identifying the exact pathway of virus emergence and the importance of sampling beyond Yunnan.

SARS-CoV-2 also shows similarities to the four endemic human coronaviruses: HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63. These viruses have zoonotic origins and the circumstances of their emergence are unclear. In direct parallel to SARS-CoV-2, HCoV-HKU1, which was first described in a large Chinese city (Shenzhen, Guangdong) in the winter of 2004, has an unknown animal origin, contains a furin cleavage site in its spike protein, and was originally identified in a case of human pneumonia[9].

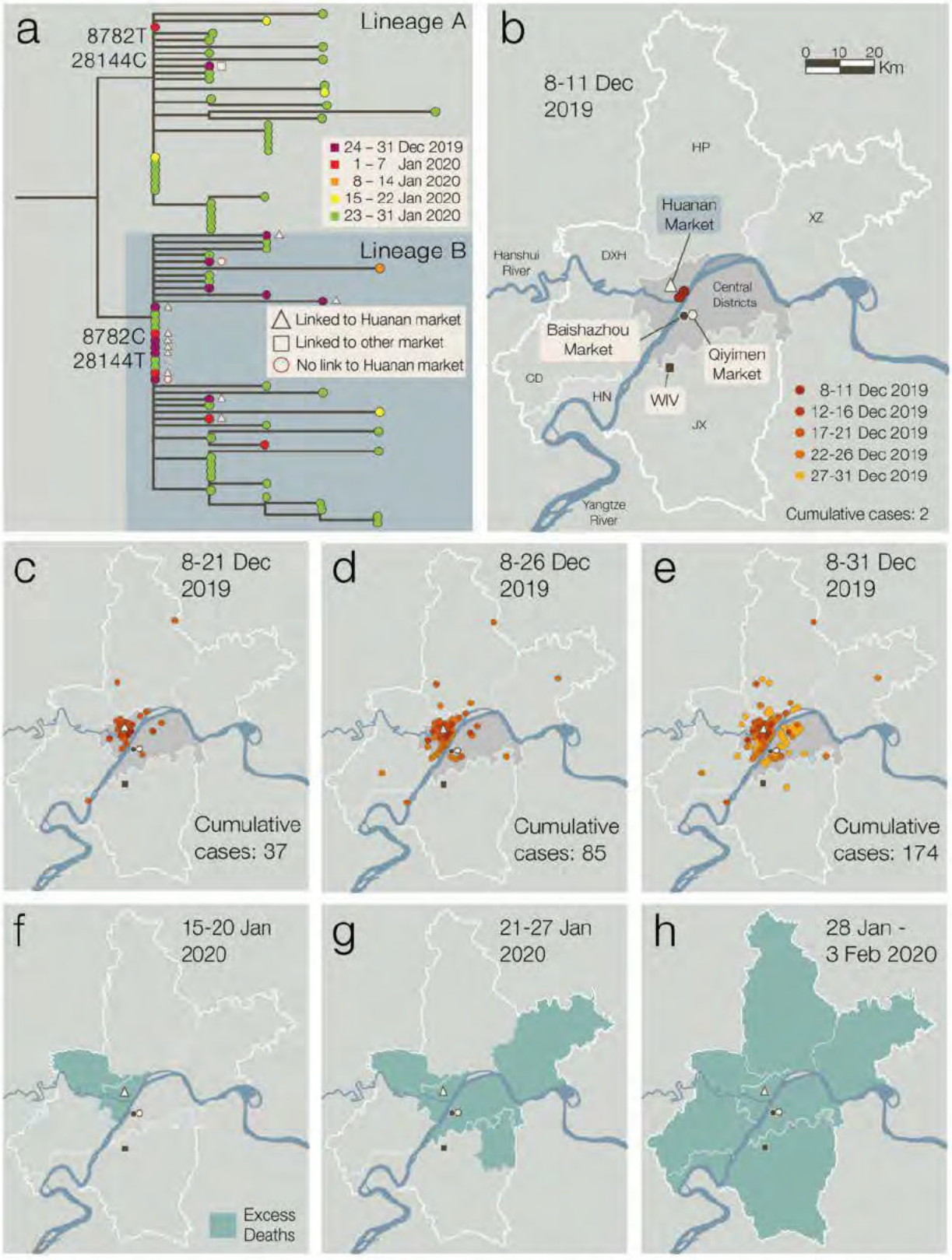

Figure 1 | Phylogenetic and epidemiological data on the early COVID-19 pandemic in Wuhan.

(**a**) Phylogenetic tree of early SARS-CoV-2 genomes sampled from Wuhan during December 2019-

January 2020. The split between lineages A and B is labelled with the coordinates and base of the two differentiating nucleotide mutations. Cases with a known association to the Huanan or other markets are denoted by symbols (reported in ref. 10). (**b**) Map of districts of Wuhan showing the location of markets, the BSL-4 campus of the Wuhan Institute of Virology (where the coronavirus work of Dr. Shi Zhengli is performed) and the earliest known cases. (**c-e**) Location of recorded COVID-19 cases in Wuhan from 8th December to 31st December 2019. Cases with a home address outside of Wuhan city are not shown. (**f-h**) Map of districts of Wuhan indicating the first record of excess deaths due to pneumonia (shaded green) from 15th January 2020. Case and excess death data were extracted and redrawn from figures provided in ref 10. For more details see **supplementary information**.

Based on epidemiological data, the Huanan market in Wuhan was an early and major epicenter of SARS-CoV-2 infection. Two of the three earliest documented COVID-19 cases were directly linked to this market selling wild animals, as were 28% of all cases reported in December 2019[10]. Overall, 55% of cases during December 2019 had an exposure to either the Huanan or other markets in Wuhan, with these cases more prevalent in the first half of that month[10]. Examination of the locations of early cases shows that most cluster around the Huanan market, located north of the Yangtze river (**Fig. 1a-e**). These districts were also the first to exhibit excess pneumonia deaths in January 2020 (**Fig. 1f-h**). There is no epidemiological link to any other locality in Wuhan, including the BSL-4 campus of the Wuhan Institute of Virology (WIV) located south of the Yangtze and the subject of considerable speculation. Although some early cases do not have a direct epidemiological link to a market[10], this is expected given high rates of asymptomatic transmission and undocumented secondary transmission events, and was similarly observed in early SARS-CoV cases in Foshan[3].

During 2019, markets in Wuhan – including the Huanan market – traded many thousands of live wild animals including high-risk species such as civets and raccoon dogs[5]. Following its closure, SARS-CoV-2 was detected in environmental samples at the Huanan market, primarily in the western section that traded in wildlife and domestic animal products, as well as in associated drainage areas[10]. While animal carcasses retrospectively tested negative for SARS-CoV-2, these were unrepresentative of the live animal species sold, and specifically did not include raccoon dogs and other animals known to be susceptible to SARS-CoV-2[5].

The earliest split in the SARS-CoV-2 phylogeny defines two lineages - denoted A and B[11] - that likely circulated contemporaneously (**Fig. 1a**). Lineage B, which became dominant globally, was observed in early cases linked to the Huanan market and environmental samples taken there, while lineage A contains a case with exposure to other markets (**Fig. 1a,b**) as well as with later cases in Wuhan and other parts of China[10]. This phylogenetic pattern is consistent with the emergence of SARS-CoV-2 involving one or more contacts with infected animals and/or traders, including multiple spill-over events, as potentially infected or susceptible animals were moved into or between Wuhan markets via shared supply chains and sold for human consumption[5]. The potential emergence of SARS-CoV-2 across multiple markets again mirrors SARS-CoV in which high levels of infection, seroprevalence and genetic diversity in animals were documented at both the Dongmen market in Shenzhen[4,12] and the Xinyuan market in Guangzhou[13,14].

Viruses closely related to SARS-CoV-2 have been documented in bats and pangolins in multiple localities in South-East Asia, including in China, Thailand, Cambodia, and Japan[15,16], with serological evidence for viral infection in pangolins for more than a decade[17]. However, a significant evolutionary gap exists between SARS-CoV-2 and the closest related animal viruses: their genetic distances of approximately 4% (~1,150 mutations) equates to decades of evolutionary divergence[18]. Widespread genomic recombination also complicates the assignment of which viruses are closest to SARS-CoV-2. Although *Rhinolophus* bat virus RaTG13 collected in Yunnan has the highest average genetic similarity to SARS-CoV-2, a history of recombination means that three other bat viruses – RmYN02, RpYN06 and PrC31 – are closer in most of the virus genome (particularly ORF1ab) and thus share a more recent common ancestor with SARS-CoV-2[15,16,19]. None of these closer viruses were collected by the WIV. This demonstrates beyond reasonable doubt that RaTG13 is not the progenitor of SARS-CoV-2, with or without laboratory manipulation or experimental mutagenesis.

Although no bat reservoir nor intermediate animal host for SARS-CoV-2 has been identified to date, initial cross-species transmission events are very likely to go undetected. Most SARS-CoV-2 index case infections are unlikely to have resulted in sustained onward transmission[20] and only a very small subset of spillover events from animals to humans result in major outbreaks. Indeed, the animal origins of many well-known human pathogens, including Ebola virus, Hepatitis C virus, poliovirus, and the coronaviruses HCoV-HKU1 and HCoV-NL63, are yet to be identified,

while it took over a decade to discover bat viruses with >95% similarity to SARS-CoV and able to use hACE-2 as a receptor[21].

Could SARS-CoV-2 have escaped from a laboratory?

There are precedents for laboratory incidents leading to isolated infections and transient transmission chains, including SARS-CoV[22]. Aside from the 1977 A/H1N1 influenza pandemic that likely originated from a large-scale vaccine challenge trial[23], there are no documented examples of human epidemics or pandemics resulting from research activity.

The emergence of SARS-CoV-2 differs markedly from documented laboratory escapes that, with the exception of Marburg virus[24], have been of readily identifiable viruses capable of human infection and associated with sustained work in high titer cultures[25–27]. No previous epidemic has been caused by the escape of a novel virus and there is no data to suggest that the WIV—or any other laboratory—were working on SARS-CoV-2, or any virus close enough to be the progenitor, prior to the COVID-19 pandemic. Viral genomic sequencing without cell culture, which was routinely performed at the WIV, represents a negligible risk as viruses are inactivated during RNA extraction[28] and no case of laboratory escape has been documented following the sequencing of viral samples.

Known laboratory outbreaks have been traced to both workplace and family contacts of index cases and to the laboratory of origin[25–27,24]. Despite extensive contact tracing of early cases during the COVID-19 pandemic, there have been no reported cases related to any laboratory staff at the WIV and all staff in the laboratory of Dr. Shi Zhengli were reported to be seronegative for SARS-CoV-2 when tested in March 2020[10]. During a period of high influenza transmission and other respiratory virus circulation[29] reports of illnesses would need to be confirmed as caused by SARS-CoV-2 to be relevant. Epidemiological modeling suggests that the number of hypothetical cases needed to result in multiple hospitalized COVID-19 patients prior to December 2019 is incompatible with observed clinical, genomic, and epidemiological data[20].

The WIV possesses an extensive catalogue of samples derived from bats and has reportedly successfully cultured three SARSr-CoVs from bats, all of which are genetically distinct from SARS-CoV-2[30–32]. These viruses were isolated from fecal samples through serial amplification in

VeroE6 cells, a process that consistently results in the loss of the SARS-CoV-2 furin cleavage site[33–39]. It is therefore highly unlikely that these techniques would result in the isolation of a SARS-CoV-2 progenitor with an intact furin cleavage site. No published work indicates that other methods, including the generation of novel reverse genetics systems, were used at the WIV to propagate infectious SARSr-CoVs based on sequence data from bats. Gain-of-function research would be expected to utilize an established SARSr-CoV genomic backbone, or at a minimum a virus previously identified via sequencing. However, past experimental research using recombinant coronaviruses at the WIV has used a genetic backbone (WIV1) unrelated to SARS-CoV-2[32] and SARS-CoV-2 carries no evidence of genetic markers one might expect from laboratory experiments[40]. There is no rational experimental reason why a new genetic system would be developed using an unknown and unpublished virus, with no evidence nor mention of a SARS-CoV-2-like virus in any prior publication or study from the WIV[32,41,42], no evidence that the WIV sequenced a virus that is closer to SARS-CoV-2 than RaTG13, and no reason to hide research on a SARS-CoV-2-like virus prior to the COVID-19 pandemic. Under any laboratory escape scenario SARS-CoV-2 would have to have been present in a laboratory prior to the pandemic, yet no evidence exists to support such a notion and no sequence has been identified that could have served as a precursor.

A specific laboratory escape scenario involves accidental infection in the course of serial passage of a SARSr-CoV in common laboratory animals such as mice. However, early SARS-CoV-2 isolates were unable to infect wild-type mice[43]. While murine models are useful for studying infection *in vivo* and testing vaccines, they often result in mild or atypical disease[44–48]. These findings are inconsistent with a virus selected for increased pathogenicity and transmissibility through serial passage through rodents. Although SARS-CoV-2 has since been engineered[49] and adapted by serial passage[50–52], specific mutations in the spike protein, including N501Y, are necessary for such adaptation in mice[51,52]. Notably, N501Y has arisen convergently in multiple SARS-CoV-2 variants of concern in the human population, presumably being selected to increase ACE2 binding affinity[53–56]. If SARS-CoV-2 resulted from attempts to adapt a SARSr-CoV for study in animal models, it would likely have acquired mutations like N501Y for efficient replication in that model, yet there is no evidence to suggest such mutations existed early in the pandemic. Both the low pathogenicity in commonly used laboratory animals and the absence of genomic markers associated with rodent adaptation indicate that SARS-

CoV-2 is highly unlikely to have been acquired by laboratory workers in the course of viral pathogenesis or gain-of-function experiments.

Evidence from genomic structure and ongoing evolution of SARS-CoV-2

Considerable attention has been devoted to claims that SARS-CoV-2 was genetically engineered or adapted in cell culture or "humanized" animal models to promote human transmission[57]. Yet, since its emergence, SARS-CoV-2 has experienced repeated sweeps of mutations that have increased viral fitness[58,59]. The first clear adaptive mutation, the D614G substitution in the spike protein, occurred early in the pandemic[60,61]. Recurring mutations in the receptor binding domain of the spike protein, including N501Y, K417N/T, L452R, and E484K/Q—constituent mutations of the variants of concern—similarly enhance viral infectivity[54,55,62] and ACE2 binding[53,63], refuting claims that the SARS-CoV-2 spike protein was optimized for binding to human ACE2 upon its emergence[56]. Further, some pangolin-derived coronaviruses have receptor binding domains that are near-identical to SARS-CoV-2 at the amino acid level[40,64] and bind to human ACE2 even more strongly than SARS-CoV-2, showing that there is capacity for further human adaptation[65]. SARS-CoV-2 is also notable for being a host generalist virus[66], capable of efficient transmission in multiple mammalian species, including mink, tigers, cats, gorillas, dogs, raccoon dogs, ferrets, and large outbreaks have been documented in mink with spill-back to humans[67] and to other animals[68]. Combined, these findings show that no specific human "pre" adaptation was required for the emergence or early spread of SARS-CoV-2, and the claim that the virus was already highly adapted to the human host[57], or somehow optimized for binding to human ACE2, is without validity.

The genesis of the polybasic (furin) cleavage site in the spike protein of SARS-CoV-2 has been subject to recurrent speculation. Although the furin cleavage site is absent from the closest known relatives of SARS-CoV-2[40], this is unsurprising as the lineage leading to this virus is poorly sampled and the closest bat viruses have divergent spike proteins due to recombination[15,16,18]. Furin cleavage sites are commonplace in other coronavirus spike proteins, including some feline alphacoronaviruses, MERS-CoV, most but not all strains of mouse hepatitis virus, as well as in endemic human betacoronaviruses such as HCoV-OC43 and HCoV-HKU1[69–71]. A near identical nucleotide sequence is found in the spike gene of the bat coronavirus HKU9-1[72], and both SARS-CoV-2 and HKU9-1 contain short palindromic sequences immediately upstream of this sequence that are indicative of natural recombination break-points via template switching[72]. Hence, simple

evolutionary mechanisms can readily explain the evolution of an out-of-frame insertion of a furin cleavage site in SARS-CoV-2 (**Fig. 2**).

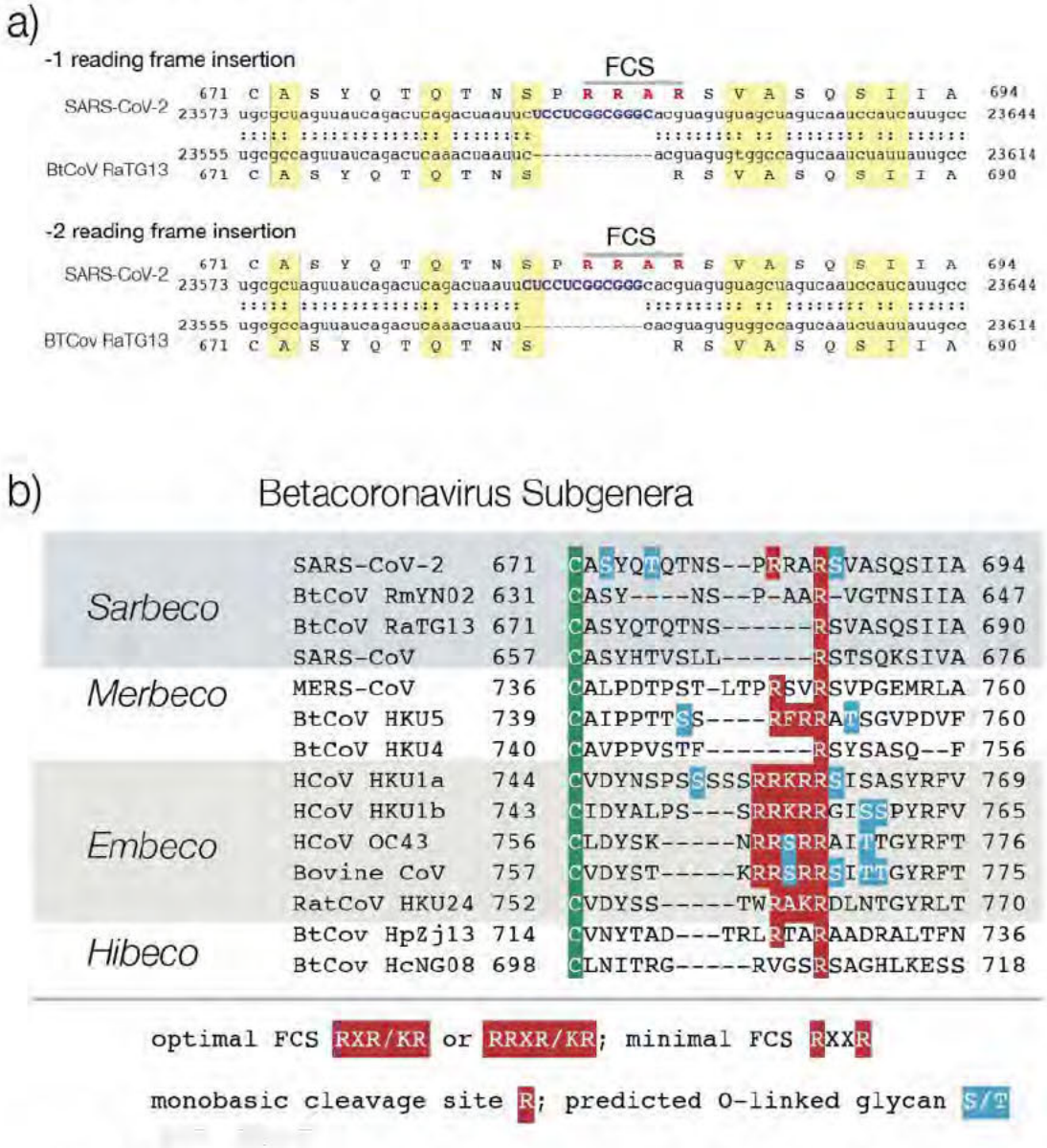

Figure 2 | Evolution of the furin cleavage site (FCS) in the spike protein of betacoronaviruses. (**a**) Sequence alignment of the region around the FCS in SARS-CoV-2 (NCBI accession MN908947) and bat coronavirus RaTG13 (NCBI accession MN996532) showing that the former was the result of an out-of-frame nucleotide sequence insertion. (**b**) Amino acid sequence alignment of the FCS region in representative members of the different subgenera of betacoronaviruses, highlighting the evolutionary volatility of this site and that the relevant amino acid motif (RRAR) in SARS-CoV-2 is functionally suboptimal. The residues predicted to be O-linked glycans are also marked. For more details see **supplementary information**.

The SARS-CoV-2 furin cleavage site (containing the amino acid motif RRAR) does not match its canonical form (R-X-R/K-R), is suboptimal compared to those of HCoV-HKU1 and HCoV-OC43, lacks either a P1 or P2 arginine (depending on the alignment), and was caused by an out-of-frame insertion (**Fig. 2**). The RRAR and RRSR S1/S2 cleavage sites in feline coronaviruses (FCoV) and cell-culture adapted HCoV-OC43, respectively, are not cleaved by furin[69]. There is no logical reason why an engineered virus would utilize such a poor furin cleavage site, which would entail such an unusual and needlessly complex feat of genetic engineering. The only previous studies of artificial insertion of a furin cleavage site at the S1/S2 boundary in the SARS-CoV spike protein utilized an optimal 'RRSRR' sequence in pseudotype systems[73,74]. Further, there is no evidence of prior research at the WIV involving the artificial insertion of complete furin cleavage sites into coronaviruses.

The recurring P681H/R substitution in the proline (P) residue preceding the SARS-CoV-2 furin cleavage site improves cleavage of the spike protein and is another signature of ongoing human adaptation of the virus[75]. The SARS-CoV-2 furin site is also lost under standard cell culture conditions[34,76], as is true of HCoV-OC43[73]. The presence of two CGG codons for arginines in the SARS-CoV-2 furin cleavage site is similarly not indicative of genetic engineering[77]. Although the CGG codon is rare in coronaviruses, it is observed in SARS-CoV, SARS-CoV-2 and other human coronaviruses at comparable frequencies[77]. Further, if low-fitness codons had been artificially inserted into the virus genome they would have been quickly selected against during SARS-CoV-2 evolution, yet both CGG codons are more than 99.8% conserved among the >1,800,000 near-complete SARS-CoV-2 genomes sequenced to date, indicative of strong functional constraints (**supplementary information, Table S1**).

Conclusions

As for the vast majority of human viruses, the most parsimonious explanation for the origin of SARS-CoV-2 is a zoonotic event. The documented epidemiological history of the virus is comparable to previous animal market-associated outbreaks of coronaviruses with a simple route for human exposure. The contact tracing of SARS-CoV-2 to markets in Wuhan exhibits striking similarities to the early spread of SARS-CoV to markets in Guangdong, where humans infected early in the epidemic lived near or worked in animal markets. Zoonotic spillover by definition selects for viruses able to infect humans. The laboratory escapes documented to date

have almost exclusively involved viruses brought into laboratories specifically because of their known human infectivity.

There is currently no evidence that SARS-CoV-2 has a laboratory origin. There is no evidence that any early cases had any connection to the WIV, in contrast to the clear epidemiological links to animal markets in Wuhan, nor evidence that the WIV possessed or worked on a progenitor of SARS-CoV-2 prior to the pandemic. The suspicion that SARS-CoV-2 might have a laboratory origin stems from the coincidence that it was first detected in a city that houses a major virological laboratory that studies coronaviruses. Wuhan is the largest city in central China with multiple animal markets and is a major hub for travel and commerce, well connected to other areas both within China and internationally. The link to Wuhan therefore more likely reflects the fact that pathogens often require heavily populated areas to become established[20].

We contend that there is substantial body of scientific evidence supporting a zoonotic origin for SARS-CoV-2. While the possibility of a laboratory accident cannot be entirely dismissed, and may be near impossible to falsify, this conduit for emergence is highly unlikely relative to the numerous and repeated human-animal contacts that occur routinely in the wildlife trade. Failure to comprehensively investigate the zoonotic origin through collaborative and carefully coordinated studies would leave the world vulnerable to future pandemics arising from the same human activities that have repeatedly put us on a collision course with novel viruses.

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From: Mulach, Barbara (NIH/NIAID) [E]
Sent: Tue, 14 Apr 2020 10:59:35 -0400
To: Mulach, Barbara (NIH/NIAID) [E]
Subject: FW: Urgent for Dr. Fauci: China's lab for studying SARS and Ebola is in Wuhan, the outbreak's center
Attachments: Daszak Wuhan Exceprts.docx

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Thursday, January 23, 2020 9:13 PM
To: Mulach, Barbara (NIH/NIAID) [E] (b) (6); Embry, Alan (NIH/NIAID) [E] (b) (6); Bryant, Paula (NIH/NIAID) [E] (b) (6); Hewitt, Judith (NIH/NIAID) [E] (b) (6)
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; Erbelding, Emily (NIH/NIAID) [E] (b) (6); Cassetti, Cristina (NIH/NIAID) [E] (b) (6); Post, Diane (NIH/NIAID) [E] (b) (6); Degrace, Marciela (NIH/NIAID) [E] (b) (6)
Subject: RE: Urgent for Dr. Fauci: China's lab for studying SARS and Ebola is in Wuhan, the outbreak's center

Hi Barbara,
Nice talking with you a little while ago. As promised, I excepted the major portions of the Daszak award that detail the work there. (b) (5)
Hopefully this is helpful. Let me know if there is anything else you need. I'm in (b) (6) at a site visit, so if I don't respond by email quickly you can also reach me via text/call my mobile (b) (6)).

Erik

From: Mulach, Barbara (NIH/NIAID) [E] (b) (6)
Sent: Thursday, January 23, 2020 8:34 PM
To: Embry, Alan (NIH/NIAID) [E] (b) (6); Stemmy, Erik (NIH/NIAID) [E] (b) (6); Bryant, Paula (NIH/NIAID) [E] (b) (6) Hewitt, Judith (NIH/NIAID) [E] (b) (6)
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; Erbelding, Emily (NIH/NIAID) [E] (b) (6); Cassetti, Cristina (NIH/NIAID) [E] (b) (6) Post, Diane (NIH/NIAID) [E] (b) (6); Degrace, Marciela (NIH/NIAID) [E] (b) (6)
Subject: RE: Urgent for Dr. Fauci: China's lab for studying SARS and Ebola is in Wuhan, the outbreak's center

Melinda forwarded this:
https://www.niaid.nih.gov/news-events/new-coronavirus-emerges-bats-china-devastates-young-swine

Grant: R01 AI119064
PI: Peter Daszak
Title: Understanding the Risk of Bat Coronavirus Emergence
Institution: ECOHEALTH ALLIANCE, INC.

2 R01 AI110964-06	2019	Snap Abs Rev SS NoA Acctg Hist **el** **MG** Docs GF PUB Like FACTS Who HSS	DASZAK, PETER	Understanding the Risk of Bat Coronavirus Emergence

-----Original Message-----
From: Embry, Alan (NIH/NIAID) [E] (b) (6)
Sent: Thursday, January 23, 2020 8:31 PM
To: Mulach, Barbara (NIH/NIAID) [E] (b) (6); Stemmy, Erik (NIH/NIAID) [E] (b) (6)>; Bryant, Paula (NIH/NIAID) [E] (b) (6); Hewitt, Judith (NIH/NIAID) [E] < (b) (6)
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; Erbelding, Emily (NIH/NIAID) [E] (b) (6) Cassetti, Cristina (NIH/NIAID) [E] (b) (6)>; Post, Diane (NIH/NIAID) [E] (b) (6); Degrace, Marciela (NIH/NIAID) [E] (b) (6)
Subject: RE: Urgent for Dr. Fauci: China's lab for studying SARS and Ebola is in Wuhan, the outbreak's center

Received. I am not aware of any support to this lab. Will check with everyone to be sure.

-----Original Message-----
From: Mulach, Barbara (NIH/NIAID) [E] (b) (6)
Sent: Thursday, January 23, 2020 8:25 PM
To: Embry, Alan (NIH/NIAID) [E] (b) (6); Stemmy, Erik (NIH/NIAID) [E] (b) (6) Bryant, Paula (NIH/NIAID) [E] <p (b) (6); Hewitt, Judith (NIH/NIAID) [E] (b) (6)
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; Erbelding, Emily (NIH/NIAID) [E] (b) (6) Cassetti, Cristina (NIH/NIAID) [E] (b) (6)
Subject: FW: Urgent for Dr. Fauci: China's lab for studying SARS and Ebola is in Wuhan, the outbreak's center

HI Alan, Erik, Paula, and Judy,

See request below. Dr. Fauci needs to know the exact nature of any NIAID support to the Wuhan Institute of Virology/Biosafety Lab for a meeting with multiple Senators on Friday morning.

See background information in this article: https://www.dailymail.co.uk/health/article-7922379/Chinas-lab-studying-SARS-Ebola-Wuhan-outbreaks-center.html

If you have any information, please share as soon as possible.

Thanks!
Barbara

-----Original Message-----
From: Haskins, Melinda (NIH/NIAID) [E] (b) (6)

Sent: Thursday, January 23, 2020 8:18 PM
To: NIAID BUGS <BUGS@niaid.nih.gov>; Handley, Gray (NIH/NIAID) [E] (b) (6)
Erbelding, Emily (NIH/NIAID) [E] (b) (6)
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Urgent for Dr. Fauci: China's lab for studying SARS and Ebola is in Wuhan, the outbreak's center

https://www.dailymail.co.uk/health/article-7922379/Chinas-lab-studying-SARS-Ebola-Wuhan-outbreaks-center.html

Colleagues,

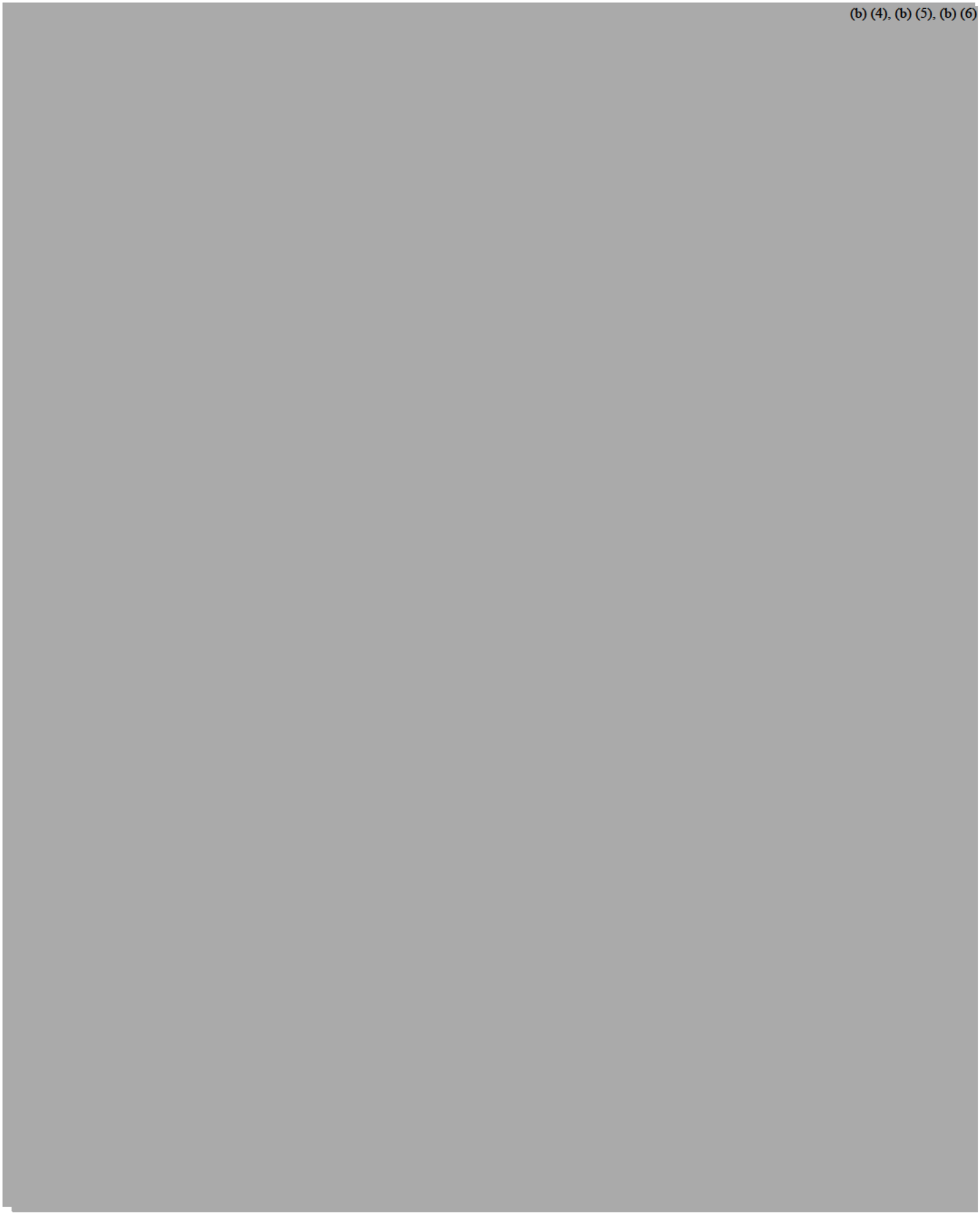
Dr. Fauci will be brief multiple Senators tomorrow morning on our novel coronavirus response at the request of Senator Lamar Alexander, who has an interest in public health matters and China. Would you please confirm the exact nature or our support to the Wuhan Institute of Virology/Biosafety Lab. You'll want to read the Daily Mail article above.

Thanks for the quick response!

Melinda

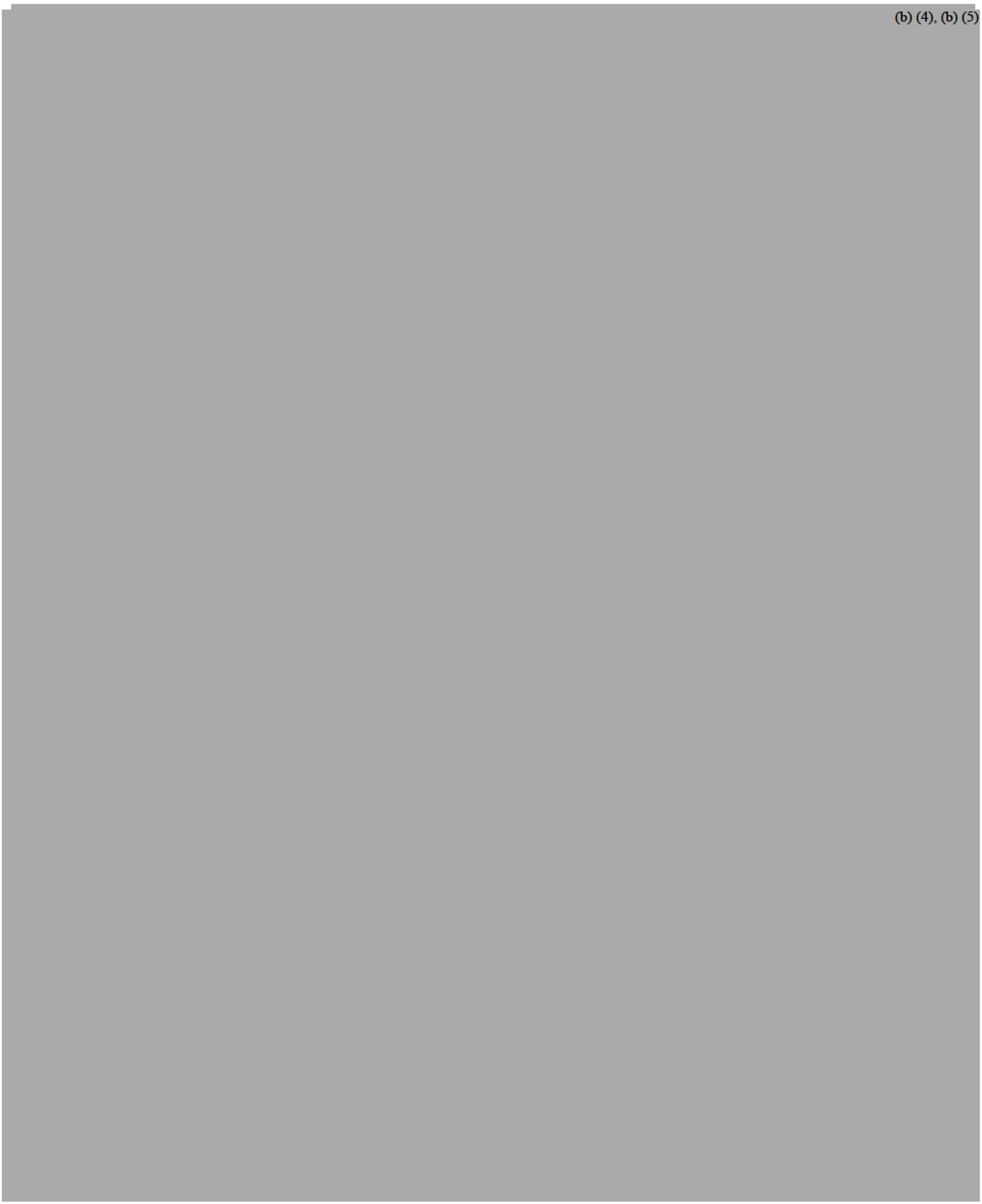
Sent from my iPhone

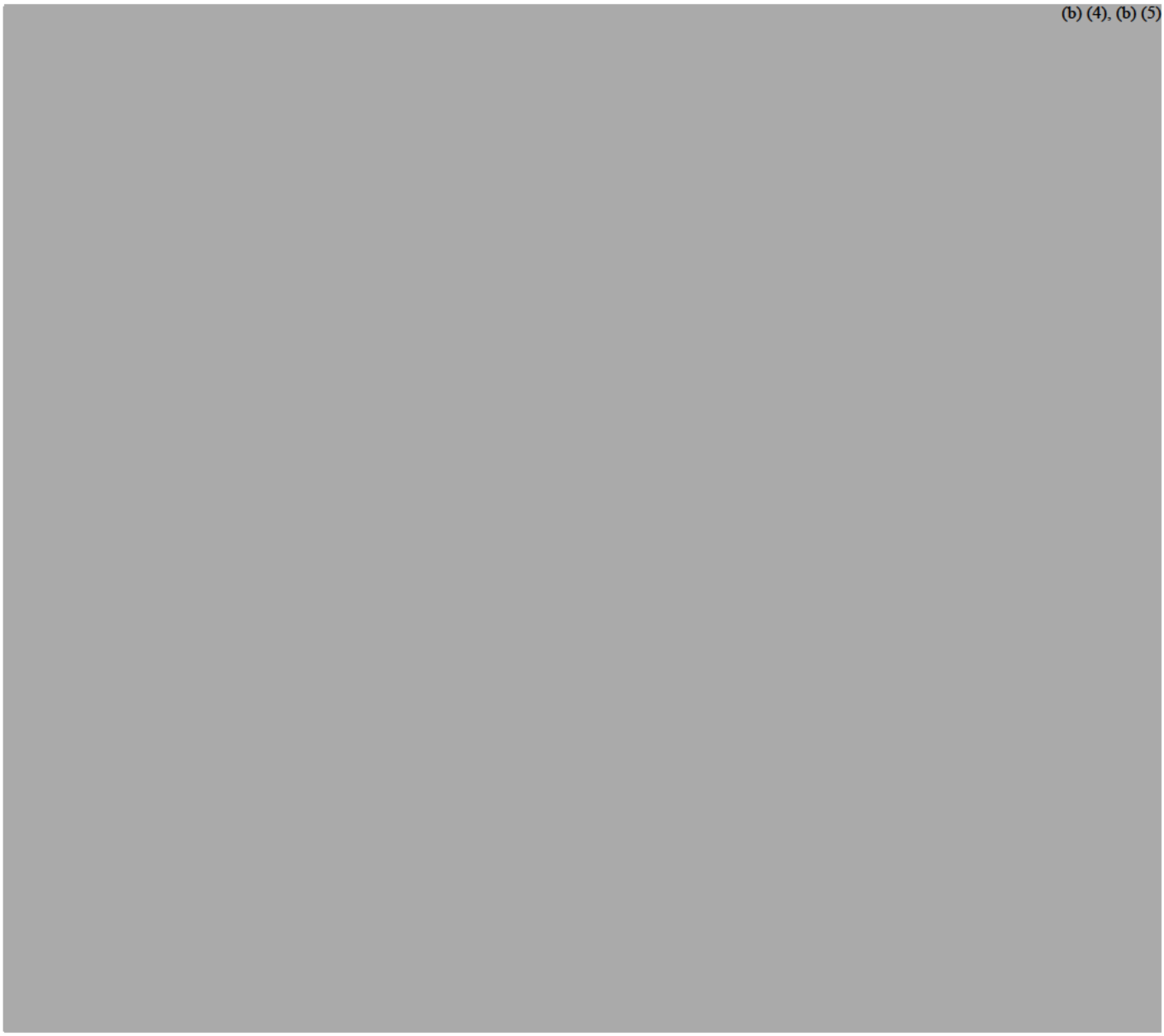
(b) (4), (b) (5)

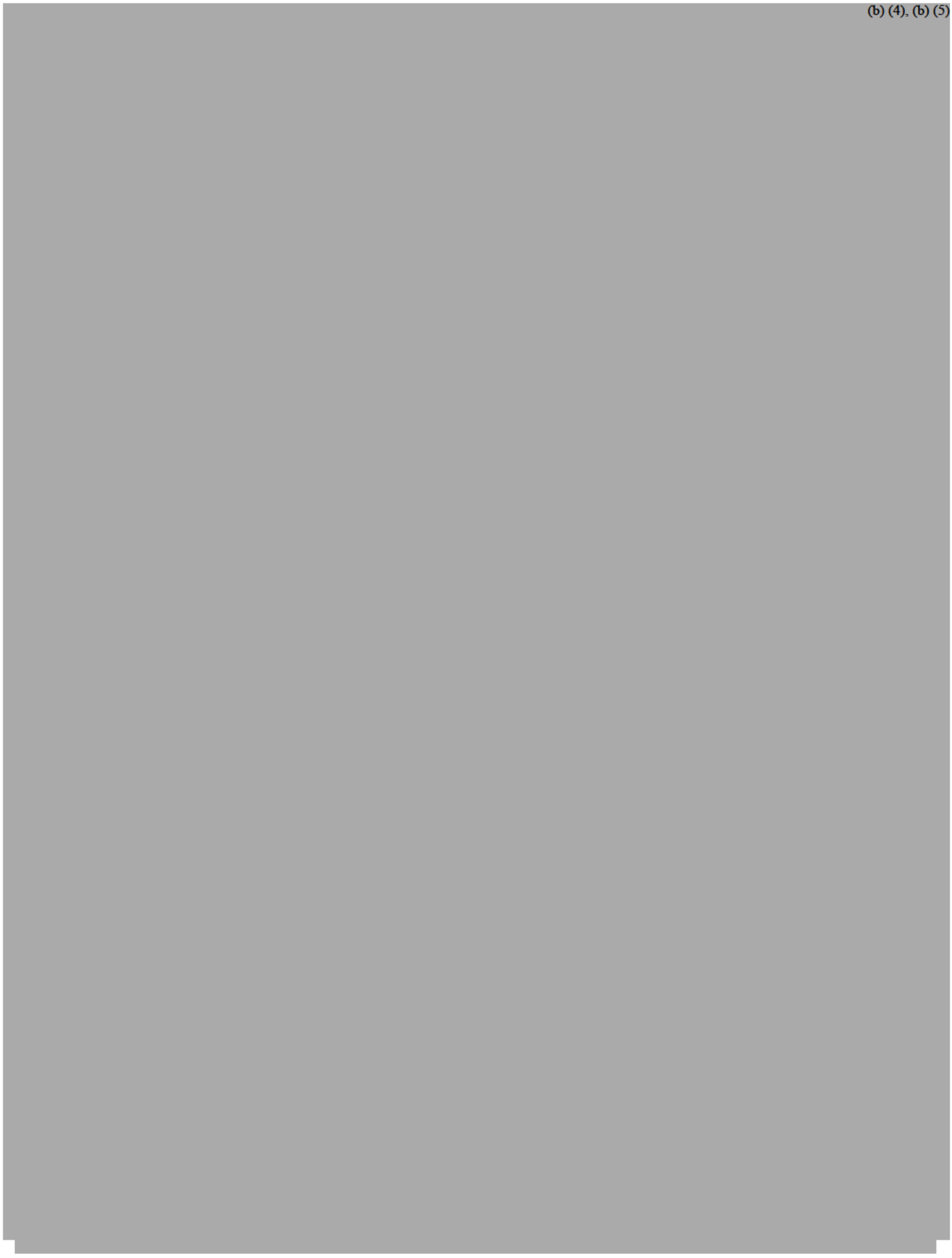


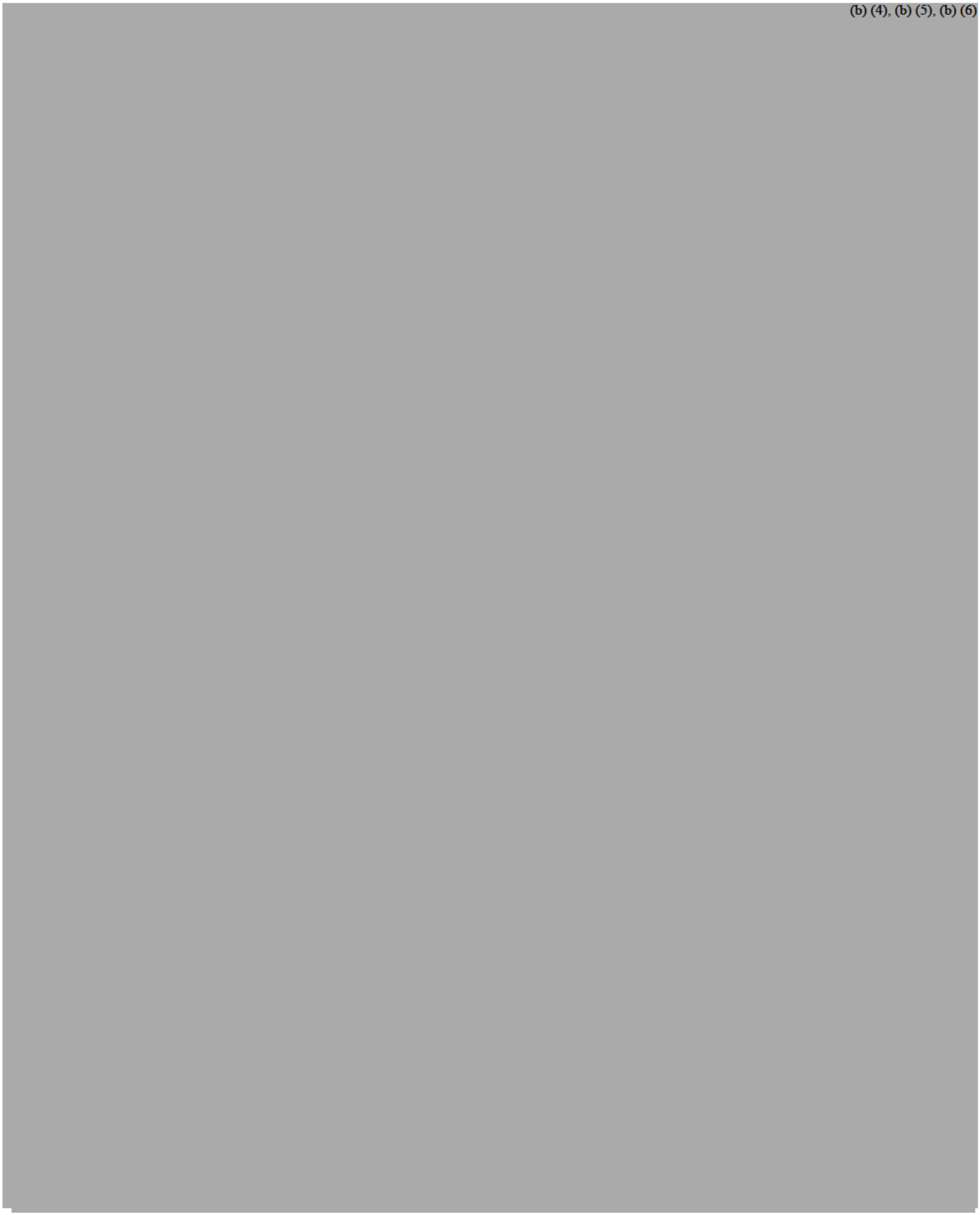

(b) (5)

[redacted]

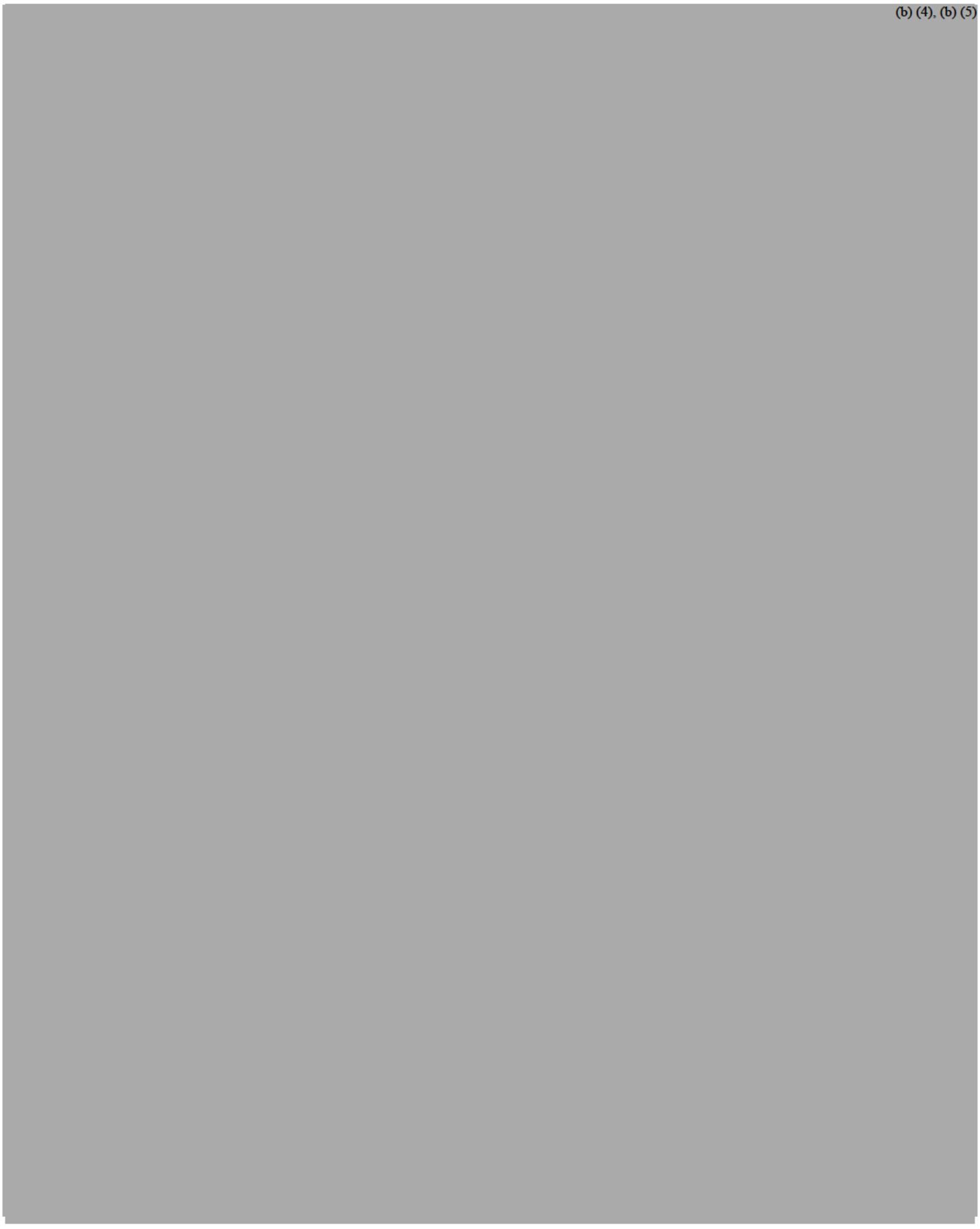


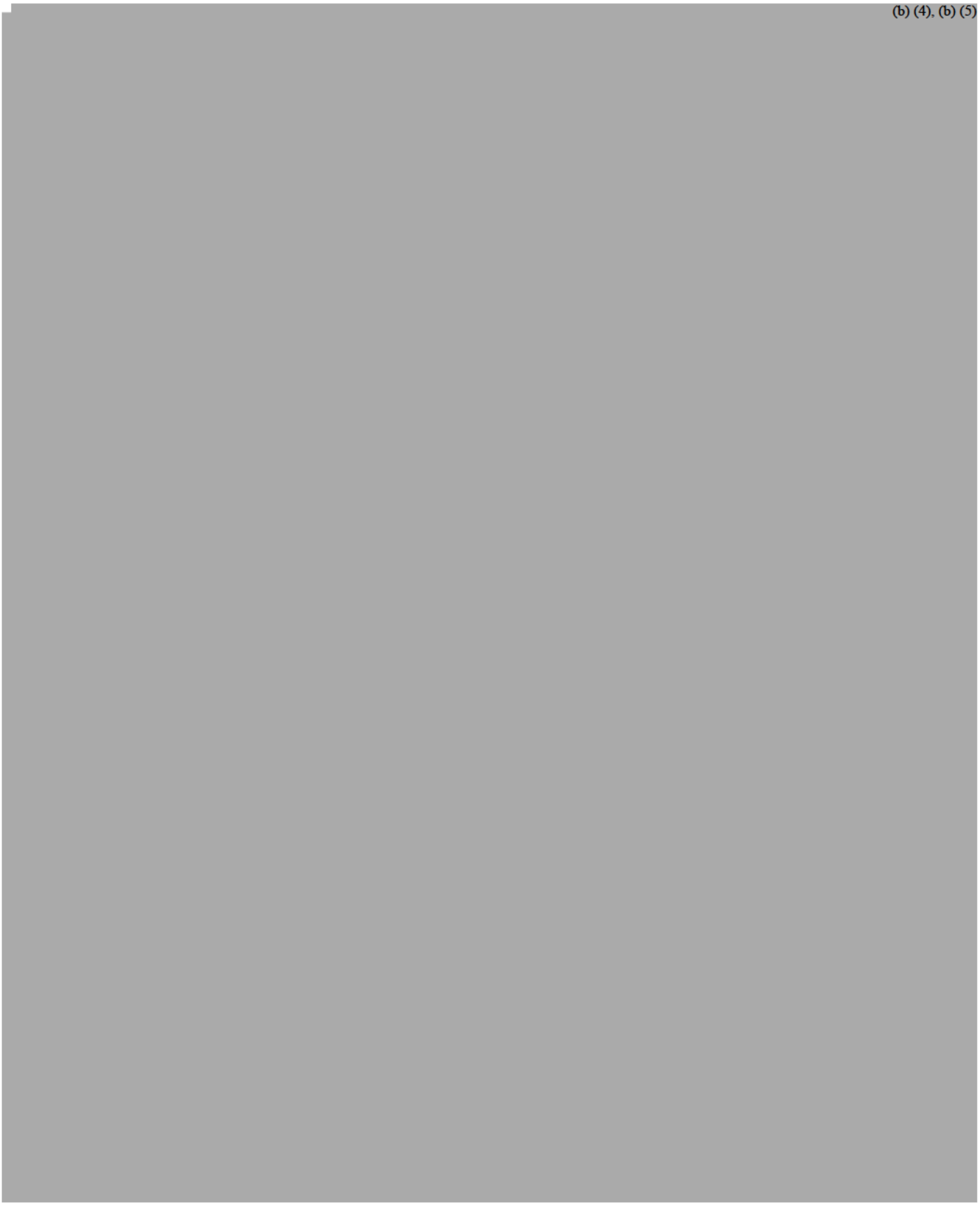

(b) (4), (b) (5)

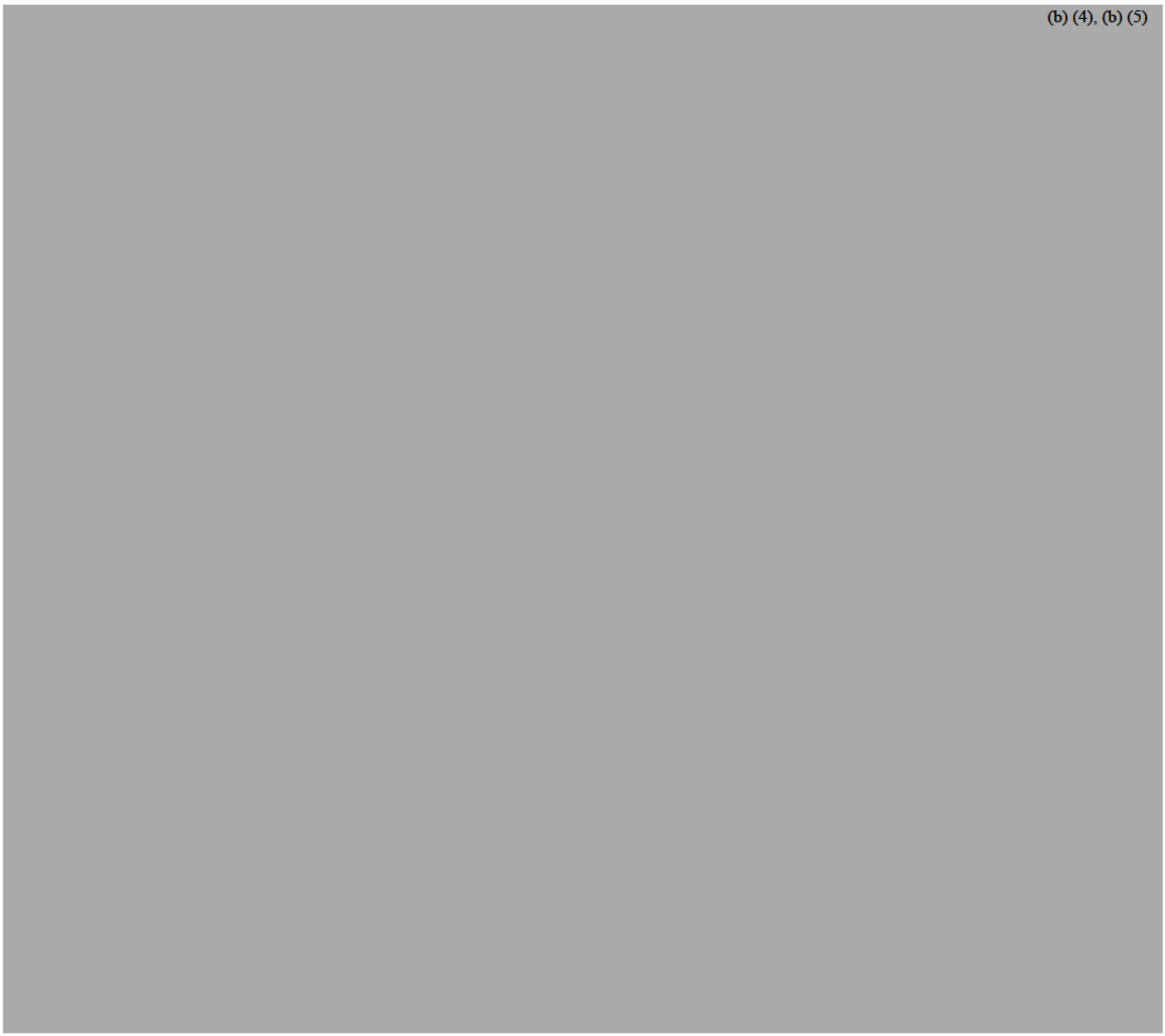





(b) (5)




(b) (4), (b) (5)


(b) (4), (b) (5)

Program Details

Project Title:
ECOHEALTH ALLIANCE, INC. (NEW YORK, UNITED STATES)

Director:
DASZAK, PETER

Start Date: 2014/08/01
End Date: 2019/07/31

Funding Organization: NIH

novel CoVs and host receptor binding domain genes, mathematical models of transmission and evolution, and in vitro and in vivo laboratory studies of host range. Zoonotic CoVs are a significant threat to global health, as demonstrated with the emergence of pandemic severe acute respiratory syndrome coronavirus (SARS-CoV) in China in 2002, and the recent and ongoing emergence of Middle East Respiratory Syndrome (MERS-CoV). Bats appear to be the natural reservoir of these viruses, and hundreds of novel bat-CoVs have been discovered in the last two decades. Bats, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a large scale human-wildlife interface, and high risk of future emergence of novel CoVs. This project aims to understand what factors increase the risk of the next CoV emerging in people by studying CoV diversity in a critical zoonotic reservoir (bats), at sites of high risk for emergence (wildlife markets) in an emerging disease hotspot (China). The three specific aims of this project are to: 1. Assess CoV spillover potential at high risk human-wildlife interfaces in China. This will include quantifying the nature and frequency of contact people have with bats and other wildlife, serological and molecular screening of people working in live markets and highly exposed to wildlife, screening wild-caught and market-sampled bats from 30+ species for CoVs using molecular assays, and genomic characterization and isolation of novel CoVs. 2. Develop predictive models of bat CoV emergence risk and host range. A combined modeling approach will include phylogenetic analyses of host receptors, and novel CoV genes (including functional receptor binding domains), a fused ecological and evolutionary model to predict host range and viral sharing, and mathematical matrix models to examine evolution and transmission dynamics. 3. Test predictions of CoV inter-species transmission. Predictive models of host range (i.e. emergence potential) will be tested experimentally using reverse genetics, pseudovirus and receptor binding assay, and virus infection experiments across a range of cell cultures from different species and humanized mice.

Collaborations:

Funding Organization	Research Organization	Program **	Director	Country	City
NIH	KING CHULALONGKORN MEMORIAL HOSPITAL	Understanding the Risk of Bat Coronavirus Emergence	WACHARAPLUKSADEE SUPAPORN	THAILAND	BANGKOK
NIH	INSTITUT PASTEUR IN CAMBODIA	Understanding the Risk of Bat Coronavirus Emergence	DUONG, VEASNA	CAMBODIA	PHNOM PENH
NIH	NATIONAL ANIMAL HEALTH CENTER	Understanding the Risk of Bat Coronavirus Emergence	THEPPANGNA, WATTHANA	LAOS	VIENTIANE CAPITAL
NIH	GUANGDONG ENTOMOLOGICAL INSTITUTE	Understanding the Risk of Bat Coronavirus Emergence	ZHU, GUANJIN	CHINA	GUANGZHOU
NIH	CENTER FOR DISEASE CONTROL AND PREVENTION OF GUANGDONG	Understanding the Risk of Bat Coronavirus Emergence	XE, CHANGWEN	CHINA	Guangzhou
NIH	YUNNAN INSTITUTE OF ENDEMIC DISEASES CONTROL AND PREVENTION	Understanding the Risk of Bat Coronavirus Emergence	ZHANG, YUN-ZH	CHINA	Dali
NIH	WUHAN INSTITUTE OF VIROLOGY, CHINESE ACADEMY OF SCIENCES	Understanding the Risk of Bat Coronavirus Emergence	SHI, ZHENGLI	CHINA	WUHAN

From: Crawford, Chase (NIH/NIAID) [E] (b) (6)
Sent: Monday, April 13, 2020 5:41 PM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: Auchincloss, Hugh (NIH/NIAID) [E] (b) (6) Harper, Jill (NIH/NIAID) [E] (b) (6) NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Request for information: Senate Qs - Wuhan Institute of Virology

HI BUGS,

(b) (5)

Thanks,
Chase
(b) (6)

From: LaMontagne, Karen (NIH/OD) [E] (b) (6)
Sent: Monday, April 13, 2020 4:23 PM
To: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Senate Qs - Wuhan Institute of Virology

Hi, NIAID,

Separately, we have heard from the offices of Senators Rubio and Braun about these linked articles:

White Coat Waste
Daily Mail
Washington Examiner

Both offices have asked if there's any information we can share with them related to this matter. Thanks in advance for anything you can provide.

Karen

From: Michelle Mitchell (b) (6)
Date: Monday, April 13, 2020 at 3:42 PM
To: Karen LaMontagne (b) (6)
Subject: Sen. Rubio question - NIH funding Wuhan virus lab

Hey Karen,

(b) (5)

Thank you,

MM

From: Justin Goodman <justin@whitecoatwaste.org>
Sent: Monday, April 13, 2020 2:36 PM
To:
Subject: Laura- NIH funding Wuhan virus lab

I hope you had a nice weekend and are staying safe and healthy. I wanted to make sure you saw that our taxpayer watchdog group just exposed that **the National Institutes of Health (NIH) has been sending tax dollars to the controversial Wuhan Institute of Virology for years, including for dangerous lab experiments on coronavirus-infected bats captured from caves.** The Daily Mail, Washington Examiner, Drudge and others ran stories about the troubling find over the weekend.

We're working with Rep. Matt Gaetz (R-FL) and others on a sign-on letter about this and would love to work with you and Senator Rubio as well to ensure no more tax dollars are shipped to the Wuhan Institute of Virology.

I'd be happy to send over more info if you're interested and answer any questions you may have.

Thanks for looking,

Justin

Justin Goodman, M.A.
Vice President, Advocacy and Public Policy
White Coat Waste Project

*Taxpayers shouldn't be forced to pay $20 billion+ for **wasteful** government animal experiments.*

PO Box 26029
Washington, DC 20001

Phone: 860.882.2492
Donate | Blog | Web | Twitter | Facebook

From: Stemmy, Erik (NIH/NIAID) [E]
To: Girma, Tseday (NIH/NIAID) [E]
Subject: RE: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER
Date: Friday, August 2, 2019 12:31:00 PM

Hi Tseday,

(b) (5) Thanks again for sorting this out!

Erik

From: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Sent: Friday, August 2, 2019 11:39 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Subject: RE: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

Hi Erik,

(b) (5)

Thanks,
Tseday

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E49
Rockville, MD 20852
Phone: (b) (6)
Email:
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)>
Sent: Wednesday, July 31, 2019 12:00 PM
To: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Subject: RE: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

Thank you Tseday! Let me know if you need anything else from me.

Erik

From: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Sent: Wednesday, July 31, 2019 11:57 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Subject: RE: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

Good morning,

I am waiting for Donna's (our division coordinator) guidance regarding this change – I will let you know.

(b) (5)

Thanks,
Tseday

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E49
Rockville, MD 20852
Phone: (b) (6)
Email: (b) (6)
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Friday, July 26, 2019 3:34 PM
To: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Subject: Re: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

(b) (5)

Erik

On Jul 26, 2019, at 3:32 PM, Girma, Tseday (NIH/NIAID) [E] (b) (6)> wrote:

Good afternoon,

Thank you for the heads up about this. I didn't receive this letter prior to this. (b) (5)

(b) (5)

Thanks,
Tseday

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E49
Rockville, MD 20852
Phone: (b) (6)
Email: (b) (6)
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

expressly made on behalf of NIAID by one of its representatives.***

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Friday, July 26, 2019 2:50 PM
To: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Subject: Re: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

Hi Tseday,

(b) (5)

Thanks,
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
To: Rodriguez, Elizabeth (NIH/NIAID) [E]
Subject: RE: Award Error for R01AI110964-06 PI Daszak
Date: Friday, August 2, 2019 12:31:00 PM

Great! I'll let the GMS know. Thanks again!

Erik

From: Rodriguez, Elizabeth (NIH/NIAID) [E] (b) (6)
Sent: Friday, August 2, 2019 12:30 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: DMID GrantOps <DMIDGrantOps@niaid.nih.gov>
Subject: RE: Award Error for R01AI110964-06 PI Daszak

Dear Erik,

(b) (5)

You're most welcome-glad I could help. Please let us know if you need any additional info.
Liz

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Friday, August 2, 2019 11:52 AM
To: Rodriguez, Elizabeth (NIH/NIAID) [E] (b) (6)
Cc: DMID GrantOps <DMIDGrantOps@niaid.nih.gov>
Subject: RE: Award Error for R01AI110964-06 PI Daszak

Hi Liz,

(b) (5)

Thanks for your help!
Erik

From: Rodriguez, Elizabeth (NIH/NIAID) [E] (b) (6)
Sent: Friday, July 26, 2019 11:09 AM

To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: DMID GrantOps <DMIDGrantOps@niaid.nih.gov>
Subject: RE: Award Error for R01AI110964-06 PI Daszak

Dear Dr. Erik Stemmy,

(b) (5)

(b) (5) Please let us know if we can be of further assistance.

Thank You,
Liz

Elizabeth Rodriguez, MS
Health Specialist, DMID Grant Ops
OSCPO/DMID/NIAID/NIH
5601 Fishers Lane, Room 7G46
Rockville, MD 20852
Phone: (b) (6)

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 25, 2019 12:31 PM
To: DMID GrantOps <DMIDGrantOps@niaid.nih.gov>
Subject: Award Error for R01AI110964-06 PI Daszak

Hi GrantOps,

(b) (5)

Thanks!
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b) (6)
Email: (b) (6)

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the

publication.

****

From: Aleksei Chmura
To: Girma, Tseday (NIH/NIAID) [E]
Cc: Peter Daszak; Stemmy, Erik (NIH/NIAID) [E]
Subject: Re: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER
Date: Monday, July 8, 2019 3:04:13 PM

Tseday,

I am just checking back with you about our FWA number. Let me know, if I should contact OHRP to get a confirmation or any necessary documentation.

Cheers!

-Aleksei

On Jul 8, 2019, at 07:58, Girma, Tseday (NIH/NIAID) [E] (b) (6)> wrote:

Thanks for sending me this. I will look into why its showing as none on my screen.

Thanks,
Tseday

From: Aleksei Chmura (b) (6)
Sent: Monday, July 8, 2019 7:39 AM
To: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Cc: Peter Daszak (b) (6)>; Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Subject: Re: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

Tseday,

This is via our current, active R01AI110964 - 05 award IRB with Hummingbird (IRB00009289).

Please see attached screen shot from OHRP. Should I get them to email you to confirm?

Cheers!

-Aleksei

<image001.png>

Aleksei Chmura, PhD
Chief of Staff

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

(b) (6) (office)
(mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Jul 8, 2019, at 07:33, Girma, Tseday (NIH/NIAID) [E] (b) (6)> wrote:

Good morning,

No – I couldn't find any record associated with that FWA in OHRP website. Please follow up with them and let me know the status.

Thanks,
Tseday

From: Aleksei Chmura (b) (6)
Sent: Monday, July 8, 2019 7:23 AM
To: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Cc: Peter Daszak (b) (6) Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Subject: Re: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

Dear Tseday,

Can you find our number as follows: FWA00022431?

Cheers!

-Aleksei

On Jul 8, 2019, at 07:21, Girma, Tseday (NIH/NIAID) [E] (b) (6)> wrote:

Good morning,

Thank you for submitting the IIA document for North Carolina performance site. While reviewing your application, I noticed that you entered 'None' in eRA commons for Human subjects Federal Wide Assurance (FWA). Per policy, "Institutions that are awarded funds for human subjects research are considered to be engaged in human subjects research and must have an approved FWA even if another institution performs the human subjects activities through a subaward." For the full list of certification and assurance requirements, go to the Human Subjects Research Requirements SOP. I also check the OHRP website, I wasn't able to look up your FWA info.

Please let us know if you established FWA with OHRP, if not, you will have to do it ASAP. We are not able to issue an award without an FWA.

Thank you,
Tseday Girma

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E24
Rockville, MD 20852
Phone: (b) (6)
Email: (b) (6)
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer

accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

From:	Aleksei Chmura
To:	Stemmy, Erik (NIH/NIAID) [E]; Girma, Tseday (NIH/NIAID) [E]
Cc:	Peter Daszak
Subject:	Re: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER
Date:	Wednesday, June 19, 2019 3:13:22 PM
Attachments:	EHAL FY19 Prov Rates Agrmt.pdf EHA- rates FY2020.pdf

Dear Tseday and Erik,

Please find our latest provisional rate agreement for FY'19 and our FY'20 rate proposal as well. We are expecting DOD/USN to come back to us with our new next month.

Many thanks!

-Aleksei

Aleksei Chmura, PhD
Chief of Staff

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From: "Girma, Tseday (NIH/NIAID) [E]" (b) (6) >
Date: June 18, 2019 at 4:25:14 PM EDT
To: (b) (6)
Cc: "Stemmy, Erik (NIH/NIAID) [E]" (b) (6)
Subject: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER

Good afternoon,

Thank you for your recent JIT submissions. The most recent F&A Rate Agreement that was provided is dated 11/14/2018 and the F&A rate of (b) (4). expired on 06/30/17.

Did you have any documentation that you can send us that shows you could use the expired rate. Otherwise, we will have to put a restriction on the award until we receive a new F&A Rate Agreement.

Please send this information ASAP but no later than 06/20/2019

DEPARTMENT OF THE NAVY
OFFICE OF NAVAL RESEARCH
875 NORTH RANDOLPH STREET
SUITE 1425
ARLINGTON, VA 22203-1995

IN REPLY REFER TO:

Agreement Date: July 9, 2018

NEGOTIATION AGREEMENT

INSTITUTION: **ECOHEALTH ALLIANCE, INC.**
460 WEST 34TH ST. 17TH FLR
NEW YORK, NY 10001-2320

The Indirect Cost and Fringe Benefits rates contained herein are for use on grants, contracts and/or other agreements issued or awarded to EcoHealth Alliance, Inc. by all Federal Agencies of the United States of America, in accordance with the provisions and cost principles mandated by 2 CFR Part 200. These rates shall be used for forward pricing and billing purposes for EcoHealth Alliance, Inc. for Fiscal Year 2019.

Section I: RATES - TYPE: PROVISIONAL (PROV)

Indirect Rates:

TYPE	FROM	TO	RATE	BASE	APPLICABLE TO	LOCATION
Prov.	07/01/18	06/30/19	(b) (4)	(a)	All	All

Fringe Benefits Rates:

TYPE	FROM	TO	RATE	BASE	APPLICABLE TO	LOCATION
Prov.	07/01/18	06/30/19	(b) (4)	(b)	All	All

DISTRIBUTION BASES

(a) Total direct costs excluding capital expenditures (buildings, individual items of equipment; alterations and renovations), the portion of each subaward in excess of $25,000, participant support costs, and flow-through funds.

(b) Salaries and Wages.

SECTION II - GENERAL TERMS AND CONDITIONS

A. **LIMITATIONS:** Use of the rates set forth under Section I is subject to any statutory or administrative limitations and is applicable to a given grant, contract or other agreement only to the extent that funds are available and consistent with any and all limitations of cost clauses or provisions, if any, contained therein. Acceptance of any or all of the rates agreed to herein is predicated upon all the following conditions: (1) that no costs other than those incurred by the recipient\contractor were included in its indirect cost pool as finally accepted and that all such costs are legal obligations of the recipient\contractor and allowable under governing cost principles; (2) that the same costs that have been treated as indirect costs are not claimed as direct costs; (3) that similar types of costs, in like circumstances, have been accorded consistent accounting treatment;

(4) that the information provided by the recipient\contractor, which was used as the basis for the acceptance of the rates agreed to herein and expressly relied upon by the Government in negotiating the said rates, is not subsequently found to be materially incomplete or inaccurate.

B. **ACCOUNTING CHANGES**: The rates contained in Section I of this agreement are based on the accounting system in effect at the time this agreement was negotiated. Changes to the method(s) of accounting for costs, which affects the amount of reimbursement resulting from the use of these rates, require the written approval of the authorized representative of the cognizant negotiating agency for the Government prior to implementation of any such changes. Such changes include but are not limited to changes in the charging of a particular type of cost from indirect to direct. Failure to obtain such approval may result in subsequent cost disallowances.

C. **PROVISIONAL RATES:** The provisional rates contained in this agreement are subject to unilateral amendment by the Government or bilateral amendment by the contracting parties at any time.

D. **USE BY OTHER FEDERAL AGENCIES**: The rates set forth in Section I hereof were negotiated in accordance with and under the authority set forth in 2 CFR Part 200. Accordingly, such rates shall be applied to the extent provided in such regulations to grants, contracts, and/or other agreements to which 2 CFR Part 200 is applicable, subject to any limitations in part A of this section. Copies of this document may be provided by either party to other Federal agencies to provide such agencies with documentary notice of this agreement and its terms and conditions.

E. **SPECIAL REMARKS**: The Government's agreement with the rates set forth in Section I is not an acceptance of the EcoHealth Alliance, Inc.'s accounting practices or methodologies. Any reliance by the Government on cost data or methodologies submitted by EcoHealth Alliance, Inc. is on a non-precedence-setting basis and does not imply Government acceptance.

Accepted:

FOR ECOHEALTH ALLIANCE, INC.:

(b) (6)

ARMINE ARUSTAMYAN
Chief Financial Officer

07-11-2018
Date

FOR THE U.S. GOVERNMENT:

KERSEY.SHEA.DELORES.1049331149

Digitally signed by KERSEY.SHEA.DELORES.1049331149
DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USN, cn=KERSEY.SHEA.DELORES.1049331149
Date: 2018.07.24 16:59:32 -04'00'

SHEA D. KERSEY
Contracting Officer

July 24, 2018
Date

For information concerning this agreement contact:
Shea Kersey
Office of Naval Research

Phone: (b) (6)
E-mail:

ECOHEALTH ALLIANCE, INC.
YEAR ENDED JUNE 30, 2018

Schedule of Indirect Costs and Calculation of indirect Costs Rate

Schedule A

			Indirect Costs
Salaries			836,247
Payroll taxes and emloyees benefits			311,491
Total salaries and related expenses			1,147,738
Professional Fees			191,643
Field Work Costs			502
Meetings and Conferences			66,653
Travel Expense			33,891
Occupancy & Insurance			666,153
Printing			20,855
Postage			2,576
Supplies			34,981
Telephone			30,545
Memberships/Subscriptions			21,576
Miscellaneous Expenses			218
Depreciation			20,681
Information Technology			37,952
Total Other than salaries and related expenses			1,128,226
Total Expenses	**A**	**$**	**2,275,964**
Distribution Base	**B**	**$**	**6,915,741**
Indirect Costs Rate			(b) (4)

See indipendent auditor's report

The accompanying notes are integral part of these notes

ECOHEALTH ALLIANCE, INC AND
WILDLIFE PRESERVATION TRUST
INTERNATIONAL, INC.

CONSOLIDATED STATEMENT OF FUNCTIONAL EXPENSES

YEAR ENDED JUNE 30, 2018

Schedule B

	Audited TOTAL	Exclusions	Notes	Adjusted Total Co	Indirect	Fund Raising	Program
Salaries	$ 4,521,242			$ 4,521,242	$ 836,247	$ 46,905	$ 3,638,090
Payroll taxes and emloyees benefits	1,606,748			1,606,748	311,491	27,521	1,267,736
Total salaries and related expenses	6,127,991	-		6,127,991	1,147,738	74,426	4,905,826
Professional Fees	541,460	22,438	1	519,022	191,643	83,504	243,875
Grant to Other Organizations	50,000	50,000	2	-			
Subcontracts	6,768,985	6,768,985	2	-			-
Field Work Costs	455,548	188,734	3	266,814	502	3,246	263,066
Meetings and Conferences	271,374	35,515	4	235,859	66,653	5,132	164,074
Travel Expense	869,470			869,470	33,891	37,661	797,918
Occupancy & Insurance	666,153			666,153	666,153		0
Printing	63,931			63,931	20,855	18,371	24,706
Postage	20,116			20,116	2,576	3,905	13,636
Supplies	37,742			37,742	34,981	-	2,762
Telephone	70,633			70,633	30,545	-	40,088
Memberships/Subscriptions	50,082			50,082	21,576	6,893	21,613
Miscellaneous Expenses	6,495	4,294	5	2,200	218	-	1,982
Depreciation	22,335			22,335	20,681	-	1,654
Catering and Faculity Rental	99,748	30,686	6	69,062		69,062	-
Information Technology	194,270	23,978	3	170,292	37,952	28,398	103,942
Investment Fees	16,765	16,765	7	-			-
Total Other than salaries and related expenses	10,205,109	7,141,395		3,063,715	1,128,226	256,173	1,679,316
Total Expenses	**$ 16,333,100**	**$ 7,141,395**		**$ 9,191,705**	**$ 2,275,964**	**$ 330,599**	**$ 6,585,142**

Notes - Excluded from indirect and direct costs

1. In-Kind and Donated Services
2. Sub-Receipent exceeding $25,000 and Pass-through to Partners over $25,000
3. Equipment over $5,000
4. Particpants Costs
5. Bad Debt expense
6. Rental expenses
7. Investment Fees

See indipendent auditor's report

The accompanying notes are integral part of these notes

ECOHEALTH ALLIANCE, INC.
YEAR ENDED JUNE 30, 2018

Summary Fringe Benefits Allocations

Schedule C-2

Federal Agency	Program	Federal Contract Number	Reference Number		Salaries and Wages	Fringe Benefits
Federal Awards						
National Science Foundation (NSF)	Pass-through from Arizona State University	141374	7015	067	$ 4,525	$ 1,054
National Science Foundation (NSF)	EcoHealth Net	DEB-08010000	7022	851	18,350	7,156
DoD - Defense Threat Reduction Agency - DTRA	Global Rapid Identification System	HDTRA1-15C-0041	7096	068	181,234	63,277
DoD - Defense Threat Reduction Agency - DTRA	Spillover of Henipaviruses and Filoviruses at Agricultural	HDTRA1-14-1-0037	7096	103	34,083	13,323
DoD - Defense Threat Reduction Agency - DTRA	Understanding the Risk of Bat-Borne Zoonotic Disease Emergence	HDTRA1-17-1-0064	7096	105	119,628	44,715
DoD - Defense Threat Reduction Agency - DTRA	Rift Valley Fever in South Africa	HDTRA1-14-1-0029	7096	294	182,691	56,971
U.S. Department of Health and Human Services (DHHS - NIH)	Bat Coronavirus in China	1R01AI110964-01	7012	049	163,575	55,511
U.S. Department of Health and Human Services (DHHS)	Columbia University Center for Excellence	5U19AI109761-05	7023	262	35,935	9,173
United States Agency for International Development (USAID)	Land Use Change	AID-486-A-13-00005	7129	302	94,709	36,030
United States Agency for International Development (USAID)	Pass Through UC Davis Emerging Pandemic Threat Program	AID-OAA-A-14-00102	711X	306	2,377,644	846,137
U. S Department of Homeland Security (DHS)	Ground Truth	HSHQDC-16-C-00113	7137	097	129,445	47,907
U. S Department of Homeland Security (DHS)	IBIS: Inbound Bio-event Information System	HSHQDC-17-C-B0031	7138	099	84,380	23,996
Sub-Total Federal					**$ 3,426,199**	**$ 1,205,251**
Non-Federal Awards						
Non-Federal Awards	Predict and Prevent pandemics		7138	107	67,451	17,849
Non-Federal Awards	Predict and Prevent pandemics		7092	850	26,270	10,133
Non-Federal Awards	Predict and Prevent pandemics		7098	850	2,680	1,050
Non-Federal Awards	Predict and Prevent pandemics		7143	104	10,979	4,296
Non-Federal Awards	Predict and Prevent pandemics		7144	106	28,450	8,253
Sub-Total Non-Federal					**$ 135,831**	**$ 41,582**
EcoHealth Alliance - General Funds	Global Rapid Identification System		7100	068	32,435	4,766
EcoHealth Alliance - General Funds	Land Use Change		7100	302	4,058	2,381
EcoHealth Alliance - General Funds	Predict and Prevent pandemics		7100	306	39,567	13,756
Sub-Total General Funds					**$ 76,060**	**$ 20,904**
EcoHealth Alliance - General Funds	Fund Raising		7100	860	46,905	27,521
Indirect Costs pool	Indirect Costs pool		7100	860	836,248	311,491
Grant Total					**$ 4,521,242**	**$ 1,606,749**

ECOHEALTH ALLIANCE, INC.
YEAR ENDED JUNE 30, 2018

Summary Fringe Benefits

Schedule C-1 - Summary

Account Description	Fringe Beenfits, USD	Less Direct Charges to Grants	Net Fringe Benefits	
Health Insurance	783,091		783,091	
Health Insurance payments in lieu of benefit	8,917		8,917	
COBRA Recovery (Exp Red)	(13,528)		(13,528)	
Vision Care	2,713		2,713	
Washington Unemployment Insurance	60		60	
Life Insurance ST/LT Disability/Dental	129,606		129,606	
NYS Unemployment Ins (NYSUI)	13,538		13,538	
Alaska Unempolyment Insurance Expense	0		0	
NYS Disability Insurance	1,016		1,016	
Pension Expense	312,180		312,180	
Tuition Reimbursement expense	18,062	7,656	10,406	
NYS Commuter Tax	17,500		17,500	
Social Security Employor Expense	306,788		306,788	
Workers Compensation	26,340		26,340	
FSA Fees	466		466	
Total Benefits	**$ 1,606,749**	**$ 7,656**	**$ 1,599,093**	A
Salaries and Wages			**$ 4,521,242**	B
Fringe Benefits Rates			**35.4%**	A/B

ECOHEALTH ALLIANCE, INC.
YEAR ENDED JUNE 30, 2018

Schedule of Federal Awards

Schedule E

Schedule F Reference	Federal Agency	Direct Federal / Pass-Through	Type of Award	Award Amount	Award Period	Federal Contract Number	Pass-through	Indirect Costs Limitations
	National Science Foundation (NSF)							
A	EcoHealth Net	Direct Federal	Research Subaward Agreement	$499,897	9-1-2016 to 8-31-2021	DEB-08010000		No
B	Pass-through from Arizona State University	Pass- Through	Grant	$162,024	9-1-2014 to 8-31-2017	141374	15-588	No
	U.S. Department of Defense (DOD)							
	Defense Threat Reduction Agency - DTRA	Direct Federal						
C	Global Rapid Identification System		DOD Contract (FAR)	$4,479,678	4-9-2015 to 9-30-2017	HDTRA1-15C-0041		No
D	Rift Valley Fever in South Africa	Direct Federal	Grant	$4,936,359	5-28-2014 to 5-27-2019	HDTRA1-14-1-0029		No
E	Henipaviruses and Filoviruses at Agricultural and Hunting Human-Animal Interfaces in Malaysia	Direct Federal	Grant	$2,408,373	5-1-2017 to 4-30-2020	HDTRA1-14-1-0037		No
F	Understanding the Risk of Bat-Borne Zoonotic Disease Emergence in Western Asia	Direct Federal	Grant	$2,881,913	10-1-2017 to 9-30-2022	HDTRA1-17-1-0064		No
	U.S. Department of Health and Human Services (DHHS)							
	National Institute of Health							
G	Bat Coronavirus in China	Direct Federal	Grant (Research)	$3,086,735	6-1-2014 to 5-31-2019	1R01AI110964-01		No
H	Pass-through from Columbia University Center for Excellence	Pass- Through	Research Subaward Agreement	$345,003	3-7-2014 to 2-28-2019	5U19AI109761-05	6(GG008377-39)	No
	United States Agency for International Development (USAID)							
I	Land Use Change		Cooperative Agreement	$2,499,147	10-15-2013 to 5-30-2018	AID-486-A-13-00005		No
J	Emerging Pandemic Threat Program	Pass- Through	Cooperative Agreement	$47,651,611	10-1-2014 to 9-30-2019		AID-OAA-A-14-00102	No
	U. S Department of Homeland Security (DHS)							
K	IBIS: Inbound Bio-event Information System	Direct Federal	Contract	$413,761	10-30-2017 to 10-28-2018	HSHQDC-17-C-B0031		No
L	Ground Truth	Direct Federal	Fixed Fee Contract	$271,272	9-30-2016 to 9-29-2018	HSHQDC-16-C-00113		N/A

ECOHEALTH ALLIANCE, INC.
YEAR ENDED JUNE 30, 2018

Schedule of Direct Costs by Awards and Applied Indirect

Schedule F

			A	B	C	D	E	F	G	H	I	J	K	L				
	Total Audited	**Indirect**	**NSF - Grant**	**NSF- Grant**	**DOD- Cost Reimbursement Contract**	**DOD- Grant**	**DOD- Grant**	**DOD- Grant**	**DHHS-Grant**	**DHHS-Grant**	**USAID -Grant**	**USAID - Grant**	**DHS- Cost Reimbursement Contract**	**DHS- Fixed Fee**	**Total Fedeal Grants**	**Total Non-Federal Grants**	**EHA Internal Funds**	**Fund Raising - EHA Internal Funds**
			DEB-08010000	**141374**	**HDTRA1-15C-0041**	**HDTRA1-14-1-0029**	**HDTRA1-14-1-0037**	**HDTRA1-17-1-0064**	**1R01AI110964-01**	**5U19AI109761-05**	**AID-486-A-13-00005**	**AID-OAA-A-14-00102**	**HSHQDC-17-C-B0031**	**HSHQDC-16-C-00113**				
Salaries	$ 4,521,242	$ 836,247	$ 18,350	$ 4,525	$ 181,234	$ 182,691	$ 34,083	$ 119,628	$ 163,575	$ 35,935	$ 94,709	$ 2,377,644	$ 129,445	$ 84,380	$ 3,426,199	$ 135,831	$ 76,060	$ 46,905
Payroll taxes and emloyees benefits	1,606,748	311,491	7,156	1,054	63,277	56,971	13,323	44,715	55,511	9,173	36,030	846,137	47,907	23,996	1,205,251	41,582	20,903	27,521
Total salaries and related expenses	6,127,991	1,147,738	25,506	5,579	244,511	239,662	47,406	164,343	219,086	45,108	130,739	3,223,781	177,352	108,376	4,631,450	177,413	96,963	74,426
Professional Fees	541,460	191,643	120		5,000	5,104	44	2,996	37,512		10,669	185,969	6	15,000	262,419	1,279	2,615	83,504
Grant to Other Organizations	50,000	-													-	50,000	-	-
Subcontracts	6,768,985	-				555,964	445,916	54,293	258,575		55,435	5,398,803			6,768,985		(0)	-
Field Work Costs	455,548	502				13,911	169,935	39,387	214		519	227,497			451,464		336	3,246
Meetings and Conferences	271,374	66,653	24,651		9	9,658		11,780	13	108	306	115,219	45	14	161,803	23,110	14,676	5,132
Travel Expense	869,470	33,891	1,402	2,347		32,829	5,976	41,655	13,592	90	39,629	601,993	612	2,579	742,704	43,488	11,726	37,661
Occupancy & Insurance	666,153	666,153													-		0	-
Printing	63,931	20,855				3,939	24	127	1,375		232	17,252	198		23,147	389	1,169	18,371
Postage	20,116	2,576	2,260			158	20		63			8,144			10,644		2,992	3,905
Supplies	37,742	34,981							2,762						2,762		(0)	-
Telephone	70,633	30,545	70			5,031	220	111	8,548	1,969	7,387	15,736		61	39,134	954	0	-
Memberships/Subscriptions	50,082	21,576				491		27	1,096		2,464	2,218		4,684	10,979	322	10,312	6,893
Miscellaneous Expenses	6,495	218													-		6,276	-
Depreciation	22,335	20,681													-		1,654	-
Catering and Faculity Rental	99,748	-													-		-	99,748
Information Technology	194,270	37,952	239		6,824	1,432	333	308	30,016	2,113	5,271	39,726	17,173	185	103,621	321	23,978	28,398
Investment Fees	16,765	-													-		16,765	-
Total Other than salaries and related expenses	10,205,109	1,128,226	28,742	2,347	11,834	628,516	622,469	150,684	353,767	4,280	121,912	6,612,556	18,034	22,523	8,577,663	119,864	92,499	286,859
Total expenses	**$ 16,333,100**	**$ 2,275,964**	**$ 54,249**	**$ 7,926**	**$ 256,344**	**$ 868,179**	**$ 669,875**	**$ 315,027**	**$ 572,853**	**$ 49,388**	**$ 252,651**	**$ 9,836,337**	**$ 195,385**	**$ 130,899**	**$ 13,209,113**	**$ 297,276**	**$ 189,462**	**$ 361,285**
Direct Costs Base			**$ 29,598**	**$ 7,926**	**$ 256,344**	**$ 312,215**	**$ 54,023**	**$ 241,882**	**$ 314,278**	**$ 49,388**	**$ 197,216**	**$ 4,437,534**	**$ 195,385**	**$ 130,899**	**$ 6,226,689**	**$ 223,299**	**$ 135,155**	**$ 330,599**
Indirect Costs Allocation			**$ 9,741**	**$ 2,608**	**$ 84,363**	**$ 102,750**	**$ 17,779**	**$ 79,603**	**$ 103,429**	**$ 16,254**	**$ 64,904**	**$ 1,460,392**	**$ 64,301**	**$ 43,079**	**$ 2,049,203**	**$ 73,488**	**$ 44,479**	**$ 108,800**

ECOHEALTH ALLIANCE, INC.
YEAR ENDED JUNE 30, 2018

GOVERNMENT PARTICIPATION IN INDIRECT COST POOLS

Schedule G

Awards		Indirect Costs Allocation	% of Base
Federal Grants	$	1,857,460	82%
Federal Contracts - Cost Reimbursements	$	148,664	7%
Federal - Fixed Fee	$	43,079	2%
Sub-Total Federal	**$**	**2,049,203**	**90%**
Non-Federal Awards	$	73,488	3%
Internal Funds	$	153,280	7%
Sub-Total Non-Federal	**$**	**226,767**	**10%**
Total Indirect Costs	**$**	**2,275,964**	**100%**

ECOHEALTH ALLIANCE, INC.
YEAR ENDED JUNE 30, 2018

RECONCILIATION OF TOTAL PAYROLL TO IRS 941 FORMS

Schedule H

Direct Salaries and Wages		3,684,995
Indirect Salaries and Wages		836,247
Total Salaries and Wages		**$ 4,521,242**
941 Reporting		
	July 1 - September 30, 2017	1,158,912
	October 1 - December 31, 2017	1,078,878
	January 1 - March 31, 2018	1,074,560
	April 1 - June 30, 2018	1,064,332
Total Salaries reported at 941		**$ 4,376,682**
Adjustments to Reconcile		
	Stipents Paid by Check	3,500
	Health Insurance payments in lieu of benefits	(661)
	Non-Taxable FSA, Dependent Care and Transit	141,721
Total Adjusments		**$ 144,560**
Total Adjusted		**$ 4,521,242**

Please let me know

Thanks,
Tseday

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E49
Rockville, MD 20852
Phone: (b) (6)
Email: (b) (6)
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

From: Girma, Tseday (NIH/NIAID) [E]
To: (b) (6)
Cc: Stemmy, Erik (NIH/NIAID) [E]
Subject: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER
Date: Tuesday, June 11, 2019 2:23:55 PM

Good afternoon,

Thank you for submitting the JIT for the above mentioned grant. Regarding the other support submitted for Amy Sims, Peng Zhou and Ben Hu, When I add the efforts of all the grants in the other support (including some of the grants listed under pending that have a to be paid status), the total active support will be higher than (b) (6), (b) (4) including the effort for the above mentioned grant. While an individual may be affiliated with a number of organizations, the combination of appointments can be no higher than (b) (6), (b) (4).

Please indicate how much effort will be taken from which grants. We need specifics that shows the individuals will not go over (b) (6), (b) (4) at the time award. Please send revised other support.

Please send me the information ASAP but no later than Wednesday, 06/12/2019

Thanks,
Tseday

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E49
Rockville, MD 20852
Phone: (b) (6)
Email: (b) (6)
NIAID, National Institutes of Health, DHHS

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From:	era-notify@mail.nih.gov
To:	NIAID FCTS; Stemmy, Erik (NIH/NIAID) [E]; Bernabe, Gayle (NIH/NIAID) [E]; Girma, Tseday (NIH/NIAID) [E]
Subject:	FACTS: State Department Clearance Request Approved
Date:	Monday, May 20, 2019 10:00:48 PM

*** This is an automated notification - Please do not reply to this message. ***

Project Number: R01AI110964-06
PI Name: PETER DASZAK
Project Title: Understanding the Risk of Bat Coronavirus Emergence

Country: SINGAPORE
SDCR Initiated By: Gayle Bernabe
SDCR Status: Approved
Action Comment:

If you have any questions, please contact the eRA Help Desk at http://grants.nih.gov/support/index.html OR call 1-866-504-9552 (tty: 301-451-5939) OR helpdesk@od.nih.gov.

From: Stemmy, Erik (NIH/NIAID) [E]
To: Girma, Tseday (NIH/NIAID) [E]; Bernabe, Gayle (NIH/NIAID) [E]
Subject: RE: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA (Sites/research objectives clarification needed)
Date: Friday, May 24, 2019 2:56:00 PM

HI Tseday and Gayle,
I think I've addressed the other questions you had. [redacted (b) (5)] Let me know if you need anything else.

Erik

From: Girma, Tseday (NIH/NIAID) [E]
Sent: Wednesday, May 22, 2019 6:35 PM
To: Bernabe, Gayle (NIH/NIAID) [E] [redacted (b) (6)]; Stemmy, Erik (NIH/NIAID) [E] [redacted (b) (6)]
Subject: RE: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA (Sites/research objectives clarification needed)

Good afternoon,

[redacted (b) (5)]

Thanks,
Tseday

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E49
Rockville, MD 20852
Phone: [redacted (b) (6)]
Email: [redacted (b) (6)]
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

From: Bernabe, Gayle (NIH/NIAID) [E]
Sent: Monday, May 6, 2019 3:27 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Girma, Tseday (NIH/NIAID) [E] (b) (6)
Subject: Re: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA (Sites/research objectives clarification needed)

Hi Erik,

(b) (5)

Thanks again,
Gayle

From: "Stemmy, Erik (NIH/NIAID) [E]" (b) (6)
Date: Monday, May 6, 2019 at 3:01 PM
To: "Bernabe, Gayle (NIH/NIAID) [E]" (b) (6)
Cc: "Girma, Tseday (NIH/NIAID) [E]" (b) (6)
Subject: RE: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA (Sites/research objectives clarification needed)

Hi Gayle,

(b) (5)

Erik

From: Bernabe, Gayle (NIH/NIAID) [E]
Sent: Monday, May 6, 2019 2:24 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Girma, Tseday (NIH/NIAID) [E]

Subject: Re: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA (Sites/research objectives clarification needed)

Hi Erik,

(b) (5)

Kind regards,
Gayle

On May 6, 2019, at 1:01 PM, Stemmy, Erik (NIH/NIAID) [E] (b) (6)> wrote:

Hi Gayle,

(b) (5)

Thanks!
Erik

From: Bernabe, Gayle (NIH/NIAID) [E]
Sent: Monday, May 6, 2019 12:20 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6); Girma, Tseday (NIH/NIAID) [E] (b) (6)
Subject: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA (Sites/research objectives clarification needed)

Good afternoon,

(b) (5)

(b) (5)

Attached is more detailed information about Research Objectives. Please let me know if you have any questions.

Thank you for your time.

Kind regards,
Gayle

Gayle Bernabe, MPH
Regional Program Officer-East/SE Asia and the Pacific
Office of Global Research (OGR)
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Department of Health and Human Services
5601 Fishers Ln Rm 1E MSC 9802
Bethesda, MD 20892-9802 [For courier deliveries: 20852]
Phone: (b) (6)
Fax: (301) 480-2954
Email: (b) (6)

From: Littleton, Ashley (NIH/NIAID) [C] <(b) (6)
Sent: Thursday, May 2, 2019 9:41 AM
To: Girma, Tseday (NIH/NIAID) [E] (b) (6)>; NIAID State Dept Clearance <StateDeptClearance@mail.nih.gov>
Cc: Stemmy, Erik (NIH/NIAID) [E] (b) (6); Bernabe, Gayle (NIH/NIAID) [E] (b) (6)
Subject: RE: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA OGR POC: Gayle Bernabe

Hello,

We have received your request and it will be reviewed by Gayle Bernabe at OGR.

Thanks
Ashley

Ashley A. Littleton
Operations Coordinator ©

National Institutes of Health
Department of Health and Human Services
National Institute of Allergy and Infectious Diseases

Office of Global Research(OGR)
5601 Fishers Lane, 1E41
Rockville, MD 20892
Main phone: (b) (6)
Direct line: (b) (6)
Cell phone:
(b) (6)

Disclaimer:

The information in this e-mail and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the original intended recipient. If you have received this e-mail in error please inform the sender and delete it from your mailbox or any other storage devices. National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are sender's own and not expressly made on behalf of the NIAID by one of its representatives.

From: Girma, Tseday (NIH/NIAID) [E]
Sent: Thursday, May 2, 2019 9:32 AM
To: NIAID State Dept Clearance <StateDeptClearance@mail.nih.gov>
Cc: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Subject: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA

The following request for foreign clearance is ready for review.

Grant Number:	**2R01AI110964 - 06**
P.I. :	**DASZAK, PETER**
Applicant Organization:	**ECOHEALTH ALLIANCE, INC**
Foreign Country:	**China**
GMS:	**Tseday Girma**
PO:	**Stemmy, Erik**

Thanks,

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E24
Rockville, MD 20852
Phone: (b) (6)
Email: (b) (6)
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

From:	Littleton, Ashley (NIH/NIAID) [C]
To:	Girma, Tseday (NIH/NIAID) [E]; NIAID State Dept Clearance
Cc:	Stemmy, Erik (NIH/NIAID) [E]; Bernabe, Gayle (NIH/NIAID) [E]
Subject:	RE: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA OGR POC: Gayle Bernabe
Date:	Thursday, May 2, 2019 9:40:50 AM

Hello,

We have received your request and it will be reviewed by Gayle Bernabe at OGR.

Thanks
Ashley

Ashley A. Littleton
Operations Coordinator ©

National Institutes of Health
Department of Health and Human Services
National Institute of Allergy and Infectious Diseases

Office of Global Research(OGR)
5601 Fishers Lane, 1E41
Rockville, MD 20892
Main phone: (b) (6)
Direct line: (b) (6)
Cell phone (b) (6)

Disclaimer:

From: Girma, Tseday (NIH/NIAID) [E]
Sent: Thursday, May 2, 2019 9:32 AM
To: NIAID State Dept Clearance <StateDeptClearance@mail.nih.gov>
Cc: Stemmy, Erik (NIH/NIAID) [E] (b) (6)>
Subject: Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA

The following request for foreign clearance is ready for review.

Grant Number:	**2R01AI110964 - 06**
P.I. :	**DASZAK, PETER**
Applicant Organization:	**ECOHEALTH ALLIANCE, INC**
Foreign Country:	**China**
GMS:	**Tseday Girma**
PO:	**Stemmy, Erik**

Thanks,

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E24
Rockville, MD 20852
Phone: (b) (6)
Email:
NIAID, National Institutes of Health, DHHS

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From:	Girma, Tseday (NIH/NIAID) [E]
To:	NIAID State Dept Clearance
Cc:	Stemmy, Erik (NIH/NIAID) [E]
Subject:	Foreign Clearance Request: Grant Number: 2 R01 AI 110964 - 06 PI Name: DASZAK, PETER - FOREIGN COUNTRY - CHINA
Date:	Thursday, May 2, 2019 9:31:39 AM

The following request for foreign clearance is ready for review.

Grant Number:	**2R01AI110964 - 06**
P.I. :	**DASZAK, PETER**
Applicant Organization:	**ECOHEALTH ALLIANCE, INC**
Foreign Country:	**China**
GMS:	**Tseday Girma**
PO:	**Stemmy, Erik**

Thanks,

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E24
Rockville, MD 20852
Phone: (b) (6)
Email:
NIAID, National Institutes of Health, DHHS

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From: Girma, Tseday (NIH/NIAID) [E]
To: Stemmy, Erik (NIH/NIAID) [E]
Subject: Grant Number: 2R01AI110964 - 06 PI Name: DASZAK, PETER
Date: Tuesday, April 30, 2019 8:59:25 AM

Good morning,

This is in regards to the foreign collaboration in China. I see that it is entered into FACTS but the state department clearance request hasn't been initiated.

Please initiate the clearance so I can forward it to OGR. If this is entered fore tracking purposes only, please make sure it is marked accordingly.

Thanks,
Tseday

Tseday Girma, MPA
Grants Management Specialist
National Institutes of Allergy and Infectious Diseases
5601 Fishers Lane, Room 4E24
Rockville, MD 20852
Phone: (b) (6)
Email:
NIAID, National Institutes of Health, DHHS

Effective January 1, 2017, NIH closeout documentation policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.

From: Stemmy, Erik (NIH/NIAID) [E]
To: Peter Daszak
Cc: Aleksei Chmura
Subject: RE: R01 renewal application 2 R01 AI110964-06
Date: Tuesday, February 26, 2019 1:34:00 PM

Hi Peter,
Yes, that's very good news. Your renewal application did score within payline. There's still a ways to go before an award would be made, so nothing is set until the notice of award goes out.

Best,
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone (b) (6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b) (6)
Sent: Tuesday, February 26, 2019 1:19 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Aleksei Chmura (b) (6)
Subject: R01 renewal application 2 R01 AI110964-06
Importance: High

Hi Erik – I just want to check with you that I read my ERA commons score correctly. From what I see the renewal proposal scored percentile of (b) (4) and impact score of (b) (4). Is that correct from your memory of it?

If so, that would mean I'm within the normal funding range, but I want to check because I don't want to be too optimistic!

The number is: 2 R01 AI110964-06

Cheers,

Peter

Peter Daszak
President

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

Tel. (b) (6)
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Timmerman, Michelle (NIH/CSR) [E]
To: (b) (6)
Cc: Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: NIH Pre-approval needed for 2R01AI110964-06
Date: Wednesday, November 14, 2018 1:53:39 PM

NIAID has provided permission to submit this application to CSR. The application is now compliant with the $500K policy. It is being assigned for additional administrative review and peer review

From: Timmerman, Michelle (NIH/CSR) [E]
Sent: Friday, November 09, 2018 2:14 PM
To: (b) (6)
Cc:
Subject: NIH Pre-approval needed for 2R01AI110964-06

Dear Dr. Daszak:
Your application to PA-18-484 entitled "Understanding the Risk of Bat Coronavirus Emergence" (2R01AI110964-06) has been received by the Division of Receipt and Referral in the Center for Scientific Review at the NIH. This application is subject to the NIH's policy regarding applications requesting $500,000 or more in subtotal direct costs for any year. Please see the Application Guide instructions for the R&R Budget Form (https://go.usa.gov/xPTR9), part 2.3.7.2 of the Grants Policy Statement it references (https://go.usa.gov/xPNCA) and the original announcement NOT-OD-02-004 (https://go.usa.gov/xPTRX). Please also see Section IV.7 "Requests of $500,000 or more for direct costs in any year" in the Funding Opportunity Announcement.

The policy has the following requirements:

- The PD/PI must contact and obtain agreement from an appropriate Institute/Center to accept assignment of the application, at least six weeks in advance of submission.
- The PD/PI must include a cover letter identifying the program staff member or the Institute/Center that has agreed to accept the assignment of the application.
- The Institute or Center that is willing to accept the application must notify the Center for Scientific Review (CSR), Division of Receipt and Referral (DRR) directly, that the application is acceptable.

Your application does not meet these requirements. Please see Total Direct Costs less Consortium F&A" in your application, which shows the requested budget of $515,358 in each year. Please also see the warning in your Commons account that states "ESubmission warning:Direct cost requests of $500k or more a year need approval to accept assignment from Institute/Center staff, except for RFAs or PAs with budgetary limits. Applications without such approval may be delayed or not accepted for review. (020.52.2)".

In order for your application to be considered for the current receipt cycle, we must receive notification of acceptance from an NIH Institute/Center by the end of the day November 16, 2018. If notification has not been received by then, your application will be withdrawn without review.

NIH policies on post-submission materials (NOT-OD-15-039) and late applications (NOT-OD-17-066) prohibit changing or correcting the budget or submitting budget forms on this

application at this point.

Please acknowledge the receipt of this email. If you have questions about this policy, do not hesitate to contact me.

Sincerely,
Michelle M. Timmerman, Ph.D.
Associate Director
Division of Receipt and Referral
Center for Scientific Review/NIH/DHHS
(b) (6)

From: Stemmy, Erik (NIH/NIAID) [E]
To: Peter Daszak
Cc: Aleksei Chmura; Hongying Li; Graham, Adam (NIH/NIAID) [E]
Subject: Re: NIH Pre-approval needed for 2R01AI110964-06
Date: Wednesday, November 14, 2018 1:44:14 PM

No problem. Thank you and Aleksei for being so responsive. I think we have everything we need so let me know if you don't hear from CSR.

Erik

Sent from my iPhone

On Nov 14, 2018, at 1:27 PM, Peter Daszak <(b) (6)> wrote:

> Thanks for your help with this Erik, it's really much appreciated!
>
> Cheers,
>
> Peter
>
> **Peter Daszak**
> *President*
>
> EcoHealth Alliance
> 460 West 34th Street – 17th Floor
> New York, NY 10001
>
> Tel. (b) (6)
> Website: www.ecohealthalliance.org
> Twitter: @PeterDaszak
>
> *EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*
>
> ---
>
> **From:** Stemmy, Erik (NIH/NIAID) [E] (b) (6)
> **Sent:** Wednesday, November 14, 2018 1:21 PM
> **To:** Aleksei Chmura
> **Cc:** Peter Daszak; Hongying Li; Graham, Adam (NIH/NIAID) [E]
> **Subject:** Re: NIH Pre-approval needed for 2R01AI110964-06

Thank you! I'll route it to our Fanta folks and let you know if we need anything else.

Erik

Sent from my iPhone

On Nov 14, 2018, at 1:17 PM, Aleksei Chmura (b) (6) wrote:

> Dear Erik,
>
> Please view the attached letter (PDF). Let me know, if it will be correct.
>
> Many thanks!
>
> -Aleksei

Aleksei Chmura, PhD
Chief of Staff

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

(b) (6) (office)
(mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Nov 14, 2018, at 12:17, Stemmy, Erik (NIH/NIAID) [E] (b) (6) > wrote:

> Hi Aleksei,
> In looking in to this further, I will need you to write a letter stating the original budget submission was in error and that if the application receives a fundable score you will reduce the budget to be below the $500k cap for all 5 years of the award. Please also include the new estimated total DC budgets for all years of the award.

The letter should be signed by the PI and the business official for EcoHealth. I'll need this ASAP. Would you be able to get it to me this afternoon?

Erik

Sent from my iPhone

On Nov 13, 2018, at 11:06 AM, Aleksei Chmura [redacted] (b) (6) wrote:

> Erik,
>
> Just to clarify, Michelle is telling us that she needs your approval for us to upload our under-$500k-per-year budget.
>
> Cheers!
>
> -Aleksei

On Nov 13, 2018, at 10:17, Aleksei Chmura [redacted] (b) (6) wrote:

> Hi, Erik,
>
> Thanks for this, Erik! I just left you a voice message and if you would please contact Michelle Timmerman [redacted] (b) (6) we are at the ready to upload and/or submit our budget.
>
> Cheers,
>
> -Aleksei

On Nov 13, 2018, at 10:01, Stemmy, Erik (NIH/NIAID) [E] [redacted] (b) (6) > wrote:

> Thanks Aleksei. Let me know if you need my help with anything else.
>
> Erik
>
> Sent from my iPhone

On Nov 11, 2018, at 7:08 PM, Aleksei Chmura [redacted] (b) (6) wrote:

Dear Erik,

I have responded to Michelle from DRR and asked about the process to upload or submit our revised budget. ASSIST does not currently permit me to modify our submitted budget, so I will await her instructions. We have modified our renewal proposal budget below the $500k per year cap and have it at the ready.

Many thanks!

-Aleksei

Aleksei Chmura, PhD
Chief of Staff &
Authorized Organizational Representative

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

[redacted] (b) (6) (office)
[redacted] (mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

Begin forwarded message:

From: "Timmerman, Michelle (NIH/CSR) [E]" [redacted] (b) (6)

Subject: RE: NIH Pre-approval needed for 2R01AI110964-06
Date: November 9, 2018 at 17:17:52 EST
To: Aleksei Chmura
(b) (6)
Cc: Peter Daszak
(b) (6) 李泓萤

Dear Aleksei:
Unfortunately, this type of change is prohibited by NIH's policies about post submission materials and late submission. I also don't see a reason NIH's late submission policy accepts, like recent review service of the PD/PI. Unfortunately, if an IC does not provide the pre-approval this application will be withdrawn.
You may also wish to view the NIH Guide (https://grants.nih.gov/funding/searchguide/index.html) to see if NIAID participates in other R01 funding opportunity announcements with an upcoming due date and May 2019 Advisory Council.
Sincerely,
Michelle

From: Aleksei Chmura
(b) (6)
Sent: Friday, November 09, 2018 4:19 PM
To: Timmerman, Michelle (NIH/CSR) [E]
(b) (6)
Cc: Peter Daszak
(b) (6)
Subject: Re: NIH Pre-approval needed for 2R01AI110964-06

Dear Michelle,

Thank you for your help earlier. We since spoke with Erik Stemmy our Program Officer at NIAID. Though he said he would have approved it, it would take 6-weeks through NIAID internal approval processes. Unfortunately, we had not realized that we were required to notify them of our intention to increase our renewal budget above the $500 per year cap in advance of

submission. Therefore, NIAID will be unable to make the 18th November deadline.

Is there any way to extend the due-date to accommodate the NIAID 6-week timeline?

If not, then we as per Erik's recommendation, we will proceed with updating our budget to $500k in direct costs per year. How may we do this in ASSIST or otherwise?

Sincerely,

-Aleksei

Aleksei Chmura, PhD
Chief of Staff &
Authorized Organizational Representative

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

(b) (6) (office)
(mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Nov 9, 2018, at 14:14,
Timmerman, Michelle (NIH/CSR)
[E]
(b) (6)
wrote:

Dear Dr. Daszak:

Your application to PA-18-484 entitled "Understanding the Risk of Bat Coronavirus Emergence" (2R01AI110964-06) has been received by the Division of Receipt and Referral in the Center for Scientific Review at the NIH. This application is subject to the NIH's policy regarding applications requesting $500,000 or more in subtotal direct costs for any year. Please see the Application Guide instructions for the R&R Budget Form (https://go.usa.gov/xPTR9), part 2.3.7.2 of the Grants Policy Statement it references (https://go.usa.gov/xPNCA) and the original announcement NOT-OD-02-004 (https://go.usa.gov/xPTRX). Please also see Section IV.7 "Requests of $500,000 or more for direct costs in any year" in the Funding Opportunity Announcement.

The policy has the following requirements:

- The PD/PI must contact and obtain agreement from an appropriate Institute/Center to accept assignment of the application, at least six weeks in advance of submission.
- The PD/PI must include a cover letter identifying the program staff member or the Institute/Center that has agreed to accept the assignment of the application.
- The Institute or Center that is willing to accept the application must notify the Center for

Scientific Review (CSR), Division of Receipt and Referral (DRR) directly, that the application is acceptable.

Your application does not meet these requirements. Please see Total Direct Costs less Consortium F&A" in your application, which shows the requested budget of $515,358 in each year. Please also see the warning in your Commons account that states "ESubmission warning:Direct cost requests of $500k or more a year need approval to accept assignment from Institute/Center staff, except for RFAs or PAs with budgetary limits. Applications without such approval may be delayed or not accepted for review. (020.52.2)".

In order for your application to be considered for the current receipt cycle, we must receive notification of acceptance from an NIH Institute/Center by the end of the day November 16, 2018. If notification has not been received by then, your application will be withdrawn without review.

NIH policies on post-submission materials (NOT-OD-15-039) and late applications (NOT-OD-17-066) prohibit changing or correcting the budget or submitting budget forms on this application at this point.

Please acknowledge the receipt of this email. If you have questions about this policy, do not hesitate to contact me.

Sincerely,
Michelle M. Timmerman, Ph.D.
Associate Director
Division of Receipt and Referral
Center for Scientific
Review/NIH/DHHS
(b) (6)

<NIAID COV 2019 Budget Letter 2R01AI110964-06.pdf>

From: Stemmy, Erik (NIH/NIAID) [E]
To: Aleksei Chmura
Cc: Peter Daszak; Graham, Adam (NIH/NIAID) [E]; 李泓萤; Alison Andre
Subject: RE: NIH Pre-approval needed for 2R01AI110964-06
Date: Friday, November 9, 2018 3:55:00 PM

I think the best thing is to check back in with Michelle Timmerman at R&R. I'm not sure if you need to withdraw and resubmit the application, or if you can edit the budget after submission. On the program side we don't have access to those systems.

Erik

From: Aleksei Chmura (b) (6)
Sent: Friday, November 9, 2018 3:09 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Peter Daszak (b) (6) Graham, Adam (NIH/NIAID) [E] (b) (6) Alison Andre
Subject: Re: NIH Pre-approval needed for 2R01AI110964-06

Dear Erik,

I am sorry that we did not realize that. We would like to proceed with having our application reviewed this round and will modify our Direct Costs to $500k per year.

Should we modify our budget in ASSIST or via another method?

Many thanks for the rapid reply!

-Aleksei

> On Nov 9, 2018, at 14:58, Stemmy, Erik (NIH/NIAID) [E] (b) (6) wrote:
>
> Hi Aleksei,
> I didn't know that your group was planning to request a budget over $500k for the renewal. Unfortunately, it won't be possible to get that approved before Nov 18th. There is a lengthy internal review process, and we require at least 6 weeks to process it. I've pasted a link below to NIAID's Big Grant SOP that has some additional information. If you would still like your application to be reviewed this round, the only option would be to reduce the budget to be below the cap, even through the out years of the award.

Let me know how you'd like to proceed. If you want to pursue the large budget we can work to get it reviewed before the next receipt date.
Erik

Big Grant SOP: https://www.niaid.nih.gov/research/big-grants-sop

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone: (b) (6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Aleksei Chmura (b) (6)
Sent: Friday, November 9, 2018 2:50 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Peter Daszak < (b) (6) Graham, Adam (NIH/NIAID) [E]
(b) (6) Alison Andre
Subject: Re: NIH Pre-approval needed for 2R01AI110964-06

Dear Erik,

We just spoke with Michelle Timmerman from the Department of Receipt and Referral. She said that as Program Officer at NIAID, you will need to fill out an "awaiting receipt of application form" to approve our renewal request in excess of the $500 per year budget cap. She also mentioned that we should update her as to the progress through NIAID internal approval and that ultimately it will be send over to her at DRR.

Apologies if I have the incorrect terms here. Please let me know, if there is anything further that we may do.

Sincerely,

-Aleksei

Aleksei Chmura, PhD
Chief of Staff

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

(b) (6) (office)
(mobile)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

On Nov 9, 2018, at 14:14, Timmerman, Michelle (NIH/CSR) [E] (b) (6) > wrote:

Dear Dr. Daszak:
Your application to PA-18-484 entitled "Understanding the Risk of Bat Coronavirus Emergence" (2R01AI110964-06) has been received by the Division of Receipt and Referral in the Center for Scientific Review at the NIH. This application is subject to the NIH's policy regarding applications requesting $500,000 or more in subtotal direct costs for any year. Please see the Application Guide instructions for the R&R Budget Form (https://go.usa.gov/xPTR9), part 2.3.7.2 of the Grants Policy Statement it references (https://go.usa.gov/xPNCA) and the original announcement NOT-OD-02-004 (https://go.usa.gov/xPTRX). Please also see Section IV.7 "Requests of $500,000 or more for direct costs in any year" in the Funding Opportunity Announcement.

The policy has the following requirements:

- The PD/PI must contact and obtain agreement from an

appropriate Institute/Center to accept assignment of the application, at least six weeks in advance of submission.

- The PD/PI must include a cover letter identifying the program staff member or the Institute/Center that has agreed to accept the assignment of the application.
- The Institute or Center that is willing to accept the application must notify the Center for Scientific Review (CSR), Division of Receipt and Referral (DRR) directly, that the application is acceptable.

Your application does not meet these requirements. Please see Total Direct Costs less Consortium F&A" in your application, which shows the requested budget of $515,358 in each year. Please also see the warning in your Commons account that states "ESubmission warning:Direct cost requests of $500k or more a year need approval to accept assignment from Institute/Center staff, except for RFAs or PAs with budgetary limits. Applications without such approval may be delayed or not accepted for review. (020.52.2)".

In order for your application to be considered for the current receipt cycle, we must receive notification of acceptance from an NIH Institute/Center by the end of the day November 16, 2018. If notification has not been received by then, your application will be withdrawn without review.

NIH policies on post-submission materials (NOT-OD-15-039) and late applications (NOT-OD-17-066) prohibit changing or correcting the budget or submitting budget forms on this application at this point.

Please acknowledge the receipt of this email. If you have questions about this policy, do not hesitate to contact me.

Sincerely,
Michelle M. Timmerman, Ph.D.
Associate Director
Division of Receipt and Referral
Center for Scientific Review/NIH/DHHS
(b) (6)

From: Graham, Adam (NIH/NIAID) [E]
To: Aleksei Chmura
Cc: Stemmy, Erik (NIH/NIAID) [E]; 李泓萤; Dr. Peter Daszak
Subject: RE: IRB Question from Grant Number: 5R01AI110964 - 05 PI Name: DASZAK , PETER
Date: Wednesday, October 10, 2018 8:04:39 AM
Attachments: image001.png

Hi Aleksei,

You would need to obtain an updated IRB approval from your institution and provide notification of that approval to us. We would not need the Chinese approval, as your IRB approval includes review of that.

Thanks,

Adam Graham
Grants Management Specialist
DHHS, NIH, NIAID, GMP
Room 4E40, MSC 9833
5601 Fishers Lane
Bethesda, MD 20892
(b) (6)

***Effective January 1, 2017**, NIH closeout policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.*

From: Aleksei Chmura (b) (6)
Sent: Wednesday, October 3, 2018 9:46 AM
To: Graham, Adam (NIH/NIAID) [E] (b) (6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b) (6); Dr. Peter Daszak (b) (6)
Subject: IRB Question from Grant Number: 5R01AI110964 - 05 PI Name: DASZAK , PETER

Dear Adam,

We have an online, anonymous survey we would like to conduct with individuals at the wildlife-domestic animal interface in southern China under our award. We are teasing out the different risk and motivating factors for Coronavirus and bat exposure as per our specific aim 2 in our

NIH-001070
NIH
National Institute of
Allergy and
Infectious Diseases

proposal. The IRB of our Chinese/local partner the Wuhan School of Public Health has reviewed our proposed study and approved it. Both the Wuhan School of Public Health and EcoHealth Alliance (our institution) have active FWAs. This proposed online, anonymous survey is not part of our current, approved IRB protocol under our award.

Our understanding is that we will also need USA IRB approval (as we have had with all our other human research) and that having a China-only IRB approval will not be sufficient for our proposed online survey. We wanted to confirm with you before proceeding with a new US IRB approval. This will affect our participant enrollment numbers too. How would we proceed with this - just forward to you our US and China IRB approval notifications or is this done via Research.gov? If it will be easier, both Hongying Li and I are available for a phone call anytime tomorrow or Friday.

Many thanks,

-Aleksei

Aleksei Chmura, PhD
Chief of Staff

EcoHealth Alliance
460 West 34th Street
Ste. 1701
New York, NY 10001

(b) (6) (direct)
(mobile)
Aleksei MacDurian (Skype)

www.ecohealthalliance.org

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EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.

Begin forwarded message:

From: "Graham, Adam (NIH/NIAID) [E]" <(b) (6)
Subject: Grant Number: 5R01AI110964 - 05 PI Name: DASZAK , PETER
Date: July 5, 2018 at 15:25:16 EDT
To: (b) (6)
"Stemmy, Erik (NIH/NIAID) [E]" (b) (6)
(b) (6)
Cc: "Linde, Emily (NIH/NIAID) [E]" (b) (6)
"Glowinski, Irene (NIH/NIAID) [E]"
"Erbelding, Emily (NIH/NIAID) [E]"
"Ford, Andrew (NIH/NIAID) [E]" (b) (6), "Khurana, Dhana (NIH/NIAID) [E]" (b) (6)

Good afternoon,

Attached is a letter notifying you that the GoF Research Funding Pause has been lifted via the HHS P3CO Framework and that the GoF term-of-award was removed when the next last Type 5 notice-of-award was issued.

Please let us know if you have any questions.

Adam Graham
Grants Management Specialist
DHHS, NIH, NIAID, GMP
Room 4E40, MSC 9833
5601 Fishers Lane
Bethesda, MD 20892
(b) (6)

***Effective January 1, 2017**, NIH closeout policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.*

From: Peter Daszak
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Aleksei Chmura; Alison Andre; Coomes, Stephanie (NIH/NIAID) [E]
Subject: RE: AI110964 Site in Laos
Date: Thursday, September 27, 2018 2:47:40 PM

Hi Erik,

Unfortunately, we weren't able to set up a field site in Laos properly – it's a tough country to work in with a very slow bureaucracy...

Let me know if the group are passing through any other sites and I'll be able to either put you in touch with our on-the-ground staff, or with other groups that are of interest...

Cheers,

Peter

Peter Daszak
President

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

Tel. (b) (6)
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Stemmy, Erik (NIH/NIAID) [E](b) (6)
Sent: Thursday, September 27, 2018 1:43 PM
To: Peter Daszak
Cc: Aleksei Chmura; Alison Andre; Coomes, Stephanie (NIH/NIAID) [E]
Subject: AI110964 Site in Laos

Hi Peter,

It was nice seeing you a few weeks ago in Beijing! I have a question for you about your site in Laos for your grant AI110964. Can you give me an idea of the work they are doing there, and the facilities they are in? Some folks from HHS leadership may be traveling through Southeast Asia, and we've been asked to identify sites for possible site visits in Laos. I know that the work there isn't a major part of the award, but wanted to check with you to see if this might be one to suggest. I would imaging the sampling sites are likely remote, but thought the labs at the National Animal Health Laboratory might be a suggestion. Please let me know what you think.

Thanks!
Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone (b) (6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

****

From: Stemmy, Erik (NIH/NIAID) [E]
To: Ford, Andrew (NIH/NIAID) [E]
Cc: Strickler-Dinglasan, Patricia (NIH/NIAID) [C]
Subject: RE: 5R01AI110964-05 GoF-P3CO Replacement Term
Date: Thursday, June 21, 2018 2:57:00 PM

Hi Andrew,
Sorry, I was in training last week and missed this in the shuffle. I think the language is fine (b) (5)

Sorry for the delay!
Erik

From: Ford, Andrew (NIH/NIAID) [E]
Sent: Thursday, June 21, 2018 2:45 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Strickler-Dinglasan, Patricia (NIH/NIAID) [C] (b) (6); Ford, Andrew (NIH/NIAID) [E] (b) (6)
Subject: FW: 5R01AI110964-05 GoF-P3CO Replacement Term

Hey Erik,

(b) (5)

Thanks,
Andrew

From: Ford, Andrew (NIH/NIAID) [E]
Sent: Thursday, June 14, 2018 9:12 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6); Khurana, Dhana (NIH/NIAID) [E] (b) (6)
Cc: Ford, Andrew (NIH/NIAID) [E] < (b) (6) Strickler-Dinglasan, Patricia (NIH/NIAID) [C] (b) (6)
Subject: 5R01AI110964-05 GoF-P3CO Replacement Term

Dear Erik and Dhana,

Attached is a draft letter to the EcoHealth Alliance noting that NIAID re-reviewed the proposed research in accordance with the HHS P3CO Framework and revised the terms and conditions of the award to reflect that should certain growth parameters be met, the NIAID Program Officer and Grants Management Specialist are to be notified immediately and that further research may require review by DHHS in accordance with the HHS P3CO Framework.

(b) (5)

(b) (5)

[redacted]

After your review we can proceed to have the letter signed and sent.

I would be happy to discuss any questions.

Thanks,
Andrew

Andrew Q. Ford, Ph.D.
Office of Scientific Coordination and Program Operations
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
5601 Fishers Lane Room 7G64
Rockville, MD 20892
(b) (6)
[redacted]

From:	Peter Daszak
To:	Stemmy, Erik (NIH/NIAID) [E]
Cc:	Smith, Philip (NIH/NIAID) [E]; Aleksei Chmura; Hongying Li; Alison Andre
Subject:	5R01AI110964 Coronavirus Year 4 Report
Date:	Wednesday, April 25, 2018 9:27:31 PM

Dear Erik,

I just wanted to send you a pdf of our Year 4 Report which I submitted last week. We've had some fantastic results this past year and I've put these in the report summary, but also included the discovery of SADS-CoV as another key findings. As you'll see, we're on track to hit all the major goals of the project by the end of Yr5, including human questionnaires and sampling, risk modeling, more in-depth viral characterization and discovery.

I know you've likely not had chance to read the report yet, but I also wanted to check-in with you soon about submitting a new proposal/renewal to build on the work we've done. You suggested that this would be good timing when we met last summer, and right now I'm looking at the November 5th 2018 deadline (renewal). I have a couple of questions on this: First, I've not submitted a renewal before the end of a current R01, and just want to check that this is the standard procedure. Our R01 officially ends in May 31st 2019. Secondly, I wanted to check in which study section would be best for this. The original proposal went to CFRS-Clinical Research and Field Studies of Infectious Diseases. I've asked Alison to set up a time for us to have a quick chat sometime in the next few weeks if possible.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001
Tel. (b) (6)
www.ecohealthalliance.org
@PeterDaszak
@EcoHealthNYC

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: Stemmy, Erik (NIH/NIAID) [E]
To: Tibbals, Melinda (NIH/NIAID) [C]
Subject: RE: number of participants in R01AI110964
Date: Thursday, October 26, 2017 2:49:00 PM

Hi Melinda,

[redacted] (b) (5) Let me know if you need more info.

Thanks!
Erik

From: Tibbals, Melinda (NIH/NIAID) [C]
Sent: Thursday, October 26, 2017 2:33 PM
To: Stemmy, Erik (NIH/NIAID) [E] [redacted] (b) (6)
Subject: number of participants in R01AI110964

Hi, Erik,

[redacted] (b) (5)
[redacted] will be enrolled in R01AI110964, UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE, Peter Daszak.

[redacted] (b) (5)

Irene is asking for the info by 3:00ish.
Thanks, Melinda

Melinda Tibbals, RAC, CCRA
NIH/NIAID/DMID/RDB, STIB [C]
5601 Fishers Lane
For FedEx, UPS, and other courier services: Rockville, MD 20852
For US Postal Services: Rockville, MD 20892-9825
Tel: [redacted] (b) (6)
Cell [redacted]
[redacted] (b) (6)

From: Normil, Carine (NIH/NIAID) [C]
To: Aleksei Chmura
Cc: Stemmy, Erik (NIH/NIAID) [E]; Dr. Peter Daszak; Smith, Philip (NIH/NIAID) [E]; Alison Andre
Subject: RE: Publication compliance for Grant Number: 5R01AI110964 - 04 PI Name: DASZAK, PETER
Date: Thursday, June 1, 2017 12:50:56 PM

Thank you, Aleksei! This information is very much appreciated.

Best,
Carine

Carine Normil

Grants Management Specialist (Contractor)

Grants Management Program, DEA, NIAID, NIH, HHS
5601 fishers Lane, Rm 4G46, Bethesda , Maryland 20892

Phone: (b) (6)

Fax: (301)-493-0597
Email (b) (6)

NIH National Institute of Allergy and Infectious Diseases

From: Aleksei Chmura (b) (6)
Sent: Wednesday, May 31, 2017 10:59 AM
To: Normil, Carine (NIH/NIAID) [C] (b) (6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b) (6) Dr. Peter Daszak (b) (6); Smith, Philip (NIH/NIAID) [E] (b) (6); Alison Andre (b) (6)
Subject: Re: Publication compliance for Grant Number: 5R01AI110964 - 04 PI Name: DASZAK, PETER
Importance: High

Dear Carine,

Please find the attached documentation of this publication being in compliance with NIH Public Access Policy.

Many thanks most,

Sincerely,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

(b) (6) (direct)
(mobile)
Aleksei MacDurian (Skype)

www.ecohealthalliance.org

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On 23 May 2017, at 13:12, Normil, Carine (NIH/NIAID) [C] (b) (6) wrote:

Good afternoon:

Your progress report for the above referenced award has a non-compliant publication. Please take the necessary steps to bring "*Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi ZL. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 November 28;503(7477):535-8. PubMed PMID: 24172901*" into compliance with the NIH Public Access Policy.

To comply with the policy, please reply to this email and provide a PDF generated report from My NCBI that includes evidence of compliance (PMCID number) for this publication. If you believe the above referenced publication does not fall under the Public Access Policy, please provide a brief explanation. A response is appreciated by **June 15, 2017**.

If you have questions about the Policy, feel free to contact me via email at (b) (6) or send a note to PublicAccess@nih.gov.

Best regards,
Carine

Carine Normil

Grants Management Specialist (Contractor)

Grants Management Program, DEA, NIAID, NIH, HHS
5601 fishers Lane, Rm 4G46, Bethesda , Maryland 20892

Phone (b) (6)

Fax: (301)-493-0597
Email: (b) (6)

<image001.jpg>

From: Stemmy, Erik (NIH/NIAID) [E]
To: Smith, Philip (NIH/NIAID) [E]
Subject: RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER
Date: Tuesday, April 25, 2017 12:26:00 PM

Thanks Philip, will do.

Erik

From: Smith, Philip (NIH/NIAID) [E]
Sent: Tuesday, April 25, 2017 12:17 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Subject: RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Hi Erik,

(b) (5)

If you have any additional questions, please let me know.

Thanks,

Philip Smith
☎: (b) (6)
✉:

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Tuesday, April 25, 2017 11:58 AM
To: Smith, Philip (NIH/NIAID) [E] (b) (6)
Subject: RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Hi Philip,
I'm completing the checklist for this award had had a couple of questions... (b) (5) Please let me know what you think.

Thanks!
Erik

From: Stemmy, Erik (NIH/NIAID) [E]
Sent: Thursday, March 16, 2017 1:23 PM
To: Aleksei Chmura (b) (6)
Cc: Smith, Philip (NIH/NIAID) [E] (b) (6)
Subject: RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Hello Aleksei,
I've been processing the new foreign clearances you requested and couple of additional questions came up for the work in Myanmar. Could you respond to the questions below?

Thank you!
Erik

-Will Dr Daszak (or other EcoHealth staff) plan to spend time directly in country in Myanmar? If so, please provide an approximate % of time.
-How long do you anticipate the sampling will continue? That is, through the remainder of the R01, or a shorter amount of time?
-Can you confirm the total amount of US$ to be sent to Myanmar for the work?

From: Aleksei Chmura (b) (6)
Sent: Wednesday, March 01, 2017 12:55 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Smith, Philip (NIH/NIAID) [E]
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Fantastic to hear, Erik!

I quite understand and no rush.

Much appreciated,

-Aleksei

> On Mar 1, 2017, at 17:51, Stemmy, Erik (NIH/NIAID) [E] (b) (6)> wrote:
>
> Hi Aleksei,
> Very sorry for my slow response, I've been swamped lately. I think I have everything I need to finish with the foreign clearance, and will let you know if I need anything else.
>
> Erik
>
> Erik J. Stemmy, Ph.D.
> Program Officer
> Respiratory Diseases Branch

Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone (b) (6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

****

From: Aleksei Chmura (b) (6)
Sent: Wednesday, March 1, 2017 12:31 PM
To: Greer, Jenny (NIH/NIAID) [E] (b) (6)
Cc: Smith, Philip (NIH/NIAID) [E] Stemmy, Erik (NIH/NIAID) [E]
(b) (6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Thanks, Jenny!

Apologies. I will chase up with Philip and Erik.

Cheers,

-Aleksei

> On Mar 1, 2017, at 17:28, Greer, Jenny (NIH/NIAID) [E]
> (b) (6) wrote:
>
> Aleksei,
>
> Thanks for your email. It looks like I did receive your email of February 18. However, as I mentioned, I am no longer the GMS assigned to this grant. You will need to touch base with Philip Smith for any updates on your request. I've copied him on this email for your convenience.

All the best,

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone (b) (6)
Email:

***Effective January 1, 2017**, NIH closeout policy has changed (see NOT-OD-17-022). NIH is no longer accepting Final Progress Reports (FPR). Grantees must now report final project outcomes using the new F-RPPR. For instructions on how to submit the new F-RPPR please see instructions on the NIH RPPR Page.*

From: Aleksei Chmura (b) (6)
Sent: Wednesday, March 01, 2017 5:07 AM
To: Greer, Jenny (NIH/NIAID) [E] (b) (6)
Subject: Fwd: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Hi, Jenny.

I received an out-of-office message from Erik last month, so just wanted to see if you also received my email and PDF attachment (below) to Erik and if there were anything else required for now. No rush - I am just checking-in.

Cheers!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

(b) (6) direct)
mobile)
Aleksei MacDurian (Skype)

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EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

Begin forwarded message:

From: Aleksei Chmura
(b) (6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER
Date: February 18, 2017 at 03:18:23 GMT
To: "Stemmy, Erik (NIH/NIAID) [E]"
(b) (6)
Cc: "Greer, Jenny (NIH/NIAID) [E]"
(b) (6) "Smith, Philip (NIH/NIAID) [E]" (b) (6)

Dear Erik,

Please find our responses in the attached PDF. If you need any additional details, please let me know.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

(b) (6) (direct)
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On Feb 15, 2017, at 08:52, Stemmy, Erik (NIH/NIAID) [E] (b) (6) wrote:

Hi Aleksei,
I know you said nothing will be changing from your currently approved animal studies, but it would be helpful for me in preparing the foreign clearance request if you could write a few concise sentences about the new animal work addressing the following points:

- Kind or species of animal and number to be used
- Location of the source of the animals, if known
- A brief description of the sampling (blood draw, swab, etc)
- Location from where the animals will be obtained (source)
- If possible, what will be done with the animals after the project ends (e.g., euthanized)

Let me know if you have any questions.
Thanks!
Erik

From: Aleksei Chmura (b) (6)
Sent: Monday, February 13, 2017 4:23 PM
To: Greer, Jenny (NIH/NIAID) [E] (b) (6)
Cc: Stemmy, Erik (NIH/NIAID) [E] (b) (6); Smith, Philip (NIH/NIAID) [E] (b) (6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Super! Thanks, Jenny.

Erik and Philip - please let me know, if you have any questions or require additional details. We look forward to your responses.

Sincerely,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

(b) (6) (direct)
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Aleksei MacDurian (Skype)

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On Feb 13, 2017, at 16:18,
Greer, Jenny (NIH/NIAID) [E]
(b) (6) wrote:

Aleksei,

Thank you for your email. I am copying Erik on this response so he can make sure he has everything needed to initiate a request for each of these foreign sites. I am also copying Philip Smith, the grants management specialist assigned to this grant for this fiscal year. Please don't hesitate to contact either of them with any questions you may have.

Please note that this response does not constitute approval and it will take at least 3 weeks for a final determination to be made.

Thanks again! And have a great afternoon!

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49,
MSC 9833
Bethesda, MD 20892-9824
Phone (b) (6)
Email:

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From: Aleksei Chmura (b) (6)

Sent: Friday, February 10, 2017 2:54 PM

To: Greer, Jenny (NIH/NIAID) [E]
(b) (6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Jenny,

I am just following up with item 1 and 1a from your email below. As per Peter's email (also below), we would like to request prior approval for collecting non-human animal samples in 7 countries: Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Thailand, and Vietnam.

No new animals will be introduced nor any new field procedures, we have submitted IACUC protocol modification - for geographic locations only - and will provide approval dates as soon as they are available.

No work will be conducted until we have your approval and IACUC approval.

Testing would be conducted locally and if any samples were to be transferred to China these would be only extracted viral DNA - and not the original sample material.

Samples will be collected by either our current China field team personnel working directly with our collaborators in these countries or by respective in-country personnel and require no more than 10% budget modification total (from already budgeted China fieldwork) for any non-China in-country work.

Here is the list of our local in-country contacts and institutions:

Cambodia
Veasna Duong
Institut Pasteur du Cambodge
No. 5 Monivong Boulevard
P.O Box. 983, Phnom Penh, Cambodia
(b) (6)

Indonesia
Joko Pamungkas
Primate Research Center at Bogor Agricultural University
JalanLodayaII/5,Bogor16151, Indonesia
(b) (6)

Lao People's Democratic Republic
Watthana Theppangna
National Animal Health Laboratory
Department of Livestock and Fisheries
Ministry of Agriculture and Forestry, Vientiane, Lao PDR
(b) (6)

Malaysia
Tom J. Hughes
Conservation Medicine, Ltd.
Suite 4A, Level 4, Main Office Tower
Financial Park Complex, Jalan Merdeka, 87000
Federal Territory of Labuan, Malaysia
(b) (6)

Myanmar
Aung Than Toe
San Pya Clinic
20/256, Insein Road
Yangon 11051, Myanmar

(b) (6)

Thailand
Supaporn Wacharapluesadee
Neuroscience Center for
Research and Development
King Chulalongkorn Memorial
Hospital
Rama 4 Road
Patumwan, Bangkok, Thailand
10330
(b) (6)

Vietnam
Nguyen Huu Nam
Faculty of Animal and
Veterinary Science
Hanoi Agricultural University
Trauquy, Gialâm, Hanoi,
Vietnam
(b) (6)

If it will be easier to have a quick chat about this, I am happy to call anytime. Also, if this request should be sent more formally as a letter attachment, we can do that rapidly as well.

I hope you and yours had a lovely Holiday and are surviving the blizzard!

Cheers,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

(b) (6) rect)
obile)

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On Aug 1, 2016, at 12:39, Greer, Jenny (NIH/NIAID) [E] (b) (6) wrote:

Thank you for your email. To answer your questions:

1. To do any work in countries other than China, you will need to request prior approval from NIH. To do so, submit a formal request, including the names, institutions, and full contact information of any institutions with which you will collaborate for such

activities. Be sure to indicate whether animal or human research will be conducted and what funds, if any, will be going into these countries. The approval process for new foreign sites takes at least 3 weeks.

1a . If you are introducing new animals into the project, then there may be additional requirements from the Office of Laboratory Animal Welfare (OLAW). Again, you would need to submit a formal request, providing a scientific justification for the inclusion of new species on the project, and, if appropriate, a new Vertebrate Animal Section. If additional IACUC approvals are required, you will need to provide us with the IACUC approval dates

(but **not** a copy of the actual approval).

2. These individuals are not listed in the Notice of Award as key personnel, so, from a grants management perspective, you do not need to get prior approval for this change. That said, if this change or other such personnel changes would have a significant impact on the scope of the project or the science itself, you would need to at least run it by your Program Officer. And if it is determined that personnel changes would cause a scope

change, then you would need grants management approval as well.

3. I do not know what you are asking here. It looks like we have approved both the Wuhan University and ECNU for work on this project. Therefore, no additional prior approval is required for changes unless otherwise specified in the NIH Grants Policy Statement (eg, a change of scope).

Please don't hesitate to contact me with any additional questions. I will be available until 2:30 eastern and then again on Wednesday.

All the best,

Jenny

Jenny Greer
Grants Management
Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane,
Room 4E49, MSC
9833
Bethesda, MD
20892-9824
Phone: (b) (6)
Email: (b) (6)

behalf of NIAID by one of its representatives.

From: Aleksei MacDurian

(b) (6)

Sent: Sunday, July 31, 2016 6:06 AM

To: Greer, Jenny (NIH/NIAID) [E]

(b) (6)

Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Jenny,

Since you were not cc'ed on the original email, I wanted to follow up with you on three things from Dr. Daszak's email to Erik (included below):

1) Do we need to formally request permission to sample species of bats and other high-risk [rodents and carnivore] hosts in countries that neighbor China (Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia). Under

this award our current US and China IACUC approved protocol via Tufts University and Wuhan Institute of Virology permits us to sample these species in these regions.

2) We provided Dr. Noam Ross' CV with our Year 2 Report. Dr. Ross has replaced Dr. Hosseini who is no longer working on this project. Do we need to do anything else for this? I have attached his Biosketch here for reference.

3) Our Human surveillance work and local IRB approval have all been through the Wuhan University School of Public Health (WUSPH) in China (DUNS No. 529049295). We would like now - in Years 3 - 5 of our award to subcontract directly with them rather than with the institution on our current budget: East China Normal University (ECNU) School of Life Sciences. The Wuhan University School of Public

Health budget amount would be the same annual amount as currently budgeted for East China Normal University in these same years.

It may be easier to briefly chat about these questions via telephone. If so, you may reach me at (b) (6) anytime.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

(b) (6)
direct)
mobile)
(Skype)

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On Fri, May 13, 2016 at 5:55 PM,

Peter Daszak
(b) (6)
wrote:

> Dear Erik,
>
> I just wanted to let you know that we submitted our Year 2 Report yesterday (attached as a pdf).
>
> It's been a pretty productive year, and some of the highlights include: collecting samples from 15 bat genera in southern China with 280 (12%) testing positive for coronaviruses; SARS-like coronaviruses being detected in*Rhinolophus* spp. bats in both Yunnan and Guangdong provinces; 7 published papers from work under our award (including one in *J. Virol.* and one in press at *J. Virol*); 218 quantitative interviews with samples and 47 qualitative coded interviews conducted transcribed and

translated.

In the report, I highlight the reduced amount of wildlife in the local markets within Southern China compared to that we've seen before, as well as the continued expansion of the Chinese wildlife trade within SE Asia so that it is now a largescale international activity. It means that SL-CoVs we find in the wildlife trade would likely have an origin in adjacent countries. Given that our collaborators and field team in China have great contacts in these countries, and EHA also has field teams in many of them, we would like to conduct short field trips to assess markets, identify wildlife in them, and sample species of bats and other high-risk hosts in countries that neighbor China

(Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia). All samples collected would still be tested at the Wuhan Institute of Virology in China. Is there a formal process to ask for permission for this, or is the report and this email appropriate?

I also wanted to let you know about a recent personnel change. Since Dr. Parviez Hosseini has moved to the US Department of State as an Information Advisor earlier this year, we hired another senior researcher Noam Ross to conduct data analysis and spatial mapping. Our Year 2 report includes his CV. Noam has great enthusiasm and l

am eager to see his work on our data collected to date. He has already been out to China is hitting the ground running!

We have had great successes this past year and I'd be happy to discuss any of them with you, if you'd like.

Cheers,

Peter

Peter Daszak
President

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

(b) (6) (direct)
(fax)
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and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

<NIH-NIAID_5R01AI110964 Additional Site Q and A.PDF>

From: Aleksei Chmura
To: Greer, Jenny (NIH/NIAID) [E]
Cc: Stemmy, Erik (NIH/NIAID) [E]; Smith, Philip (NIH/NIAID) [E]
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER
Date: Monday, February 13, 2017 4:24:29 PM

Super! Thanks, Jenny.

Erik and Philip - please let me know, if you have any questions or require additional details. We look forward to your responses.

Sincerely,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

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mobile)
(Skype)

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On Feb 13, 2017, at 16:18, Greer, Jenny (NIH/NIAID) [E] (b) (6) wrote:

Aleksei,

Thank you for your email. I am copying Erik on this response so he can make sure he has everything needed to initiate a request for each of these foreign sites. I am also copying Philip Smith, the grants management specialist assigned to this grant for this fiscal year. Please don't hesitate to contact either of them with any questions you may have.

Please note that this response does not constitute approval and it will take at least 3 weeks for a final determination to be made.

Thanks again! And have a great afternoon!

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone (b) (6)
Email:

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From: Aleksei Chmura (b) (6)
Sent: Friday, February 10, 2017 2:54 PM
To: Greer, Jenny (NIH/NIAID) [E] (b) (6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Jenny,

I am just following up with item 1 and 1a from your email below. As per Peter's email (also below), we would like to request prior approval for collecting non-human animal samples in 7 countries: Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Thailand, and Vietnam.

No new animals will be introduced nor any new field procedures, we have submitted IACUC protocol modification - for geographic locations only - and will provide approval dates as soon as they are available.

No work will be conducted until we have your approval and IACUC approval.

Testing would be conducted locally and if any samples were to be transferred to China these would be only extracted viral DNA - and not the original sample material.

Samples will be collected by either our current China field team personnel working directly with our collaborators in these countries or by respective in-country personnel and require no more than 10% budget modification total (from already budgeted China fieldwork) for any non-China in-country work.

Here is the list of our local in-country contacts and institutions:

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Veasna Duong
Institut Pasteur du Cambodge
No. 5 Monivong Boulevard
P.O Box. 983, Phnom Penh, Cambodia
(b) (6)

Indonesia
Joko Pamungkas
Primate Research Center at Bogor Agricultural University
JalanLodayaII/5,Bogor16151, Indonesia
(b) (6)

Lao People's Democratic Republic
Watthana Theppangna
National Animal Health Laboratory
Department of Livestock and Fisheries
Ministry of Agriculture and Forestry, Vientiane, Lao PDR
(b) (6)

Malaysia
Tom J. Hughes
Conservation Medicine, Ltd.
Suite 4A, Level 4, Main Office Tower
Financial Park Complex, Jalan Merdeka, 87000
Federal Territory of Labuan, Malaysia
(b) (6)

Myanmar
Aung Than Toe
San Pya Clinic
20/256, Insein Road
Yangon 11051, Myanmar
(b) (6)

Thailand
Supaporn Wacharapluesadee
Neuroscience Center for Research and Development
King Chulalongkorn Memorial Hospital
Rama 4 Road
Patumwan, Bangkok, Thailand 10330
(b) (6)

Vietnam

Nguyen Huu Nam
Faculty of Animal and Veterinary Science
Hanoi Agricultural University
Trauquy, Gialâm, Hanoi, Vietnam
(b) (6)

If it will be easier to have a quick chat about this, I am happy to call anytime. Also, if this request should be sent more formally as a letter attachment, we can do that rapidly as well.

I hope you and yours had a lovely Holiday and are surviving the blizzard!

Cheers,

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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On Aug 1, 2016, at 12:39, Greer, Jenny (NIH/NIAID) [E] (b) (6) wrote:

Thank you for your email. To answer your questions:

1. To do any work in countries other than China, you will need to request prior approval from NIH. To do so, submit a formal request, including the names, institutions, and full contact information of any institutions with which you will collaborate for such activities. Be sure to indicate whether animal or human research will be conducted and what funds, if any, will be going into these countries. The approval process for new foreign sites takes at least 3 weeks.

1a . If you are introducing new animals into the project, then there may be additional requirements from the Office of Laboratory Animal Welfare (OLAW). Again, you would need to submit a formal request,

providing a scientific justification for the inclusion of new species on the project, and, if appropriate, a new Vertebrate Animal Section. If additional IACUC approvals are required, you will need to provide us with the IACUC approval dates (but **not** a copy of the actual approval).

2. These individuals are not listed in the Notice of Award as key personnel, so, from a grants management perspective, you do not need to get prior approval for this change. That said, if this change or other such personnel changes would have a significant impact on the scope of the project or the science itself, you would need to at least run it by your Program Officer. And if it is determined that personnel changes would cause a scope change, then you would need grants management approval as well.
3. I do not know what you are asking here. It looks like we have approved both the Wuhan University and ECNU for work on this project. Therefore, no additional prior approval is required for changes unless otherwise specified in the NIH Grants Policy Statement (eg, a change of scope).

Please don't hesitate to contact me with any additional questions. I will be available until 2:30 eastern and then again on Wednesday.

All the best,

Jenny

Jenny Greer
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane, Room 4E49, MSC 9833
Bethesda, MD 20892-9824
Phone (b) (6)
Email:

From: Aleksei MacDurian (b) (6)
Sent: Sunday, July 31, 2016 6:06 AM
To: Greer, Jenny (NIH/NIAID) [E] (b) (6)
Subject: Re: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Dear Jenny,

Since you were not cc'ed on the original email, I wanted to follow up with you on three things from Dr. Daszak's email to Erik (included below):

1) Do we need to formally request permission to sample species of bats and other high-risk [rodents and carnivore] hosts in countries that neighbor China (Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia). Under this award our current US and China IACUC approved protocol via Tufts University and Wuhan Institute of Virology permits us to sample these species in these regions.

2) We provided Dr. Noam Ross' CV with our Year 2 Report. Dr. Ross has replaced Dr. Hosseini who is no longer working on this project. Do we need to do anything else for this? I have attached his Biosketch here for reference.

3) Our Human surveillance work and local IRB approval have all been through the Wuhan University School of Public Health (WUSPH) in China (DUNS No. 529049295). We would like now - in Years 3 - 5 of our award to subcontract directly with them rather than with the institution on our current budget: East China Normal University (ECNU) School of Life Sciences. The Wuhan University School of Public Health budget amount would be the same annual amount as currently budgeted for East China Normal University in these same years.

It may be easier to briefly chat about these questions via telephone. If so, you may reach me at + (b) (6) anytime.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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

(b) (6)
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(Skype)

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On Fri, May 13, 2016 at 5:55 PM, Peter Daszak [redacted (b) (6)] wrote:

> Dear Erik,
>
> I just wanted to let you know that we submitted our Year 2 Report yesterday (attached as a pdf).
>
> It's been a pretty productive year, and some of the highlights include: collecting samples from 15 bat genera in southern China with 280 (12%) testing positive for coronaviruses; SARS-like coronaviruses being detected in*Rhinolophus* spp. bats in both Yunnan and Guangdong provinces; 7 published papers from work under our award (including one in *J. Virol.* and [redacted (b) (4)]; 218 quantitative interviews with samples and 47 qualitative coded interviews conducted transcribed and translated.
>
> In the report, I highlight the reduced amount of wildlife in the local markets within Southern China compared to that we've seen before, as well as the continued expansion of the Chinese wildlife trade within SE Asia so that it is now a largescale international activity. It means that SL-CoVs we find in the wildlife trade would likely have an origin in adjacent countries. Given that our collaborators and field team in China have great contacts in these countries, and EHA also has field teams in many of them, we would like to conduct short field trips to assess markets, identify wildlife in them, and sample species of bats and other high-risk hosts in countries that neighbor China (Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia). All samples collected would still be tested at the Wuhan Institute of Virology in China. Is there a formal process to ask for permission for this, or is the report and this email appropriate?
>
> I also wanted to let you know about a recent personnel change. Since Dr. Parviez Hosseini has moved to the US Department of State as an Information Advisor earlier this year, we hired another senior researcher Noam Ross to conduct data analysis and spatial mapping. Our Year 2 report includes his CV. Noam has great enthusiasm and I am eager to see his work on our data collected to date. He has already been out to China is hitting the ground running!
>
> We have had great successes this past year and I'd be happy to discuss any of them with you, if you'd like.

Cheers,

Peter

Peter Daszak
President

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

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From: Aleksei Chmura
To: Stemmy, Erik (NIH/NIAID) [E]
Cc: Smith, Philip (NIH/NIAID) [E]
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning
Date: Tuesday, April 18, 2017 10:29:47 AM

Thanks, Erik!

-Aleksei

On Apr 18, 2017, at 12:53, Stemmy, Erik (NIH/NIAID) [E] (b) (6) wrote:

> Thanks Aleksei. I've passed your response along and will let you know if there are any follow up questions.
>
> Best,
> Erik
>
> ---
>
> **From:** Aleksei Chmura (b) (6)
> **Sent:** Monday, April 17, 2017 1:38 PM
> **To:** Stemmy, Erik (NIH/NIAID) [E] (b) (6)
> **Cc:** Normil, Carine (NIH/NIAID) [C] (b) (6); Dr. Peter Daszak (b) (6); Smith, Philip (NIH/NIAID) [E] (b) (6); 李泓萤 (b) (6)
> **Subject:** Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning
>
> Dear Erik,
>
> As per Peter, the work is planned to supplement that done by PREDICT and hopefully to collaborate with the PREDICT team if possible. The aim is for the Co-investigator (Zhengli Shi) and her field team to coordinate with the PREDICT Myanmar field team and co-leads to ensure that there is no duplication of effort (the NIAID group will not use the PREDICT protocols), and that there is the opportunity for cross-training. Samples will be collected from bats and tested by PCR for SARS-like Coronaviruses, then for positive samples, to do a series of further characterization of the viruses using the techniques Zhengli has developed in her lab (spike protein binding assays etc.).
>
> Samples collected will also be made available to the Myanmar lab so that the PREDICT protocols can be run in-country.
>
> Please let me know, if you have any further questions.
>
> Cheers,

Aleksei Chmura
Senior Coordinator of Operations

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irect)
nobile)
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On 13 Apr 2017, at 12:26, Stemmy, Erik (NIH/NIAID) [E] (b) (6) wrote:

Thanks Aleksei!

One additional item. In processing the foreign clearance for Myanmar, the State Department requested a little bit more information on how the project relates to the PREDICT work. Specifically, they've asked:

"could you ask the PI to clarify how they are working with the USAID funded PREDICT Project – it is our understanding that ECO-Health is a partner in PREDICT and the sampling methods, etc. described are similar to activities in PREDICT (it may be that the PR is going to be doing additional testing on already collected samples, but that is not clear from the information provided)."

It sounds like they just want to clarify whether the sampling work is in addition to the PREDICT work. Will this be specific sampling for MERS beyond what is already being done?

Best,
Erik

From: Aleksei Chmura [(b) (6)
Sent: Wednesday, April 12, 2017 7:42 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Cc: Normil, Carine (NIH/NIAID) [C] (b) (6); Peter Daszak (b) (6) Smith, Philip (NIH/NIAID) [E]
Subject: Re: eRA Commons: RPPR for Grant 5R01AI110964-04 Submitted to NIH with a Non-Compliance warning

Dear Erik,

The non-compliant paper referenced above has been uploaded in NIHMS and should be updated in Peter's My NCBI as soon as NIHMS approves it.

Many thanks!

-Aleksei

Aleksei Chmura
Senior Coordinator of Operations

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On Thu, Apr 13, 2017 at 12:10 AM, <era-notify@mail.nih.gov> wrote:

> *** This is an automated notification - Please do not reply to this message. ***
>
> Dear Grantee,
>
> The progress report for the above-reference award includes citation(s) that are out of compliance with the NIH Public Access Policy. Compliance with the NIH Public Access Policy is a legal requirement and a term and condition of all NIH awards. This award will be delayed until all publications arising from it are in compliance with the policy.
> The Authorized Organization Representative (AOR) or PD/PI with delegated Progress Report Submit Authority must provide verification that all publications are in compliance with the NIH Public Access Policy, to the Grants Management Specialist (GMS). The Public Access compliance verification may be submitted either using the new Progress Report Additional Material (PRAM) link on the eRA Commons Status page or via email.
>
> Instructions for SO/AOR verification:
>
> - Verify that the PD/PI has used My NCBI to enter publications and/or update compliance status.
> - Include a My NCBI PDF report demonstrating all the formerly non-compliant public access citations are now compliant. To process your award, every citation in the report should be either complete, in process or exempt N/A).

Please
see http://publicaccess.nih.gov/citation_methods.htm for more information about acceptable compliance statuses for public access papers. We have more information about My NCBI athttp://publicaccess.nih.gov/communications.htm.

- If unable to provide verification, provide a justification for why the specific publication(s) cannot be brought into compliance.

NIH awardees are responsible for ensuring that evidence of compliance is included in all NIH applications, proposals and reports. If you have questions about the Policy, please check the NIH Public Access Website or send an email to PublicAccess@nih.gov.

For any further questions about this email, call the eRA Help Desk at 1-866-504-9552 or refer to http://grants.nih.gov/support for additional methods of contact.

Please access Commons at http://public.era.nih.gov/commons/.

For more information please visit http://era.nih.gov/

From:	era-notify@mail.nih.gov
To:	NIAID FCTS; Stemmy, Erik (NIH/NIAID) [E]; Bernabe, Gayle (NIH/NIAID) [E]; Greer, Jenny (NIH/NIAID) [E]
Subject:	FACTS: State Department Clearance Request Approved
Date:	Friday, March 17, 2017 5:14:30 AM

*** This is an automated notification - Please do not reply to this message. ***

Project Number: R01AI110964-03
PI Name: PETER DASZAK
Project Title: Understanding the Risk of Bat Coronavirus Emergence

Country: THAILAND
SDCR Initiated By: Gayle Bernabe
SDCR Status: Approved
Action Comment: We note that the PIs include USAID Regional Development Mission for Asia partners currently undertaking similar work in the same countries through the Emerging Pandemic Threats PREDICT 2 project. The distinction between that project and this one is not immediately clear.

If you have any questions, please contact the eRA Help Desk at http://grants.nih.gov/support/index.html OR call 1-866-504-9552 (tty: 301-451-5939) OR helpdesk@od.nih.gov.

From: Stemmy, Erik (NIH/NIAID) [E]
To: Peter Daszak; Alison Andre
Cc: Aleksei Chmura; Smith, Philip (NIH/NIAID) [E]; Evelyn Luciano
Subject: RE: Out of Office RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER
Date: Thursday, March 16, 2017 2:11:00 PM

Thank you Peter!

Erik

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases NIAID/NIH/HHS
5601 Fishers Lane, Room 8E18
Bethesda, MD 20892-9825
Phone (b) (6)
Email:

Getting ready to publish? Share the good news with your program officer asap! NIAID may be able to help publicize your article. And, remember to list your NIAID grant or contract number in the publication.

From: Peter Daszak (b) (6)
Sent: Thursday, March 16, 2017 2:10 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6); Alison Andre (b) (6)
Cc: Aleksei Chmura (b) (6) Smith, Philip (NIH/NIAID) [E] (b) (6) Evelyn Luciano (b) (6)
Subject: RE: Out of Office RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Hi Erik,

I've just returned from travel and we'll get answers to you on this by Monday COB.

Cheers,

Peter

Peter Daszak
President

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From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Thursday, March 16, 2017 1:33 PM
To: Alison Andre
Cc: Peter Daszak; Aleksei Chmura; Smith, Philip (NIH/NIAID) [E]
Subject: FW: Out of Office RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Hello Alison,
I received an out of office message from Aleksei. I am working on new foreign clearances for the grant referenced above, and need some additional information for the site in Myanmar. Would you be able to help address the questions below?

Thank you,
Erik

-Will Dr Daszak (or other EcoHealth staff) plan to spend time directly in country in Myanmar? If so, please provide an approximate % of time.
-How long do you anticipate the sampling will continue? That is, through the remainder of the R01, or a shorter amount of time?
-Can you confirm the total amount of US$ to be sent to Myanmar for the work?

From: Aleksei Chmura (b) (6)
Sent: Thursday, March 16, 2017 1:23 PM

To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)

Subject: Out of Office RE: Year 2 Report for 5R01AI110964 - 02 PI Name: DASZAK, PETER

Thank you for your email.

I will be out of the office and traveling until 20 March 2017. During this time, I may not have regular access to emails and voice messages. If you should need immediate assistance, please contact Alison Andre at (b) (6) Otherwise, I will respond to your message as soon as possible.

Sincerely,

--

Aleksei Chmura
Senior Coordinator of Operations

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From:	Peter Daszak
To:	Fenton, Matthew (NIH/NIAID) [E]; Erbelding, Emily (NIH/NIAID) [E]; Cassetti, Cristina (NIH/NIAID) [E]; Linde, Emily (NIH/NIAID) [E]; Post, Diane (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Cc:	Aleksei Chmura
Subject:	FW: Regarding 2R01AI110964-06
Date:	Sunday, April 11, 2021 5:14:05 PM
Attachments:	Response to NIH April 2021 re. reactivation and suspension of 2R01AI110964.pdf
Importance:	High

Dear all,

I'm just forwarding my response (attached letter and email chain below) to Michael Lauer re. the 10 conditions imposed on the grant to EcoHealth Alliance.

I've tried to stick to a logical argument, but I'm also mindful of the dozens of FoIA requests targeting EHA and myself and that previous letters have been leaked to the press, so have made sure all details are laid out. I do not aim to make this letter public, of course and am sending this to you confidentially.

As per my email to Dr. Lauer below, the intent of this letter is to demonstrate in good faith what we believe are reasonable efforts to address these conditions and to state the limits of what is possible. The goal is full and rapid reinstatement of our funding – not only because of the damage this has already done to our organization and my personal safety, but more importantly because coronaviruses are likely continuing to spill over into people in the region and our research may help reduce that risk.

Thanks for all your help and support and I will let you know what I hear back from the Director's Office in due course.

Cheers,

Peter

Peter Daszak
President

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EcoHealth Alliance

Dr. Michael Lauer
Deputy Director for Extramural Research,
NIH, Bethesda, MD.

Response to the Reinstatement and immediate suspension of 2R01AI110964 "Understanding the Risk of Bat Coronavirus Emergence"

April 11th 2021

Dear Dr. Lauer,

I am responding your letters of 7/8/2020 and 10/3/2020 regarding the reinstatement and immediate suspension of NIH grant 2R01AI110964 "*Understanding the Risk of Bat Coronavirus Emergence*", that was terminated "for convenience" on 4/24/2020. In particular, this letter addresses the conditions you state would need to be fulfilled in order for us to have access to the funds to continue this work.

As you know, we had not set up any subcontracts to the Wuhan Institute of Virology under this renewal R01. Immediately following NIH's letter on 4/19/2020 that the WIV was being 'investigated', we suspended all plans for contractual work with WIV. This termination of a funded relationship with the institute makes it extraordinarily difficult and more likely impossible to provide the information requested about an autonomous foreign organization – as would also be the case for a domestic one - that our organization neither works with currently, nor has control over.

Additionally, our collaborative work with the Wuhan Institute of Virology prior to your grant termination letter of 4/24/2020 and that planned in the suspended grant, is wholly unrelated to many of the conditions listed below. These conditions also pertain to certain events and situations that in no way involve EcoHealth Alliance or are not under our control. Thus, most of the conditions below are either unrelated to EcoHealth Alliance's planned research in our highly rated, approved and funded grant application, and/or to the biosafety of our continued research funded by the suspended grant when it is reinstated in full.

Furthermore, in our recent correspondence with NIH regarding the latest in a series of FoIA requests, we were informed (1/26/2021 – see email correspondence at the end of this letter) by an NIH staff member Garcia-Malene Gorka that "any indication from my program that there is an ongoing investigation into WIV can now be disregarded, as we recently confirmed there are no pending investigations into that organization." Because this was the explanation in your initial letter of 4/19/2020 for the decisions from your office regarding restrictions on, termination of, then reinstatement and suspension of our grant, we believe that these decisions should now be reassessed.

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Despite our concerns about the relevance, fairness, or ability to fulfil the conditions as set forth in detail below, I have made extensive efforts to satisfy NIH's broad concerns, and have provided details of how these are relevant to each condition below. This includes serving as an expert on the WHO-China joint Mission on the Animal Origins of COVID-19, which involved 1 month on the ground in China (including 2 weeks locked in quarantine), at great personal burden and risk to me, to our organization, and to my family. I undertook this mission at a time when I have had increasing levels of personal attack and harassment, including a white-powder letter to my home address a few weeks after the details of our grant termination went public, and death threats that begun at the same time and continue to this day. It is clear in the wording of these attacks that many are a direct result of dangerous conspiracy theories inadvertently amplified by NIH's grant termination, and repeated in the conditions listed below. This type of harassment has accelerated to the point that personal security guards are now stationed at my home address, where I have also had to install invasive equipment and set up procedures to protect my family against expected violent attacks. Additionally, I now meet regularly with FBI agents and others at my home to monitor these threats. As I am sure you appreciate, this has a significant toll on my work, my personal life and my family.

Below, I detail our response to each of the conditions placed on our suspended grant, in an effort to provide as much information as possible and to explain the limitations on what we can do to respond. I look forward to your reply and hope that these will allow NIH to lift the suspension on funding so that we can continue our work to help protect our nation, indeed the global population, against future coronavirus pandemics. Should you wish, I feel certain we may discuss these points without legal counsel in a scientist-to-scientist conversation, as you have suggested verbally to others at NIH, and they have conveyed to me.

1. Provide an aliquot of the actual SARS-CoV-2 virus that WIV used to determine the viral sequence. We believe this condition is effectively impossible for us to fulfil, for the following reasons. Firstly, there is no scientific nor administrative rationale for us to attempt to obtain a SARS-CoV-2 aliquot given that it is not part of our funded collaboration with WIV. Secondly, EcoHealth Alliance scientists do not have any capacity to work on such an aliquot (EHA does not conduct virological laboratory work on SARS-CoV-2) in the USA. This further reduces the validity of a scientific basis for this request to WIV. Thirdly, EcoHealth Alliance scientists were not part of the work that WIV conducted to determine the viral sequence of SARS-CoV-2, and this was not part of our (then active) R01 funded collaboration. This is publicly stated by the lack of EHA authors listed on the paper and the lack of acknowledgement of our grant as a funding source for this work. This publicly discounts any claim of sample ownership or control. Fourthly, the collaborative research laid out in our now-suspended grant does not include the shipping of human viral isolates out of China. Finally, during the last 16 months, there has been a series of vitriolic attacks from the US Government accusing China of bioengineering and releasing SARS-CoV-2 or of otherwise allowing COVID to become pandemic. Given these attacks, and WIV's status as a government entity, it seems to us incredulous that any request, particularly without scientific rationale, from a US non-profit to a Chinese Government laboratory for an active sample of a pathogenic human virus would likely be successful. We note that 1) to our knowledge China has not supplied such an aliquot to any formal request from a government; and 2) that if circumstances were reversed and a Chinese non-

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governmental institution requested a similar pathogenic viral aliquot from a US government BSL-4 laboratory, this would also be unlikely to be fulfilled.

While we understand that it may be of scientific interest to some US-based researchers to analyze this viral sequence, this scientific interest could easily be satisfied without the need for an aliquot. The full genome of this viral sequence was uploaded to a freely accessible database on January 10 2020, and has been used widely by scientists in the USA (included those funded by NIH) and around the world in their work. Furthermore, isolates of the virus from patients in Thailand and Australia during early 2020 are essentially the same, and have been shared extensively.

2. Explain the apparent disappearance of Huang Yanling, a scientist / technician who worked in the WIV lab but whose lab web presence has been deleted.

International experts on the WHO COVID-19 origins mission, including myself, asked direct questions on this issue to staff at WIV, including the Director of the institute, the P4 Lab Director, Dr. Shi and others. The response from all was consistent, as stated in the WHO mission report published 3/30/2020: "This person according the WIV staff was an alumnus who graduated in 2015 and was now working in a different province and did not accept to talk with media. The person had been contacted and tested and ascertained to be healthy."

Given that the WHO team was not given access to this individual, and that China's personal privacy laws are preclude our ability to insist on a meeting, it is difficult to see how a request from a US non-profit would have been approved. It seems at the least to be significantly outside the remit of a US-based non-profit organization to inquire further about the whereabouts of a citizen of a foreign country who has never to our knowledge been involved in our work, and over whom we have no control, influence, nor legal responsibility.

Finally, while many conspiracy theorists have suggested that the lack of a web presence of this person suggests some nefarious activity, there are dozens of unremarkable and routine reasons why a person may be removed from a web listing of employees or students. Not least of these is when a staff member leaves an institution, or a student graduates.

3. Provide the NIH with WIV's responses to the 2018 U.S. Department of State cables regarding safety concerns.

We believe that WIV senior staff comments reported in the WHO COVID origins mission report directly address this request in that they publicly state that no significant safety issues were found in their laboratory prior to, or following, the emergence of COVID. Any questions regarding the safety of the WIV also need to be put into the context of the widely published history of this lab as being built to international safety engineering standards, adhering to international safety practice standards indicated in the BMBL, and with lead WIV staff trained in safety in the United States by a known authority running the BSL-4 lab at the University of Texas Medical Branch in Galveston (as reported in the U.S. Dept of State cables). Furthermore, no verifiable evidence of safety issues have been reported prior to, or following the U.S. Dept of State cables.

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Regarding the U.S. Dept. of State cables, these do not in fact provide evidence of safety concerns at the laboratory. Neither do they convincingly imply safety issues. In fact, they may be simply interpreted as a request for funding from a diplomatic mission set up to further joint US-China research. It is important to note that initially only very limited phrases from these cables were selectively leaked by a Washington Post reporter in an opinion piece that did not verify nor quote direct sources. This opinion piece is demonstrably incomplete in its reporting, however it has been widely cited as providing evidence of safety issues at WIV (https://www.washingtonpost.com/opinions/2020/04/14/state-department-cables-warned-safety-issues-wuhan-lab-studying-bat-coronaviruses/). I have some detailed knowledge of the background to these cables because the diplomatic visit to WIV that they report was a direct result of our NIH-funded work. As part of EcoHealth Alliance's work in China over the past 15 years, including that funded by NIAID, I visited the US Embassy in Beijing regularly and was involved in discussions with US Embassy staff to set up a field visit to the WIV in order to generate goodwill between the US and China at a time when President Trump was planning a state visit. I did this out of a sense of duty to our government, and to the NIH so that our project could help foster goodwill between our countries, as well as provide an indication of the importance of NIH's work. Following the US Embassy staff mission, I was told by people privy to the cable's contents that the articles were positive and supportive of the work we were doing under NIAID funding, and that the trip was a success.

Now that the full text of these cables (embedded at the end of this letter) has been released with minor redactions (https://news.slashdot.org/story/20/07/20/0611205/full-text-of-us-state-department-cables-finally-released-showing-safety-in-chinese-lab), it seems that this more positive interpretation is justified. As you can see in the excerpts below, the request for more laboratory technician support could be reasonably interpreted as simply a request for the funding for more laboratory technician support, rather than a statement that the lab was unsafe, particularly given that the visit was set up as part of an effort to further develop US-China collaborative research opportunities. Furthermore, the cables are extremely positive about the importance of the collaborative work we were conducting with WIV under NIAID funding:

"**REDACTED** noted that the new lab has a serious shortage of appropriately trained technicians and investigators needed to safely operate this high-containment laboratory. University of Texas Medical Branch in Galveston (UTMB), which has one of several well-established BSL-4 labs in the United States (supported by the National Institute of Allergy and Infectious Diseases (NIAID of NIH)), has scientific collaborations with WIV, which may help alleviate this talent gap over time. Reportedly, researchers from GTMB are helping train technicians who work in the WIV BSL-4 lab. Despite this they would welcome more help from U.S. and international organizations as they establish "gold standard" operating procedures and training courses for the first time in China."

"The ability of WIV scientists to undertake productive research despite limitations on the use of the new BSL-4 facility is demonstrated by a recent publication on the origins of SARS. Over a five-year study **REDACTED** (and their research team) widely sampled bats in Yunnan province with funding support from NIAID/NIH, USAID, and several Chinese funding agencies. The study results were published in PLoS

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Pathogens online on Nov. 30, 2017 (1), and it demonstrated that a SARS-like coronaviruses isolated from horseshoe bats in a single cave contain all the building blocks of the pandemic SARS-coronavirus genome that caused the human outbreak. These results strongly suggest that the highly pathogenic SARS-coronavirus originated in this bat population. Most importantly, the researchers also showed that various SARS-like coronaviruses can interact with ACE2, the human receptor identified for SARS coronavirus. This finding strongly suggests that SARS-like coronaviruses from bats can be transmitted to humans to cause SARS-like disease. From a public health perspective, this makes the continued surveillance of SARS-like corona viruses in bats and study of the animal-human interface critical to future emerging coronavirus outbreak prediction and prevention."

4. Disclose and explain out-of-ordinary restrictions on laboratory facilities, as suggested, for example, by diminished cell-phone traffic in October 2019, and the evidence that there may have been roadblocks surrounding the facility from October 14-19, 2019.

The WIV staff categorically stated to the WHO mission that their lab is audited annually and no unusual events have been identified. The reports of diminished cell-phone traffic and roadblocks have not been verified or published by reliable sources. Furthermore, should hard evidence of diminished cell-phone traffic and roadblocks exist, it is not necessarily indicative of any issues related to concerns about the laboratory studies underway or safety or security incidents within the laboratory. These issues could be explained by any one of a series of issues that occur regularly in the US without nefarious connotations. For example, they could be due to roadwork or other infrastructure repair or maintenance, technical problems with cell-phone transmission, or rerouting of traffic as regularly occurs in Washington DC and other cities due to transport of visiting dignitaries or other events. Finally, there is no credible reason to think that any request a US non-profit might make to the Chinese government for an explanation of traffic or cell-phone issues would result in any response.

5. Explain why WIV failed to note that the RaTG13 virus, the bat-derived coronavirus in its collection with the greatest similarity to SARS-CoV-2, was actually isolated from an abandoned mine where three men died in 2012 with an illness remarkably similar to COVID-19, and explain why this was not followed up.

Since your letter of 7/8/2020, it has been widely reported that WIV scientists have published an addendum to their original paper in *Nature* that described SARS-CoV-2 and compared it phylogenetically to RaTG13. In this follow-up publication, they explain the rationale for conducting work in this mine, and any potential connection to the miner's illnesses and deaths. Importantly, they state that serological results in their lab at the time of the incident did not show that these miners were positive for SARSr-CoVs as some media articles have suggested. They then re-tested the miner samples in 2020 using a range of assays, and found no evidence of SARS-related CoV, nor of SARS-CoV-2 specific antibodies or nucleic acid. During the meeting of the WHO mission team with WIV staff, they were asked a series of questions about the miner's illnesses. The responses were that, while symptoms identified were similar to COVID in that they had pneumonia (a common occupational hazard for miners), their symptoms were also similar to other bacterial or fungal pneumonias. This, and the lack of evidence for SARSr-CoV infection, led them to conclude that SARS or COVID infection was not the cause of these miner's illnesses.

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6. Additionally, EcoHealth Alliance must arrange for WIV to submit to an outside inspection team charged to review the lab facilities and lab records, with specific attention to addressing the question of whether WIV staff had SARS-CoV-2 in their possession prior to December 2019. The inspection team should be granted full access to review the processes and safety of procedures of all of the WIV fieldwork (including but not limited to collection of animals and biospecimens in caves, abandoned man-made underground cavities, or outdoor sites). The inspection team could be organized by NIAID, or, if preferred, by the U.S. National Academy of Sciences.

The WHO mission was negotiated at the very highest levels as the legitimate way to proceed in an investigation of COVID-19 origins, particularly with such critical geopolitical ramifications from this pandemic. Given the intensity of political attacks and conspiracy theories around this lab, it is unreasonable to expect that the Chinese government or WIV would respond to a request from a US non-profit for an outside inspection team. The 11 international expert members of the WHO team included authorities on epidemiology, animal-origin viral infections and One Health. Members of this team have extensive experience conducting lab audits (e.g. Dr. Peter Ben Embarek), running laboratories dealing with human clinical samples (e.g. Drs. Dominic Dwyer, Thea Fischer), and commissioning, managing and accrediting laboratories in foreign countries (myself, Dr. Fabian Leendertz). The WHO-China Joint Study report details the field site visits to multiple labs in Wuhan, including the WIV and summarizes our findings. This includes information on the management of the WIV, safety at the labs, audits and training and testing of staff. I acted in good faith to try to conform to the WHO terms of reference while ensuring that as much information on the laboratory was provided in the report. This information specifically addresses one of your questions above, with categorical statements from WIV senior staff that they did not have SARS-CoV-2 in their possession prior to December 2019.

After returning to the USA, and in the weeks prior to the publication of the report, I worked hard to make sure this critical information was shared as rapidly as possible with the US Government and agencies, including by:

- Briefing Drs. Anthony Fauci and Clifford Lane of NIAID on the findings of the mission;
- Presenting a full talk about the work to the NIAID COVID PI group that meets weekly
- Briefing FBI and other US Government intelligence agency staff
- Briefing members of the US NASEM Forum on Microbial Threats
- Briefing staff on the White House National Security Council
- Briefing staff on the House Committee for Science, Space, and Technology

7. Lastly, EcoHealth Alliance must ensure that all of its subawards are fully reported in the Federal Subaward Reporting System

This has been done and all subawards fully reported as soon as we could once you notified us of this requirement in your letter of 7/8/2020.

8. Provide copies of all EcoHealth Alliance – WIV subrecipient agreements as well as any other documents and information describing how EcoHealth Alliance monitored WIV's compliance with the terms and conditions of award, including with respect to biosafety.

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As we related in response to your letter of 4/19/2020 that asked us to suspend work with WIV, we had not yet set up a subcontract with WIV for the period of this award, therefore no such subrecipient agreements exist. Our plan was to monitor WIV's compliance as we had in the 5 years prior, by means of semi-annual meetings with the lead investigator and assessments of compliance against all conditions of the award. Additionally, following the NIH's termination, then reinstatement and suspension of our funding, we have contracted with a leading lab biosafety contractor based in Southeast Asia (Dr. Paul Selleck) who has extensive experience commissioning, accrediting and auditing BSL-2, -3, and -4 labs, and has worked for over a decade at the BSL-4 Australian Animal Health Lab. We will be using their services where appropriate for foreign lab subcontractees to assess lab biosafety procedures and conduct audits, including following the full reinstatement of 2R01AI110964. Finally, we have appointed a Senior Field Veterinarian who will oversee all EcoHealth Alliance fieldwork in the region and ensure continued compliance with biosafety when conducting animal capture, sampling and sample handling. We have done this at EcoHealth Alliance's own expense, despite our unblemished record on biosafety, to pre-empt calls for further sanctions against our work given the continued attacks against EcoHealth Alliance in the press after the termination of our NIH grant.

9. Describe EcoHealth's efforts to evaluate WIV's risk of noncompliance with Federal statutes, regulations, and the terms and conditions of the subaward.

Over a 15-year period of collaboration with WIV, we have found no evidence to suggest that there was any element of noncompliance with any of the conditions of the grants or contracts covering our collaboration. Our interactions with all staff at the institute have been professional, respectful, open, and with a focus on the science at a very high level. This has contributed to a relationship built on trust and one that is entirely comparable to our scientific collaborations with laboratories in the US, Europe, Australia, Thailand and over 20 other countries. We continue to believe that this laboratory is highly competent and is an extremely low risk for undisclosed accidental release of virus, and there is no verifiable indication as to why we should not continue to believe so. We would of course consider a change in this assessment if significant and verifiable evidence of lab biosafety issues or breach of other Federal statutes are brought forth, but to date we are aware of none.

10. Provide copies of all WIV biosafety reports from June 1, 2014 through May 31, 2019.

Given the intense geopolitical pressure around the accusations that WIV intentionally or accidentally released SARS-CoV-2 (something which the WHO mission deemed 'extremely unlikely'), obtaining such information is not a plausible option at present.

11. Additional information, re. Lack of ongoing investigation into Wuhan Institute of Virology by NIH:

From: Garcia-Malene, Gorka (NIH/OD) [E] (b) (6)
Sent: Tuesday, January 26, 2021 12:20:51 PM
To: [REDACTED]
Cc: [REDACTED] Bartok, Lauren (NIH/NIAID) [E]; NIH FOIA
Subject: [EXT] FW: FOIA Case No. 55702 re: EcoHealth Alliance & Grant No. R01AI110964-6

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
New York, NY 10018
(b) (6)
EcoHealthAlliance.org

Good afternoon, **[REDACTED]** –

I'd like to insert myself into the unfolding FOIA conversation in hopes of providing some helpful context. Our records show that this competing renewal has in fact been funded. In addition, any indication from my program that there is an ongoing investigation into WIV can now be disregarded, as we recently confirmed there are no pending investigations into that organization. If we can agree on the above, all that would remain is to receive your proposed redactions to the records sought under the FOIA request.

Please let me know if there are any questions. I look forward to facilitating the Pre-Disclosure Notification process as efficiently as possible.

Best regards.

Gorka Garcia-Malene | FOIA Officer for the National Institutes of Health

From: [REDACTED]
Sent: Monday, January 25, 2021 5:21 PM
To: Bartok, Lauren (NIH/NIAID) (b) (6)
Cc: [REDACTED]
Subject: FOIA Case No. 55702 re: EcoHealth Alliance & Grant No. R01AI110964-6

Dear Ms. Bartok:

As you may recall, this firm represents EcoHealth Alliance, Inc. ("EcoHealth Alliance"), with respect to certain FOIA requests, including the instant request, FOIA Case No. 55702. The instant request seeks the same documents sought last year in FOIA Case No. 53996, regarding the research project *Understanding the Risk of Bat Coronavirus Emergence*, funded under grant 2R01AI110964. A copy of our prior letter regarding FOIA 53996 is available via the link provided below using the password **[REDACTED]**. On the grounds set forth in the letter, FOIA 53996 was denied in its entirety.

Likewise, FOIA 55702 should be denied and the grant documents should be withheld. First, grant 2R01AI110964-06 remains an unfunded competing renewal grant that is the subject of a pending first-level appeal and, thus, the materials are not subject to disclosure under NIH Grants Policy Statement §2.3.11.2.2. Moreover, in the context of the appeal, NIH has made multiple requests for further information regarding The Wuhan Institute of Virology ("WIV"), which requests indicate that a law enforcement investigation concerning WIV remains ongoing. Second, as demonstrated by the recent attack on the US Capital fueled by disinformation and conspiracy theories, the need to protect the privacy of EcoHealth Alliance's employees and affiliates is more important than ever. Last, while EcoHealth Alliance did not initially identify that the grant proposal contained confidential-commercial and propriety information, this is not dispositive. Moreover, since the

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filing of the renewal application, there has been a global COVID-19 pandemic, which has sparked international and highly competitive research in the area of bat coronaviruses.

At the very least, the responsive documents will require significant redactions. While the grant documents were previously reviewed and redacted in connection with FOIA 53996, we require a further opportunity to review the documents to confirm, *inter alia*, that all personnel information has been removed given the heightened risk of harm in this unprecedented political environment. Accordingly, EcoHealth Alliance respectfully requests a forty-five (45) day extension of time to respond to FOIA 55702, to allow sufficient time for EcoHealth Alliance to conduct a further review of the responsive documents and provide an updated letter response that incorporates recent developments and specific justifications for additional redactions.

Please confirm that NIH will deny FOIA 55702 in its entirety or that NIH is agreeable to EcoHealth Alliance's request for an extension of time to provide a particularized response to FOIA 55702. Please also confirm NIH's receipt of this email.

Thank you.

Best,
[REDACTED]

FOIA Case No. 53996 - EcoHealth Alliance's Letter Response to FOIA Request, dated June 5, 2020 (With Exhibits)
[REDACTED]

[REDACTED]

Tarter Krinsky & Drogin LLP
1350 Broadway | New York | NY | 10018
www.tarterkrinsky.com | LinkedIn
COVID-19 RESOURCE CENTER

12. Publicly released details of U.S. Department of State Cables regarding visit to Wuhan Institute of Virology, as cited in condition #3 above. These are available from a number of sources, including the Washington Post and (https://news.slashdot.org/story/20/07/20/0611205/full-text-of-us-state-department-cables-finally-released-showing-safety-in-chinese-lab).

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MRN:	18 BEIJING 138
Date/DTG:	Jan 19, 2018 / 190739Z JAN 18
From:	AMEMBASSY BEIJING
Action:	WASHDC, SECSTATE *ROUTINE*
E.O.:	13526
TAGS:	SHLH, ETRD, ECON, PGOV, CN
Captions:	SENSITIVE
Reference:	17 WUHAN 48
Subject:	China Opens First Bio Safety Level 4 Laboratory

1. (SBU) **Summary and Comment:** The Chinese Academy of Sciences (CAS) has recently established what is reportedly China's first Biosafety Level 4 (BSL-4) laboratory in Wuhan. This state-of-the-art facility is designed for prevention and control research on diseases that require the highest level of biosafety and biosecurity containment. Ultimately, scientists hope the lab will contribute to the development of new antiviral drugs and vaccines, but its current productivity is limited by a shortage of the highly trained technicians and investigators required to safely operate a BSL-4 laboratory and a lack of clarity in related Chinese government policies and guidelines. (b)(5)

(b)(5)

(b)(5) **End Summary and Comment.**

China Investing in Infectious Disease Control

2. (U) Between November 2002 and July 2003, China faced an outbreak of Severe Acute Respiratory Syndrome (SARS), which, according to the World Health Organization, resulting in 8,098 cases and leading to 774 deaths reported in 37 countries. A majority of cases occurred in China, where the fatality rate was 9.6%. This incident convinced China to prioritize international cooperation for infectious disease control. An aspect of this prioritization was China's work with the Jean Merieux BSL-4 Laboratory in Lyon, France, to build China's first high containment laboratory at Wuhan's Institute of Virology (WIV), an institute under the auspices of the Chinese Academy of Sciences (CAS). Construction took 11 years and $44 million USD, and construction on the facility was completed on January 31, 2015. Following

two years of effort, which is not unusual for such facilities, the WIV lab was accredited in February 2017 by the China National Accreditation Service for Conformity Assessment. It occupies four floors and consists of over 32,000 square feet. WIV leadership now considers the lab operational and ready for research on class-four pathogens (P4), among which are the most virulent viruses that pose a high risk of aerosolized person-to-person transmission.

Unclear Guidelines on Virus Access and a Lack of Trained Talent Impede Research

3. (SBU) In addition to accreditation, the lab must also receive permission from the National Health and Family Planning Commission (NHFPC) to initiate research on specific highly contagious pathogens. According to some WIV scientists, it is unclear how NHFPC determines what viruses can or cannot be studied in the new laboratory. To date, WIV has obtained permission for research on three viruses: Ebola virus, Nipah virus, and Xinjiang hemorrhagic fever virus (a strain of Crimean Congo hemorrhagic fever found in China's Xinjiang Province). Despite this permission, however, the Chinese government has not allowed the WIV to import Ebola viruses for study in the BSL-4 lab. Therefore, WIV scientists are frustrated and have pointed out that they won't be able to conduct research project with Ebola viruses at the new BSL-4 lab despite of the permission.

(b)(6)

(b)(6) Thus, while the BSL-4 lab is ostensibly fully accredited, its utilization is limited by lack of access to specific organisms and by opaque government review and approval processes. As long as this situation continues, Beijing's commitment to prioritizing infectious disease control - on the regional and international level, especially in relation to highly pathogenic viruses, remains in doubt.

(b)(6) noted that the new lab has a serious shortage of appropriately trained technicians and investigators needed to safely operate this high-containment laboratory. University of Texas Medical Branch in Galveston (UTMB), which has one of several well-established BSL-4 labs in the United States (supported by the National Institute of Allergy and Infectious Diseases (NIAID of NIH)), has scientific collaborations with WIV, which may help alleviate this talent gap over time. Reportedly, researchers from GTMB are helping train technicians who work in the WIV BSL-4 lab. Despite this, (b)(6) they would welcome more help from U.S. and international organizations as they establish "gold standard" operating procedures and training courses for the first time in China. As China is building more BSL-4 labs, including one in Harbin Veterinary Research Institute subordinated to the Chinese Academy of Agricultural Sciences (CAAS) for veterinary research use (b)(6) the training for technicians and investigators working on dangerous pathogens will certainly be in demand.

Despite Limitations, WIV Researchers Produce SARS Discoveries

6. (SBU) The ability of WIV scientists to undertake productive research despite limitations on the use of the new BSL-4 facility is demonstrated by a recent publication on the origins of SARS. Over a five-year study, (b)(6) (and their research team) widely sampled bats in Yunnan province with funding support from NIAID/NIH, USAID, and several Chinese funding agencies. The study results were published in PLoS Pathogens online on Nov. 30, 2017 (1), and it demonstrated that a SARS-like coronaviruses isolated from horseshoe bats in a single cave contain all the building blocks of the pandemic SARS-coronavirus genome that caused the human outbreak. These results strongly suggest that the highly pathogenic SARS-coronavirus originated in this bat population. Most importantly, the researchers also showed that various SARS-like coronaviruses can interact with ACE2, the human receptor identified for SARS-coronavirus. This finding strongly suggests that SARS-like coronaviruses from bats can be transmitted to humans to cause SARS-like disease. From a public health perspective, this makes the continued surveillance of SARS-like coronaviruses in bats and study of the animal-human interface critical to future emerging coronavirus outbreak prediction and prevention. (b)(5) (b)(5) WIV scientists are allowed to study the SARS-like coronaviruses isolated from bats while they are precluded from studying human-disease causing SARS coronavirus in their new BSL-4 lab until permission for such work is granted by the NHFCP.

1. Hu B, Zeng L-P, Yang X-L, Ge X-Y, Zhang W, Li B, et al. (2017) Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS Pathog 13(11): e1006698. https://doi.org/10.1371/journal.ppat.1006698

Signature:	BRANSTAD
Drafted By:	(b)(6)
Cleared By:	
Approved By:	
Released By:	
Info:	CHINA POSTS COLLECTIVE *ROUTINE*
Dissemination Rule:	Archive Copy

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SBU

We await your response at the earliest opportunity.

Yours sincerely,

Dr. Peter Daszak
President

(t) (b) (6) (e) (b) (6)
cc. Dr. Aleksei A. Chmura (Chief-of-Staff)

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
New York, NY 10018
(b) (6)
EcoHealthAlliance.org

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

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From: Peter Daszak (b) (6)
Sent: Sunday, April 11, 2021 4:36 PM
To: 'Lauer, Michael (NIH/OD) [E]' < (b) (6)
Cc: Aleksei Chmura < (b) (6) 'Lauer, Michael (NIH/OD) [E]' (b) (6)
Subject: Regarding 2R01AI110964-06
Importance: High

Dear Dr. Lauer,

Please find attached a detailed response to your two previous letters.

I hope you will take our response in the way it was intended – a good faith effort to address as far as is reasonably possible the general concerns that NIH has expressed to us, with a goal of rapid and full removal of the suspension on funding for this critically important work.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
520 Eighth Avenue, Suite 1200
New York, NY 10018-6507
USA

Tel.: (b) (6)
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

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

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From: Lauer, Michael (NIH/OD) [E] (b) (6)
Sent: Wednesday, March 10, 2021 5:37 AM
To: Peter Daszak < (b) (6)
Cc: Aleksei Chmura (b) (6) >; Lauer, Michael (NIH/OD) [E] (b) (6)

Subject: Re: Regarding 2R01AI110964-06

Dear Dr. Daszak

Attached please find two letters that I sent you previously.

Sincerely,
Michael S Lauer, MD

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
1 Center Drive, Building 1, Room 144
Bethesda, MD 20892
Phone (b) (6)
Email:

From: Peter Daszak (b) (6)
Date: Thursday, March 4, 2021 at 10:02 PM
To: "Lauer, Michael (NIH/OD) [E]" (b) (6)
Cc: Aleksei Chmura (b) (6) ***[Peter Daszak:]*** REDACTED>
Subject: Regarding 2R01AI110964-06

Dear Dr. Lauer,

I spoke yesterday with my program officer and other NIAID staff regarding our grant on the risk of coronavirus emergence (2R01AI110964-06) that includes collaboration with scientists at the Wuhan Institute of Virology, China. ***[Peter Daszak:]*** **REDACTED** joined the meeting and told me about his conversation with you about the conditions currently in place on our grant and my efforts to address some of them via my recent work in Wuhan with the WHO. He also commented that you would be willing to talk with me, as PI of this award, about a pathway to reinstate this grant. I would very much value this and am emailing to see if we can arrange a time that's suitable for you, perhaps next week if possible?

I'm cc'ing my assistant **REDACTED**, who can help arrange a suitable time, and also our Chief of Staff Aleksei Chmura, who I would hope could join us, as someone who can access any relevant information on this award, and gained his own Ph.D as part of our original R01 work in China. I want to reassure you that I would not request to talk with legal counsel or bring them into a conversation, and that this would be a discussion with scientists focused on the goals of the grant, focused on research to protect us all against further coronavirus spillover.

Sincerely,

Peter

Peter Daszak
President

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460 West 34th Street
New York, NY 10001
USA

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Website: www.ecohealthalliance.org

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

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From:	Lauer, Michael (NIH/OD) [E]
To:	Matthew R.Torsiello
Cc:	Linde, Emily (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Andrew N. Krinsky; Nels T. Lippert; Black, Jodi (NIH/OD) [E]; Erbelding, Emily (NIH/NIAID) [E]; Bulls, Michelle G. (NIH/OD) [E]; Peter Daszak; Aleksei Chmura; Lauer, Michael (NIH/OD) [E]
Subject:	Re: EcoHealth Alliance re Suspension of NIH Grant No. 2R01 AI 110964-6
Date:	Friday, August 14, 2020 5:17:14 AM
Attachments:	image001.png EcoHealth Alliance - Letter to NIH re Grant Suspension 8-13-2020 (with Exhibits)[2].pdf

Dear Mr. Torsiello – letter received.

Thank you, Mike

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
1 Center Drive, Building 1, Room 144
Bethesda, MD 20892
Phone (b) (6)
Email

From: "Matthew R.Torsiello" (b) (6)
Date: Thursday, August 13, 2020 at 5:54 PM
To: "Lauer, Michael (NIH/OD) [E]" (b) (6)
Cc: "Linde, Emily (NIH/NIAID) [E]" (b) (6), "Stemmy, Erik (NIH/NIAID) [E]" (b) (6) "Andrew N. Krinsky" (b) (6), "Nels T. Lippert" (b) (6) "Black, Jodi (NIH/OD) [E]" (b) (6), "Erbelding, Emily (NIH/NIAID) [E]" (b) (6) "Bulls, Michelle G. (NIH/OD) [E]" (b) (6), Peter Daszak (b) (6), Aleksei Chmura (b) (6), "Linde, Emily (NIH/NIAID) [E]" (b) (6)
Subject: EcoHealth Alliance re Suspension of NIH Grant No. 2R01 AI 110964-6

Dr. Lauer:

Please see the attached letter from Andrew Krinsky on behalf of EcoHealth Alliance, Inc., regarding the decision by NIH to suspend NIH Research Grant 2R01 AI 110964-6 on or about July 8, 2020.

Please confirm receipt. Thank you.

Tarter Krinsky & Drogin LLP
1350 Broadway
New York, NY 10018
P (b) (6)
F 212.216.8001
www.tarterkrinsky.com

Andrew N. Krinsky, *Partner*
(b) (6), *Direct Dial*
(b) (6)

August 13, 2020

Via Email, Certified Mail, & FedEx
(b) (6)

Michael S. Lauer, MD
NIH Deputy Director for Extramural Research
National Institutes of Health
National Institute of Allergy and Infectious Diseases
1 Center Drive, Building 1, Room 144
Bethesda, Maryland 20892

Re: Suspension of NIH Grant 2R01 AI 110964-6

Dear Dr. Lauer:

This firm represents EcoHealth Alliance, Inc. ("EcoHealth Alliance"), in connection with the post-award decision by the National Institute of Allergy and Infectious Diseases ("NIAID"), an Institute within the National Institute of Health ("NIH"), under the Department of Health and Human Services ("HHS"), on July 8, 2020, to suspend grant 2R01 AI 110964-6 (the "Suspension:"), which funds the project *Understanding the Risk of Bat Coronavirus Emergence* (the "Project").

This letter constitutes EcoHealth Alliance's initial response to the Suspension, which was due to purported concerns regarding the safety of unspecified research being conducted at the Wuhan Institute of Virology ("WIV") and for EcoHealth Alliance's alleged failure to report certain subawards in connection with grant 2R01 AI 110964-6 (the "Grant").[1] As set forth in more detail below, the Suspension is unjustified as WIV has no connection to the Project or EcoHealth Alliance's current research and EcoHealth Alliance had not issued any subawards in connection with the Grant at the time of the Suspension. Moreover, NIAID is not authorized under 45 CFR §§ 75.371, 75.205, and 75.207, entitled *Specific Award Conditions*, to impose, *inter alia*, conditions that consist of demands for information regarding entities that are neither subrecipients of grant funds nor project affiliates.[2] Accordingly, EcoHealth Alliance hereby demands that the Suspension be withdrawn and all funding in the HHS Payment Management System be released immediately.

BACKGROUND

A. EcoHealth Alliance

EcoHealth Alliance is a prolific New York-based nonprofit institution dedicated to protecting the health of people, animals, and the environment from emerging zoonotic diseases. For more than a decade, EcoHealth Alliance has been conducting cutting edge scientific research

[1] A copy of my prior letter, dated May 22, 2020, regarding NIH's termination of the Grant, is attached hereto as Exhibit 1.
[2] Notwithstanding NIH's lack of authority to impose extraneous conditions on the Grant and Project, EcoHealth Alliance has made a good faith effort to respond to NIH's questions regarding WIV.

to identify hundreds of new coronaviruses ("CoVs") in bats and to study the capacity of these viruses to infect human cells. The purpose of this research is to identify high risk populations so international actors can leverage their resources to address potential pandemics. In cooperation with a global network of over seventy partners, including academic institutions, intergovernmental and governmental agencies, infectious disease surveillance laboratories, and other international and national organizations in over thirty countries, EcoHealth Alliance's work has led to numerous scientific papers published in high impact journals. These publications have been critical in raising awareness of the threat that CoVs pose to global health, the global economy, and U.S. National Security.

EcoHealth Alliance has a long history of successful cooperation with NIH including multiple Research Project Grant R01 awards. In particular, Peter Daszak, EcoHealth Alliance's President and Chief Scientist, has been the Principal Investigator on more than five multidisciplinary R01s. As demonstrated by Dr. Daszak's research, which produced the first ever global emerging disease "hotspots" map that identified locations in the world where viruses with pandemic potential are most likely to emerge, EcoHealth Alliance is uniquely qualified to assist in both identifying the origins of severe acute respiratory syndrome coronavirus 2 ("SARS-CoV-2") and developing and implementing strategies to combat coronavirus disease 2019 ("COVID-19").

Significantly, at this time, EcoHealth Alliance is working with several countries including, *inter alia*, Bangladesh, Côte d'Ivoire, Indonesia, Liberia, Malaysia, Republic of Congo, and Thailand to distribute PPE and provide critical reagents to test for and contain COVID-19. Notably, this effort is being supported by both the United States Department of State and the United States Agency for International Development. EcoHealth Alliance is also assisting the U.S. Geological Survey, the U.S. Fish & Wildlife Service, the International Union for Conservation of Nature, the World Health Organization, the World Organization for Animal Health, and the World Bank Group to place the COVID-19 pandemic in historical context, assess the risk of COVID-19 resurgence and spillover impacts, and determine best practices and cost-effective solutions to combat the virus. In sum, EcoHealth Alliance's research agenda is more consequential than ever.

B. NIH Issues EcoHealth Alliance A Five-Year Research Grant To Continue The Project

NIH issued EcoHealth Alliance an initial five-year research award for the Project in 2014. In 2019, EcoHealth Alliance submitted a renewal application to NIH through NIAID that contained a revised scope of work, research goals, and proposed collaborators and sought to extend the Project for an additional five years. Upon filing of its renewal application, the Project was ranked as an "extremely high priority" (in the top 3%) by NIAID during its external review process. In light of its success, the absence of any allegation that EcoHealth Alliance had violated the terms and conditions of its prior awards, and the importance of EcoHealth Alliance's continued research, on July 24, 2019, NIH reauthorized grant R01 AI 110964 and issued EcoHealth Alliance a notice of award in the amount of $733,750.00 funded under grant 2R01 AI 110964-6.[3]

[3] A copy of the notice of award, dated July 24, 2019, is attached hereto as Exhibit 1-A.

C. EcoHealth Alliance Informs HHS That WIV Is Not A Subrecipient Of Grant Funds And Agrees Not To Collaborate With WIV In Connection With The Project

On April 19, 2020, Michael S. Lauer, MD, NIH Deputy Director for Extramural Research, sent a letter to EcoHealth Alliance on behalf of NIH regarding WIV. The letter stated that, given allegations that COVID-19 "was precipitated by the release from WIV of the coronavirus responsible for COVID-19", NIH was pursuing suspension of WIV from participating in Federal programs. However, Dr. Lauer assured EcoHealth Alliance that "[t]his suspension of the sub-recipient does not affect the remainder of [EcoHealth Alliance's] grant assuming that no grant funds are provided to WIV following receipt of this email during the period of suspension." [4]

On April 21, 2020, Dr. Daszak of EcoHealth Alliance responded by email to Dr. Lauer stating that he could "categorically state that no funds from [sic] 2R01 AI 110964-6 have been sent to Wuhan Institute of Virology, nor has any contract been signed." Dr. Daszak further represented that EcoHealth Alliance would comply with all NIAID requirements. Dr. Lauer acknowledged (1) that no monies from grant 2R01 AI 110964-6 had gone to WIV and no contract between EcoHealth Alliance and WIV had been signed and (2) EcoHealth Alliance's agreement that it would not provide any funds to WIV until and unless directed otherwise by NIH.[5]

D. NIH Unlawfully Terminates The Grant "For Convenience"

Notwithstanding NIH's representation that suspension of WIV would not affect EcoHealth Alliance's ongoing research, the Grant, or the Project, on April 24, 2020, NIH notified EcoHealth Alliance by letter that, effective immediately, the Grant and Project had been terminated (the "Termination"). The purported grounds for the Termination were: (1) convenience; (2) NIH's discretion not to award a grant, or to award a grant at a particular funding level; and (3) NIH's belief that the Project outcomes did not align with the program goals and agency priorities.[6] As a result of the Termination, EcoHealth Alliance was notified by HHS that it was required to submit a Final Research Performance Progress Report for the Project.

E. EcoHealth Alliance Files A First-Level Appeal Of The Termination

On May 22, 2020, by letter, EcoHealth Alliance filed a first-level appeal of the Termination on NIH, pursuant to NIH Grants Policy Statement Section 8.7 and 42 CFR 50, Subpart D (the "Appeal"). (Ex. 1). In its Appeal, EcoHealth Alliance argued, *inter alia*, that: (1) NIH research grants are not subject to termination for convenience; (2) NIH's discretion to award a grant at a particular funding level did not authorize NIH to issue a post-award decision to terminate a duly awarded grant during the budget period; (3) the research goals of the Project and the NIAID are substantially identical; and (4) there was no rational basis to terminate the Grant for cause.

---

[4] A copy of the NIAID's letter regarding WIV, dated April 19, 2020, is attached hereto as Exhibit 1-B.
[5] A copy of the email correspondence between NIH and EcoHealth Alliance is attached hereto as Exhibit 1-C.
[6] A copy of the NIAID's letter regarding the Termination, dated April 24, 2020, is attached hereto as Exhibit 1-D.

F. NIAID Withdraws The Termination But Suspends The Grant Due To Alleged Safety Concerns At WIV And For EcoHealth's Purported Failure To Report Subawards

Lacking a rational basis for its decision to terminate the Grant, on July 8, 2020, Dr. Lauer notified EcoHealth Alliance by letter that NIAID had withdrawn its termination of the Grant supporting the Project.[7] However, citing "bio-safety concerns" at WIV and EcoHealth Alliance's purported failure to report unspecified subawards, NIAID proceeded to immediately suspend the Grant and the Project, pursuant to 45 CFR § 75.371 and NIH Grants Policy Statement Section 8.5.2, leaving the status of the Project effectively unchanged. In addition, the Suspension seeks to impose on EcoHealth Alliance the outrageous obligation to provide NIH with information and materials in the custody and control of WIV and to somehow facilitate access by an USFG "inspection team" to WIV, as a condition for lifting the Suspension.[8]

ARGUMENT

In the Suspension, NIAID identifies two and only two grounds for its decision to suspend the Grant and the Project: (1) purported safety concerns regarding WIV; and (2) EcoHealth Alliance's purported failure to report unspecified subawards. As set forth in detail herein, EcoHealth Alliance is not conducting any research or otherwise collaborating with WIV in connection with the Project. Moreover, EcoHealth Alliance had not issued any subawards in connection with the Grant at the time of the Suspension. Accordingly, the Suspension should be withdrawn immediately.[9]

A. NIH's Purported Concern That WIV Poses A Threat To Public Health And Welfare Is Not A Basis To Suspend The Grant Or The Project As WIV Is Not A Current Subrecipient Of Grant Funds And Has No Connection To The Project

Under 45 CFR §§ 75.207, 75.205, and 75.371 and NIH Grants Policy Statement Section 8.5.2, NIAID may take one or more enforcement actions where a grant recipient has failed to materially comply with the terms and conditions of the award. Under 45 CFR 75.374, the HHS awarding agency must provide the non-Federal entity an opportunity to object and provide information challenging any suspension or termination action. Given the exclusion of WIV from the Project, and NIH's failure to identify any other safety concerns, there is no basis for NIAID to suspend the Grant or to impose additional conditions.

At all relevant times, EcoHealth Alliance has duly monitored the activities of its subrecipients as necessary to ensure that any subawards were used for authorized purposes, in compliance with Federal statutes, regulations, and the terms and conditions of the subaward. Moreover, EcoHealth Alliance is not aware of any allegation that any subrecipient of grant 1R01 AI 110964 funds has ever used such funds for unauthorized purposes, or in violation of any Federal

[7] Please confirm that, due to the withdrawal of the Termination, EcoHealth Alliance is not required to submit a final Project report at this time.
[8] A copy of the NIAID's letter regarding the Suspension, dated July 8, 2020, is attached hereto as Exhibit 2.
[9] EcoHealth Alliance notes that the Suspension did not state any specific deadline for EcoHealth Alliance to respond to the Suspension or proposed additional conditions. Accordingly, this response is timely.

statutes, regulations, or the terms and conditions of the subject subaward. Furthermore, NIH has never accused EcoHealth Alliance of any act that posed a risk to public welfare and safety.

Significantly, WIV is the only organization identified in the Suspension as posing a risk to public welfare and safety. As stated in my prior letter on May 22, 2020, regarding the now admittedly unlawful termination of the Grant, at NIH's express request, no Grant funds have been distributed to WIV and no contract has been signed between EcoHealth Alliance and WIV in connection with the Project. Thus, the allegation that WIV's independent research at its facility poses unspecified bio-safety concerns should have no bearing on the Project, which was in strict compliance with NIH Grants Policy Statement §§ 4 and 4.1.24, and the terms and conditions of the Notice of Award (Ex. 1-A), at the time of the Suspension.

To reiterate, WIV is not a subrecipient of any Grant funds and will not be involved in the Project in any capacity. (*see* Ex. 1-C-7). Significantly, NIAID explicitly told EcoHealth Alliance that it could exclude WIV and continue the Project without jeopardizing the Grant so long as "no grant funds [were] provided to WIV." (Ex. 1-B).

B. EcoHealth Alliance Has Duly Reported All Issued Subawards And Was In Compliance With The Transparency Act At The Time Of The Suspension

Contrary to NIAID's assertion that EcoHealth Alliance failed to report unspecified subawards, EcoHealth Alliance did not issue or sign any subawards in connection with the 2019 Grant or before July 8, 2020. Accordingly, the reporting requirements of the Federal Funding Accountability and Transparency Act (the "FFATA") did not apply at the time of the Suspension.

Regarding the Project period between 2014 and 2019, EcoHealth Alliance duly complied with all NIAID-system-only financial reporting requirements. While EcoHealth Alliance had not entered the FFATA reporting information in the Federal Subaward Reporting System ("the FSRS"), all subawards issued in connection with the 2014 Project and the 2019 Project are now fully reported in the FSRS. Notably, no one at NIAID or NIH ever contacted or otherwise notified EcoHealth Alliance that it was not in compliance. As EcoHealth Alliance has taken appropriate corrective action that fully resolves its alleged non-compliance with the FFATA, pursuant to NIH Grants Policy Statement Section 8.5.2, the Suspension should be withdrawn.

C. HHS Has No Authority To Impose New Conditions That Are Wholly Unrelated To The Project And EcoHealth Alliance's Ongoing Research

Under 45 CFR § 75.207, NIAID may impose additional specific award conditions under the following circumstances: when the applicant or recipient has a history of failure to comply with the general or specific terms and conditions of a Federal award; when an applicant or recipients fails to meet expected performance goals; and when an applicant or recipient is not otherwise responsible. Allowed conditions include: (1) requiring payments as reimbursements rather than advance payments; (2) withholding authority to proceed to the next phase until receipt of evidence of acceptable performance within a given period of performance; (3) requiring additional, more detailed financial reports; (4) requiring additional project monitoring (5) requiring the non-Federal entity to obtain technical or management assistance; or (6) establishing additional

prior approvals. (45 CFR § 75.207[b]). The purpose of these additional conditions are to encourage the award recipients to comply with the original terms and conditions of the award, applicable statutes, and regulations.

There is no statute or NIH Grants Policy Statement provision that authorizes NIAID to impose additional conditions that consist of demands for information and materials regarding entities that are neither current subrecipients of grant funds nor connected to the research project funded by the subject grant. This makes sense, given that the purpose of imposing additional conditions is to ensure that research funded under a particular grant is conducted safely and in compliance with applicable laws.

Here, NIH's First, Second, Third, Fourth, Fifth, and Sixth proposed conditions, which require that EcoHealth Alliance, *inter alia*, provide information and materials regarding WIV, are wholly unrelated to the safety and efficacy of Project and EcoHealth Alliance's ongoing research as WIV is not a subrecipient of Grant funds (*see* Ex. 1-C-6, 7 and 8). Moreover, certain conditions, including the Sixth condition that "EcoHealth Alliance must arrange for WIV to submit to an outside inspection team charged to review the lab facilities and lab records, with specific attention to addressing the question of whether WIV staff had SARS-CoV-2 in their possession prior to December 2019" seek to impose impossible obligations. EcoHealth Alliance has no authority to grant NIAID access to the WIV lab facilities and is not conducting any research with WIV in connection with the Project. Whether or not EcoHealth Alliance is able to provide responses to the proposed conditions regarding WIV will not affect the safety of EcoHealth Alliance's current research, which will not involve WIV.

Without waiving any objections, in the interest of cooperation, EcoHealth Alliance has made a good faith effort to provide responses to the additional conditions (the "Requests") based on information now known to Peter Daszak, EcoHealth Alliance's President and Chief Scientist.[10]

CONCLUSION

Every single outbreak of a novel virus has been accompanied by the allegation that the subject virus was created in a lab, including, *inter alia*, HIV, Ebola, and now SARS-CoV-2. There is no credible evidence to support these theories. By comparison, we know that seventy-five percent of new emerging diseases originate in wildlife. Every species of wildlife carry these viruses, an estimated 1.7 million of which are still unknown. While many of these viruses are benign, occasionally a lethal virus will emerge that can directly infect humans. EcoHealth Alliance is a valuable resource. The instant request to resume the Project funded by the Grant presents HHS with the opportunity to support proven research regarding the threat of zoonotic disease emergence and to support scientists who are working to determine whether certain vaccines and drugs can kill the SARS-CoV-2 virus to save our lives.

At this time, EcoHealth Alliance is in compliance with all of the terms and conditions of the award including the FFATA, there is no public health concern posed by EcoHealth Alliance's

[10] A copy of EcoHealth Alliance's Objections and Responses to the Requests is attached hereto as Exhibit 3.

resumption of the Project, which will not involve WIV in any capacity (*see* NIH Grants Policy Sections 4 and 4.1.24), and EcoHealth Alliance has hereby provided, to the best of its ability, the information and materials requested by NIH in the Suspension. Accordingly, the Suspension should be withdrawn and all funding in the HHS Payment Management System should be released immediately.

Please note that this letter is not intended to provide an exhaustive list of all possible grounds for vacating the Suspension and may not reflect all arguments and claims that EcoHealth Alliance will assert in the event that it is required to file a first-level appeal or other action or proceeding concerning any future adverse determination by NIAID affecting the Grant or the Project. All of EcoHealth Alliance's rights and remedies to seek review of any adverse determination are expressly reserved.

Should you wish to present evidence in an effort to refute any of the factual assertions made in this letter, and/or to engage in good faith negotiations regarding appropriate terms and conditions for the resumption of funding for grant 2R01 AI 110964-6, we are prepared to review such evidence and to participate in such negotiations.

We await your response to this letter.

Very truly yours,

(b) (6)

Andrew N. Krinsky

cc: (*by email*)

Dr. Erik Stemmy
Ms. Emily Linde
(b) (6)

Exhibit 1

Tarter Krinsky & Drogin LLP
1350 Broadway
New York, NY 10018
P (b) (6)
F 212.216.8001
www.tarterkrinsky.com

Andrew N. Krinsky, *Partner*
(b) (6), *Direct Dial*
(b) (6)

May 22, 2020

Via Email, Certified Mail, & FedEx
(b) (6)

Michael S. Lauer, MD
NIH Deputy Director for Extramural Research
National Institutes of Health
National Institute of Allergy and Infectious Diseases
1 Center Drive, Building 1, Room 144
Bethesda, Maryland 20892

Re: Termination of NIH Grant 2R01 AI 110964-6

Dear Dr. Lauer:

This firm represents EcoHealth Alliance, Inc. ("EcoHealth Alliance") with regard to the post-award decision by the National Institute of Allergy and Infectious Diseases ("NIAID"), an Institute within the National Institute of Health ("NIH"), under the Department of Health and Human Services ("HHS"), to terminate the project *Understanding the Risk of Bat Coronavirus Emergence*, funded under grant R01 AI 110964, on April 24, 2020 (the "Termination").

This letter, pursuant to NIH Grants Policy Statement Section 8.7 and 42 CFR 50, Subpart D, constitutes EcoHealth Alliance's first-level appeal of the Termination, which was "for convenience." As set forth in more detail below, the Termination is not authorized under the NIH Grants Policy Statement, arbitrary and capricious and an indefensible attack on public health and welfare given that it undermines a pivotal 10-year research project involving the origins, spread and threat of emerging bat coronaviruses during the peak of an unprecedented worldwide coronavirus pandemic. Accordingly, EcoHealth Alliance hereby demands that grant 2R01 AI 110964-6 be reinstated immediately.

BACKGROUND

A. EcoHealth Alliance

EcoHealth Alliance is a prominent New York-based nonprofit institution dedicated to protecting the health of people, animals, and the environment from emerging zoonotic diseases. For more than a decade, EcoHealth Alliance has been conducting cutting edge scientific research to identify hundreds of new coronaviruses ("CoVs") in bats and to study the capacity of these viruses to infect human cells. The purpose of this research is to identify high risk populations so international actors can leverage their resources to address potential pandemics. In cooperation with a global network of over seventy partners, including academic institutions, intergovernmental

and governmental agencies, infectious disease surveillance laboratories, and other international and national organizations in over thirty countries, EcoHealth Alliance's work has led to numerous scientific papers published in high impact journals. These publications have been critical in raising awareness of the threat that CoVs pose to global health, the global economy, and U.S. National Security.

EcoHealth Alliance has a long history of successful cooperation with NIH including multiple Research Project Grant R01 awards. In particular, Peter Daszak, EcoHealth Alliance's President and Chief Scientist, has been the Principal Investigator on five multidisciplinary R01s. All of these projects used modeling, epidemiology, laboratory, and field science to test hypotheses on the emergence of wildlife-origin viral zoonoses, including SARS-CoV, the Nipah and Hendra viruses, Avian influenza, and other bat-origin viruses. EcoHealth Alliance, a 501(c)(3) organization, is unique in that it goes one step further by leveraging its research goals to create an alliance of international collaborators that can advocate for real-world changes to protect high risk populations.

Notably, in collaboration with virologists in China, EcoHealth Alliance isolated and characterized SARSr-CoVs from bats that use the same human host cell receptor (ACE2) as SARS-CoV. This work provided critical reagents and resources that have advanced scientific understanding of virus-host binding and contributed to vaccine development. For example, the genetic sequences of the bat viruses that EcoHealth Alliance discovered under its NIH research funding, which were published online (Genbank & GISAID), have been used to test the effectiveness of the drug Remdesivir against not only SARS-CoV, but also MERS, and other potentially zoonotic or pre-pandemic bat CoVs. Significantly, this type of testing can be performed without the need for viral cultures or shipping viruses internationally.

B. NIH Awards And Extends EcoHealth Alliance Research Grant R01 AI 110964

In 2014, NIH issued EcoHealth Alliance a five-year research award for the project *Understanding the Risk of Bat Coronavirus Emergence*, funded under grant R01 AI 110964 (the "Project"). EcoHealth Alliance received additional awards for the Project each year between 2015 and 2018. Between 2015 and 2019, the Project resulted in the publication of more than twenty papers.

In 2019, EcoHealth Alliance submitted a renewal application to NIH through NIAID to extend the Project period for an additional five years. Upon filing of its renewal application, the Project was ranked as an "extremely high priority" (in the top 3%) by NIAID during its external review process. In light of its success and the importance of EcoHealth Alliance's work, on July 24, 2019, NIH reauthorized grant R01 AI 110964 and increased EcoHealth Alliance's funding. EcoHealth Alliance was issued a notice of award in the amount of $733,750.00 (the "2019 Award"). The notice of award also extended the Project period for an additional five years to 2024. A copy of the notice of award is attached hereto as Exhibit A.

C. EcoHealth Alliance Agrees Not To Fund The Wuhan Institute Of Virology

During the pendency of the Project, in December of 2019, China reported a cluster of cases of pneumonia in Wuhan, Hubei Province. It was later determined that the cause of this pneumonia

was a novel CoV, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease (COVID-19). Thereafter, SARS-CoV-2 spread to nearly every country throughout the world. In response, EcoHealth Alliance has prioritized its efforts in conducting research that will be integral to developing an effective strategy to combat SARS-CoV-2.

On April 19, 2020, Michael S. Lauer, MD, NIH Deputy Director for Extramural Research, sent a letter to EcoHealth Alliance on behalf of NIH regarding a laboratory in China, the Wuhan Institute of Virology ("WIV"). WIV was a prior sub-recipient of a small portion of the R01 AI 110964 grant funds. The letter stated that, given allegations that COVID-19 "was precipitated by the release from WIV of the coronavirus responsible for COVID-19", NIH was pursuing suspension of WIV from participating in Federal programs. However, Mr. Lauer assured EcoHealth Alliance that "[t]his suspension of the sub-recipient does not affect the remainder of [EcoHealth Alliance's] grant assuming that no grant funds are provided to WIV following receipt of this email during the period of suspension." A copy of the letter is attached hereto as Exhibit B.

On April 21, 2020, Dr. Daszak of EcoHealth Alliance responded by email to Dr. Lauer stating that he could "categorically state that no funds from [sic] 2R01 AI 110964-6 have been sent to Wuhan Institute of Virology, nor has any contract been signed." Dr. Daszak further represented that EcoHealth Alliance would comply with all NIAID requirements. Dr. Lauer acknowledged (1) that no monies from grant 2R01 AI 110964-6 had gone to WIV and no contract between EcoHealth Alliance and WIV had been signed and (2) EcoHealth Alliance's agreement that it would not provide any funds to WIV until and unless directed otherwise by NIH. A copy of the email correspondence between NIH and EcoHealth Alliance is attached hereto as Exhibit C.

D. NIH Abruptly Terminates Research Grant 2R01 AI 110964-6 "For Convenience"

Notwithstanding NIH's representation that suspension of WIV would not affect the remainder of EcoHealth Alliance's 2019 Award, on April 24, 2020, NIH notified EcoHealth Alliance by letter that, effective immediately, the 2019 Award had been terminated by NIAID. The stated grounds for the Termination were: (1) convenience; (2) NIH's discretion not to award a grant, or to award a grant at a particular funding level; and (3) NIH's belief that the Project outcomes did not align with the program goals and agency priorities. A copy of the Termination is attached hereto as Exhibit D.

ARGUMENT

A. NIH Research Grants Are Not Subject To Termination For Convenience

"Termination for convenience" refers to the exercise of the government's right to bring to an end the performance of all or part of the work provided for under a contract prior to the expiration of the contract "when it is in the Government's interest" to do so. Federal agencies typically incorporate clauses in their procurement contracts which give them the right to terminate for convenience. Here, there is no clause in the terms and conditions applicable to the 2019 Award, or in the NIH Grants Policy Statement, that permits NIAID or NIH to issue a post-award decision to terminate a NIH research grant award "for convenience."

Moreover, the unprecedented assertion by NIH that active research grants can be terminated "for convenience" during the subject budget period renders Section 8.5.2 of the NIH Grants Policy Statement meaningless. *See, e.g., Li v. Eddy*, 324 F.3d 1109, 1110 (9th Cir. 2003) (rejecting suggested statutory interpretation on the grounds that the interpretation ran squarely against the canon of construction that courts interpret statutes so as not to render any section meaningless). Section 8.5.2 of the NIH Grants Policy Statement governs, *inter alia*, modification or termination of an award for misconduct. If NIH grants were terminable for convenience, NIH could always choose to terminate for convenience to avoid (1) the "for cause" restriction on grant terminations and (2) the labor intensive task of enforcing compliance through disallowing costs, withholding further awards, or wholly suspending the grant, pending corrective action.

B. NIH's Discretion Not To Award A Grant, Or To Award a Grant At A Particular Funding Level, Does Not Authorize A Post-Award Decision To Terminate

NIH's discretion regarding the "decision not to award a grant, or to award a grant at a particular funding level" does not give NIH the authority to issue a post-award decision terminating a duly awarded grant during the budget period. This purported discretion, which is based on language in the last paragraph of NIH Grants Policy Statement Section 2.4.4, entitled *Disposition of Applications*, concerns NIH's authority to reject incomplete or otherwise undesirable grant applications in the first instance only. The provisions of Section 2, generally, have no bearing on post-award decisions affecting duly approved grants for which specified funds have already been allocated. As the 2019 Grant in the amount of $733,750.00 was awarded to EcoHealth Alliance on July 24, 2019, NIH's authority to deny initial grant applications does not allow NIH to terminate the 2019 Grant.

C. The Research Goals Of EcoHealth Alliance And NIAID Are Virtually Identical

NIH's contention that the Project's outcomes do not align with the agency's priorities is demonstrably false. First, the Project was ranked as "extremely high priority" on external review by NIAID less than nine months ago, before the discovery of SARS-CoV-2. Since this discovery, NIH has promulgated new grants seeking applicants to conduct research on the same issues covered by the Project and the 2019 Award.

In addition, there is substantial overlap between the four strategic research priorities on page 1 of NIAID's Strategic Plan for COVID-19 Research, published April 22, 2020, and the three Specific Aims of the Project. Both NIAID and EcoHealth Alliance seek to: (1) improve fundamental knowledge of SARS-Cov-2; (2) develop methods to assess the rate of infection and disease incidence; (3) contribute to the development of an effective vaccine; and (4) increase public health preparedness. Copies of the Project's Specific Aims and the NIAID Strategic Plan's four strategic research priorities for COVID-19 research are attached hereto as Exhibit E.

D. There Is No Rational Basis To Terminate The 2019 Award For Cause

The grounds and procedures for suspension and termination of awards are specified in NIH Grants Policy Statement Section 8.5.2 and 45 CFR Parts 75.371 through 75.373. Notably, Section

8.5.2 provides, *inter alia*, that NIH will generally suspend (rather than immediately terminate) a grant and allow the recipient an opportunity to take appropriate corrective action before NIH makes a termination decision. Through this lens, 45 CFR 75.372 provides that NIH may terminate a Federal award, in whole or in part, if: (1) the non-Federal entity fails to comply with the terms and conditions of the award; (2) for cause; (3) by the HHS awarding agency or pass-through entity with the consent of the non-Federal entity; or (4) by the non-Federal entity upon written notice to the HHS awarding agency setting forth the reasons for such termination, and other information. None of the foregoing predicate conditions exist here.

As of the date of the Termination, EcoHealth Alliance had not received any notice from NIH, NIAID, or HHS that it either failed to comply with any of the terms or conditions of the 2019 Award, or committed any misconduct in connection with the award. To the contrary, in email correspondence following EcoHealth Alliance's representation that it had not and would not give any funds from the 2019 Award to WIV, Aleksei Chmura, EcoHealth Alliance's Chief of Staff, memorialized the mutual agreement between NIH and EcoHealth Alliance that EcoHealth Alliance was in compliance with all requests. (Ex. C, p. 1). To be clear, EcoHealth Alliance clearly and unequivocally stated that it had not and will not distribute any funds from the 2019 Award to WIV.

In sum, there is no statutory, regulatory, or contractual basis for NIAID's termination of the Project, *Understanding the Risk of Bat Coronavirus Emergence*, funded under grant 2R01 AI 110964-6. However, please note that this letter is not intended to provide an exhaustive list of all possible grounds for reversal of the Termination and may not reflect all arguments and claims that EcoHealth Alliance will assert in the event that a formal second-level appeal of the Termination is required.

Should you wish to present evidence in an effort to refute any of the factual assertions made in this letter and/or to engage in good faith negotiations regarding appropriate terms and conditions for the resumption of funding for grant 2R01 AI 110964-6, we are prepared to review such evidence and to participate in such negotiations.

We await your response to this letter.

Very truly yours,

(b) (6)

Andrew M. Krinsky

cc: (*by email*)

Dr. Erik Stemmy (b) (6)
Ms. Emily Linde (

Exhibit A

Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/24/2019

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 2R01AI110964-06
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Dr. Daszak, Peter
PD/PI
460 West 34th Street
Suite 1701
New York, NY 100012320

Award e-mailed to: (b) (6)

Period Of Performance:
Budget Period: 07/24/2019 – 06/30/2020
Project Period: 06/01/2014 – 06/30/2024

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $733,750 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tseday G Girma
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 2R01AI110964-06

[redacted]

[redacted]

Approved Budget	$733,750
Total Amount of Federal Funds Obligated (Federal Share)	$733,750
TOTAL FEDERAL AWARD AMOUNT	$733,750
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$733,750

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
6	$733,750	$733,750
7	$709,750	$709,750
8	$709,750	$709,750
9	$709,750	$709,750
10	$709,750	$709,750

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI110964B
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019	2020	2021	2022	2023
AI	8472364	$733,750	$709,750	$709,750	$709,750	$709,750

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414B / **Released**: (b) (6) 07/18/2019
Award Processed: 07/24/2019 12:03:26 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R01AI110964-06

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 2R01AI110964-06

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 2R01AI110964-06

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

[redacted]

****

The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

[redacted]

****

This award reflects current Federal policies regarding Facilities & Administrative (F&A) Costs for foreign grantees including foreign sub-awardees, and domestic awards with foreign sub-awardees. Please see: Chapter 16 Grants to Foreign Organizations, International Organizations, and Domestic Grants with Foreign Components, Section 16.6 "Allowable and Unallowable Cost" of the NIH Grants Policy.

****

This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

****

The budget period anniversary start date for future year(s) will be **July** 1.

****

Dissemination of study data will be in accord with the Recipient's accepted genomic data sharing plan as stated in the page(s) **203** of the application. Failure to adhere to the sharing plan as mutually agreed upon by the Recipient and the NIAID may result in Enforcement Actions as described in the NIH Grants Policy Statement.

****

This award is subject to the Clinical Terms of Award referenced in the NIH Guide for Grants and Contracts, July 8, 2002, NOT AI-02-032. These terms and conditions are hereby incorporated by reference, and can be accessed via the following World Wide Web address: https://www.niaid.nih.gov/grants-contracts/niaid-clinical-terms-award All submissions required by the NIAID Clinical Terms of Award must be forwarded electronically or by mail to the responsible NIAID Program Official identified on this Notice of Award.

****

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

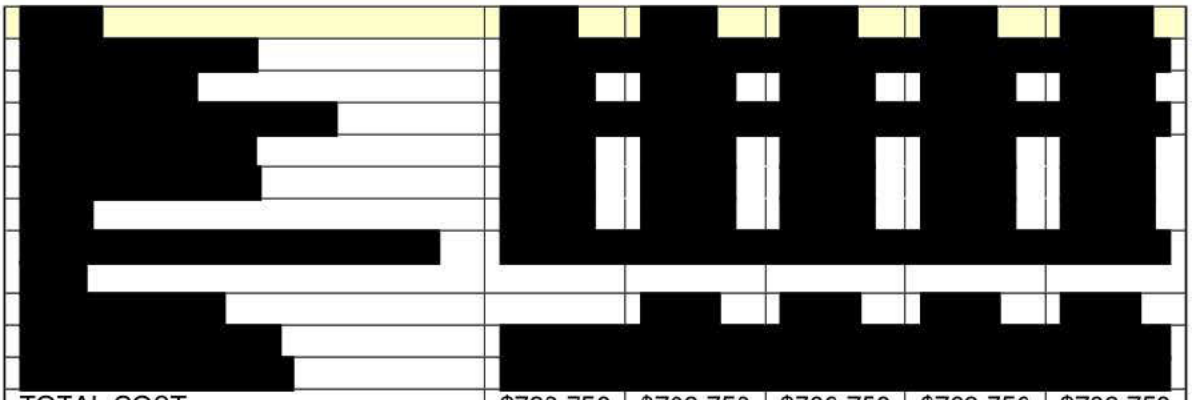
The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Tseday G Girma
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: (b) **Phone**: (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 2R01AI110964-06

INSTITUTION: ECOHEALTH ALLIANCE, INC.



TOTAL COST	$733,750	$709,750	$709,750	$709,750	$709,750

Facilities and Administrative Costs	Year 6	Year 7	Year 8	Year 9	Year 10
F&A Cost Rate 1	32%	32%	32%	32%	32%
F&A Cost Base 1	$438,711	$363,711	$363,711	$363,711	$363,711
F&A Costs 1	$140,388	$116,388	$116,388	$116,388	$116,388

Exhibit B

Date: April 19, 2020

From: Michael S Lauer, MD
NIH Deputy Director for Extramural Research

Lauer, Michael (NIH/OD) [E] Digitally signed by Lauer, Michael (NIH/OD) [E] Date: 2020.04.19 10:47:40 -04'00'

To: Kevin Olival, PhD
Vice-President for Research
EcoHealth Alliance
(b) (6)

Naomi Schrag, JD
Vice-President for Research Compliance, Training, and Policy
Columbia University
(b) (6)

Subject: Project Number 2R01AI110964-06

Dear Dr. Olival and Ms. Schrag:

EcoHealth Alliance, Inc. is the recipient, as grantee, of an NIH grant entitled "Understanding the Risk of Bat Coronavirus Emergence." It is our understanding that one of the sub-recipients of the grant funds is the Wuhan Institute of Virology ("WIV"). It is our understanding that WIV studies the interaction between corona viruses and bats. The scientific community believes that the coronavirus causing COVID-19 jumped from bats to humans likely in Wuhan where the COVID-19 pandemic began. There are now allegations that the current crisis was precipitated by the release from WIV of the coronavirus responsible for COVID-19. Given these concerns, we are pursuing suspension of WIV from participation in Federal programs.

While we review these allegations during the period of suspension, you are instructed to cease providing any funds from the above noted grant to the WIV. This temporary action is authorized by 45 C.F.R. § 75.371(d) ("Initiate suspension or debarment proceedings as authorized under 2 C.F.R. part 180"). The incorporated OMB provision provides that the funding agency may, through suspension, immediately and temporarily exclude from Federal programs persons who are not presently responsible where "immediate action is necessary to protect the public interest." 2 C.F.R. § 180.700(c). It is in the public interest that NIH ensure that a sub-recipient has taken all appropriate precautions to prevent the release of pathogens that it is studying. This suspension of the sub-recipient does not affect the remainder of your grant assuming that no grant funds are provided to WIV following receipt of this email during the period of suspension.

Exhibit C

From: Lauer, Michael (NIH/OD) [E] (b) (6)
Sent: Sunday, April 19, 2020 11:00 AM
To: (b) (6) Naomi Schrag (b) (6)
Cc: Black, Jodi (NIH/OD) [E] (b) (6)
Subject: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06
Importance: High

Dear Dr. Olival and Ms. Schrag

Please see attached.

Many thanks, Mike

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
1 Center Drive, Building 1, Room 144
Bethesda, MD 20892
Phone (b) (6)
Email:

2 Kevin Olival email on 20 April 2020

From: Kevin Oliva (b) (6) >
Subject: Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06
Date: April 20, 2020 at 4:12:28 PM EDT
To: "Lauer, Michael (NIH/OD) [E]" (b) (6) >
Cc: Naomi Schrag (b) (6) >, "Black, Jodi (NIH/OD) [E]" (b) (6)

Dear Mike,

I received the attached letter, however please note:

1. I am not the PI on this award. You should contact Dr. Peter Daszak (b) (6) who is the PI and leading this project for EcoHealth Alliance.
2. Columbia University is not involved in this NIH project, and it is not clear to me why Naomi and Columbia University were included.

Thank you,
Kevin

Kevin J. Olival, PhD
Vice President for Research

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

(b) (6) (direct)
(mobile)
1.212.380.4465 (fax)
www.ecohealthalliance.org

3 Michael Lauer email on 20 April 2020

Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06

Lauer, Michael (NIH/OD) [E] (b) (6)

Mon 4/20/2020 4:31 PM

To: Kevin Olival (b) (6) Peter Daszak (b) (6)

Cc: Naomi Schrag (b) (6) Black, Jodi (NIH/OD) [E] (b) (6) Lauer, Michael (NIH/OD) [E] (b) (6);

Importance: High

2 attachments

Screen Shot 2020-04-20 at 4.23.38 PM.png; EcoHealth Alliance re AI grant 4 19 20.pdf;

Thank you Kevin

- We need to work with a senior responsible business official – usually PI's and senior business officials are different people.
- When I looked you up on the web, I see the Columbia logo (see attached screenshot). Specifically, it appears to be Columbia University > Ecology, Evolution, and Environmental Biology > EcoHealth Alliance (labeled as an "Affiliation/Department"). Thus the web profile makes it look to me as if EcoHealth Alliance is linked to Columbia University.
- In any case, I'm looping in Dr. Daszak.
- We need to know all sites in China that have been in any way linked to this award (Type 1 and Type 2). We have data in NIH, but we want to make absolutely sure that we're of the same understanding.

We greatly appreciate your prompt attention to this matter.

Best, Mike

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
1 Center Drive, Building 1, Room 144
Bethesda, MD 20892
Phone (b) (6)
Email:

Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06

4 Michael Lauer email on 20 April 2020

Lauer, Michael (NIH/OD) [E] (b) (6)

Mon 4/20/2020 6:34 PM

To:Naomi Schrag (b) (6) >; Kevin Olival (b) (6) Peter Daszak (b) (6)

Cc:Black, Jodi (NIH/OD) [E] (b) (6) Lauer, Michael (NIH/OD) [E] (b) (6) ;

1 attachment

Screen Shot 2020-04-20 at 4.23.38 PM.png;

Thanks Naomi – not the impression an observer would get looking at the website (see screen shot), but we understand about the grant.

If they "are entirely separate entities" then why does Columbia identify EcoHealth Alliance as an "Affiliation/Department" on its website.

Maybe with the label "Affiliation/Department" you would have a clearly visible disclaimer that says, "EcoHealth Alliance is not affiliated with nor a department of Columbia"? – although even that is internally contradictory.

Best, Mike

From: Naomi Schrag (b) (6),
Date: Monday, April 20, 2020 at 5:19 PM
To: "Lauer, Michael (NIH/OD) [E]" (b) (6), Kevin Olival (b) (6), (b) (6)
Cc: Naomi Schrag (b) (6), "Black, Jodi (NIH/OD) [E]" (b) (6)
Subject: RE: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06

Dear Dr. Lauer,
Columbia and EcoHealth Alliance are entirely separate entities. Some individuals affiliated with EcoHealth Alliance do have adjunct appointments in Columbia's Ecology, Evolution, and Environmental Biology ("E3B") department, but we are not aware of any Columbia involvement with the referenced grant, and have found no agreement or record in our grants system to the contrary.

We would be happy to answer any additional questions. Thank you.
Sincerely,
Naomi Schrag

Naomi J. Schrag

Vice President for Research Compliance, Training and Policy
Office of Research Compliance and Training
475 Riverside Drive, Suite 840
New York, New York 10115
(b) (6)
www.researchcompliance.columbia.edu

RE: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06

5 Peter Daszak email on 21 April 2020

Peter Daszak

Tue 4/21/2020 1:32 AM

To: Lauer, Michael (NIH/OD) [E] (b) (6); Naomi Schrag (b) (6); Kevin Olival (b) (6)

Cc: Black, Jodi (NIH/OD) [E] (b) (6)

Dear Michael Lauer & Jodi Black – I now have your email and will deal with it directly with you and your staff. Naomi is correct that there is no involvement of Columbia University in this grant. I'm sure NIH has records to confirm that.

From this moment on, I will not cc any staff at Columbia as part of this discussion, and I hope you will also honor that. Respecfully, the discussion of whether or not EHA is an affiliate of CU is entirely irrelevant to the request that you contacted us about, and should remain a private matter between EcoHealth Alliance and Columbia University.

I'll look over your email and respond tomorrow.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street
New York, NY 10001
USA

Tel.: + (b) (6)
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

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

RE: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06

6 Peter Daszak email on 21 April 2020

Peter Daszak

Tue 4/21/2020 7:03 PM

To:Lauer, Michael (NIH/OD) [E] (b) (6)

Cc:Black, Jodi (NIH/OD) [E] (b) (6) Aleksei Chmura (b) (6)
Stemmy, Erik (NIH/NIAID) [E] (b) (6) (b) (6)

Importance: High

1 attachment

EcoHealth Alliance re AI grant 4 19 20.pdf;

Dear Michael – Confirming receipt of your email. I'm also cc'ing the following people so they're aware of this request:

1. Our AOR – Dr. Aleksei Chmura, who has access to all our records
2. My Program Officer for this award, Dr. Erik Stemmy & the Division Director (DMID), Dr. Emily Erberding, so they are informed and aware of the request and our response.

That said we need some time to go through the request for information and will provide this as quickly as we can.

However, **I can categorically state that no funds form 2R01AI110964-06 have been sent to Wuhan Institute of Virology, nor has any contract been signed.** Furthermore, we will comply with NIAID requirements, of course.
Concerning the request for information on all of the sites linked to this award in China, you should be aware that these are documented in our progress reports over the course of the grant. As you can understand we are under enormous pressure to generate data related to the current pandemic, and we do not want to divert staff to this effort. We are hoping the previously filed reports will satisfy this request.

We are well aware of the political concerns over the origins of this outbreak. Our collaboration with Wuhan Institute of Virology has been scientific and we have been consistently impressed with the scientific capabilities of that laboratory and its research staff. Our joint work has led to a series of critical papers published in high impact journals that served to raise awareness of the future threat coronaviruses pose for global health and therefore US national security. Scientific insights with epidemiological significance have been jointly published and our relationship has always been open and transparent and with one concern only, scientific validity. We are concerned that current actions may jeopardize 15 years of fruitful collaboration with colleagues in Wuhan, who are working at the leading edge to design vaccines and drugs that could help us fight this new threat in future years. It is quite remarkable that of the 5 vaccine candidates listed by WHO that are already in human trials, 3 have been developed in China. That said, we of course will

do all we can to make sure any further questions from NIH or any Federal agency are addressed to our fullest knowledge.

Yours sincerely,

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street
New York, NY 10001
USA

Tel.: + (b) (6)
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

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

7 Michael Lauer email on 21 April 2020

From: **Lauer, Michael (NIH/OD) [E]** (b) (6)
Subject: Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06
Date: April 21, 2020 at 19:28
To: Peter Daszak (b) (6)
Cc: Black, Jodi (NIH/OD) [E] (b) (6), Aleksei Chmura (b) (6), Stemmy, Erik (NIH/NIAID) [E] (b) (6), Erbelding, Emily (NIH/NIAID) [E] (b) (6)v, Lauer, Michael (NIH/OD) [E] (b) (6)

ML

Many thanks Peter for your response.

We note that:

- No monies have gone to WIV on the Type 2 award and no contract has been signed.
- You agree that you will not provide any funds to WIV until and unless directed otherwise by NIH.
- All foreign sites for the Type 1 and Type 2 awards have been documented in the progress reports submitted to NIH.

We appreciate your working with us.

Best, Mike

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
1 Center Drive, Building 1, Room 144
Bethesda, MD 20892
Phone (b) (6)
Email:

8 Aleksei Chmura email on 21 April 2020

From: **Aleksei Chmura** (b) (6)
Subject: Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06
Date: April 23, 2020 at 13:50
To: Lauer, Michael (NIH/OD) [E] (b) (6)
Cc: Peter Daszak (b) (6) Black, Jodi (NIH/OD) [E] (b) (6) v, Erik Stemmy (b) (6)
Erbelding, Emily (NIH/NIAID) [E] (b) (6)

(b) (6)

Dear Mike,

I read that we are in agreement and in compliance with all requests. Please let us know if anything further is required. We will continue in our usual close communication with our Program Officer Erik Stemmy.

Sincerely,

-Aleksei

Aleksei Chmura
Chief of Staff &
Authorized Organizational Representative

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

(b) (6) (office)
(mobile)
www.ecohealthalliance.org

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

From: **Lauer, Michael (NIH/OD) [E]** (b) (6)
Subject: Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06
Date: April 23, 2020 at 13:59
To: Aleksei Chmura (b) (6)
Cc: Peter Daszak (b) (6), Black, Jodi (NIH/OD) [E] (b) (6), Stemmy, Erik (NIH/NIAID) [E] (b) (6), Erbelding, Emily (NIH/NIAID) [E] (b) (6), Lauer, Michael (NIH/OD) [E] (b) (6) Compliance Review ComplianceReview@mail.nih.gov

ML

Many thanks Aleksei.

9 Michael Lauer email on 21 April 2020

Best, Mike

From: **Lauer, Michael (NIH/OD) [E]** (b) (6)
Subject: PLEASE READ -- Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06
Date: April 24, 2020 at 16:47
To: Aleksei Chmura (b) (6) Peter Daszak (b) (6)
Cc: Black, Jodi (NIH/OD) [E] (b) (6), Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Erbelding, Emily (NIH/NIAID) [E] (b) (6), Linde, Emily (NIH/NIAID) [E] (b) (6)
Lauer, Michael (NIH/OD) [E] (b) (6) Bulls, Michelle G. (NIH/OD) [E] (b) (6)

ML

Dear Dr. Chmura and Dr. Daszak

Please see attached.

10 Michael Lauer email on 24 April 2020

Sincerely,
Michael S Lauer, MD

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
1 Center Drive, Building 1, Room 144
Bethesda, MD 20892
Phone (b) (6)
Email:

From: **Aleksei Chmura** (b) (6)
Subject: Re: PLEASE READ -- Re: Please read and acknowledge receipt -- Actions needed regarding 2R01AI110964-06
Date: April 27, 2020 at 23:57
To: Lauer, Michael (NIH/OD) [E] (b) (6)
Cc: Peter Daszak (b) (6), Black, Jodi (NIH/OD) [E] (b) (6), Erik Stemmy (b) (6)
Emily Erbelding (b) (6), Linde, Emily (NIH/NIAID) [E] (b) (6), Bulls, Michelle G. (NIH/OD) [E]
(b) (6) Alison Andre (b) (6)

(b) (6)

Dear Michael,

Could Peter and I have a quick chat with you sometime tomorrow (Tuesday) about your email, below?

Sincerely,

11 Aleksei Chmura email on 27 April 2020

-Aleksei

Aleksei Chmura, PhD
Chief of Staff

EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001

(b) (6) (office)
(mobile)
www.ecohealthalliance.org

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

Exhibit D

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

24 April 2020

Drs. Aleksei Chmura and Peter Daszak
EcoHealth Alliance, Inc.
460 W 34th St
Suite 1701
New York, NY 10001

Re: Termination of NIH Grant R01 AI 110964

Dear Drs. Chmura and Daszak:

I am writing to notify you that the National Institute of Allergy and Infectious Diseases (NIAID), an Institute within the National Institutes of Health (NIH), under the Department of Health and Human Services (HHS) has elected to terminate the project *Understanding the Risk of Bat Coronavirus Emergence*, funded under grant R01 AI110964, for convenience. This grant project was issued under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284). This grant was funded as a discretionary grant as outlined in the NIH Grants Policy Statement, which states that the decision not to award a grant, or to award a grant at a particular funding level, is at the discretion of the agency, in accordance with NIH's dual review system.

At this time, NIH does not believe that the current project outcomes align with the program goals and agency priorities. NIAID has determined there are no animal and human ethical considerations, as this project is not a clinical trial, but rather an observational study.

As a result of this termination, a total of $369,819.56 will be remitted to NIAID and additional drawdowns will not be supported. The remaining funds have been restricted in the HHS Payment Management System, effective immediately.

Please let me know if you have any questions concerning the information in this letter.

Sincerely,

Lauer, Michael (NIH/OD) [E] Digitally signed by Lauer, Michael (NIH/OD) [E]
Date: 2020.04.24 16:41:16 -04'00'

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
Email: (b) (6)

cc: Dr. Erik Stemmy
Ms. Emily Linde

Exhibit E

SPECIFIC AIMS

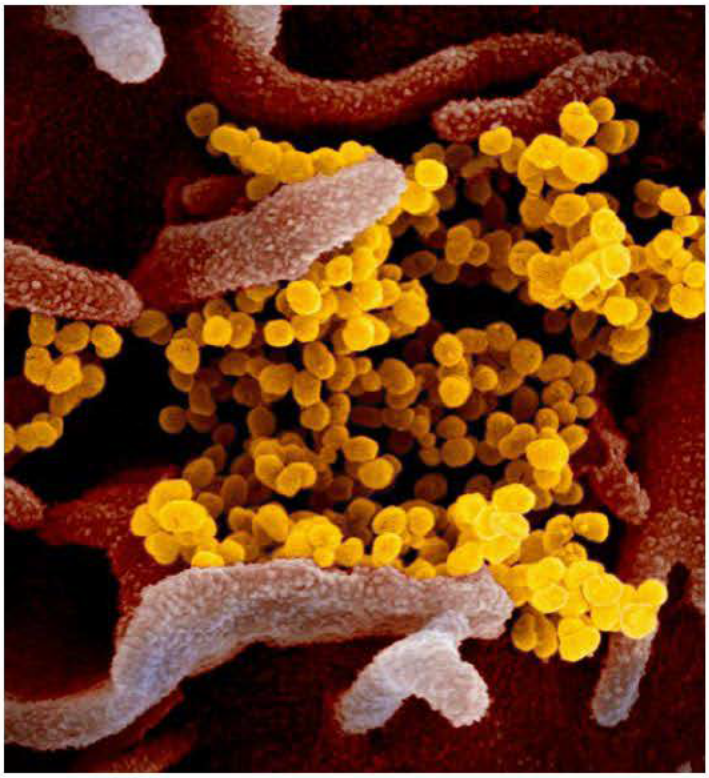
Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002, and the continuing spread of Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then we have sequenced dozens of novel SARS-related CoV (SARSr-CoV) strains. Our previous R01 work demonstrates that bats in southern China harbor an extraordinary diversity of SARSr-CoVs, some of which are able to use human ACE2 to enter into human cells, can infect humanized mouse models to cause SARS-like illness, and evade available therapies or vaccines. We found that the bat hosts of SARSr-CoVs appear to no longer be traded in wildlife markets, and that people living close to bat habitats are the primary risk groups for spillover. At one of these sites, we found diverse SARSr-CoVs containing every genetic element of the wild-type SARS-CoV genome, and serological evidence of human exposure among people living nearby. Thus, there is significant potential for future spillover of SARSr-CoVs, and of public health impacts. *Yet salient questions remain: Are there specific bat communities and sites that harbor CoV strains with higher risk for bat-to-human spillover? Which human behaviors drive risk of bat SARSr-CoV exposure that could lead to infection? Does human exposure to these viruses cause SARS-like or other illness? Can we characterize viral strain diversity, bat traits and human behaviors to assess risk of potential future CoV spillover?* **The proposed work in this renewal R01 builds on these findings** to address these issues by conducting: **1)** focused sampling of bats in southern China to **identify viral strains with high predicted risk of spillover**; **2)** community-based, and clinic-based syndromic, sampling of people to **identify spillover, and assess behavioral risk factors and evidence of illness**; and **3)** conduct *in vitro* and *in vivo* viral characterization and analyze epidemiological data **to identify hotspots of future CoV spillover risk**. This work will follow 3 specific aims:

Aim 1: Characterize the diversity and distribution of high spillover-risk SARSr-CoVs in bats in southern China. We will conduct targeted bat sampling at sites where we predict that undiscovered high risk SARSr-CoV strains exist. Bat sampling will be targeted geographically and by host species to test predictions about evolutionary diversity of SARSr-CoV. We will analyze RdRp and S protein sequences to test their capacity for spillover to people in Aim 3.

Aim 2: Community- and clinic-based surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences. We will conduct focused, targeted human surveys and sampling to identify key risk factors for SARSr-CoV spillover and evidence of illness. To maximize our opportunity of capturing human exposure to bat CoVs, we will conduct community-based surveillance in regions with high SARSr-CoV prevalence and diversity, and individuals having contact with bats. We will assess bat-CoV seropositive status against a small number of questions about human-wildlife contact and exposure. We will conduct clinic-based syndromic surveillance close to these sites to identify patients presenting with influenza-like illness and severe acute respiratory illness, assess their exposure to bats via a questionnaire, and test samples for PCR- and serological evidence of SARSr-CoV infection. We will conduct follow-up sampling to capture patients who had not yet seroconverted at the time of clinic visit.

Aim 3: *In vitro* and *in vivo* characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyses to identify the regions and viruses of public health concern. We will characterize the propensity of novel SARSr-CoVs to infect people *in vitro* using primary human airway epithelial cells and *in vivo* using the transgenic hACE2 mouse model. We will use mAb and vaccine treatments to test our hypothesis that SARSr-CoVs with 10-25% divergence in S protein sequences from SARS-CoV are likely able to infect human cells, and to evade mAb therapeutics and vaccines. We will then map the geographic distribution of their bat hosts and other ecological risk factors to identify the key 'hotspots' of risk for future spillover.

Overall, our SARSr-CoV program serves as a model platform to integrate virologic, molecular and ecologic factors contributing to CoV emergence while informing high impact strategies to intervene and prevent future pandemics. This includes providing critical reagents, therapeutic interventions and recombinant viruses for future SARSr-CoV pandemic and public health preparedness.



This scanning electron microscope image shows SARS-CoV-2 (yellow), the virus that causes COVID-19, isolated from a patient in the United States, emerging from the surface of cells (pink) cultured in the lab. Credit: NIAID-RML

NIAID STRATEGIC PLAN FOR COVID-19 RESEARCH

FY2020 – FY2024

April 22, 2020

National Institute of Allergy and Infectious Diseases

Table of Contents

Executive Summary

The National Institute of Allergy and Infectious Diseases (NIAID) at the United States (U.S.) National Institutes of Health (NIH) is committed to safeguarding the health of Americans and people around the world by accelerating research efforts to prevent, diagnose, and treat COVID-19 and characterize the causative agent of this disease, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This *NIAID Strategic Plan for COVID-19 Research* builds on current trans-NIAID efforts to better understand SARS-CoV-2 pathogenesis, transmission, and mechanisms of protective immunity by expanding resources and activities that support rapid development of biomedical tools to more effectively combat this disease and pandemic. Given the urgency of the public health response, studies that inform efforts to control virus spread and mitigate morbidity and mortality, including therapeutic and vaccine development, are the priority. In addition, it is essential to develop rapid, accurate, point-of-care diagnostics—a critical asset to mitigating the spread of COVID-19.

Box 1
NIAID Strategic Plan for COVID-19 Research Mission

Conduct and support research on SARS-CoV-2 and COVID-19 to accelerate the development of safe and effective medical countermeasures that decrease disease incidence, mitigate morbidity and prevent mortality.

The *NIAID Strategic Plan for COVID-19 Research* aligns with the priorities set by U.S. Government–wide task forces for the development of medical countermeasures. NIAID actively participates in COVID-19 task forces to identify opportunities, ensure open communication, encourage resource sharing, and avoid duplication of effort. The plan is structured around four strategic research priorities:

1. **Improve fundamental knowledge of SARS-CoV-2 and COVID-19,** including studies to characterize the virus and how it is transmitted and understand the natural history, epidemiology, host immunity, disease immunopathogenesis, and the genetic, immunologic, and clinical associations with more severe disease outcomes. This includes accelerating the development of small and large animal models that replicate human disease.

2. **Support the development of diagnostics and assays**, including point-of-care molecular and antigen-based diagnostics for identifying and isolating COVID-19 cases and serologic assays to better understand disease prevalence in the population. Diagnostics also will be essential for evaluating the effectiveness of candidate countermeasures.

3. **Characterize and test therapeutics**, including identifying and evaluating repurposed drugs and novel broad-spectrum antivirals, virus-targeted antibody-based therapies (including plasma-derived intravenous immunoglobulin (IVIG) and monoclonal antibodies), and host-directed strategies to combat COVID-19.

4. **Develop safe and effective vaccines against SARS-CoV-2,** including support of clinical trial testing.

To accelerate research, NIAID will leverage current resources and global collaborations, including existing research programs and clinical trials networks. NIAID's research response to COVID-19 will build on experience with diseases caused by other zoonotic coronaviruses (CoVs), including severe acute

respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). NIAID will pursue public-private partnerships to facilitate the translation of research outcomes into life-saving public health interventions. Working with pharmaceutical companies, NIAID has already initiated Phase 1 clinical trials for candidate COVID-19 vaccines and therapeutics. A concerted effort will be made to include minority populations, as well as at-risk and vulnerable populations, in all aspects of NIAID-sponsored research to address health disparities between diverse groups. Characterization of the fundamental virology of SARS-CoV-2 and the immunological response to infection will inform future studies and facilitate the development of effective medical countermeasures. With collaboration from all agencies within the U.S. government and other key U.S. and global partners, NIAID will rapidly disseminate these results so that the information can be translated into clinical practice and public health interventions to combat the pandemic. As such, NIAID has already implemented open sharing of scientific data through publicly available websites and will continue to promote the prompt disclosure of SARS-CoV-2 and COVID-19 research data by the scientific community.

Research Plan

Priority 1: Improve fundamental knowledge of SARS-CoV-2 and COVID-19

Developing effective medical and public health countermeasures against a newly emergent virus like SARS-CoV-2 will require a better understanding of the complex molecular and immune mechanisms underlying infection and disease. Studies that delineate the viral lifecycle and host immune responses to infection can lead to the identification of novel targets for intervention against SARS-CoV-2 infection and COVID-19. Early studies suggest that the clinical manifestations of COVID-19 can vary significantly, and disease severity can range from mild to critical. Thus, a detailed understanding of the clinical course of disease, as well as the clinical, virologic, immunological, and genetic predictors of disease severity, are needed. Gaps also exist in our understanding of the dynamics of disease transmission in different populations over time, including the role of pediatric and elderly populations in viral spread, and the potential seasonality of viral circulation.

Objective 1.1: Characterize fundamental SARS-CoV-2 virology and immunological host response to infection

- **Support the development and distribution of reagents and viral isolates to researchers.** NIAID will continue to support both intramural and extramural researchers by developing reagents and assays for virus characterization and immunological analyses. NIAID will continue to accelerate SARS-CoV-2 research by sourcing viral isolates and clinical specimens for the research community and placing them in repositories to help advance research and countermeasure development. In addition, NIAID will place other critical reagents needed for assay development (e.g., pseudovirions and antigens) in publicly available repositories for distribution.

- **Characterize virus biology and immunological responses to disease.** A comprehensive understanding of the

Box 2
Priority 1: Improve fundamental knowledge of SARS-CoV-2 and COVID-19

Objective 1.1: Characterize fundamental SARS-CoV-2 virology and immunological host response to infection
Objective 1.2: Evaluate disease dynamics through natural history, transmission, and surveillance studies
Objective 1.3: Develop animal models that recapitulate human disease

biological processes involved in SARS-CoV-2 infection and the pathogenesis of COVID-19 are paramount to developing new medical countermeasures to fight the spread of disease. Building on prior research related to MERS and SARS coronaviruses, early studies confirmed several critical features of SARS-CoV-2 infection, including the primary host receptor, angiotensin converting enzyme 2 (ACE-2), and the structure of the virus receptor-binding domain. Studies that delineate the viral lifecycle and host immune responses to infection can lead to the identification of novel targets for intervention against SARS-CoV-2 infection and COVID-19. Understanding the function of essential viral proteins will be necessary for improving diagnostic and immunological assays, *in vitro* and *in vivo* models, and other resources needed to advance safe and effective medical countermeasure development. In addition, evaluating the dynamics of host-pathogen interactions at the molecular and cellular levels will be critical to advancing our understanding of viral pathogenesis and immune responses that contribute to SARS-CoV-2 infection.

- **Determine viral evolution and molecular epidemiology.** With a newly emergent virus like SARS-CoV-2, studies to characterize genetic diversity, including those that assess the potential for the virus to evolve and escape host immunity, are pivotal for understanding disease progression and transmission dynamics and may have implications for countermeasure development. Viral genomic analysis matched with patient clinical data will be important to identify biomarkers of virulence and establish paradigms of sequence diversity. In addition, evaluating viral sequence associations with disease outcomes, immune status, and viral replication will provide crucial data to accelerate the development of effective medical countermeasures.

- **Develop low-containment assays to study virus neutralization.** Studies using non-infectious pseudovirions can be conducted in labs without BSL-3 capacity, making them an important tool to enhance understanding of SARS-CoV-2 infection. This capability would enable researchers without high-containment infrastructure to study the dynamics of virus neutralization *in vitro*.

- **Research into optimal public health prevention and mitigation modalities**. Clinical trials including family members of a COVID-19 positive individual can be devised to evaluate transmission, prevention, and other mitigation measures within the household.

Objective 1.2: Evaluate disease dynamics through natural history, transmission, and surveillance studies

- **Characterize disease incidence through surveillance studies.** Clinical manifestations of COVID-19 can vary greatly, ranging from asymptomatic or mildly symptomatic to the development of pneumonia, acute respiratory distress syndrome, and even death.[1] The variation in clinical presentation of COVID-19, combined with the challenges in diagnostic capacity, have made accurate initial assessments of disease incidence a formidable challenge. However, rapid point-of-care and point-of-need molecular tests, which became available in March 2020, will enable hospitals and other healthcare facilities to make informed decisions regarding patient isolation and care. Studies that leverage existing high-throughput diagnostic capacity along with these rapid tests will advance our understanding of disease incidence across the nation and will be a critical component of strategies to implement effective medical countermeasures. Combining these studies with broad serosurveillance studies across existing surveillance networks, including blood bank studies, would

[1] Wu Z and McGoogan JM. *JAMA* 2020 Feb 24. Epub. PMID 32091533.

provide a more complete picture of the scope of disease and the dynamics of infection. Detailed knowledge of host genetics and the human responses to infection across the lifespan will not only provide insights into new approaches for diagnosis, treatment, and prevention, but also may elucidate why individuals respond to SARS-CoV-2 in different ways. Reports to date suggest that COVID-19 resolves in most cases,[2] implying that the immune system can keep the infection from progressing to severe disease in many individuals. However, additional research is needed to better understand why some people progress to severe disease, which will lend critical insights to medical countermeasure development.

- **Assess the dynamics of disease transmission.** Our current understanding of COVID-19 transmission is limited. While recent studies have suggested timeframes for virus survival in aerosols and on surfaces,[3] the contributions of different routes of transmission and the dynamics of animal-to-human and human-to-human transmission remain unclear. The diverse clinical presentations of COVID-19, including a high prevalence of asymptomatic cases, add further complexity to understanding transmission dynamics. Providing a clearer picture of the natural history of viral shedding is a priority, both in acute cases and in asymptomatic infection. Given the challenges of accurately diagnosing asymptomatic individuals because they do not present for treatment, determining the role they play in transmission would provide valuable insights. Elucidating the role of pediatric cases in the spread of SARS-CoV-2 is particularly important. Although pediatric COVID-19 cases are generally asymptomatic or have less severe clinical manifestations than those of adults, the role that children play in spreading the virus is unknown. Additionally, studies to identify potential animal reservoirs and better understand transmission from animals to humans are a research priority, as these reservoirs may lead to future virus introductions and re-emergence of disease in humans. Virus transmission depends on a complex interplay of host, viral, and environmental factors that contribute to disease incidence and spread. Identifying the factors that maintain the disease transmission cycle is critical to developing effective medical countermeasures and public health interventions that will prevent future pandemics.

- **Determine disease progression through natural history studies.** Delineating the natural history of COVID-19 will inform immunopathogenesis, viral tropisms and length of shedding, immune phenotypes, and both protective immunity and host susceptibility. Disease assessment using longitudinal cohort studies, including among high-risk populations such as healthcare workers and the elderly, are important to better understand disease pathogenesis and immune responses to infection. Biomarkers identified from these studies may provide valuable insights into predictors of disease severity.

Objective 1.3: Develop animal models that recapitulate human disease

- **Develop small and large animal models that replicate SARS-CoV-2 pathogenesis.** Developing animal models that recapitulate human disease is a vital early step toward understanding disease pathogenesis and testing the efficacy of medical countermeasures. Small animal models enable rapid, scalable analyses that are particularly valuable for screening countermeasure candidates for efficacy and addressing issues concerning vaccine-induced immune enhancement. Among the small animal models being tested, transgenic mice expressing the human ACE-2 receptor are a promising candidate. In parallel, development and characterization of large animal models, including non-human primates (NHPs) that mimic human COVID-19, are a pivotal step to advance promising

[2] ibid.

[3] van Doremalen *N et al. N Engl J Med* 2020 Mar 17. Epub. PMID 32182409.

countermeasure candidates. Previous experience with related coronavirus diseases such as MERS and SARS suggests that replicating human disease, particularly its more severe manifestations, in an animal model may be challenging. Fundamental research assessing animal models ranging from mice to NHPs is already underway. NIAID will continue to support the development of small and large animal model candidates to better understand this emerging infection and investigate optimal ways to treat and prevent COVID-19. NIAID also will ensure that validated animal models are made available to the scientific community for evaluating priority countermeasures.

Priority 2: Support the development of diagnostics and assays

Availability of rapid, accurate Food and Drug Administration (FDA)-cleared or authorized diagnostics will increase testing capacity and are critical for identifying and rapidly isolating cases, tracking spread of the virus, managing patient care, and supporting clinical trials. Molecular tests specifically designed to detect SARS-Cov-2 RNA in clinical samples are able to detect low levels of pathogen in clinical samples and offer robust specificity in differentiating SARS-CoV-2 from other related viruses. Continuing to improve the speed and accuracy of molecular and antigen-based diagnostics and making them available at point- of-care will be paramount to accelerating the ability to mitigate disease spread in the current outbreak and any future outbreaks. The development of serologic assays would further bolster surveillance efforts, including the ability to identify individuals who may have resolved prior infection with SARS-CoV-2.

Objective 2.1: Accelerate the development and evaluation of diagnostic platforms

- **Support the development, characterization and availability of reagents for diagnostic validation.** NIAID will support this effort through the development and testing of reagents for diagnostic validation that will be made available through NIAID-sponsored repositories.

Box 3 **Priority 2: Support the development of diagnostics and assays**
Objective 2.1: Accelerate the development and evaluation of diagnostic platforms *Objective 2.2: Develop assays to increase understanding of infection and disease incidence*

- **Support the development of new rapid diagnostics.** NIAID will provide funding to support the development of new rapid diagnostics, including molecular tests and novel antigen detection tests with improved sensitivity, if deemed feasible based on natural history studies.

- **Support the evaluation of promising diagnostics.** In some cases, stakeholders that develop potential diagnostic tests do not have the infrastructure needed to rigorously validate those tests against clinical samples. NIAID will support the testing of promising diagnostics and provide the capacity for evaluating them with live virus samples using our biocontainment laboratories.

Objective 2.2: Develop assays to increase understanding of infection and disease incidence

- **Develop and validate SARS-CoV-2 serological assays.** Serological tests, which detect host antibodies to infectious agents, do not detect the presence of a pathogen directly but can be used as a surrogate marker of infection. Developing more effective serologic tests would help provide information on the extent of asymptomatic infections and cumulative disease incidence, for example through serosurveillance studies. NIAID, with the Centers for Disease Control and

Prevention and the FDA, is developing tests that identify antibodies to SARS-CoV-2 proteins to determine seroprevalence rates and potentially help distinguish antibody responses in individuals receiving vaccines. NIAID will support the development and validation of additional serological assays for serosurveillance studies and as tools for testing the efficacy of promising vaccine or therapeutic candidates.

Priority 3: Characterize and test therapeutics

Currently, there are no FDA-approved or licensed therapeutics specific for coronaviruses. While traditional development pathways for therapeutics can take years, the urgency of the current outbreak underscores the need for rapid development and testing of promising therapeutics. Possible avenues for developing therapeutics include the evaluation of broad-spectrum antiviral agents (antivirals) that have shown promise for other coronaviruses and the identification of novel monoclonal antibodies (mAbs). For broad-spectrum antivirals, Phase 2/2b testing of the RNA polymerase inhibitor developed by Gilead, remdesivir, is already underway. Additional studies will be critical to identify promising therapeutic candidates and to advance them through clinical trial testing. To optimize findings during the pandemic, multiple clinical trials will be conducted in parallel among various populations, including both inpatient and outpatient studies.

Objective 3.1: Identify promising candidates with activity against SARS-CoV-2

- **Screen protease inhibitor and nucleotide analogue class agents and other small molecules with documented activity against other coronaviruses SARS-CoV-2**. Screening drugs that are already licensed by the FDA for other indications and might be efficacious against SARS-CoV-2 infection may provide a route to identifying a therapeutic for use in the current pandemic. Broad-spectrum antivirals that are already FDA approved or in clinical development for other indications—including those previously targeting SARS-CoV-1 and MERS CoV—can be evaluated for their potential activity against SARS-CoV-2 infections. Approved therapeutics for other infectious diseases also are being evaluated as possible treatments for COVID-19. By leveraging their existing efficacy, safety, and manufacturability data, the time to development and production can be reduced. NIAID also will continue working with partners to screen compound libraries for potential activity against SARS-CoV-2. For these studies, priority will be given to compounds based on *in vitro* screening data and the existence of human safety data.

Box 4
Priority 3: Characterize and test therapeutics

Objective 3.1: Identify promising candidates with activity against SARS-CoV-2
Objective 3.2: Conduct treatment studies to advance high-priority therapeutic candidates

- **Identify viral targets for therapeutic development**. Advances in structural biology technology enable researchers to map key viral structures at an unprecedented level. The Structural Genomics Centers for Infectious Diseases (SGCID) apply state-of-the-art, high-throughput technologies and methodologies, including computational modeling, x-ray crystallography, nuclear magnetic resonance imaging, and cryogenic electron microscopy, to experimentally characterize the three dimensional atomic structure of proteins that play an important biological role in human pathogens and infectious diseases. NIAID will continue to support use of this powerful technology to identify viral targets of SARS-CoV-2 for therapeutics or vaccines.

- **Identify novel mAbs for use as therapy or prophylaxis.** Data from early studies indicate that well-characterized convalescent plasma may provide a treatment benefit in COVID-19.[4] Therefore, IVIG derived from convalescent plasma may also hold promise for treatment. Moreover, peripheral blood mononuclear cells and plasma are being used to identify novel neutralizing antibodies. Through collaborations with structural biologists, binding properties can be quickly assessed. Paired with assessment of neutralization activity, the most promising mAbs will be identified for further characterization in animal models and human trials.

Objective 3.2: Conduct treatment studies to advance high-priority therapeutic candidates

- **Characterize and evaluate host-directed strategies for treatment of disease.** Experience with other coronaviruses indicates that infection of the respiratory tract is rapid and damage is primarily mediated by the host inflammatory response.[5] These conditions may make it difficult to modify COVID-19 with pathogen-directed therapeutics. Instead, host-directed strategies that target the immune response may exert a beneficial therapeutic effect. Host-directed strategies, including immune-modulating agents, will be investigated as potential therapeutic candidates.

- **Conduct clinical trials to demonstrate safety and efficacy of lead therapeutic candidates.** Many potential therapeutic candidates have been identified and are being tested in clinical trials.
    - In March 2020, NIAID launched a multicenter, adaptive, randomized controlled clinical trial to evaluate the safety and efficacy of the investigational antiviral drug remdesivir (GS-5734) for the treatment of COVID-19 in hospitalized adults with laboratory-confirmed SARS-CoV-2 infection and evidence of lung involvement. The trial builds on recent studies by NIAID scientists showing that remdesivir can improve the disease course in rhesus macaques when administered promptly after viral challenge with the MERS CoV.[6] The trial is also adaptive, allowing for additional arms should other therapeutics warrant assessment for efficacy.
    - NIAID is finalizing the protocol for the Big Effect Trial (BET), in which putative therapeutics that have existing human data and are readily available will be tested in patients hospitalized with lower respiratory tract disease. Each potential intervention will be given to approximately 75 patients and evaluated for mitigating disease symptoms. Candidate therapeutics that meet the criteria in this initial study will be further evaluated in larger clinical trials for which the infrastructure is already in place.
    - As mentioned above, identification of novel mAbs for therapy or prophylaxis is another strategic priority. These mAbs should be safe, highly effective, amenable to fast manufacturing, and easy to administer. They will be tested in clinical trials to develop immunotherapies for the prevention and early treatment of COVID-19, potentially in high-risk populations including healthcare workers.

- **Conduct outpatient studies for mild COVID-19 cases**. In cases of mild COVID-19 that do not require hospitalization, outpatient studies could be extremely valuable for testing promising, orally administered FDA-approved drugs that have existing safety data. The antiviral activity of hydroxychloroquine and azithromycin against SARS-CoV-2 has been the focus of many early

[4] Roback JD and Guarner J. *JAMA* 2020 Mar 27. Epub. 32219429.

[5] Newton AH et al. *Semin Immunopathol*. 2016;38(4):471-82. PMID 26965109.

[6] de Wit E et al. *Proc Natl Acad Sci USA* 2020;117(12):6771-6. PMID 32054787.

therapeutic studies.[7,8,9] Testing of these and other candidates, including protease inhibitors and other molecules, in outpatient studies may provide critical efficacy data and could identify an existing drug or drug combination that is safe and effective against COVID-19.

- **Conduct outpatient studies in high-risk populations.** High-risk populations, including health care workers, the elderly or individuals with chronic conditions, are a critical target for the development of therapeutics. Conducting studies in patients with mild cases of COVID-19 among these high-risk groups would be of interest for identifying the benefits of early treatment strategies to mitigate the impact of infection. Therapeutic candidates that have once a day dosing could also be considered for pre-exposure prophylaxis (PrEP) in some of these populations.

Priority 4: Develop safe and effective vaccines against SARS-CoV-2

Developing a safe and effective SARS-CoV-2 vaccine is a priority for preventing future outbreaks of the virus. As vaccine candidates for MERS-CoV, SARS-CoV-1 and other coronaviruses have previously been developed, NIAID investigators and the scientific community are well poised to use similar approaches in the current pandemic. NIAID will leverage its broad intramural and extramural infrastructure to advance vaccine candidates through Phase 1 safety and dosing clinical trials, with considerations for Phase 2/2b clinical trials for the most promising candidates.

Objective 4.1: Advance promising vaccine candidates through clinical trial testing

- **Conduct a Phase 1 clinical trial of (mRNA) platform candidate mRNA-1273.** Given the urgency of the response effort to develop a safe and effective vaccine, NIAID is prioritizing promising vaccine candidates that can be rapidly produced and tested. NIAID, in collaboration with the biotechnology company Moderna, is conducting a Phase 1 clinical trial of a vaccine candidate that uses a messenger RNA (mRNA) vaccine platform expressing a NIAID-designed recombinant spike protein of SARS-CoV-2. The trial is being conducted at NIAID-funded clinical research sites, with the first enrolled individual receiving the vaccine on March 16, 2020.

- **Prepare for a pivotal Phase 2/2b clinical trial of candidate mRNA-1273. Preparing for the likelihood of a seasonal recurrence of SARS-CoV-2 is imperative to the public health response.** Given the theoretical risk of vaccine-enhanced respiratory disease, large Phase 2 trials are unlikely to launch until this possibility is evaluated in animal models. Planning for those animal studies is underway, and, assuming favorable results, a Phase 2/2b study could be launched later in 2020. This represents a historically fast timeline for the development and testing of a vaccine candidate. Additionally, these studies will provide information on correlates of immunity that will help accelerate the advancement of other vaccine candidates. If the mRNA-1273 vaccine candidate shows protection against SARS-CoV-2 infection in a Phase 2/2b trial, NIAID will work with government partners to ensure that the vaccine is manufactured in sufficient quantities to allow prompt distribution to those at highest risk of acquiring disease.

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[7] Gautret P et al. *Int J Antimicrob Agents*. 2020 Mar 20:105949. Epub. PMID 32205204.

[8] Molina JM et al. 2020 Med Mal Infect. 2020 Mar 30. pii:S0399-077X(20)30085-8. Epub. PMID 32240719.

[9] Chen Z et al. medRxiv 2020:2020.03.22.20040758.
https://www.medrxiv.org/content/10.1101/2020.03.22.20040758v2

Box 5.
Priority 4: Develop safe and effective vaccines against SARS-CoV-2

Objective 4.1: Advance promising vaccine candidates through clinical trial testing
Objective 4.2: Advance vaccine development through assay and reagent development
Objective 4.3: Advance vaccine development through adjuvant characterization and development

- **Investigate additional candidates through NIAID vaccine programs.** Although promising candidates may show efficacy in preclinical studies, many do not translate into effective vaccines in clinical trials. Therefore, it is crucial to support multiple promising preclinical candidates in the research and development pipeline. To that end, NIAID is advancing multiple additional SARS-CoV-2 vaccine candidates through its Rocky Mountain Laboratories (RML), including approaches that have shown promise against coronaviruses that cause SARS and MERS. Building on previous research to develop a MERS-CoV vaccine, scientists at RML are collaborating with Oxford University investigators to develop a SARS-CoV-2 vaccine that uses a chimpanzee adenovirus vector. RML investigators also are partnering with the biopharmaceutical company CureVac on an mRNA vaccine candidate and collaborating with the University of Washington on a universal coronavirus vaccine development. By leveraging its extensive expertise and research infrastructure, NIAID will continue working with partners and collaborators to advance promising SARS-CoV-2 vaccine candidates.

- **Leverage existing vaccine approaches to target SARS-CoV-2.** NIAID is pursuing multiple strategies to develop a COVID-19 vaccine. Building on past research on emerging pathogens, especially MERS-CoV and SARS-CoV-1 (the virus that causes SARS), NIAID is using previously developed vaccine platforms to rapidly assess the potential of SARS-CoV-2 vaccine candidates. This approach has already resulted in several promising strategies that may be leveraged for SARS-CoV-2, including vaccination using recombinant spike protein, chimpanzee adenovirus vaccine vector, virus-like particles, and live attenuated virus. In addition, NIAID is funding the development of novel vaccine candidates that will be efficacious across the lifespan, including in the elderly.

Objective 4.2: Advance vaccine development through assay and reagent development

- **Develop critical reagents to support vaccine development**. Appropriate tools are needed to identify the most promising vaccine candidates and advance the development of lead candidates as rapidly as possible. To accelerate the vaccine pipeline, NIAID is generating master and working SARS-CoV-2 virus stocks and other reagents critical for developing SARS-CoV-2 immune assays, developing quantitative tests for characterizing SARS-CoV2 assay material, developing a quantitative SARS-CoV-2-specific ELISA, developing virus-specific neutralization assays, and developing quantitative assays for assessing SARS-CoV-2 viral load.

Objective 4.3: Advance vaccine development through adjuvant characterization and development

- **Provide adjuvants to support vaccine development**. Adjuvants are vaccine components that improve vaccine efficacy by inducing long-lived protective immunity. Selection of appropriate adjuvants is crucial for developing safe and effective vaccines. NIAID is working with multiple collaborators to provide adjuvants to the research community for use in SARS-CoV-2 vaccine candidates. These adjuvants are at various stages of development and include compounds that

specifically improve vaccine efficacy in elderly individuals or modulate host immunity toward protective responses while limiting or preventing harmful inflammatory responses.

Conclusion

The sudden emergence and rapid global spread of the novel coronavirus SARS-CoV-2 has created a daunting public health challenge. To address this challenge, NIAID is focusing its considerable expertise and emerging infectious disease resources to facilitate the development of medical countermeasures including diagnostics, therapeutics, and vaccines. The resulting discoveries will not only help mitigate the current pandemic, but also inform prevention, diagnosis, and treatment of future emerging infectious diseases.

A comprehensive strategy requires a coordinated effort among governmental, academic, private, and community-based organizations. The *NIAID Strategic Plan for COVID-19 Research* defines the areas of COVID-19 research within the NIAID mission and outlines the institute's research priorities and goals. This strategic plan builds on many other national efforts and represents a commitment from multiple U.S. government agencies to improve coordination of COVID-19 research and discovery efforts and the development of medical countermeasures.

Exhibit 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

8 July 2020

Drs. Aleksei Chmura and Peter Daszak
EcoHealth Alliance, Inc.
460 W 34th St
Suite 1701
New York, NY 10001

Re: NIH Grant R01AI110964

Dear Drs. Chmura and Daszak:

In follow-up to my previous letter of April 24, 2020, I am writing to notify you that the National Institute of Allergy and Infectious Diseases (NIAID), an Institute within the National Institutes of Health (NIH), under the Department of Health and Human Services (HHS), has withdrawn its termination of grant R01AI110964, which supports the project *Understanding the Risk of Bat Coronavirus Emergence*. Accordingly, the grant is reinstated.

However, as you are aware, the NIH has received reports that the Wuhan Institute of Virology (WIV), a subrecipient of EcoHealth Alliance under R01AI110964, has been conducting research at its facilities in China that pose serious bio-safety concerns and, as a result, create health and welfare threats to the public in China and other countries, including the United States. Grant award R01AI110964 is subject to biosafety requirements set forth in the NIH Grants Policy Statement (e.g., NIH GPS, Section 4.1.24 "Public Health Security") and the Notice of Award (e.g., requiring that "Research funded under this grant must adhere to the [CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)]."). Moreover, NIH grant recipients are expected to provide safe working conditions for their employees and foster work environments conducive to high-quality research. NIH GPS, Section 4. The terms and conditions of the grant award flow down to subawards to subrecipients. 45 C.F.R. § 75.101.

As the grantee, EcoHealth Alliance was required to "monitor the activities of the subrecipient as necessary to ensure that the subaward is used for authorized purposes, in compliance with Federal statutes, regulations, and the terms and conditions of the subaward . . ." 45 C.F.R. § 75.352(d). We have concerns that WIV has not satisfied safety requirements under the award, and that EcoHealth Alliance has not satisfied its obligations to monitor the activities of its subrecipient to ensure compliance.

Moreover, as we have informed you through prior Notices of Award, this award is subject to the Transparency Act subaward and executive compensation reporting requirement of 2 C.F.R. Part

170. To date you have not reported any subawards in the Federal Subaward Reporting System.

Therefore, effective the date of this letter, July 8, 2020, NIH is suspending all activities related to R01AI110964, until such time as these concerns have been addressed to NIH's satisfaction. This suspension is taken in accordance with 45 C.F.R. § 75.371, Remedies for Noncompliance, which permits suspension of award activities in cases of non-compliance, and the NIH GPS, Section 8.5.2, which permits NIH to take immediate action to suspend a grant when necessary to protect the public health and welfare. This action is not appealable in accordance with 42 C.F.R. § 50.404 and the NIH GPS Section 8.7, Grant Appeals Procedures. However, EcoHealth Alliance has the opportunity to provide information and documentation demonstrating that WIV and EcoHealth Alliance have satisfied the above-mentioned requirements.

Specifically, to address the NIH's concerns, EcoHealth must provide the NIH with the following information and materials, which must be complete and accurate:

1. Provide an aliquot of the actual SARS-CoV-2 virus that WIV used to determine the viral sequence.
2. Explain the apparent disappearance of Huang Yanling, a scientist / technician who worked in the WIV lab but whose lab web presence has been deleted.
3. Provide the NIH with WIV's responses to the 2018 U.S. Department of State cables regarding safety concerns.
4. Disclose and explain out-of-ordinary restrictions on laboratory facilities, as suggested, for example, by diminished cell-phone traffic in October 2019, and the evidence that there may have been roadblocks surrounding the facility from October 14-19, 2019.
5. Explain why WIV failed to note that the RaTG13 virus, the bat-derived coronavirus in its collection with the greatest similarity to SARS-CoV-2, was actually isolated from an abandoned mine where three men died in 2012 with an illness remarkably similar to COVID-19, and explain why this was not followed up.
6. Additionally, EcoHealth Alliance must arrange for WIV to submit to an outside inspection team charged to review the lab facilities and lab records, with specific attention to addressing the question of whether WIV staff had SARS-CoV-2 in their possession prior to December 2019. The inspection team should be granted full access to review the processes and safety of procedures of all of the WIV field work (including but not limited to collection of animals and biospecimens in caves, abandoned man-made underground cavities, or outdoor sites). The inspection team could be organized by NIAID, or, if preferred, by the U.S. National Academy of Sciences.
7. Lastly, EcoHealth Alliance must ensure that all of its subawards are fully reported in the Federal Subaward Reporting System

During this period of suspension, NIH will continue to review the activities under this award, taking into consideration information provided by EcoHealth Alliance, to further asses compliance by EcoHealth Alliance and WIV, including compliance with other terms and conditions of award that may be implicated. Additionally, during the period of suspension, EcoHealth Alliance may not allow research under this project to be conducted. Further, no funds from grant R01AI110964 may be provided to or expended by EcoHealth Alliance or any subrecipients; all such charges are unallowable. It is EcoHealth Alliance's responsibility as the

recipient of this grant award to ensure that the terms of this suspension are communicated to and understood by all subrecipients. EcoHealth Alliance must provide adequate oversight to ensure compliance with the terms of the suspension. Any noncompliance of the terms of this suspension must be immediately reported to NIH. Once the original award is reinstated, NIH will take additional steps to restrict all funding in the HHS Payment Management System in the amount of $369,819. EcoHealth Alliance will receive a revised Notice of Award from NIAID indicating the suspension of these research activities and funding restrictions as a specific condition of award.

Please note that this action does not preclude NIH from taking additional corrective or enforcement actions pursuant to 45 CFR Part 75, including, but not limited to, terminating the grant award. NIH may also take other remedies that may be legally available if NIH discovers other violations of terms and conditions of award on the part of EcoHealth Alliance or WIV.

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Sincerely,

Michael S. Lauer -S

Digitally signed by Michael S. Lauer -S
Date: 2020.07.08 21:43:41 -04'00'

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
Email: (b) (6)

cc: Dr. Erik Stemmy
Ms. Emily Linde

Exhibit 3

ECOHEALTH ALLIANCE'S OBJECTIONS AND RESPONSES TO NIH'S ADDITIONAL CONDITIONS ON GRANT 2R01 AI 110964-6

EcoHealth Alliance, Inc. ("EcoHealth Alliance"), by and through its attorneys, Tarter Krinsky & Drogin LLP, hereby responds and objects to the additional conditions (the Requests") imposed on grant 2R01 AI 110964-6 on July 8, 2020, by the National Institute of Allergy and Infectious Diseases ("NIAID"), an Institute within the National Institutes of Health ("NIH"), under the Department of Health and Human Services ("HHS"), as follows:

GENERAL OBJECTIONS[1]

1. EcoHealth Alliance objects to the Requests to the extent they purport to impose obligations beyond those authorized by the NIH Grants Policy Statement and the applicable statutes and regulations.

2. EcoHealth Alliance objects to the Requests to the extent they seek information and documents that are neither relevant to the Project nor reasonably likely to affect the safety or efficacy of EcoHealth Alliance's continued research funded by grant 2R01 AI 110964-6.

3. EcoHealth Alliance objects to the Requests to the extent they seek the production of documents that are not in EcoHealth Alliance's possession, custody, or control.

4. EcoHealth Alliance objects to the Requests to the extent they are vague, ambiguous, or otherwise unclear as to the precise categories of documents and information sought.

5. EcoHealth Alliance objects to the Requests to the extent that they are overbroad, unduly burdensome, or unreasonably cumulative and duplicative.

6. EcoHealth Alliance objects to the Requests to the extent they seek documents and information concerning personal information relating to individuals not affiliated with the Project or Grant on the ground that such requests may invade the rights of privacy of such individuals.

[1] Any capitalized terms not otherwise defined herein shall have the same meaning ascribed to them in EcoHealth Alliance's letter to NIAID, dated August 12, 2020.

7. EcoHealth Alliance objects to the Requests to the extent they seek documents and information regarding transactions or occurrences that took place on or before July 1, 2019, on the ground that such requests are overbroad, and that such documents and information are not relevant to EcoHealth Alliance's continued research funded by grant 2R01 AI 110964-6.

8. EcoHealth Alliance's Responses and Objections to the Requests (including each Request therein) shall not be interpreted as implying that: (i) responsive documents or information exist, (ii) EcoHealth Alliance acknowledges the proprietary of any Request; or (iii) that any Request propounded by NIH is either factually correct or legally binding upon EcoHealth Alliance.

9. EcoHealth Alliance specifically reserves its right to amend, modify, or supplement the objections and responses provided herein.

10. These general objections ("General Objections") are hereby incorporated by reference into each and every of EcoHealth Alliance's responses to the Requests, below.

RESPONSES AND OBJECTIONS TO THE REQUESTS

1. Provide an aliquot of the actual SARS-CoV-2 virus that WIV used to determine the viral sequence.

Response to Request No. 1:

EcoHealth Alliance objects to the Request to the extent it seeks documents and information that are not in EcoHealth Alliance's possession, custody, or control. EcoHealth Alliance further objects to the Request to the extent it seeks information that is not relevant to the Project, which was granted prior to the discovery of SARS-CoV-2. Subject to and notwithstanding the foregoing and without prejudice thereto, EcoHealth Alliance responds that it has no knowledge or information regarding the actual SARS-CoV-2 virus that WIV used to determine the viral sequence.

2. Explain the apparent disappearance of Huang Yanling, a scientist / technician who worked in the WIV lab but whose lab web presence has been deleted.

Response to Request No. 2:

See General Objections. EcoHealth Alliance objects to the Request to the extent it purports to seek information or documents that are not in EcoHealth Alliance's possession, custody, or control. EcoHealth Alliance further objects to the Request to the extent it seeks information that is not relevant to the Project. EcoHealth Alliance further objects to the extent the Request seeks documents and information concerning personal information relating to individuals who are not affiliated with the Project. Subject to and notwithstanding the foregoing and without prejudice thereto, EcoHealth Alliance responds that it lacks knowledge or information regarding the alleged "disappearance of Huang Yanling" or the contention that her "lab web presence has been deleted."

3. Provide the NIH with WIV's responses to the 2018 U.S. Department of State cables regarding safety concerns.

Response to Request No. 3:

See General Objections. EcoHealth Alliance objects to the Request to the extent it purports to seek information or documents that are not in EcoHealth Alliance's possession, custody, or control. EcoHealth Alliance further objects to the Request to the extent it seeks information that is not relevant to the Project. Subject to and notwithstanding the foregoing and without prejudice thereto, EcoHealth Alliance responds that, upon information and belief, it is not in possession, custody, or control of "WIV's responses to the 2018 U.S. Department of State cables regarding safety concerns."

4. Disclose and explain out-of-ordinary restrictions on laboratory facilities, as suggested, for example, by diminished cell-phone traffic in October 2019, and the evidence that there may have been roadblocks surrounding the facility from October 14-19, 2019.

Response to Request No. 4:

See General Objections. EcoHealth Alliance objects to the Request in that it is vague, ambiguous, or otherwise unclear as to the precise categories of documents and information that are being sought and because the term "out-of-ordinary" is undefined. EcoHealth Alliance further objects to the Request to the extent it purports to seek documents or information that are not in EcoHealth Alliance's possession, custody, or control. Subject to and notwithstanding the foregoing and without prejudice thereto, EcoHealth Alliance responds that it lacks knowledge or information regarding "diminished cell-phone traffic in October 2019" and/or "roadblocks surrounding [WIV] from October 14-19, 2019."

5. Explain why WIV failed to note that the RaTG13 virus, the bat-derived coronavirus in its collection with the greatest similarity to SARS-CoV-2, was actually isolated from an abandoned mine where three men died in 2012 with an illness remarkably similar to COVID-19, and explain why this was not followed up.

Response to Request No. 5:

See General Objections. EcoHealth Alliance objects to the Request to the extent it purports to seek information or documents that are not in EcoHealth Alliance's possession, custody, or control. EcoHealth Alliance further objects to the Request to the extent it seeks information that is not relevant to the Project. Subject to and notwithstanding the foregoing and without prejudice thereto, EcoHealth Alliance responds that it lacks knowledge or information regarding the contention that "WIV failed to note that the RatG13 virus…was [] isolated from an abandoned mine where three men died in 2012" and why this was not followed up.

6. Additionally, EcoHealth Alliance must arrange for WIV to submit to an outside inspection team charged to review the lab facilities and lab records, with specific attention to addressing the question of whether WIV staff had SARS-CoV-2 in their possession prior to December 2019. The inspection team should be granted full access to review the processes and safety of procedures of all of the WIV field work (including but not limited to collection of animals and biospecimens in caves, abandoned man-made underground cavities, or outdoor sites). The inspection team could be organized by NIAID, or, if preferred, by the U.S. National Academy of Sciences.

Response to Request No. 6:

See General Objections. EcoHealth Alliance objects to the Request to the extent it seeks to impose obligations on EcoHealth Alliance that are not authorized by the NIH Grants Policy Statement or any applicable statute or regulation. EcoHealth Alliance further objects to the Request to the extent it seeks to impose obligations that are wholly unrelated to the Project or EcoHealth Alliance's ongoing research funding by the Grant. Subject to and notwithstanding the foregoing and without prejudice thereto, EcoHealth Alliance responds that, on April 19, 2020, Michael S. Lauer, MD, NIH Deputy Director for Extramural Research, sent a letter to EcoHealth Alliance on behalf of NIH that stated that EcoHealth Alliance was not allowed to collaborate with WIV regarding the Project and that it should not remit any Grant funds to WIV. On April 21, 2020, Peter Daszak of EcoHealth Alliance sent an email to Dr. Lauer that confirmed (i) no funds from the Grant had been sent to WIV, (ii) no contract had been signed between EcoHealth Alliance regarding research funded under the Grant, and (iii) EcoHealth Alliance would not provide any funds to WIV. As a result, at this time, EcoHealth Alliance is not collaborating with WIV, is not

in possession, custody, or control of WIV, and has no authority to grant NIAID and the U.S. National Academy of Sciences access the facility to conduct an inspection.

7. Lastly, EcoHealth Alliance must ensure that all of its subawards are fully reported in the Federal Subaward Reporting System.

Response to Request No. 7:

See General Objections. Subject to and notwithstanding the General Objections and without prejudice thereto, EcoHealth Alliance responds that, upon information and belief, as of the date of these responses, all of EcoHealth Alliance's subawards are fully reported in the Federal Subaward Reporting System.

Dated: New York, New York
August 13, 2020

TARTER KRINSKY & DROGIN LLP
Attorneys for EcoHealth Alliance

(b) (6)

B

Andrew N. Krinsky
1350 Broadway, 11th Floor
New York, New York 10018
Tel: (b) (6)

TO: Dr. Michael S. Lauer (b) (6)
Dr. Erik Stemmy (b) (6)
Ms. Emily Linde (b) (6)

Best,

Matthew

Matthew R.Torsiello | Associate
D: (b) (6) | F: 212-216-8001
(b) (6) | Bio

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COVID-19 RESOURCE CENTER

Affiliation	Authors	Country	Volume	Journal Issue	Journal Short-form to PubMed abstract	Journal Title ISBN	Journal Volume	Language	Page Number	PMC ID	PMID	Pub Date	Title (Link to full text in PubMed Central)	PubMed Publications in PubMed	Related Publications in Google Scholar	Articles Citing from PubMed Articles	Articles Citing from Google Scholar	Clinical Trials Cited
EcoHealth Alliance, 460 West 34th Street, New York, NY 10001, USA	Li, Hong-Ying; Zhu, Guang-Jian; Zhang, Yun-Zhi; Zhang, Li-Kun; Holmes, Edward C; Wang, Mang; Eden, John-Sebastian; Shi, Mang; Fan, Zhao-Jun; Tian, Jun-Hua; Duchêne, Sebastián; Hu, Wen-Bo; Miller, Matthew; Wang, Lin-Fa; Shi, Zheng-Li	England	1876-4061	(b) (5)	International Health	Int Health	12	eng	77-83	7610874	31840039	2020 01 31	Host-shedding data of zoonotic risk factors among rural communities in southern China	2019	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China.	Cui, Jie; Li, Fang; Shi, Zheng-Li	England	1740-1526		Nature reviews. Microbiology	Nat Rev Microbiol	17	eng	181-192	7097006	29321432	2019 03	Origin and evolution of pathogenic coronaviruses	2019	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
EcoHealth Alliance, 460 West 34th Street, 17th Floor, New York, NY, 10001, USA. zebey@ecohealthalliance.org	Eaton, Tom; Olival, Kevin J	United States	1612-9210		Ecohealth		15	eng	707-711	6290576	30374865	2018 10	Co-infection and Zoonotic Disease Risk.	2018	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
MOH Key Laboratory of Systems Biology of Pathogens, Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, People's Republic of China	Wu, Zhaoqing; Lu, Lung; Du, Jing; Yang, Li; Ren, Xiaowei; Liu, Bo; Jiang, Jiaming; Yang, Jun; Dong, Jie; Sun, Lihua; Zhu, Yibing; Li, Yahui; Zheng, Xiaobao; Zhang, Cun; Li, Xiaodong; Zhang, Shuang	England	1868-2618		Virulence	Microbiome	6	eng	198	6171737	30348186	2018 10 31	Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of	2018	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
Queensland Department of Primary Industries, Brisbane, Qld, Australia.	Field, Hume Ernest	Germany	1869-219		Zoonoses and public health	Zoonoses Public Health	65	eng	742-748	6240111	29790274	2018 09	Evidence of Australian bat lyssavirus infection in diverse Australian bat taxa.	2018	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China.	Luo, Chu-Ming; Wang, Ning; Yang, Xing-Lou; Liu, Hai-Zhou; Zhang, Wei; Li, Bei; Hu, Ben; Peng, Cheng; Geng, Qi-Bin; Zhu, Guang-Jian; Li, Fang; Shi, Zheng-Li	United States	1098-6618		Journal of virology	J Virol	92	eng		5660132	29669831	2018 07 01	Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East	2018	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China.	Zhou, Peng; Yin, Wang; Lou Chen, Yang; Xiaojian; Du, Xin-Feng; Duoss, Wen-Zhe; Fan, Zilwei; Ye-Wei, Xin-Jing-Wei; Mani, Shellenbe; Zhang, Ran; Huang, G; Dai, Li-De-Wei; Guo, Aug; Wang, Ning; Li, Shi-Yue; Yang, Xing-Lou; Huang, Bu; Wu, Zhong; Fu-E; Gao, Hai; Lau, Chu-Ming; Jiang, Ying-Ang; Miller, Maureen; Zhu, Guangjian; Daszak, Aleksan; Wang, Lin-Fa; Shi, Zheng-Li; Luo,	England	1476-4687		Nature	Nature	556	eng	255-258	7094408	29618817	2018 04	Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin.	2018	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China.	Wang, Ning; Li, Shi-Yue; Yang, Xing-Lou; Huang, Bu; Wu, Zhong; Fu-E; Gao, Hai; Lau, Chu-Ming; Jiang, Ying-Ang; Miller, Maureen; Zhu, Guangjian; Daszak, Aleksan; Wang, Lin-Fa; Shi, Zheng-Li; Luo,	China	1998-6769		Virological Sinica	Virol Sin	33	eng	351-357	6178078	29916079	2018 08	Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China.	2018	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
CAS Key Laboratory of Special Pathogens and Biosafety, Center for Emerging Infectious Diseases of Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China.	Hu, Ben; Ling, Lei-Ping; Yuan, Xing-Yi; Yang, Xing-Lou; Xu, Bing-Yi; Zhang, Wei; Li, Bei; Wang, Xin-Ru; Zhan, Bo; Jiang, Chang-Liang; Wang, Xiao-Guang; Zou, Zhe-Qing; Zhuang, Xiang; Zhang, Hai-Xiang; Luo, Dong; Tong, Yi-Gang; Tan, Wen-Jie; Shi, Zheng-Li	United States	1553-7374		PLoS pathogens	PLoS Pathog	13	eng	e1006698	5708621	28934359	2017 Dec	Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of	2017	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China.	Deng, Jin-Ping; Lu, Guo-Yi; Yang, Xiang; Yi, Wanlou; Sun, Zhou; Yu, Xiao-Ling; Xian, Yan-Ling; Chen, Yu; Zhong, Zhaohui; Liu, Huan; Xiao, Gengfu; Li, Fang; Shi, Zheng-Li	China	1869-1889		Science China. Life sciences	Sci China Life Sci	60	eng	1389-1393	7088137	28349401	2017 12	Close relationship of SARS-coronavirus-specific antibodies against bat SARS-like coronaviruses.	2017	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
EcoHealth Alliance, 460 West 34th Street, New York, New York 10001 USA.	Olival, Kevin J; Hosseini, Parviez R; Zambrana-Torrelio, Carlos; Ross, Nikolai; Bogich, Tiffany L; Daszak, Peter	England	1476-9255		Nature	Nature	546	eng	646-650	5570460	28636590	2017 06 29	Host and viral traits predict zoonotic spillover from mammals.	2017	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
EcoHealth Alliance, 460 West 34th Street, 17th Floor, New York, NY, 10001, USA. olival@ecohealthalliance.org	Olival, Kevin J; McKnight, Sara A	United States	1612-9210		Ecohealth	Ecohealth	14	eng	1-6	5380539	28197918	2017 03	Predicting the "Dormant" Herpesviruses.	2017	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
Key Laboratory of Special Pathogens and Center for Emerging Infectious Diseases, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China	Yang, Xing-Lou; Hu, Ben; Wang, Bo; Wang, Mei-Hong; Zhang, Qiao; Zhang, Wei; Wu, Li-Ju; Ma, Xing-Yi; Zhang, Yun-Zhi; Daszak, Peter; Wang, Lin-Fa; Shi, Zheng-Li	United States	1098-5514		Journal of virology	J Virol	90	eng	6338-6	4836331	27053557	2016 May	Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor	2016	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
EcoHealth Alliance, 460 W. 34th St Fl 16, 17th Floor, New York, NY, 10a, olival@ecohealthalliance.org	Olival, Kevin J	United States	1612-9210		Ecohealth	Ecohealth	13	eng	344-54	4931857	26922148	2016 Mar	To Cull, or Not to Cull, that is the Question.	2016	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China.	Wang, Wen-Hong; Zhang, Wei; Sun, Yu-Fei; Hu, Ben; Ge, Xing-Yi; Yang, Xing-Lou; Zhang, Yun-Zhi; Shi, Zheng-Li	China	1995-8250		Virological Sinica	Virol Sin	31	eng	78-80	7090618	26932971	2016 Feb	Serological Surveillance of SARS-like coronaviruses in bats by recombinant viral-like P1protein.	2016	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
Key Laboratory of Special Pathogens, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China	Zeng, Lei-Ping; Gao, Yu-Tian; Ge, Xing-Yi; Zhang, Qian; Peng, Cheng; Yang, Xing-Lou; Tian, Bing; Li, Bei; Du, Jing; Ortiz-Baez, Alexis S; Daszak, Peter; Shi, Zheng-Li	United States	1998-4146		Journal of Virology	J Virol	90	eng	6535-6545	4959218	27194764	2016 07 15	Bat Severe Acute Respiratory Syndrome-like Coronavirus WIV1 Encodes an Extra Accessory	2016	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
University of California Davis, Davis, CA, United States of America	Young, Clark C W; Olival, Kevin	United States	1932-6203		PloS one	PLoS One	11	eng	e0148257	4752487	26867652	2016	Estimating Viral Diversity in Bats.	2016	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
Key Laboratory of Special Pathogens and Center for Emerging Infectious Diseases, Wuhan Institute of Virology, Chinese Academy of Science (Wuhan), China	Hu, Ben; Ge, Xingyi; Wang, Lin-Fa; Shi, Zhengli	England	1758-4574		Virology journal	Virol J	12	eng	221	4687305	26689940	2015 Dec 23	Bat origin of human coronaviruses.	2015	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google
[illegible]	[illegible]	England	1476-4687		Nature	Nature	503	eng	535-8	3889361	24172901	2013 Nov 28	Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor.	2013	Related, PubMed	Related, Google Scholar	Cited By, PMC	Cited By, Google

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

24 April 2020

Drs. Aleksei Chmura and Peter Daszak
EcoHealth Alliance, Inc.
460 W 34th St
Suite 1701
New York, NY 10001

Re: Termination of NIH Grant R01 AI 110964

Dear Drs. Chmura and Daszak:

I am writing to notify you that the National Institute of Allergy and Infectious Diseases (NIAID), an Institute within the National Institutes of Health (NIH), under the Department of Health and Human Services (HHS) has elected to terminate the project *Understanding the Risk of Bat Coronavirus Emergence*, funded under grant R01 AI110964, for convenience. This grant project was issued under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284). This grant was funded as a discretionary grant as outlined in the NIH Grants Policy Statement, which states that the decision not to award a grant, or to award a grant at a particular funding level, is at the discretion of the agency, in accordance with NIH's dual review system.

At this time, NIH does not believe that the current project outcomes align with the program goals and agency priorities. NIAID has determined there are no animal and human ethical considerations, as this project is not a clinical trial, but rather an observational study.

As a result of this termination, a total of $369,819.56 will be remitted to NIAID and additional drawdowns will not be supported. The remaining funds have been restricted in the HHS Payment Management System, effective immediately.

Please let me know if you have any questions concerning the information in this letter.

Sincerely,

Lauer, Michael (NIH/OD) [E] Digitally signed by Lauer, Michael (NIH/OD) [E]
Date: 2020.04.24 16:41:16 -04'00'

Michael S Lauer, MD
NIH Deputy Director for Extramural Research
Email (b) (6)

cc: Dr. Erik Stemmy
Ms. Emily Linde

Date: April 19, 2020

From: Michael S Lauer, MD
NIH Deputy Director for Extramural Research

Lauer, Michael (NIH/OD) [E] Digitally signed by Lauer, Michael (NIH/OD) [E] Date: 2020.04.19 10:47:40 -04'00'

To: Kevin Olival, PhD
Vice-President for Research
EcoHealth Alliance
(b) (6)

Naomi Schrag, JD
Vice-President for Research Compliance, Training, and Policy
Columbia University
(b) (6)

Subject: Project Number 2R01AI110964-06

Dear Dr. Olival and Ms. Schrag:

EcoHealth Alliance, Inc. is the recipient, as grantee, of an NIH grant entitled "Understanding the Risk of Bat Coronavirus Emergence." It is our understanding that one of the sub-recipients of the grant funds is the Wuhan Institute of Virology ("WIV"). It is our understanding that WIV studies the interaction between corona viruses and bats. The scientific community believes that the coronavirus causing COVID-19 jumped from bats to humans likely in Wuhan where the COVID-19 pandemic began. There are now allegations that the current crisis was precipitated by the release from WIV of the coronavirus responsible for COVID-19. Given these concerns, we are pursuing suspension of WIV from participation in Federal programs.

While we review these allegations during the period of suspension, you are instructed to cease providing any funds from the above noted grant to the WIV. This temporary action is authorized by 45 C.F.R. § 75.371(d) ("Initiate suspension or debarment proceedings as authorized under 2 C.F.R. part 180"). The incorporated OMB provision provides that the funding agency may, through suspension, immediately and temporarily exclude from Federal programs persons who are not presently responsible where "immediate action is necessary to protect the public interest." 2 C.F.R. § 180.700(c). It is in the public interest that NIH ensure that a sub-recipient has taken all appropriate precautions to prevent the release of pathogens that it is studying. This suspension of the sub-recipient does not affect the remainder of your grant assuming that no grant funds are provided to WIV following receipt of this email during the period of suspension.

EcoHealth Alliance

14 November 2018

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Re: Budget Error in Renewal Proposal 2R01AI110964-06

To Whom It May Concern:

This letter pertains to our renewal proposal (2R01AI110964-06) with the title "Understanding the Risk of Bat Coronavirus Emergence" for consideration under the NIH Research Grant Program (R01, PA-18-484). Our submitted renewal proposal budget was in error. If our application receives a fundable score, we will reduce our budget to below the $500,000 per year cap in all 5 years of the award.

Our total budget should be **$3,225,898** including indirect costs. Without indirect costs our total direct cost budget should be **$2,499,944** and annually detailed as follows:

Year	Direct Cost	Indirect Cost	Total
1	$499,989	$164,835	$664,824
2	$499,989	$140,280	$640,269
3	$499,989	$140,280	$640,269
4	$499,989	$140,280	$640,269
5	$499,989	$140,280	$640,269

Please contact us, if there are any questions or further details required. Thank you very much for your consideration.

Sincerely,

(b) (6)

Dr. Peter Daszak, PI
President, EcoHealth Alliance
460 West 34th Street, Ste 1701
New York, NY 10001, USA

(b) (6)

(b) (6)

Dr. Aleksei Chmura
AOR and Chief of Staff, EcoHealth Alliance
460 West 34th Street, Ste 1701
New York, NY 10001, USA

(b) (6)

1. **Kind or species of animal and number to be used:**

Species taxa	Family, Genus or Species Name	Target numbers
Fruit bats	*e.g.: Cynopterus, Rousettus, Eonycterus spp.*	900 individuals (30 individuals from 30 different species)
Insectivorous bats	*e.g.: Rhinilophidae, Hipposideridae, Emballonuridae, Vespertillionidae, Mollsidae, Miniopteridae spp.*	
Rodents	e.g: Chinese bamboo rat (*Rhizomys sinensis*), Malayan porcupine (*Hystrix brachyura*), bandicoot (*Bandicota indica*)	900 individuals
Small Carnivores	e.g.: Raccoon dog (*Nyctereutes procyonoides*), Asian Palm civet (*Paradoxurus hemaphroditus*), ferret badger (*Melogale moschata*)	500 individuals

2. **Location of the source of the animals, if known:**

Free-ranging bat surveys and bats in wet markets: China, Malaysia, Thailand, Cambodia, Lao PDR, Myanmar, Vietnam, and Indonesia.

Other mammals: We will opportunistically sample the other aforementioned taxa that are also sold in live animal markets, trading locations or bred on farms to supply markets throughout southeast Asia. Species and numbers of animals sampled from markets will be based on animal availability.

3. **A brief description of the sampling (blood draw, swab, etc)**

Bat capture. Free-ranging bats will be captured using either a mist net or harp trap and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. Bats will be manually restrained during sampling. Bats that are fractious may be anesthetized for restraint purposes in order to maximize safety for the bat and handler. Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh feces will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from either from the cephalic vein or from the radial artery or vein using a 25-gauge needle. Bats are held for a maximum of six hours and then released following sample collection. We will euthanize 2 individuals per bat species for organ tissue banking.

Wild and captive bred rodent capture. Free-ranging rodents will be captured using box traps. Captive bred rodents (e.g. at rodent farms) will be manually captured and restrained. Traps for free-ranging rodents will be checked a minimum of every 12 hours, including once in the morning. Captive bred and wild rodent sampling procedures (including anesthesia, if necessary), will involve manual restraint, venipuncture, mucosal swabs, fecal, and urine sample collection.

Other small mammals: Anesthesia will be used to restrain small mammals such as civets and ferret badgers. Animals will be monitored continuously while recovering from anesthesia and will only be released once fully recovered from anesthesia. Animals that are sourced from markets and that may potentially be consumed, will be manually restrained without anesthesia, if possible, so that they may be returned to the vendor. Otherwise, the animal will be sampled and then euthanized via exsanguination

(cardiac puncture) while under anesthesia, then disposed of using biohazard protocols in order to prevent subsequent human or animal consumption.

4. **Location from where the animals will be obtained (source):**

Markets and surrounding caves/forest: sites will be identified along value chain routes linking southern China to southeast Asian countries that serve as sources for the Chinese market system. Specific field sites have not yet been determined.

5. **If possible, what will be done with the animals after the project ends (e.g., euthanized)**

All wild animals will be released unharmed after sampling at the capture location. While we do not anticipate any severe adverse events related to the capture or sampling of free ranging wildlife, we will observe all animals caught in traps and nets for injuries. Veterinary care of wildlife in the field is limited. Any animal with an injury that is deemed life-threatening, or significant enough to prevent survival upon release, will be humanely euthanized in accordance with the AVMA guidelines for euthanasia (2013). Any animal that is injured in the course of restraint or sampling such that it is deemed unable to survive if released or if appears to be in severe pain due to injury, will be humanely euthanized. Animals that are caught and moribund (depressed mentation, non-responsive to stimuli, emaciated and weak or exhibiting neurological signs), will be humanely euthanized.

(b) (5)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

July 05, 2018

Mr. Aleksei Chmura
EcoHealth Alliance
460 W. 34th Street – 17th Floor
New York, NY 10001

RE: 5R01AI110964-05

Dear Mr. Chmura:

On December 19, 2017, the U.S. Department of Health and Human Services (DHHS) issued the *Department of Health and Human Services Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens* (HHS P3CO Framework) (https://www.phe.gov/s3/dualuse/Documents/P3CO.pdf). The HHS P3CO Framework is responsive to and in accordance with the *Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight* (Recommended Policy Guidance) (https://www.phe.gov/s3/dualuse/Documents/P3CO-FinalGuidanceStatement.pdf) issued by the White House Office of Science and Technology Policy on January 9, 2017. Additionally, and as noted in the Recommended Policy Guidance, adoption of the HHS P3CO Framework satisfies the requirement for lifting the Research Funding Pause on certain gain-of-function (GoF) research.

The HHS P3CO Framework guides DHHS funding decisions on research that is reasonably anticipated to create, transfer, or use enhanced potential pandemic pathogens (PPPs). A PPP is a pathogen that satisfies both of the following:

- It is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations; and
- It is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.

In accordance with the HHS P3CO Framework, research involving an enhanced PPP is subject to additional HHS department-level review. NIAID re-reviewed the grant application and other information provided by you, and made the following assessment:

> The experiments to generate MERS-like or SARS-like chimeric coronaviruses, are not subject to the HHS P3CO Framework. The terms and conditions of the award have been revised to indicate that should experiments proposed in this award result in a virus with enhanced growth by more than 1 log compared to wild type strains, you must notify your NIAID Program Officer and

Grants Management Specialist immediately and that further research involving the resulting virus(es) may require review by the DHHS in accordance with the HHS P3CO Framework.

Please remember that the institution must comply in full with all terms and conditions placed on this grant.

Please let us know if you have any questions, or if you require additional information.

Sincerely,

(b) (6)

Adam Graham
Grants Management Specialist
NIAID/NIH/DHHS

(b) (6)

Erik J. Stemmy, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS

CC: Dr. Peter Daszak
Ms. Emily Linde
Dr. Emily Erbelding
Dr. Irene Glowinski
Dr. Andrew Ford

(b) (5)

(b) (5)

From:	Routh, Jennifer (NIH/NIAID) [E]
To:	Selgrade, Sara (NIH/NIAID) [E]; Deatrick, Elizabeth (NIH/NIAID) [E]; Embry, Alan (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]; Billet, Courtney (NIH/NIAID) [E]; NIAID BUGS
Cc:	NIAID OCGR NSWB; NIAID Media Inquiries; Haskins, Melinda (NIH/NIAID) [E]
Subject:	RE: For review again: Response to GoF inquiry from FactCheck.org
Date:	Thursday, July 22, 2021 4:55:08 PM
Attachments:	Response to FactCheck JR ed ae cb AOF clean_ss.docx
Importance:	High

I am adding BUGS, Andrew and Courtney to this thread so we are all on the same thread. The original response pulled from cleared language. I'd like to get this finalized ASAP. We really need to move this forward.

Jennifer Routh [E]
News and Science Writing Branch
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
NIH/HHS
31 Center Drive Room 7A17C
Bethesda, MD 20892
Direct: (b) (6)

From: Selgrade, Sara (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 4:49 PM
To: Deatrick, Elizabeth (NIH/NIAID) [E] (b) (6); Embry, Alan (NIH/NIAID) [E] (b) (6)
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>; NIAID Media Inquiries <mediainquiries@niaid.nih.gov>; Haskins, Melinda (NIH/NIAID) [E] (b) (6)
Subject: RE: For review again: Response to GoF inquiry from FactCheck.org

Thanks Elizabeth. Some edits for your consideration in the attached. I'm copying Melinda as well for her review.

Defer to Alan on whether DMID should review again.

From: Deatrick, Elizabeth (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 4:33 PM
To: Embry, Alan (NIH/NIAID) [E] (b) (6)>; Selgrade, Sara (NIH/NIAID) [E] (b) (6)
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>; NIAID Media Inquiries <mediainquiries@niaid.nih.gov>
Subject: For review again: Response to GoF inquiry from FactCheck.org

Good afternoon,

(b) (5)

(b) (5)

DMID has reviewed our response to FactCheck.org on GoF research, but recommended that we run the copy past you one more time before moving it forward in the clearance process. Please let me know if you have any notes on the attached clean document.

Best,

Elizabeth Deatrick
Technical Writer-Editor
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
(b) (6)

Please note that I am not a spokesperson for NIAID and should not be quoted as such.

From: Abbey, Lillian (NIH/NIAID) [E]
To: Ford, Andrew (NIH/NIAID) [E]
Cc: DMID Word Nerds
Subject: FW: For review: GoF Response to FactCheck.org
Date: Thursday, July 22, 2021 2:16:02 PM
Attachments: Response to FactCheck JR ed ae cb.docx

Hey Andrew,
I acknowledged Elizabeth's request since I am on comms duty from 12-5 today but wondering if you want to handle this. Please let me know.
Thanks,
Lillian

From: Deatrick, Elizabeth (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 2:07 PM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>; NIAID Media Inquiries <mediainquiries@niaid.nih.gov>
Subject: For review: GoF Response to FactCheck.org

Good afternoon,

Yesterday, we received a media inquiry from FactCheck.org regarding the exchange that Dr. Fauci had with Sen. Paul in the briefing the other day, and asking specifically about the paper that Sen. Paul cited. Would you be able to have Program review the attached response we've drafted? It uses some existing language we've used for other purposes, but we would still like to check it for accuracy

The writer's deadline has passed, but we'd still like to get it to them today, if possible.

Best,
Elizabeth Deatrick
Technical Writer-Editor
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
(b) (6)

Please note that I am not a spokesperson for NIAID and should not be quoted as such.

(b) (5)

From: Miers, Sarah (NIH/NIAID) [E]
To: Ford, Andrew (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Hauguel, Teresa (NIH/NIAID) [E]
Subject: Re: Urgent Action: Additional FLIPs for review
Date: Tuesday, October 19, 2021 1:17:57 PM

Thanks!

From: "Ford, Andrew (NIH/NIAID) [E]" (b) (6)
Date: Tuesday, October 19, 2021 at 1:14 PM
To: "Stemmy, Erik (NIH/NIAID) [E]" (b) (6), "Miers, Sarah (NIH/NIAID) [E]" (b) (6), "Hauguel, Teresa (NIH/NIAID) [E]" (b) (6)
Subject: RE: Urgent Action: Additional FLIPs for review

I added one comment to Erik's (b) (5)

Andrew Q. Ford, Ph.D.
Office of Scientific Coordination and Program Operations
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
5601 Fishers Lane Room 7G64
Rockville, MD 20892
(b) (6)

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Tuesday, October 19, 2021 1:00 PM
To: Miers, Sarah (NIH/NIAID) [E] (b) (6); Hauguel, Teresa (NIH/NIAID) [E] (b) (6); Beigel, John (NIH) [E] (b) (6); Cardemil, Cristina (NIH/NIAID) [E] (b) (6); Ford, Andrew (NIH/NIAID) [E] (b) (6)
Subject: RE: Urgent Action: Additional FLIPs for review

Hi Sarah,
Comments from me in the attached. I didn't have comments for the others.

Erik

From: Miers, Sarah (NIH/NIAID) [E] (b) (6)
Sent: Tuesday, October 19, 2021 11:42 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6) Hauguel, Teresa (NIH/NIAID) [E] (b) (6); Beigel, John (NIH) [E] (b) (6); Cardemil, Cristina (NIH/NIAID) [E] (b) (6); Ford, Andrew (NIH/NIAID) [E] (b) (6)
Subject: Urgent Action: Additional FLIPs for review
Importance: High

Hello –

We have received additional flip cards to review for Dr. Fauci for the upcoming Senate HELP hearing. Some of these cards have been reviewed before and OCGR-Leg has added questions or edits for us to review on them. Here is the list of cards:

- 2016 – Baric Nature Microbiology Paper
- _Covid – Vax Trials in Children
- 2017 PLOS Pathogens Paper
- Benefits of GOF research
- Furin Cleavage Site_CGG Repeats
- EcoHealth Alliance CoV Grant (2014-2018) Year 5 [Also going to DEA for review]
- _COVID – Pregnant Women and Vax Trials [Also going to DAIDS for review]
- Natural Immunity to SARS-CoV [Going to all Divisions for review]

Unfortunately we have a very short turnaround time for these and Emily needs to review them. Please respond to me by 2:00 PM today. My apologies for the rapid turnaround.

Thanks
Sarah

From: "Arms, Erin (NIH/NIAID) [E]" (b) (6)
Date: Tuesday, October 19, 2021 at 11:14 AM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Action by 3 pm today (10/19): Additional FLIPs for review

Hello Bugs,

Background

Thank you for your ongoing assistance to prepare FLIPs for the November 4th Senate HELP Committee hearing to discuss the ongoing Federal response to COVID-19. Attached are additional FLIPs for your review. Please note – some of the FLIPs have already been reviewed. The versions of these FLIPs included here have additional edits or questions for your review.

2016 – Baric Nature Microbiology Paper

- _Covid – Vax Trials in Children
- 2017 PLOS Pathogens Paper
- Benefits of GOF research
- Furin Cleavage Site_CGG Repeats
- EcoHealth Alliance CoV Grant (2014-2018) Year 5 [Also going to DEA for review]
- _COVID – Pregnant Women and Vax Trials [Also going to DAIDS for review]
- Natural Immunity to SARS-CoV [Going to all Divisions for review]

Action

This is an expedited request for FLIP review. Please send completed FLIPs to OCGR-Leg by **3 PM today, October 19th.** As these are for Dr. Fauci's use during the upcoming hearing, we do request that Dr. Erbelding clear DMID's updated FLIPs.

Please let us know if you have any questions or if any of the above FLIPs should be reviewed by additional Divisions.

Thanks,
Erin

Erin Arms, Ph.D.
(she, her, hers)
Public Health Analyst
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
31 Center Drive
Bldg. 31, Room 7A17H, MSC 2520
Bethesda, MD 20892-2080
(b) (6)

From:	Miers, Sarah (NIH/NIAID) [E]
To:	Hastings, Andrew (NIH/NIAID) [E]
Cc:	NIAID BUGS; NIAID OCGR Leg
Subject:	Re: For Review by 10 AM Tomorrow (10/22): Responses to QFRs for House L-HHS Appropriations hearing
Date:	Friday, October 22, 2021 10:10:11 AM
Attachments:	LHHS 5.25 - QFRs for NIAIDassignments_toDiv AQF.docx

Hi Drew, we have some edits and comments in the attached. Please let me know if you have any questions or need anything else.

Sarah

From: "Miers, Sarah (NIH/NIAID) [E]" (b) (6)
Date: Thursday, October 21, 2021 at 10:59 AM
To: "Hastings, Andrew (NIH/NIAID) [E]" (b) (6)
Cc: NIAID BUGS <BUGS@niaid.nih.gov>, NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Re: For Review by 10 AM Tomorrow (10/22): Responses to QFRs for House L-HHS Appropriations hearing

Will do, Drew

From: "Hastings, Andrew (NIH/NIAID) [E]" (b) (6)
Date: Thursday, October 21, 2021 at 10:29 AM
To: NIAID BUGS <BUGS@niaid.nih.gov>, NIAID DAIDS WOCRB <NIAIDDAIDSSCIB@niaid.nih.gov>, NIAID DAIT-OPA <DAIT-OPA@niaid.nih.gov>, NIAID DCR-OCGR <NIAIDDCR-OCGR@mail.nih.gov>, NIAID DIR-OCGR <NIAIDDIR-OCGR@mail.nih.gov>, NIAID VRC-OPA <NIAIDVRC-OPA@niaid.nih.gov>
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: For Review by 10 AM Tomorrow (10/22): Responses to QFRs for House L-HHS Appropriations hearing

Good morning,

Background:
NIAID has been assigned to respond to a few questions for the record (QFRs) from the House Labor-HHS Appropriations subcommittee on May 25th, 2021. As you may recall, Dr. Fauci accompanied Dr. Collins to the hearing along with several other IC Directors. We have been instructed to prepare these responses with information that is current as of the date of the hearing.

OCGR-Leg used previously cleared documents to prepare the attached draft responses to these QFRs.

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Action:

By 10 AM Tomorrow, Friday, October 22nd, please review these draft responses and let us know if you have any edits in track-changes.

Let me know if you have any questions.

Thanks,
Drew

Cell # (b) (6)

Andrew K. Hastings, Ph.D.
Public Health Analyst
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
Bldg. 31, Room 7A17, MSC 2520
Bethesda, MD 20892-2520
(b) (6)

From:	Miers, Sarah (NIH/NIAID) [E]
To:	Stemmy, Erik (NIH/NIAID) [E]; Hauguel, Teresa (NIH/NIAID) [E]; Beigel, John (NIH) [E]; Cardemil, Cristina (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]
Subject:	Urgent Action: Additional FLIPs for review
Date:	Tuesday, October 19, 2021 11:42:30 AM
Attachments:	2016 - Baric Nature Microbiology Paper_toDMID.docx _COVID - Vax Trials in Children_toDMID.docx 2017 PLOS Pathogens Paper_toDMID.docx Benefits of GOF research_toDMID.docx Furin Cleavage Site_CGG Repeats_toDMID.docx EcoHealth Alliance CoV Grant (2014-2018) Year 5 RPPR toDivisions.docx _COVID - Pregnant Women and Vax Trials_toDivs.docx Natural Immunity to SARS-CoV-2_toDivs.docx
Importance:	High

Hello –

We have received additional flip cards to review for Dr. Fauci for the upcoming Senate HELP hearing. Some of these cards have been reviewed before and OCGR-Leg has added questions or edits for us to review on them. Here is the list of cards:

- 2016 – Baric Nature Microbiology Paper
- _Covid – Vax Trials in Children
- 2017 PLOS Pathogens Paper
- Benefits of GOF research
- Furin Cleavage Site_CGG Repeats
- EcoHealth Alliance CoV Grant (2014-2018) Year 5 [Also going to DEA for review]
- _COVID – Pregnant Women and Vax Trials [Also going to DAIDS for review]
- Natural Immunity to SARS-CoV [Going to all Divisions for review]

Unfortunately we have a very short turnaround time for these and Emily needs to review them. Please respond to me by 2:00 PM today. My apologies for the rapid turnaround.

Thanks
Sarah

From: "Arms, Erin (NIH/NIAID) [E]" (b) (6)
Date: Tuesday, October 19, 2021 at 11:14 AM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Action by 3 pm today (10/19): Additional FLIPs for review

Hello Bugs,

Background

Thank you for your ongoing assistance to prepare FLIPs for the November 4th Senate HELP Committee hearing to discuss the ongoing Federal response to COVID-19. Attached are additional FLIPs for your review. Please note – some of the FLIPs have already been reviewed. The versions of

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these FLIPs included here have additional edits or questions for your review.

- 2016 – Baric Nature Microbiology Paper
- _Covid – Vax Trials in Children
- 2017 PLOS Pathogens Paper
- Benefits of GOF research
- Furin Cleavage Site_CGG Repeats
- EcoHealth Alliance CoV Grant (2014-2018) Year 5 [Also going to DEA for review]
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- Natural Immunity to SARS-CoV [Going to all Divisions for review]

Action

This is an expedited request for FLIP review. Please send completed FLIPs to OCGR-Leg by **3 PM today, October 19th.** As these are for Dr. Fauci's use during the upcoming hearing, we do request that Dr. Erbelding clear DMID's updated FLIPs.

Please let us know if you have any questions or if any of the above FLIPs should be reviewed by additional Divisions.

Thanks,
Erin

Erin Arms, Ph.D.
(she, her, hers)
Public Health Analyst
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
31 Center Drive
Bldg. 31, Room 7A17H, MSC 2520
Bethesda, MD 20892-2080
(b) (6)

From:	Miers, Sarah (NIH/NIAID) [E]
To:	Arms, Erin (NIH/NIAID) [E]; NIAID BUGS
Cc:	NIAID OCGR Leg
Subject:	Re: Action by 3 pm today (10/19): Additional FLIPs for review
Date:	Tuesday, October 19, 2021 2:41:53 PM
Attachments:	_COVID - Pregnant Women and Vax Trials_toDivs_cc.docx _COVID - Vax Trials in Children_to_DMID_cc.docx 2016 - Baric Nature Microbiology Paper_toDMID_es AQF.docx 2017 PLOS Pathogens Paper_toDMID_es.docx Benefits of GOF research_toDMID_es.docx EcoHealth Alliance CoV Grant (2014-2018) Year 5 RPPR toDivisions_es.docx Furin Cleavage Site_CGG Repeats_toDMID_es.docx

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Erin, (b) (5) Our edits and responses are included in the attached cards.

From: "Miers, Sarah (NIH/NIAID) [E]" (b) (6)
Date: Tuesday, October 19, 2021 at 11:38 AM
To: "Arms, Erin (NIH/NIAID) [E]" (b) (6), NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Re: Action by 3 pm today (10/19): Additional FLIPs for review

Will do, Erin

From: "Arms, Erin (NIH/NIAID) [E]" (b) (6)
Date: Tuesday, October 19, 2021 at 11:14 AM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Action by 3 pm today (10/19): Additional FLIPs for review

Hello Bugs,

Background

Thank you for your ongoing assistance to prepare FLIPs for the November 4th Senate HELP Committee hearing to discuss the ongoing Federal response to COVID-19. Attached are additional FLIPs for your review. Please note – some of the FLIPs have already been reviewed. The versions of these FLIPs included here have additional edits or questions for your review.

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- Natural Immunity to SARS-CoV [Going to all Divisions for review]

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Action

This is an expedited request for FLIP review. Please send completed FLIPs to OCGR-Leg by **3 PM today, October 19th.** As these are for Dr. Fauci's use during the upcoming hearing, we do request that Dr. Erbelding clear DMID's updated FLIPs.

Please let us know if you have any questions or if any of the above FLIPs should be reviewed by additional Divisions.

Thanks,
Erin

Erin Arms, Ph.D.
(she, her, hers)
Public Health Analyst
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
31 Center Drive
Bldg. 31, Room 7A17H, MSC 2520
Bethesda, MD 20892-2080
(b) (6)

From:	Selgrade, Sara (NIH/NIAID) [E]
To:	Erbelding, Emily (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Hauguel, Teresa (NIH/NIAID) [E]; Mulach, Barbara (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]; Fenton, Matthew (NIH/NIAID) [E]; Linde, Emily (NIH/NIAID) [E]; Ghedin, Elodie (NIH/NIAID) [E]
Cc:	NIAID OCGR Leg; Embry, Alan (NIH/NIAID) [E]; Billet, Courtney (NIH/NIAID) [E]; Folkers, Greg (NIH/NIAID) [E]; NIAID BUGS; NIAID DIR-OCGR; Holland, Steven (NIH/NIAID) [E]
Subject:	URGENT for ASF - ASAP: Review Hearing Prep Q&A
Date:	Wednesday, July 7, 2021 7:30:16 PM
Attachments:	Key Questions_070721_to Divisions.docx
Importance:	High

Hello all,

Background

As you are aware, Dr. Fauci will testify before a hearing of the Senate Health, Education, Labor, and Pensions (HELP) Committee on COVID-19 on July 20th. In preparation for this hearing, NIAID OD has assembled the attached Q&A for Dr. Fauci's reference.

Action Item

We would appreciate your review of the attached document for accuracy and completeness **ASAP.** Please note there are comments included for your consideration and input, where relevant. If there are areas where you feel information is duplicative, or other questions you think could be raised, please let us know.

Thank you for your help on this quick turnaround request for Dr. Fauci. Please let us know if you have any questions.

Thanks,
Sara

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From: Selgrade, Sara (NIH/NIAID) [E]
To: Linde, Emily (NIH/NIAID) [E]; Arms, Erin (NIH/NIAID) [E]; NIAID DEA DART
Cc: NIAID BUGS; NIAID OCGR Leg; Embry, Alan (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Subject: RE: Action ASAP: follow-up Q"s on Year 4 RPPR - FLIPs for Nov 4th Senate HELP hearing
Date: Thursday, October 21, 2021 1:35:35 PM
Attachments: Year 4 EHA.pdf

Hi Emily,

Related to this topic, we've been asked to confirm that the attached is the original year 4 RPPR. Can you let me know? It looks correct to me, but would appreciate if you could confirm.

Thanks,
Sara

From: Linde, Emily (NIH/NIAID) [E] (b) (6)
Sent: Wednesday, October 20, 2021 3:56 PM
To: Selgrade, Sara (NIH/NIAID) [E] (b) (6); Arms, Erin (NIH/NIAID) [E] (b) (6); NIAID DEA DART <dart@mail.nih.gov>
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: RE: Action ASAP: follow-up Q's on Year 4 RPPR - FLIPs for Nov 4th Senate HELP hearing

Please find attached a copy of the original RPPR (b) (5) (b) (5) and the regenerated RPPR.

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(b) (5) The publications would have pulled, automatically, any publications that are associated with the grant as captured by the NLM managed MyNCBI system and would not have included any publications previously reported. It looks like the 5 publications originally reported (two complete* and three in process) are not in the regenerated version and that five later publications (all complete* at the time of regeneration) are in the regenerated version. [* N.B. complete means that they are compliant with the NIH Public Access Reporting Policy].

I hope this helps.

Please let me know if you have additional questions.

Thanks,

Emily

From: Linde, Emily (NIH/NIAID) [E] (b) (6)
Sent: Wednesday, October 20, 2021 2:44 PM

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-05	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2017 - 05/31/2018	**Requested Budget Period:** 06/01/2018 - 05/31/2019
Report Term Frequency: Annual	**Date Submitted:** 04/13/2018
Program Director/Principal Investigator Information: PETER DASZAK , PHD BS **Phone number:** (b) (6) **Email:** (b) (6)	**Recipient Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK, NY 100012320 **DUNS:** 077090066 **EIN:** 1311726494A1 **RECIPIENT ID:** NIAID Coronavirus
Change of Contact PD/PI: N/A	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)	**Signing Official:** ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.
To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the
phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Year 4 NIAID CoV Training and Prof Devlp.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

1.Conference and University Lectures: PI Daszak, and Co-investigators Shi, Epstein, Olival, and Zhang gave invited University and Conference lectures including Harvard Univ. Columbia Univ., Tufts Univ., Mt. Sinai, the 2nd International Symposium on Emerging Viral Disease in China, the 2nd International Symposium on the Infectious Diseases of Bats in Colorado, Cell Symposia: Emerging and Re-emerging Viruses 2017 in Virginia, The International Union of Microbiological Societies 2017 National Academy of Sciences in Singapore, 2018 Borneo Quality of Life Conference in Malaysia, 2017 Chemical and Biological Defense Science and Technology (CBD S&T) in California, Prince Mahidol Award Conference in Bangkok, Collaboration for Environmental Evidence Meeting in Paris, US-China NSF Ecology and Evolution of Infectious Disease (EEID) Meeting, and others that included specific discussion of the current project and results.

2.Agency and other briefings: PI Daszak and Co-investigator Shi introduced this project and discussed new opportunities about predicting and preventing zoonoses within National Institute of Allergy and Infectious Disease Office, Defense Advanced Research Projects Agency, National Natural Science Foundation of China, Chinese Center for Disease Control and Prevention, US NASEM Forum on Microbial Threats, Chinese Academy of Sciences, and the Health Working Group at the US Embassy in Beijing.

3.Public outreach: PI Daszak and Co-investigator Shi, Epstein, Olival, have presented this work to the general public in a series of meetings over Year 4 including at Cosmos Club briefings that EcoHealth Alliances hosts in Washington DC, over 10 meetings on the China National Virome Project and the Global Virome Project in China, Europe, Australia, Southeast Asia and Latin America. Co-investigator Olival presented this work at a public event on Disease Transmission and Technologies in New York, co-investigator Ross presented this work at EcoHealth Webinar on wildlife trade network research. Zhu broadly introduced this work to the conservation and ecological research community in China through field training workshops.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces.

•To commence an in-depth analysis of data collected from the integrated biological behavioral surveillance from Yunnan, Guangxi, and Guangdong provinces, incorporating questionnaires and serological testing results.
•To initiate lab analysis of human samples collected from the passive hospital surveillance from four hospitals in Yunnan province: 1) Dali College Affiliated Hospital; 2) Dali Prefecture Hospital; 3) Kunming No. 3 People's Hospital, and 4) Chuxiong Prefecture Hospital. The goal will be to identify examples of CoV spillover events in China that may lead to illness.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk

•To repeat and continue in vivo experiments of SARSr-CoVs with spike variants on hACE-expressing transgenic mice (survival rate, histopathological analysis, etc) to evaluate the risk of cross-species infection of different SARSr-CoVs to humans;
•Continue searching for the receptor of SARSr-CoVs with deletions in the homologous region of SARS-CoV RBD (i.e. Rp3, Rs672), and SARSr-CoVs that are unable to utilize bat ACE2 (e.g. Rs4231).
•Continue the phylogeographic study of bat-CoV with newly collected samples to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south China and SE Asia.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

•Using the full-length infectious cDNA clone of MERS-CoV, chimeric viruses with the spikes of newly identified MERSr-CoVs will be constructed. The pathogenesis of these MERSr-CoVs will be tested on the human DPP4-expressing mouse model that has already been developed and validated in Y4.
•To conduct a population genetics study of Rhinolophus sinicus ACE2s, including the amplification of ACE2 genes from R. sinicus samples of different origin, test of the usage efficiency of R. sinicus ACE2s of different origins by SL-CoVs and kinetics study on the binding of SL-CoV RBD to different R. sinicus ACE2s.
•In collaboration with South China Agrricultural University, gather data on the spatial structure and barn-level mortality records to parameterize our mathematical model of virus spread that incorporates a meta-population structure in individual and use this to fit the model on a training set of farms and validate it on a hold-out set.
•Using the intra-farm transmission model, we will (a) determine the characteristics of a farm that determine the likelihood and size of an outbreak given a spillover event, and (b) determine whether SADS and PEDV outbreaks on farms can be distinguished by differing dynamics, as measured by transmission parameters in our intra-farm transmission model.

Year 4 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: R01AI110964-03

Reporting Period: 06/01/2017 – 05/31/2018

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B.2 What was accomplished under these goals?

Summary

The results of the 4th year of our R01 work are detailed below. They include:

- Completed behavioral risk survey questionnaires and biological sample data collection for 1,585 people in Yunnan, Guangxi, and Guangdong provinces.
- Preliminary analysis of behavioral survey responses exploring key risk factors relating to potential viral zoonotic disease spillover in China, indicating notable differences among the respondents in Guangdong, Guangxi, and Yunnan.
- Completed serologic testing of collected human samples for MERS-CoV, SARSr-CoV, HKU9 CoV and HKU10 CoV, showing the serologic evidence of spillover of bat SARS-related CoVs (7 people in Yunnan province) and HKU9 CoV (2 people in Guangxi province).
- Testing of samples from 671 individual bats to identify diverse alpha- and beta-coronaviruses.
- Genetic diversity and genomic characterization of beta-coronaviruses in fruit bats and characterization of the full-length genome sequence of a novel HKU9-related CoV.
- Analysis of host-virus phylogeography for all bat CoV RdRp sequences collected by our group in China from 2008-2015 (Alpha-CoVs: n = 491; Beta-CoVs: n = 326) to identify the geographic areas that are likely sources of origin/diversity for this important group of viruses.
- Identification of two novel MERS-related CoVs that use DPP4 receptor.
- *In vivo* infection of SARSr-CoVs with variants of S protein in human ACE2 (hACE2) expressing mice.
- Identification of a novel bat-origin CoV (swine acute diarrhea syndrome coronavirus, SADS-CoV) causing a multi-farm outbreak of fatal acute diarrhea in piglets in Guangdong (published in *Nature* in April 2018).
- Development of an intra-farm transmission model to understand SADS-CoV spread and help predict and prevent future outbreaks.

Specific Aim 1: Assessment of CoV spillover potential at high-risk human-wildlife interfaces

During Year 4 we completed behavioral risk surveys and biological sample collection from people at selected sites in three provinces in southern China (Guangdong, Guangxi, and Yunnan) and began analyzing the results.

Behavioral Survey

We administered 1,585 surveys in Guangdong, Guangxi, and Yunnan provinces. Questions explored respondent health-seeking behavior, experiences with unusual illnesses, contact with wildlife and livestock, and general background information. Blood samples were collected from respondents and tested for SAS-related CoVs (SARSr-CoVs) and HKU10-CoV using serological assays. Survey data was analyzed by province to examine patterns among respondent characteristics and behavioral risk factors across provinces.

Respondent General Background Information

Of the 1,585 respondents who completed the survey, 420 were from Guangdong, 412 were from Guangxi, and 753 were from Yunnan. More females than males completed the survey in all provinces. The mean age of the overall survey sample was 52 years **(Figs. 1, 2)**.

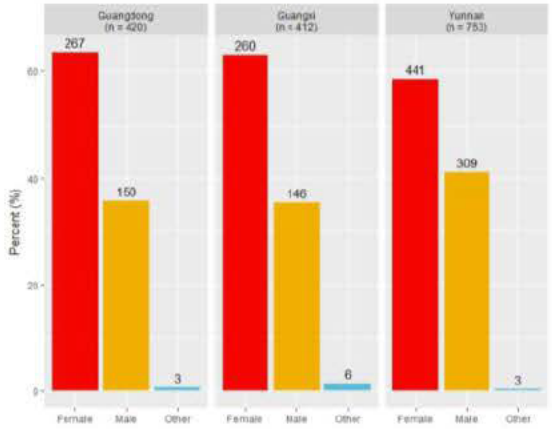


Figure 1: *Gender of respondents*

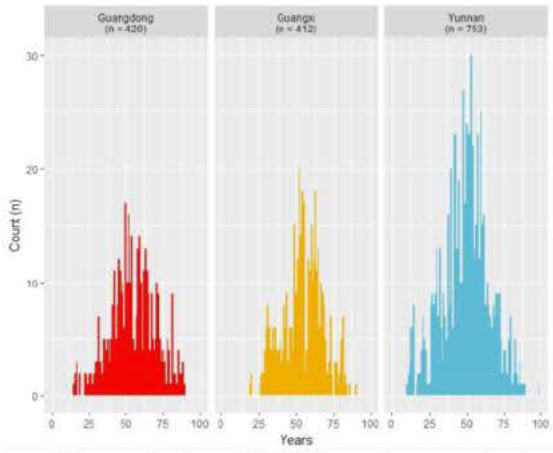


Figure 2: *Age distribution of respondents.*

Across all provinces, most respondents had lived in their respective locales for more than 5 years (96.3%) **(Fig. 3)** and earned less than 10,000 renminbi (RMB) annually (84.6%) **(Fig. 4)**. In 2016, the updated poverty standard in China was 3,000 RMB as defined by Poverty Alleviation Office of State Council. More families in Guangxi (61.8%) lived at or below the poverty level as compared to those in Guangdong (36.9%) and Yunnan (43.3%).

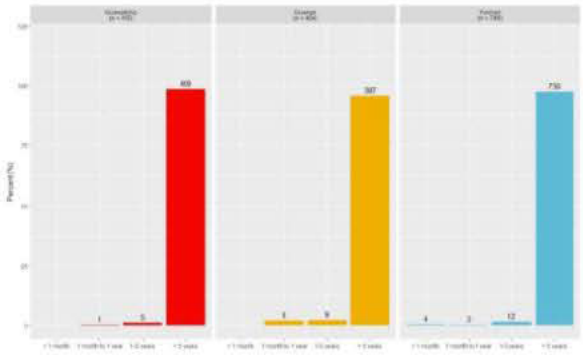


Figure 3: *Duration of residency.*

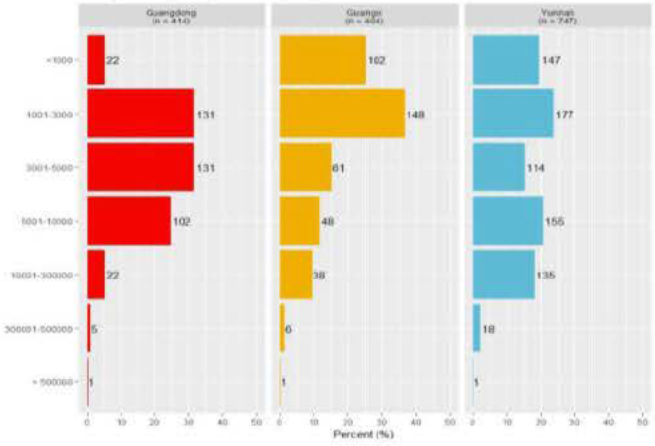


Figure 4: *Family annual per capita income (RMB).*

In Guangdong, Guangxi, and Yunnan, 73.9%, 57.0% and 69.6% of respondents, respectively, had a primary school-level education or less **(Fig. 5)**. Across all provinces the most common livelihood was crop production. In Yunnan, 699 out of 753 (92.8%) individuals from the province identified crop production as a livelihood activity. In comparison, 237 out of 420 (56.4%) individuals from Guangdong, and 260 out of 412 (63.1%) individuals from Guangxi **(Fig. 6)** named crop production as a livelihood in the last year. Respondents, however, where not restricted to defining a single livelihood, many indicated engaging in multiple types of livelihoods.

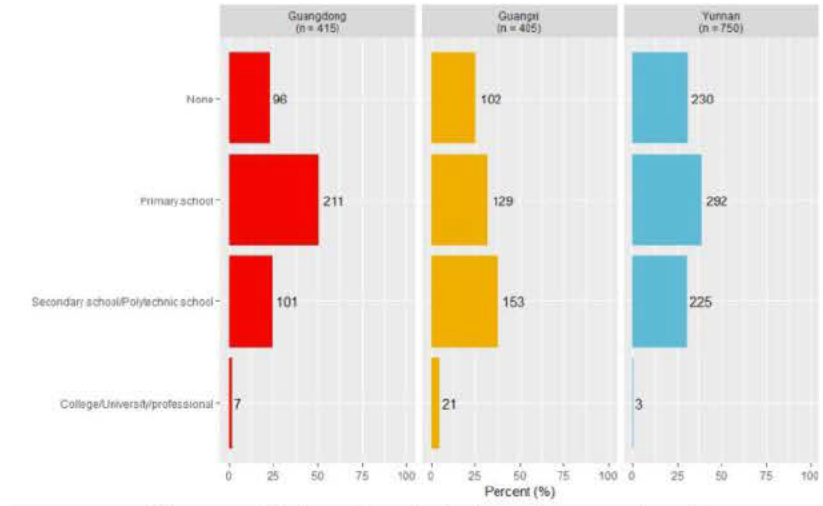


Figure 5: *Highest level of education completed*

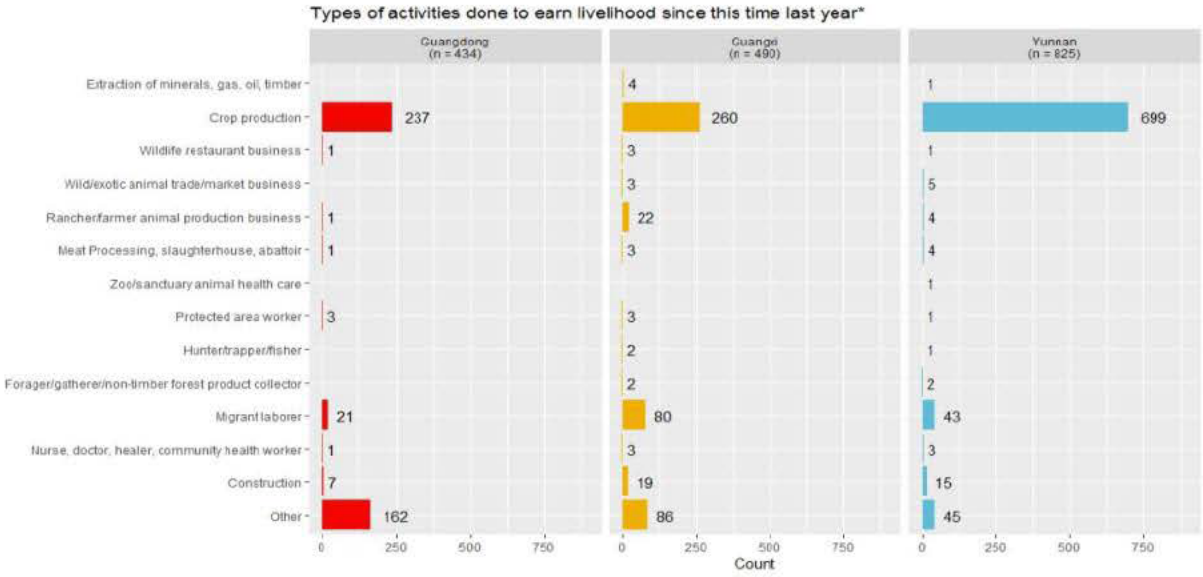


Figure 6: *Types of activities conducted to earn a livelihood since this time last year (above)*

In Guangdong, Guangxi, and Yunnan, 41.7%, 50.7% and 59.6% of respondents, respectively, indicated that they traveled outside of their village town or city in the past year. Among those who traveled, the average number of trips was 5 in Guangdong and Guangxi, and 6 in Yunnan. The average distance traveled by respondents in Guangdong and Yunnan were 113 Km and 118 Km, respectively, compared to 66 Km by respondents in Guangxi.

Health-Seeking Behavior and Experiences with Unusual Illnesses

When asked where they usually get treatment for illness or infection, the top 3 responses across all provinces in aggregate were hospitals, clinics, and pharmacies/dispensaries in descending order **(Fig. 7)**. However, within Yunnan, most respondents went to hospitals, followed by pharmacies, then clinics.

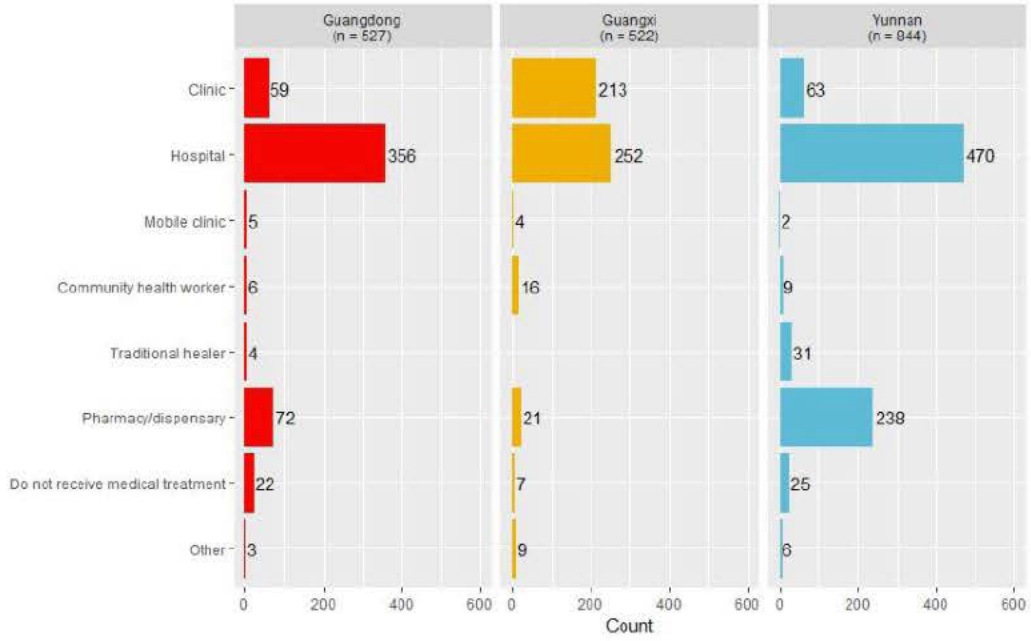
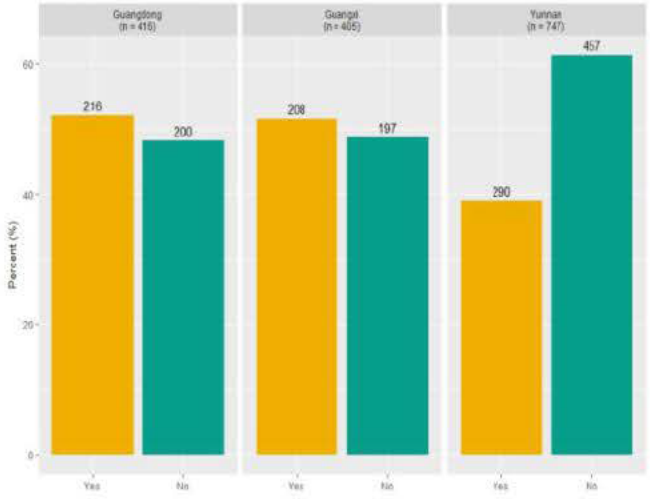


Figure 7: *Location where care was usually received for illness or infection.*

All survey respondents were asked whether they had experienced an unusual illness in their lifetime and in the past year, defined by a series of the most common symptoms associated with encephalitis, hemorrhagic fever (HF), severe acute respiratory infection (SARI), and influenza-like illness (ILI). Additional symptoms that were asked about included: fever with diarrhea or vomiting; fever with rash; and, persistent rash or sores on skin. Respondents were not restricted to selecting one illness and could provide multiple responses.

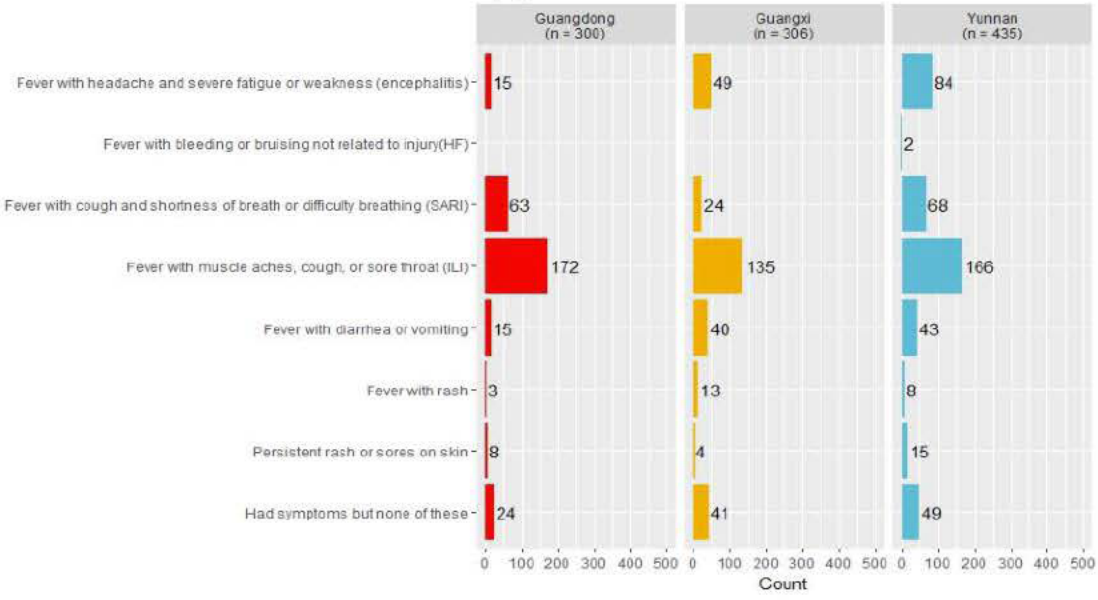
The proportion of respondents who had an unusual illness with any of the above-mentioned symptoms in their lifetime varied slightly by province. Between the three provinces, Yunnan had the fewest number of respondents who reported experiencing the symptoms provided (38.8%), compared to Guangdong and Guangxi (51.9% and 51.3%, respectively). Yunnan was also the only province where less than half of the respondents reported experiencing the symptoms provided **(Fig. 8)**.

Figure 8: *Respondent's experience of unusual illnesses.*



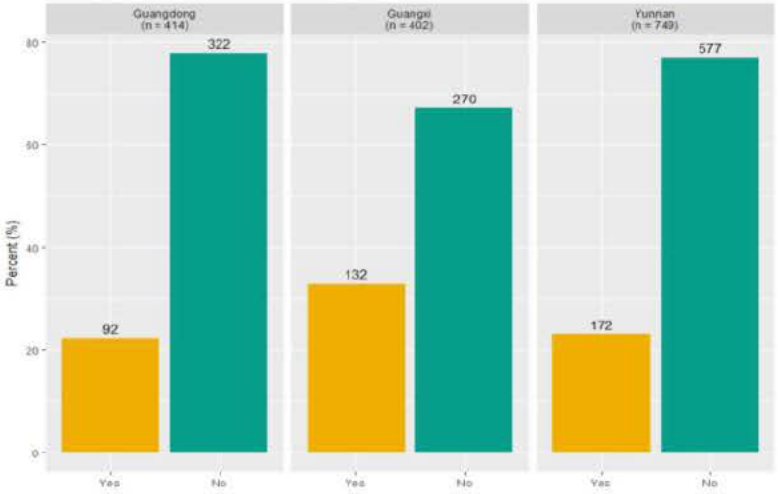
Across all three provinces, among those who had experienced any symptoms of unusual illness in their lifetimes, those associated with ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI, then by other symptoms not mentioned in the survey. In Guangxi province, the second most reported symptoms were ones associated with encephalitis, followed by other symptoms not mentioned in the survey. Similarly, in Yunnan, symptoms associated with encephalitis were the second most commonly reported, but this was followed by symptoms associated with SARI **(Fig. 9)**.

Figure 9: *Symptoms reported by people who had experienced unusual illness in their lifetime.*



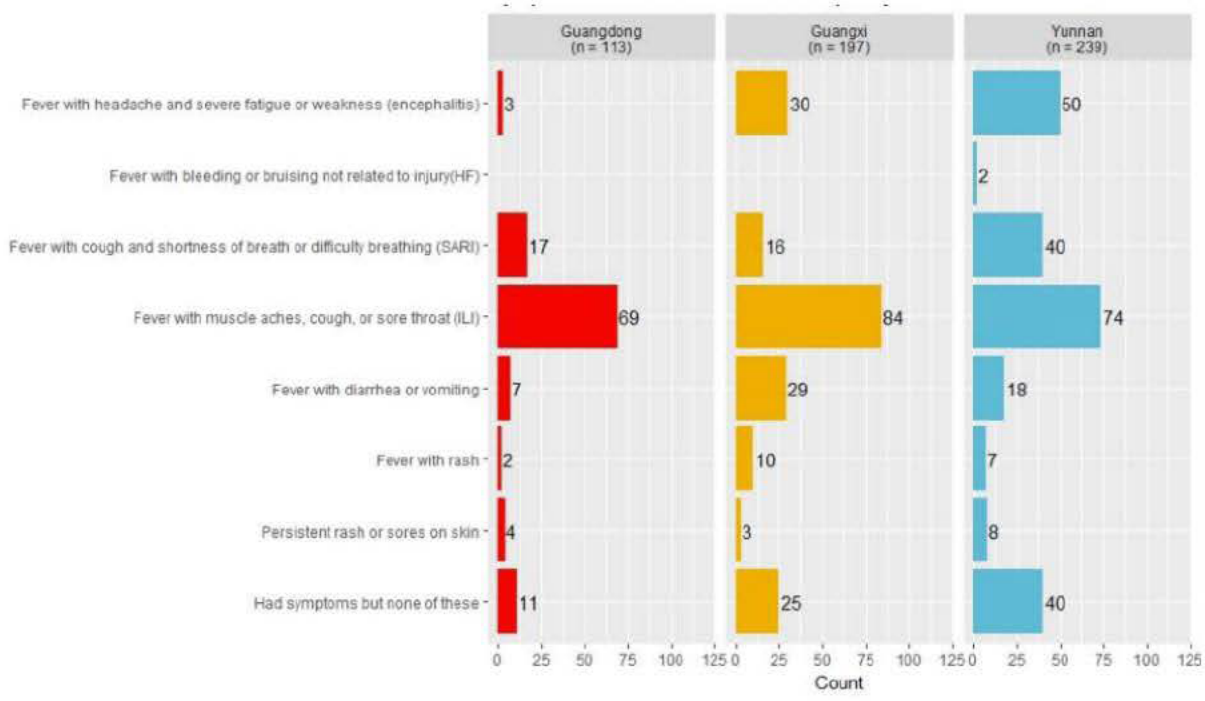
In each province, just under one-third of respondents who experienced the symptoms associated with an unusual illness in their lifetime indicated experiencing any of the symptoms in the past year – 22.2% in Guangdong, 32.8% in Guangxi and 23.0% in Yunnan **(Fig. 10)**.

Figure 10: *Whether respondents had experienced symptoms associated with an unusual illness, in the past year.*



Of the respondents who reported having symptoms of unusual illness in the past year, across all three provinces, symptoms associated ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI then by other symptoms not provided in the survey. In Guangxi, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by fever with diarrhea or vomiting. In Yunnan, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by both SARI and other symptoms not provided in survey **(Fig. 11)**.

Figure 11: *Symptoms experienced by those reporting unusual illness in the past year.*



When respondents were asked what caused the symptoms associated with unusual illness experienced in the past year, 64.4% in Guangxi (85 of 132 respondents), and 50.0% in both Guangdong and Yunnan (46 of 92 respondents and 86 of 172, respectively), said they did not know the cause **(Fig. 12)**. Only one respondent in Guangxi said their symptoms were due to

contact with animals (wild animals, specifically). Two respondents in Guangdong and one respondent in Guangxi said their symptoms were due to contact with animals (non-wild animals, specifically), whereas none of the respondents in Yunnan attributed their cause to contact with animals.

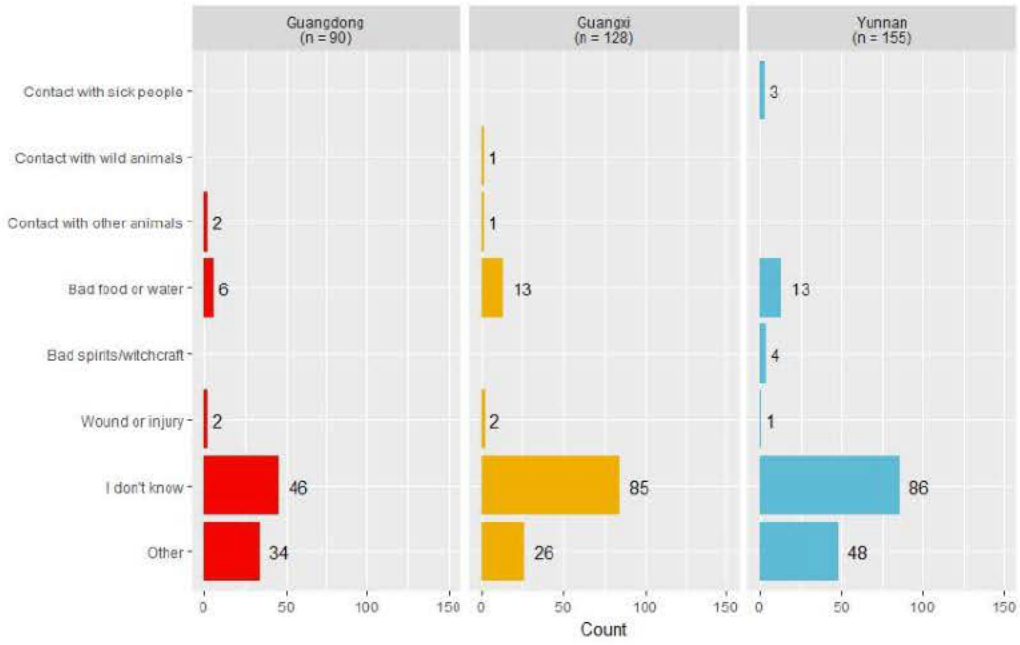
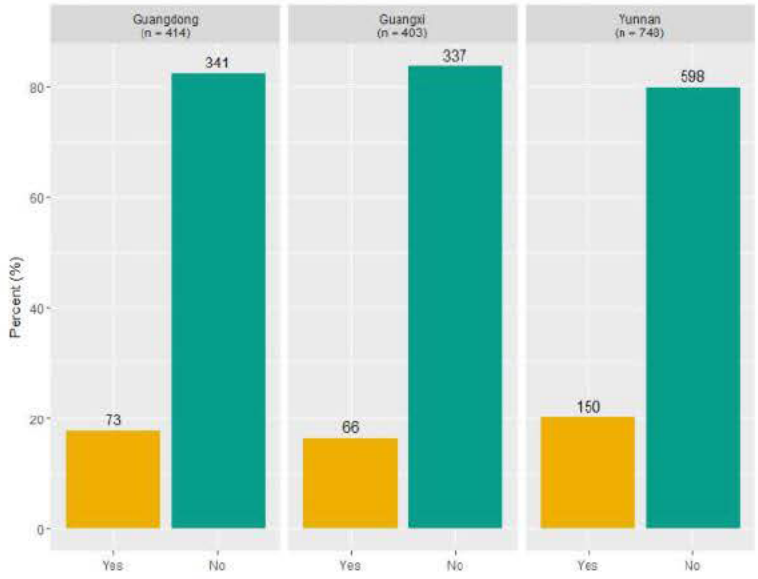


Figure 12: *Reported cause of sickness in the past year.*

Respondents reporting an unusual illness in the past year were asked if any of the people they lived with in the past year had symptoms similar to theirs, to assess possibilities of transmission among household members. Most respondents did not, across all three provinces: 82.4% in Guangdong, 83.6% in Guangxi and 79.9% in Yunnan **(Fig. 13)**.

Figure 13: *Whether household members had similar symptoms of unusual illness, in the past year*



Of the household members who experienced symptoms of unusual illness in the past year, the most commonly reported symptoms were those associated with ILI **(Fig. 14)**.

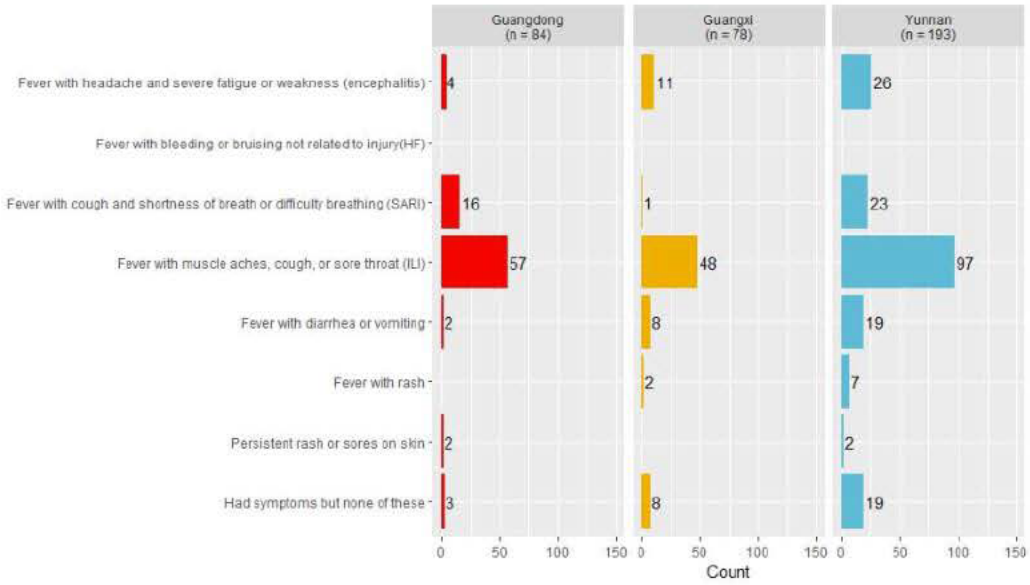


Figure 14: *Symptoms of household members who were ill, in past year.*

Respondents were also asked if any members of their household who experienced symptoms of unusual illness died as a result of their illness in the past year. Across all the three provinces, almost none had died from these illnesses **(Fig. 15)**.

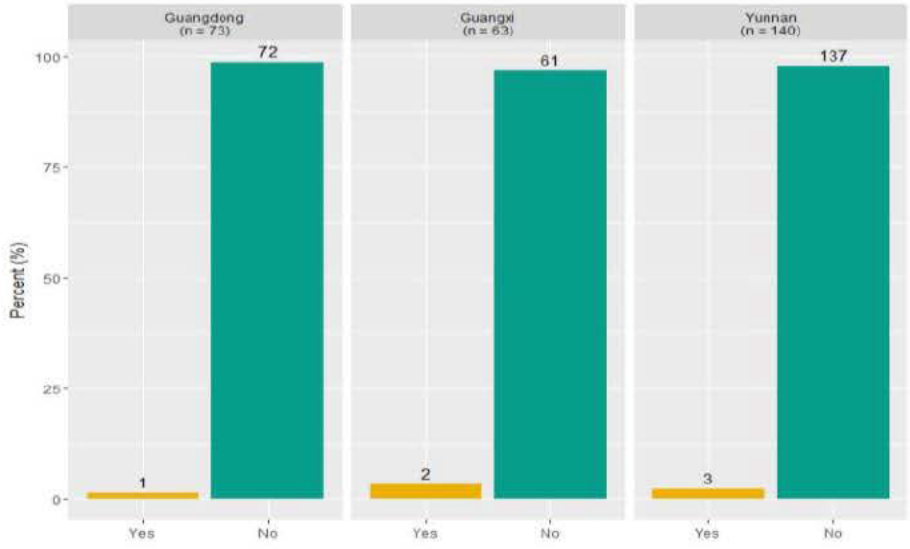


Figure 15: *Whether household members died from illness, in the past year.*

Contact with Animals

All respondents were asked about various types of animal contacts in their lifetime and in the past year. More than two-thirds of the respondents across all provinces, as well as in each of the provinces, reported raising an animal within their lifetime (71.2% in Guangdong, 77.7% in Guangxi, and 97.7% in Yunnan). More than half of the respondents in each province reported having animals come inside their dwellings (83.1 % in Guangdong, 60.2% in Guangxi, and 92.5% in Yunnan). More than half of respondents in each province reported handling live animals (51.5 % in Guangdong, 56.9% in Guangxi, and 62.9% in Yunnan) **(Table 1)**. Respondents from Yunnan had more types of contact with animals in their lifetime than those from Guangdong and Guangxi. With the exception of cooking or handling meat, organs, or blood from a recently killed animal and being scratched or bitten by an animal, the proportion of respondents from Yunnan who engaged in all types of animal activities was higher than the other provinces.

Type of animal contact (past year)	Guangdong		Guangxi		Yunnan	
	(n)	(%)	(n)	(%)	(n)	(%)
Lived with an animal as a pet	43	100 %	72	98.6 %	335	100 %
Handled live animals	212	100 %	226	98.3 %	332	99.7 %
Raised a live animal	296	100 %	312	99.4 %	518	99.8 %
Shared water source with animals for washing	47	100 %	19	95.0 %	97	100 %
Seen animal feces in or near food before you have eaten it	18	100 %	15	93.8 %	43	100 %
Eaten food after an animal has touched or damaged it	6	100 %	6	100 %	29	100 %
Animals come inside the dwelling where you live	345	100 %	239	98.0 %	493	100 %
Cooked or handled meat, organs, or blood from a recently killed animal	333	100 %	144	97.3 %	412	100 %
Eaten raw or undercooked meat or organs or blood	2	100 %	25	89.3 %	65	98.5 %
Eaten an animal that was not well/sick	--	--	1	100 %	6	100 %
Found a dead animal and collected it to eat, share, or sell	--	--	3	100 %	10	100 %
Been scratched or bitten by an animal	1	100 %	31	100 %	28	96.6 %
Slaughtered an animal	145	100 %	69	98.6 %	303	100 %
Hunted or trapped an animal	9	100 %	4	100 %	22	95.7%

Table 1: *Types of animal contact, within a respondent's lifetime.*

Respondents who reported having animal contact in their lifetime were also asked to indicate if they had the same type of animal contact in the past year **(Table 2)**. In the past year, across all three provinces and in each province, almost all respondents engaged in all contact types with the exception of eating an animal that was not well/sick, and finding a dead animal and collecting it to eat, share, or sell (0% for both in Guangdong).

Type of animal contact (lifetime)	Guangdong		Guangxi		Yunnan	
	(n)	(%)	(n)	(%)	(n)	(%)
Lived with an animal as a pet	43	10.4 %	73	18.1 %	335	62.9 %
Handled live animals	212	51.5 %	230	56.9 %	334	62.8 %
Raised a live animal	296	71.2 %	314	77.7 %	521	97.7 %
Shared water source with animals for washing	47	11.5 %	21	5.2 %	97	18.2 %
Seen animal feces in or near food before you have eaten it	18	4.4 %	16	3.9 %	43	8.1 %
Eaten food after an animal has touched or damaged it	6	1.5 %	6	1.5 %	29.0	5.4 %
Animals come inside the dwelling where you live	345	83.1 %	244	60.2 %	493	92.5 %
Cooked or handled meat, organs, or blood from a recently killed animal	333	80.4 %	148	36.7 %	413	77.5 %
Eaten raw or undercooked meat or organs or blood	2	0.5 %	28	6.9 %	68	12.8 %
Eaten an animal that was not well/sick	--	--	1	0.3 %	6	1.1 %
Found a dead animal and collected it to eat, share, or sell	--	--	3	0.7 %	10	1.9 %

***Table 2**: Types of animal contact, in past year.*

Respondents who had animal contact in the past year were asked to identify the animals involved in the interaction. *(Figs. 16-26, below: the first two figures are enlarged to show row labels, which are identical for all)*. Cats and dogs were the most common pets reported across all provinces and in each province **(Fig. 16b)**.

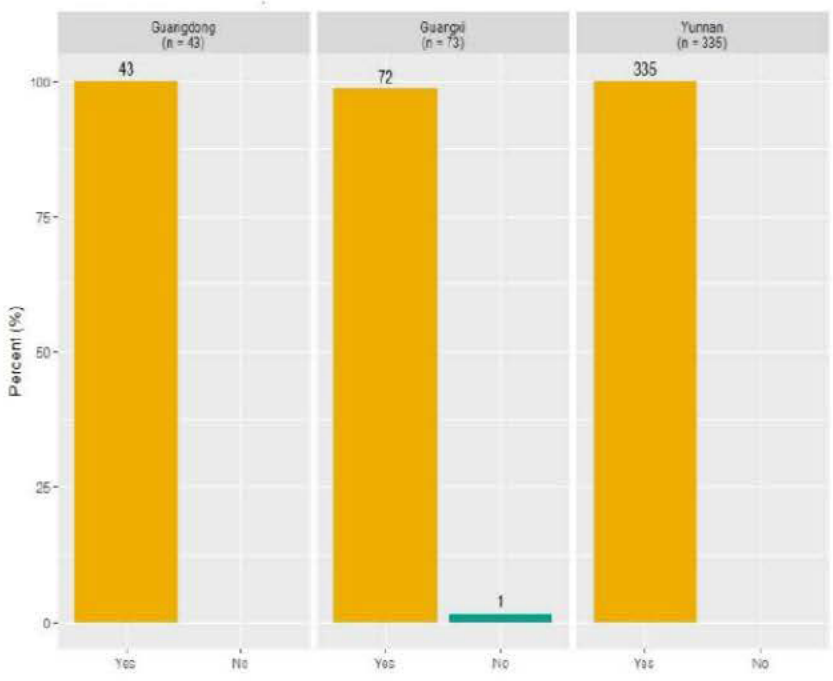
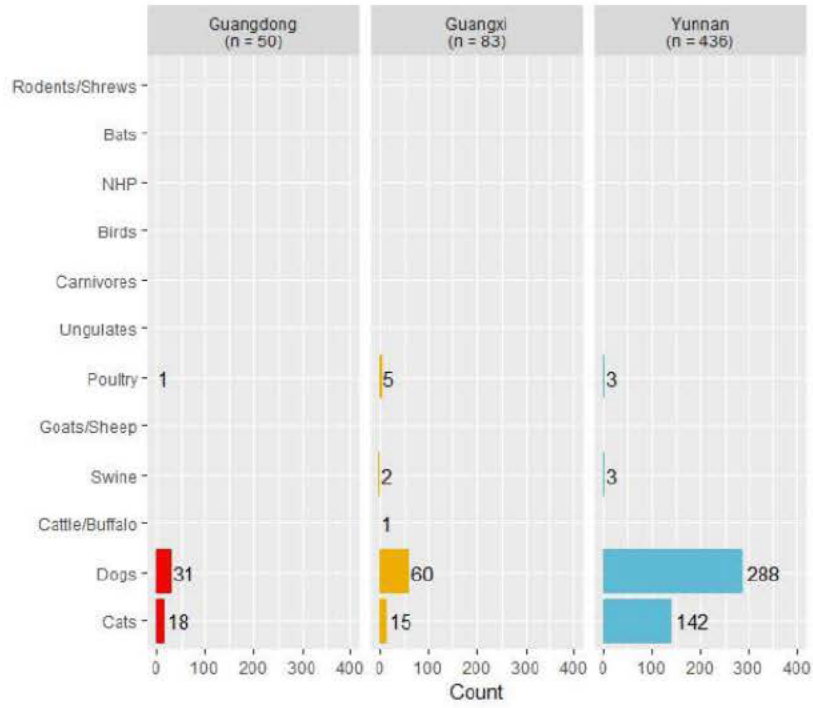


***Figure 16a (top) & b (below):** (a) Whether respondents had lived with an animal as a pet, in the past year, and (b) among those who had, types of animal kept as pets.*



Poultry was the most common type of animal handled across all provinces as well as in each province, with 96.2%, 90.3%, and 92.8% of respondents handling animals in Guangdong, Guangxi and Yunnan, respectively **(Fig. 17b)**.

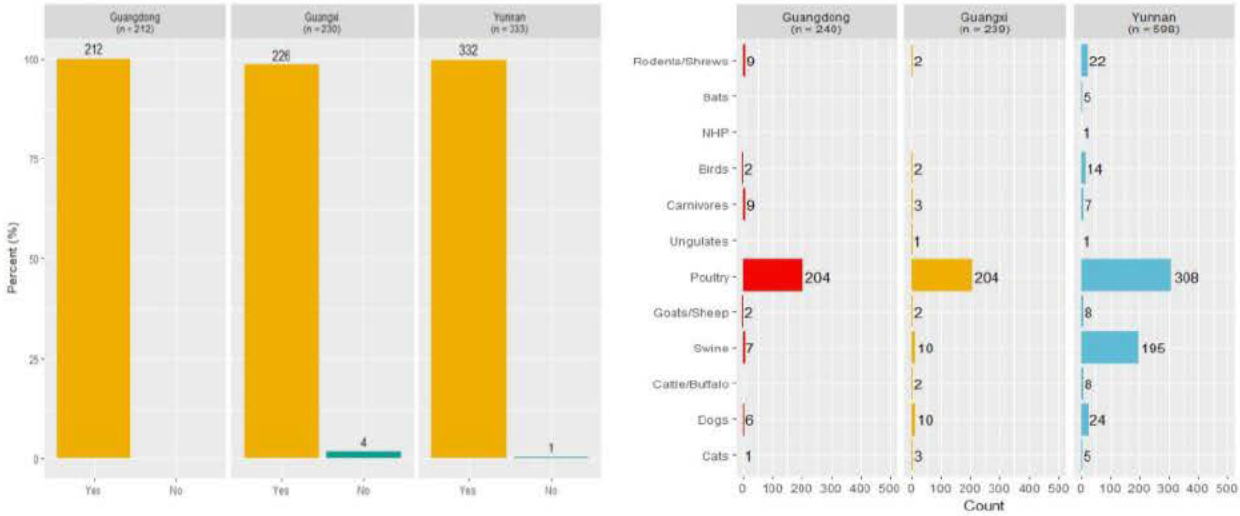
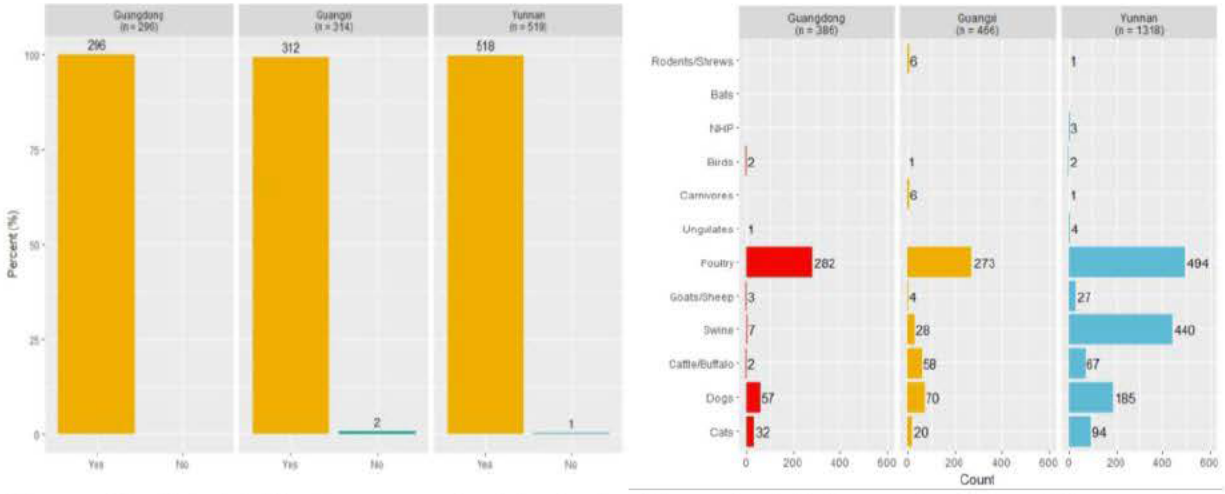


Figure 17a & b: *(a) Whether respondents had handled live animals, in the past year, and (b) among those who had, types of live animals handled.*

Poultry was also the most commonly raised animal in each of the three provinces; 95.3%, 87.5%, 95.4% in Guangdong, Guangxi, and Yunnan, respectively **(Fig. 18b)**.



Figures 18a & b: *(a) Whether respondents had raised live animals in the past year, and (b) among those who had, types of animals raised.*

In all three of the provinces, the most common type of animals found in respondent dwellings were rodents or shrews. In Guangdong and Yunnan, birds were the second most common animal type found in dwellings. In Guangxi province, birds along with poultry were the second most common animal type. Respondents in Guangdong and Yunnan reported that all 12 animal taxa had come inside their dwellings in the past year. Taxa seen in the dwellings of respondents from Guangdong and Yunnan and not Guangxi were non-human primates, ungulates, goats or sheep, swine, and cattle or buffalo **(Fig. 20b)**.

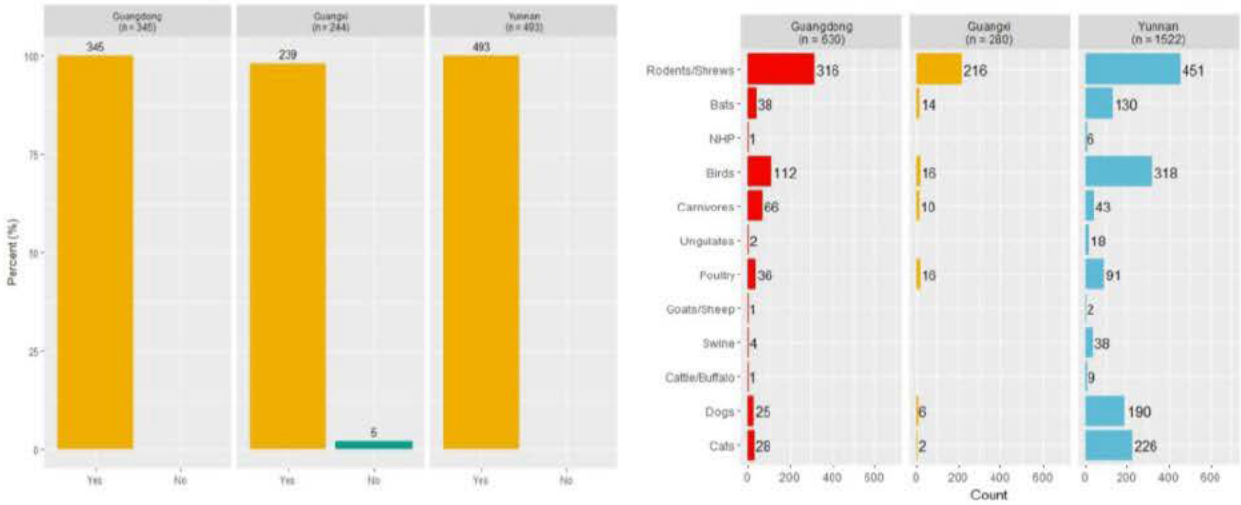


Figure 19a & b: *(a) Whether respondents had animals come inside dwelling, in the past year, and (b) among those who had, types of animals in dwelling.*

Almost all of the respondents who said they have cooked or handled meat, organs, or blood in their lifetime reported doing so in the past year. Common animal types that were cooked handled included poultry and swine in all three provinces **(Fig. 20)**.

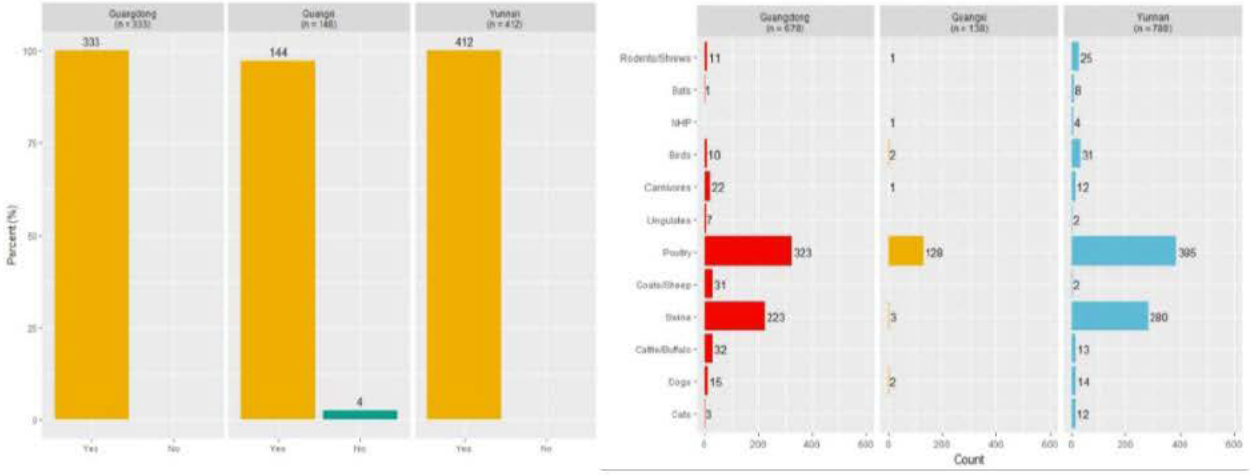


Figure 20a & b: *(a) Whether respondents had cooked or handled meat, organs or blood from a recently killed animal, in the past year, and (b) among those who had, types of animals whose meat, organs or blood was cooked or handled.*

More respondents in Yunnan reported eating raw or undercooked meat compared to respondents in Guangdong and Guangxi **(Fig. 21)**. In Yunnan, 96% of respondents who ate raw or undercooked meat in their lifetime did so in the past year. The types of animal products that were eaten raw or undercooked by respondents in Yunnan were mostly from swine. In Guangxi, the most commonly reported type of animal meat that had been eaten raw or undercooked was that of carnivores.

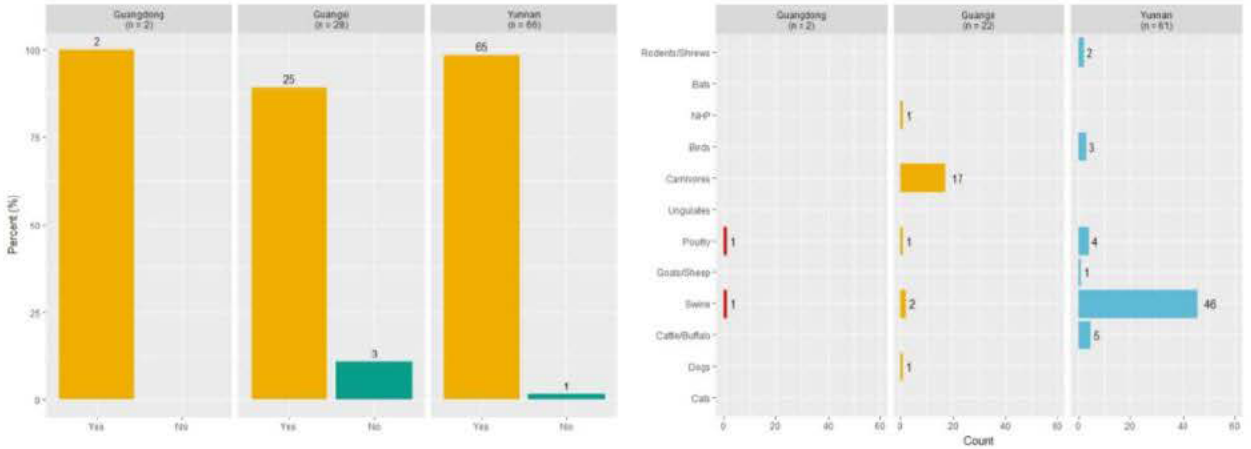


Figure 21 a & b: *(a) Whether respondents had eaten raw or undercooked meat or organs or blood, in the past year, and (b) among those who had, types of animals whose meat, organs or blood were eaten raw or undercooked.*

Across all provinces, a total of 13 respondents in Guangxi and Yunnan indicated that they collected an animal that was found dead to eat, share or sell. In Guandong, no respondents reported finding a dead animal and collecting it to eat, share, or sell. The most common type of animal collected across all provinces in aggregate was poultry. In Yunnan, poultry was the most common type of animal found dead and collected to eat, share or sell (80.0%), whereas dogs were the most common type in Guangxi (66.7%) (**Fig. 22)**.

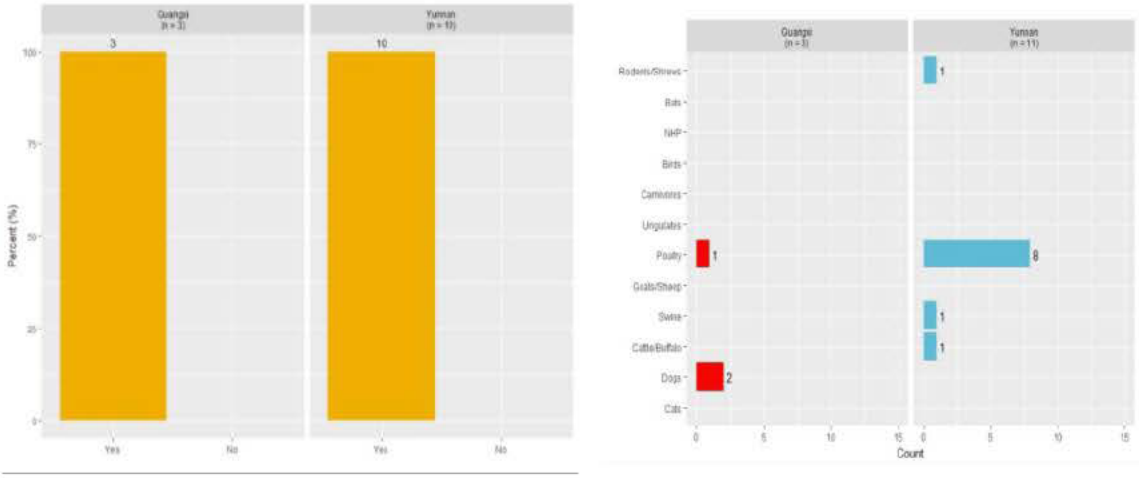


Figure 22 a & b: *(a) Whether respondents had found a dead animal and collected it to eat, share, or sell, in the past year, and (b) among those who had, types of animals that were found dead and collected to eat, share, or sell.*

In each province, almost all of the respondents who indicated being scratched or bitten by an animal in their lifetime said it occurred in the past year (100% in Guangdong, 98.6% in Guangxi, and 100% in Yunnan). In both Guangxi and Yunnan, dogs were the common type of animal that respondents said they were scratched or bitten by (64.5% in Guangxi and 50.0% in Yunnan). Cats were the second most common in Guangxi and Yunnan (9.6% in Guangxi, and 28.5% in Yunnan). Across all three provinces, only one respondent from Yunnan said that they were scratched or bitten by a bat **(Fig. 23)**.

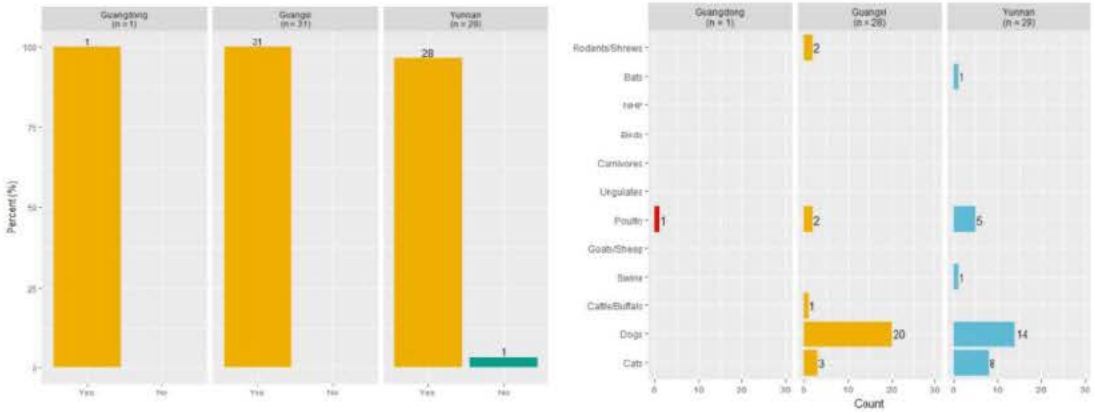


Figure 23 a & b: *(a) Whether respondents had been scratched or bitten by an animal, in the past year, and (b) among those who had, types of animals that scratched or bit respondents.*

Poultry was the most common type of animal slaughtered during the past year across all provinces as well as in each province (95.8% in Guangdong, 79.7% in Guangxi, and 94.1% in Yunnan). In addition to poultry, respondents in Yunnan also commonly only slaughtered swine (43.9%), compared to 1.4% in Guangdong and 7.3% in Guangxi **(Fig. 24)**.

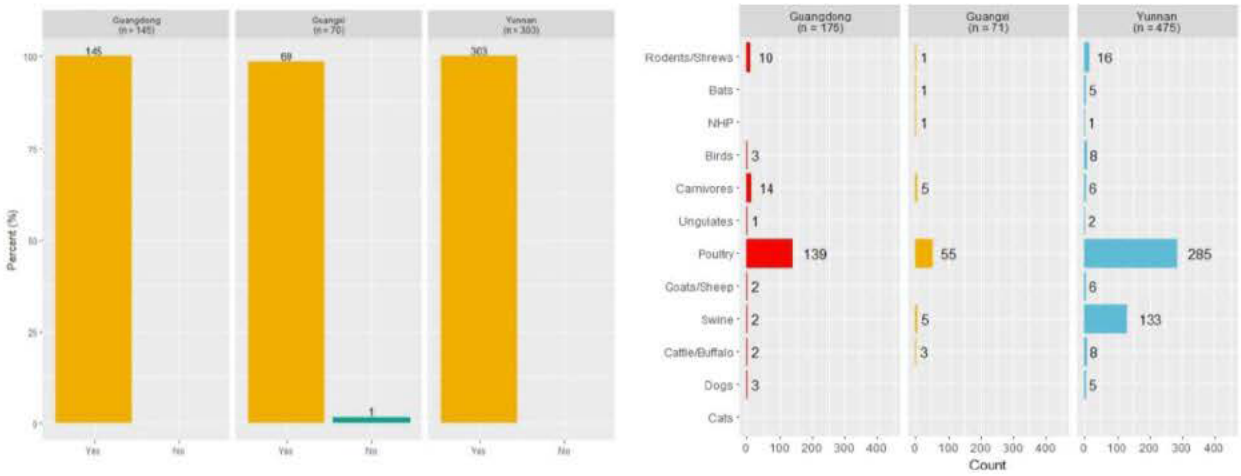


Figure 24 a & b: *(a) Whether respondents had slaughtered an animal, in the past year, and (b) among those who had, types of animals slaughtered.*

Carnivores were the most common taxa of animals hunted or trapped in the past year, in Guangdong and Guangxi. In Yunnan, rodents or shrews and birds were reported as the most common. Bats, non-human primates and dogs were animal types hunted by respondents in Yunnan but not by respondents in Guangdong and Guangxi **(Fig. 25)**.

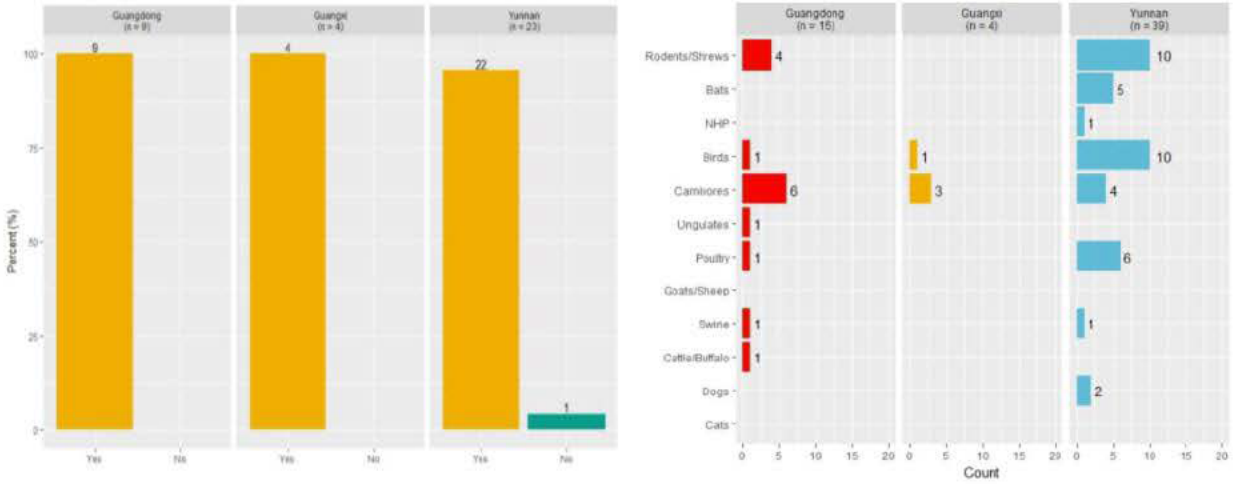


Figure 25 a & b: *(a) Whether respondents had hunted or trapped an animal, in the past year, and (b) among those who had, types of animals hunted or trapped.*

In examining bat-specific contact, across all provinces and within each province, the most common interaction with bats was finding them inside their houses. Respondents in Yunnan also hunted/trapped and handled bats, and were scratched/bitten by bats, whereas these did not occur in Guangdong or Guangxi **(Fig. 26)**.

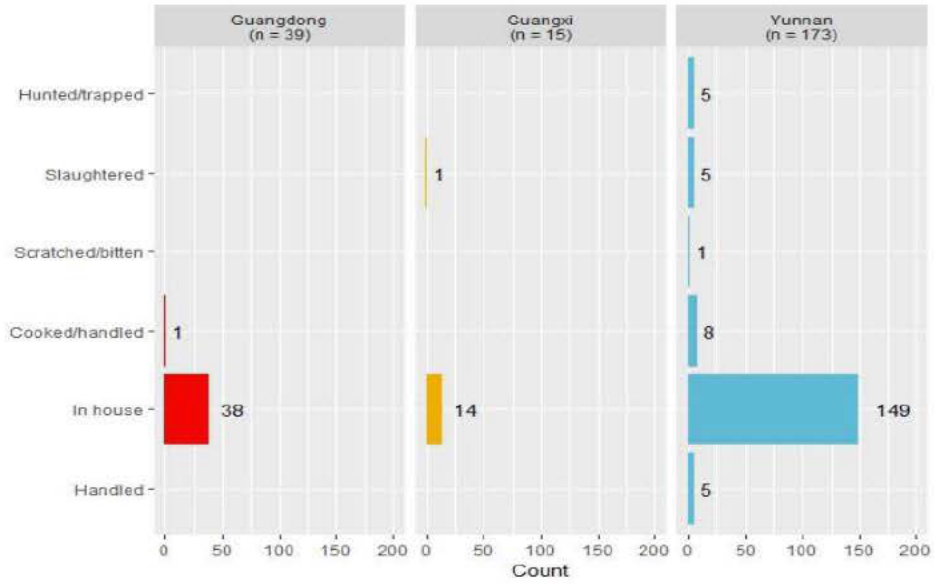


Figure 26: *Types of bat contact.*

After respondents were asked about their contact with wildlife and livestock, they were asked about their knowledge of whether animals can spread diseases and whether they were worried about diseases and disease outbreaks at wet markets. The proportion of respondents who thought that animals can spread disease was highest in Guangdong province (72.3%). In Guangxi and Yunnan, the proportion of those who thought animals could spread disease compared to those who thought that they did not were roughly equivalent – 47.5% versus 50.7% in Guangxi and 49.2% versus 49.3% in Yunnan **(Fig. 27)**.

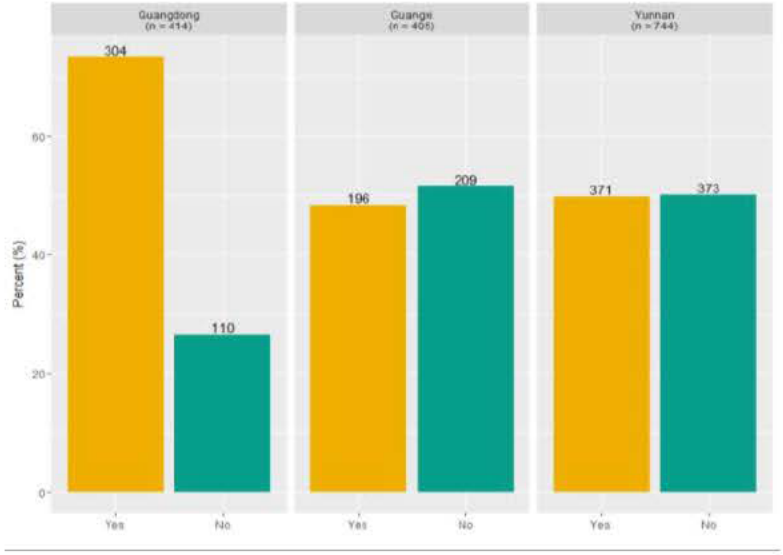


Figure 27: *Whether respondents thought that animals can spread disease.*

Similarly, when respondents were asked about whether they were worried about diseases or disease outbreaks in animals at wet markets, Guangdong had the highest proportion of respondents who said they were worried (67.3%). In both Guangxi and Yunnan, the proportion of respondents that was not worried (57.5% and 51.5%, respectively) was higher than the proportion that was worried **(Fig. 28)**

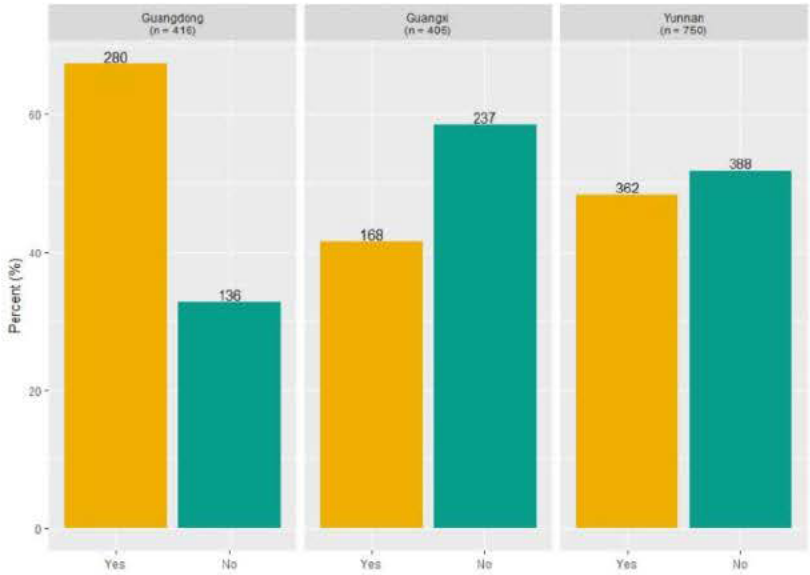


Figure 28: *Whether respondents were worried about diseases or disease outbreaks in animals at wet markets.*

Serological Evidence of Bat SARS-related CoV Infection in Humans

Respondents were asked to provide a biological sample to assess whether SARS-CoV spillover had occurred at the high-risk location where the survey has been implemented. A total of 1,530 serum samples were collected from 2016 to 2017 from individual residents in villages close to bat caves where coronaviruses were previously detected.

We developed an ELISA serology test using the purified NP protein of MERS-CoV, SARSr-CoV, HKU9 CoV and HKU10 CoV as coating antigen respectively and using Anti-Human IgG Monoclonal antibody as secondary antibody. All sera were screened for antibodies against these 4 bat-origin coronaviruses. Anti-SARSr-CoV NP IgG was detected in 10 samples, and 6 samples were positive for IgG against HKU10 NP. The 16 ELISA positive samples were further tested by confirmatory western blot, 7 samples from Yunnan province were confirmed positive for anti-SARSr-CoV, two samples (one from Guangdong province and one Guangxi province) were confirmed positive for anti-HKU10 **(Table 3)**.

Locations		Sample No.	NP Antibody Positive No.			
			HKU9 CoV	MERS CoV	SARSr-CoV	HKU10 CoV
Yunnan (2016)	Jinning	209			*6	
	Mengla	168			2 (*1)	
	Jinghong	212				2
	Lufeng	144				
Guangdong (2016)	Zengcheng	234			1	2
	Ruyuan	179				
Guangxi (2017)	Mashan	160			1	
	Guilin	224				*2
Total		1,530	0	0	*7	*2

Table 3 *Results of ELISA testing of human sera for antibodies to 4 different bat CoV species (*confirmed with western blot).*

Links Between ELISA Results and Behavior

Only one out of the seven SARS-related CoV seropositive respondents said that they had an unusual illness in their lifetime with reported symptoms similar to encephalitis or neural involvement. Two of the respondents said they had experienced symptoms in the past year with only one respondent specifying that they experienced epigastric pain and dizziness. The seven seropositive SARSr-CoV respondents reported various types of animal contacts in the past year. Three had lived with an animal as a pet, four handled a live animal, four raised a live animal, five saw animals inside their dwellings, five had cooked or handled meat, organs, or blood from a recently killed animals, one ate an animal that they knew was not well or sick, one was scratched or bitten by an animal, and four had slaughtered an animal. The only bat contact reported was by one respondent who saw a bat in their dwelling.

Both of the respondents who tested positive for HKU10-CoV antibodies said they had experienced an unusual illness in their lifetime, with symptoms associated with encephalitis and SARI. Neither respondent had experienced any symptoms of unusual illness in the past year. Both had reported handling and raising animals, with one indicating they saw animals come inside their dwelling, and one indicating cooking or handling meat, organs, or blood from a recently killed animal. No bat contact was reported by either of the respondents. Overall, five of the total nine SARS-related CoV and HKU10-CoV seropositive respondents reported being worried about disease or disease outbreaks at wet markets. Seven of the nine reported purchasing live animals from a wet market.

Specific Aim 1: Summary of Key Findings

Our analysis of the key risk factors relating to potential viral zoonotic disease spillover in China indicated some notable differences among the respondents in Guangdong, Guangxi, and Yunnan. With respect to demographic factors, Guangxi fared the lowest on key socio-economic

status indicators when compared to Guangdong and Yunnan provinces as reflected by the higher proportion of respondents in Guangxi living under the poverty level.

When assessing the type of animal contact and the associated animal taxa over the course of a respondent's lifetime, the results show that respondents in Yunnan engaged in greater contact with animals then those from Guangdong and Guangxi. For example, for 12 of the 14 animal contact types, a higher proportion of Yunnan respondents engaged in these respective activities than in Guangdong and Guangxi. Respondents in Yunnan also reported hunting bats, dogs, and non-human primates which were not reported to being hunted in Guangdong and Guangxi. Swine contact was higher in Yunnan for handling, raising, and slaughtering activities. When examining the various types of animal contact associated with bats only, our results also show that Yunnan respondents reported more varied types of contact with bats. Respondents in Yunnan indicated handling, being scratched by, slaughtering, and hunting bats, but these interactions did not occur in Guangdong or Guangxi. Additional analyses that examine predictors of animal contact in each province will be the focus of human behavioral analyses in Year 5 of the study.

Even though our sample population lives in areas that have dense and diverse bat populations, our results show an overall low proportion of respondents reporting hunting and trapping bats in all three provinces. The low proportion of hunting practice could be attributed to the success of conservation enforcement efforts undertaken by the government. These efforts may have effectively reduced the illegal practice of hunting wildlife or, as a consequence, moved the activity underground which made respondents less forthcoming about revealing their engagement in such practices. Further investigation into the potential causes is also warranted.

Our analyses also reveal differences in perceptions associated with zoonotic disease spillover between Guangdong, and Guangxi and Yunnan. For example, the proportion of respondents who thought that animals can spread disease was highest in Guangdong province at 72.3%, as compared to Guangxi (48.3%) and Yunnan (49.9%). Moreover, about two-thirds of respondents in Guangdong were worried about diseases and disease outbreaks in wet markets. These differences in perception observed in Guangdong compared to Guangxi and Yunnan could potentially be attributable to a heightened awareness of zoonotic disease emergence due to the 2001 SARS outbreak.

Finally, our serological testing results provide the first evidence ever of a bat SARSr-CoV spilling over into people in the wild. All of the SARSr-CoV positive individuals were from Yunnan province, which is the site of a cave in which we have identified a large diversity of SARSr-CoVs within the virome of which every genetic element of SARS-CoV can be identified. These findings warrant further investigations into the type of exposures that may have contributed to bat SARS-related CoVs to infect humans in this particular region. **They also highlight this region as a hotspot for SARSr-CoV future spillover risk.**

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk

Bat CoV PCR Detection and Sequencing from Live-Sampled Bat Populations

We collected rectal swab and oral swab samples from 671 individual bats from 20 species in Guangdong and Guangxi provinces in southern China in Year 4 **(Table 4)**. 671 rectal swab samples were tested for CoV RNA and 154 (23.0%) were positive **(Table 5)**.

Date of Sampling	Sampling Locations	Rectal swabs	Oral swabs
May 10th 2017	Hezhou, Guangxi	6	6
May 11-12th 2017	Chongzuo, Guangxi	67	67
May 13th 2017	Nanning, Guangxi	66	66
May 17th, 2017	Beihai, Guangxi	23	23
May 19th 2017	Chongzuo, Guangxi	36	36
May 21st 2017	Yangshan, Qingyuan, Guangdong	46	46
May 22nd, June 7 h 2017	Huidong, Huizhou, Guangdong	103	103
June 9th 2017	Nanning, Guangxi	71	71
June 9th 2017	Ningming, Chongzuo, Guangxi	63	63
September 10th 2017	Huidong, Huizhou, Guangdong	100	100
September 11th 2017	Yingde, Guangdong	90	90
Total		**671**	**671**

Table 4. *Bat samples collected for CoV surveillance in Year 4*

Species	Guangdong	Guangxi	Total
Rhinolophus sinicus	**9**/27	6	**9**/33
Rhinolphus rex		4	4
Rhinolophus pusilus	1	2	3
Rhinolophus pearsoni	5		5
Hipposideros armiger	24	8	32
Hipposideros larvatus	9	9	18
Hipposideros pomona		20	20
Hipposideros pratti	26		26
Aselliscus stoliczkanus		1	1
Miniopterus fuliginosus	1		1
Miniopterus pusillus	**29**/39		**29**/39
Myotis chinensis	**2**/27		**2**/27
Myotis daubentonii	2		2
Myotis ricketti	**86**/178		**86**/178
Pipistrellus abramus		2	2
Pipistrellus pipistrellus		2	2
Scotophilus kuhli		**24**/137	24/137
Tylonycteris pachypus		**4**/115	**4**/115
Tylonycteris robustula		3	3
Cynopterus sphinx		23	23
Total	**126/339**	**28/332**	**154/671**

Table 5*. Number of bat specimens tested and positive (bold) in Year 4*

A high prevalence of HKU6-related coronaviruses (48.3%), Scotophilus coronavirus 512 (17.5%), and coronavirus 1B (71.8%) was detected in *Myotis ricketii*, *Schotophilus khulii* and *Miniopterus pusillus*, respectively. SARS-related coronaviruses and HKU2-related coronaviruses were discovered in 4 and 5 *Rhinolophus sinicus* samples respectively from Guangdong. HKU4 coronaviruses were identified in 4 *Tylonycteris pachypus* from Guangxi **(Fig. 29)**.

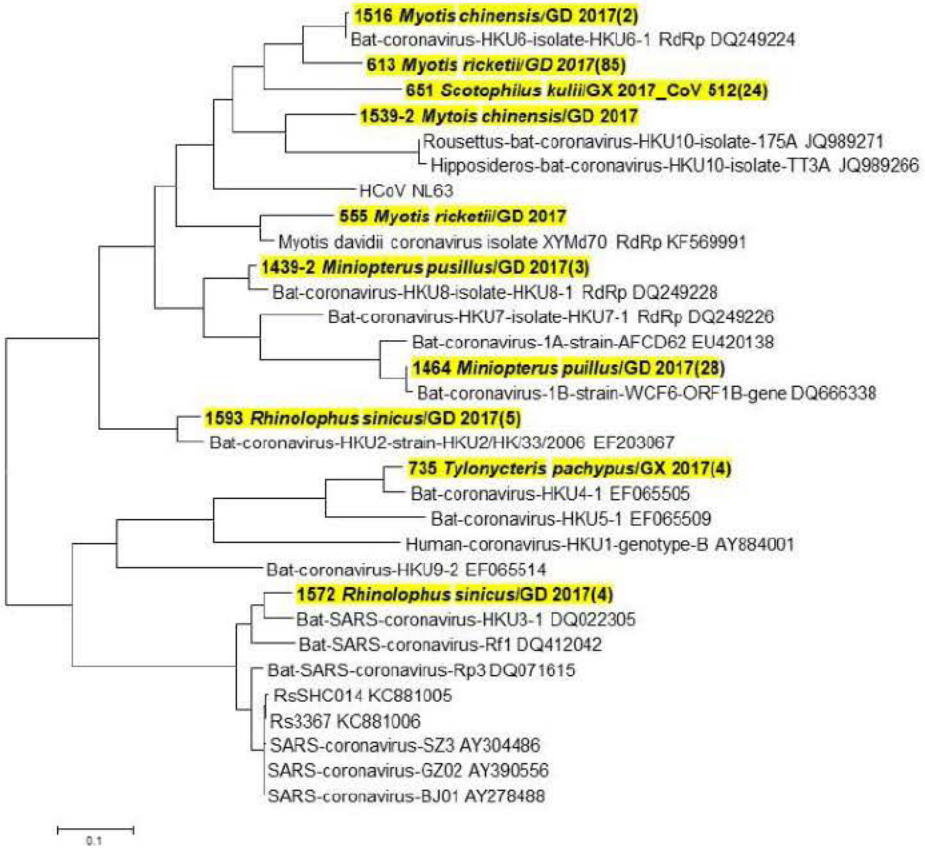


Figure 29: *Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence)*

Genetic Diversity and Genomic Characterization of Betacoronaviruses in Fruit Bats

In Year 4, we analyzed the genetic diversity of betacoronaviruses we have detected since 2009 in different species of fruit bats in Yunnan province, including *Eonycteris spelaea*, *Rousettus leschenaultia* and an unclassified *Rousettus* species. These viruses are classified into two betacoronavirus species, HKU9-CoV and GCCDC1-CoV. All HKU9-related viruses (n=46) were found in *Rousettus* spp. bats while GCCDC1-related viruses (n=13) from *E. spelaea*. Phylogenetic analysis of the full-length N gene suggests that HKU9-related CoVs are highly diverse and divided into 5 lineages with previously reported strains, and the GCCDC1-related CoVs were more similar between each other **(Fig. 30)**.

The full-length genome sequence of a novel HKU9-related CoV termed 2202 was determined. It shares 83% nt identity with other HKU9 strains, with the most divergent regions located in the S

protein, but shares only 68% aa identity with those of other HKU9 strains. Virus quantification revealed that intestine was the primary infected organ for HKU9-related CoVs while kidney and lungs could also be target tissues, suggesting potential for spillover through oral-fecal, respiratory, or uro-genitary routes.

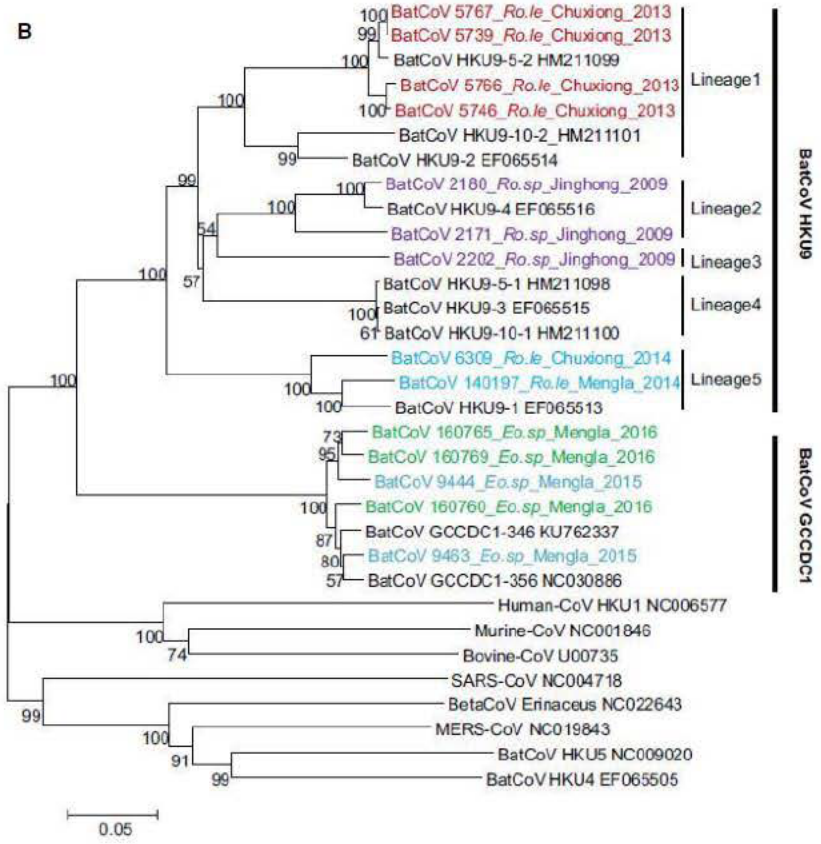


Figure 30. *Phylogenetic analysis of full-length N gene of HKU9 and GCCDC1 CoVs*

Bat Coronavirus Host-Virus Phylogeography in China

We used discrete ancestral character state reconstruction to estimate viral history and reconstructed the inferred bat host genus for each node within the phylogenetic tree **(Figs. 31, 32)**. The color of tree branches indicates the inferred ancestral host bat genus for the reconstructed phylogeny. *Rhinolophus* is the inferred ancestral host of lineages B and C (SARS-like CoVs and MERS-like CoVs, respectively). This genus played an important role in the diversification of Beta-CoVs. A larger host diversity is observed for Alpha-CoVs. Our dataset for this analysis includes all CoV RdRp sequences isolated from bat specimens collected by our team from 2008-2015 (Alpha-CoVs: n = 491 – Beta-CoVs: n = 326), including those collected under prior NIAID funding (1 R01 AI079231), funding from Chinese Federal Agencies, and a large majority from our current NIAID project. All Chinese bat CoV RdRp sequences available in GenBank were also added to our dataset (Alpha-CoVs: n = 226 – Beta-CoVs: n = 206).

Phylogenetic trees were reconstructed for Alpha- and Beta-CoVs separately using Bayesian inference (BEAST 1.8).

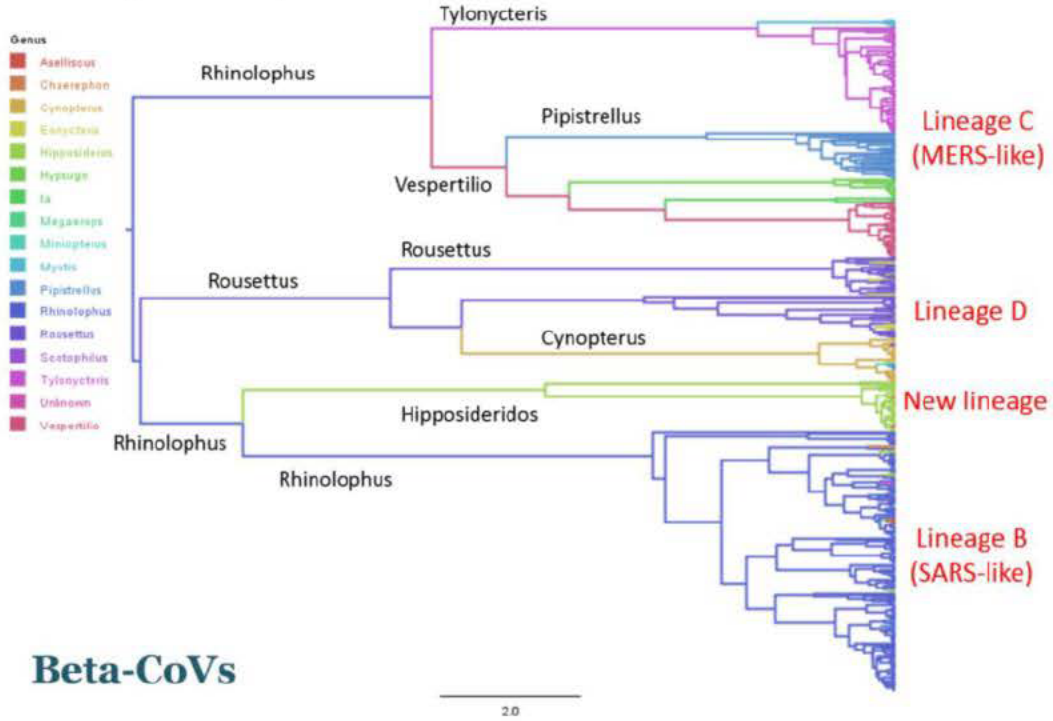


Figure 31. *Ancestral host reconstruction for Beta-CoVs, at a host genus level.*

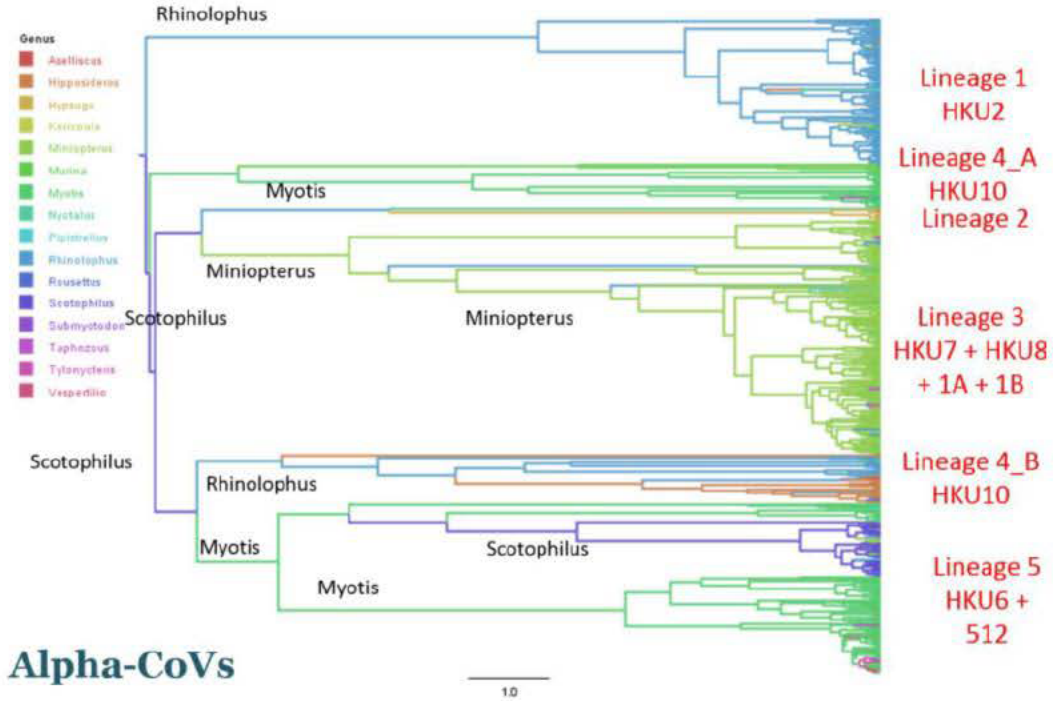


Figure 32. *Ancestral host reconstruction for Alpha-CoVs, at a host genus level.*

To better understand the geographic origins and extent of specific CoV clades, we also used discrete ancestral character state reconstruction in BEAST to reconstruct the ancestral location of each branch of the tree. We used SPREAD to visualize the tree in its geographic context and infer CoV spatial spread in China **(Fig. 33)**. These analyses allow us to identify the geographic areas that are likely sources of origin/diversity for this important group of viruses. The common ancestor of most Beta-CoVs lineages is located in Hong Kong and Guangdong. The common ancestor of most Alpha-CoV lineages was located in Yunnan province, and our results suggest they spread to other provinces from Yunnan.

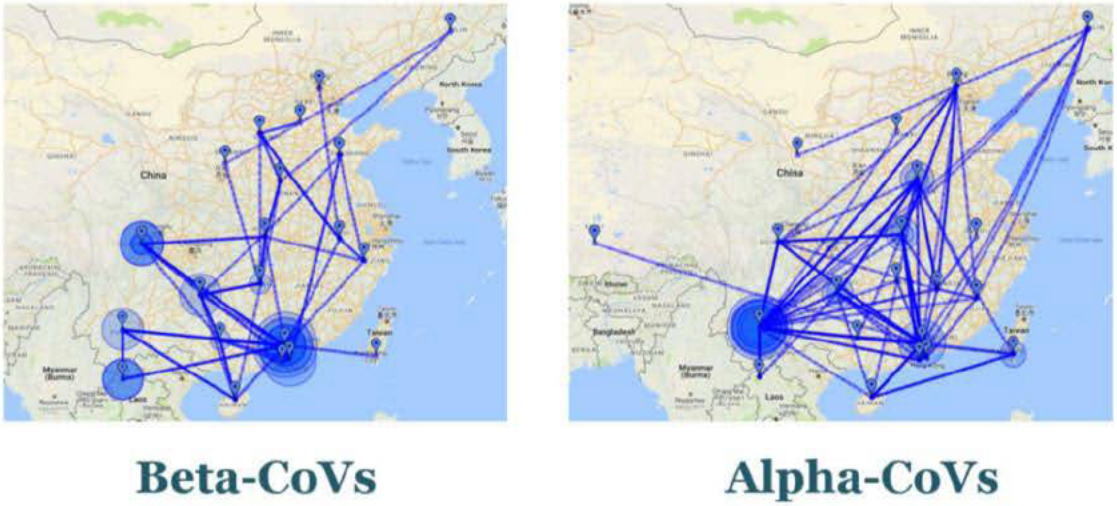


Figure 33. *Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.*

Specific Aim 3: Testing Predictions of CoV Inter-Species Transmission

Identification of two novel MERS-related CoVs that use DPP4 receptor

Two novel MERSr-CoVs, BtCoV/li/GD/2013-845 and BtCoV/li/GD/2014-422, were identified from great evening bats (*Ia io*) in Guangdong province. Phylogenetic analysis of polyprotein 1 and the E, M, and N proteins suggests that the two novel strains are more closely related to MERS-CoV than to other lineage C Beta-CoVs. Their RdRp sequences are closely related to those of MERS-CoV and other MERSr-CoVs, with 94.4–97.0% aa identities. In contrast, they are divergent from MERS-CoV and other MERSr-CoVs in the spike protein, with only 58.9–64.7% aa identities. However, in the receptor-binding domain (RBD) of the spike protein, the two novel MERSr-CoVs are identical to MERS-CoV at six out of the 13 residues that directly interact with human DPP4 receptor, making them more similar to MERS-CoV than any other known lineage C BetaCoVs **(Fig. 34a)**. Protein–protein interaction assays demonstrated that the spike proteins of the novel MERSr-CoVs bind to both human and bat DPP4 **(Fig. 34b)**. Moreover, bat cells exogenously expressing human DPP4 support the entry of the retrovirus pseudotyped with BtCoV/li/GD/2014-422 spike, while the pseudovirus fails to enter cells that do not express DPP4. The results demonstrate that the spike protein of the newly identified MERSr-CoV recognizes the human DPP4 receptor.

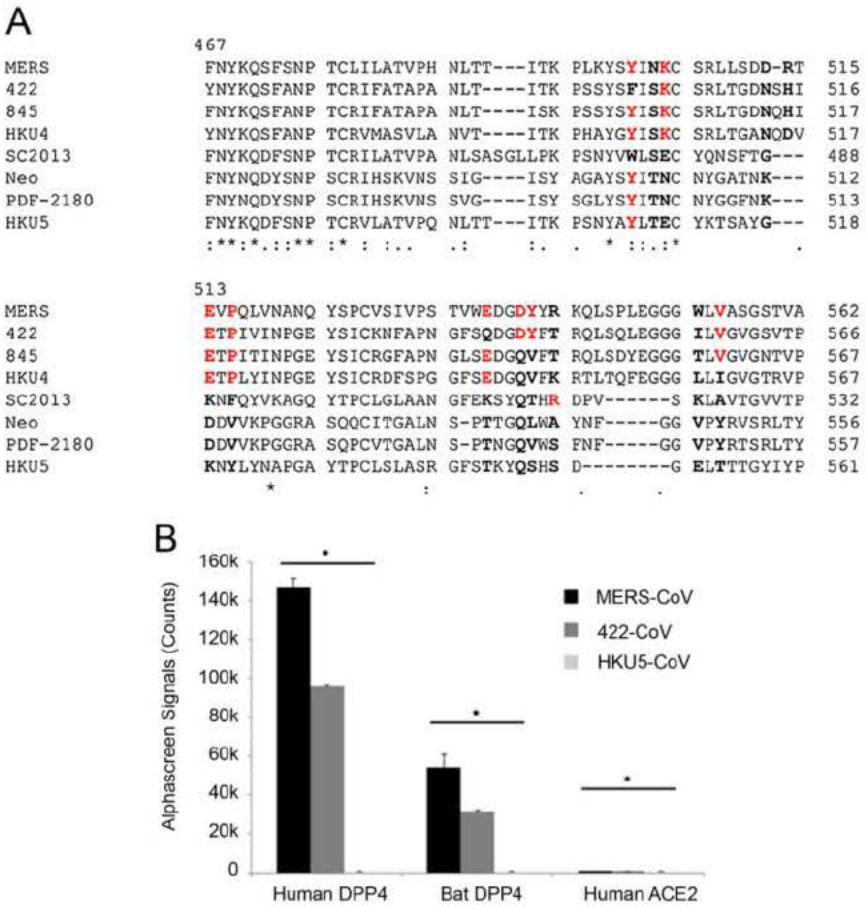


Figure 34. *BtCoV/li/GD/2014-422 RBD analysis (a) and DPP4-binding assay (b)*

In Vivo Infection of Human ACE2 (hACE2) Expressing Mice with SARSr-CoV S Protein variants

Using the reverse genetic methods we previously developed, infectious clones with the WIV1 backbone and the spike protein of SHC014, WIV16 and Rs4231, respectively, were constructed and recombinant viruses were successfully rescued. In Year 4, we performed preliminary *in vivo* infection of SARSr-CoVs on transgenic mice that express hACE2. Mice were infected with 10^5 pfu of full-length recombinant virus of WIV1 (rWIV1) and the three chimeric viruses with different spikes. Pathogenesis of the 4 SARSr-CoVs was then determined in a 2-week course. Mice challenged with rWIV1-SHC014S have experienced about 20% body weight loss by the 6th day post infection, while rWIV1 and rWIV-4231S produced less body weight loss. In the mice infected with rWIV1-WIV16S, no body weight loss was observed **(Fig. 35a)**. 2 and 4 days post infection, the viral load in lung tissues of mice challenged with rWIV1-SHC014S, rWIV1-WIV16S and rWIV1-Rs4231S reached more than 10^6 genome copies/g and were significantly higher than that in rWIV1-infected mice **(Fig. 35b)**. These results demonstrate varying pathogenicity of SARSr-CoVs with different spike proteins in humanized mice.

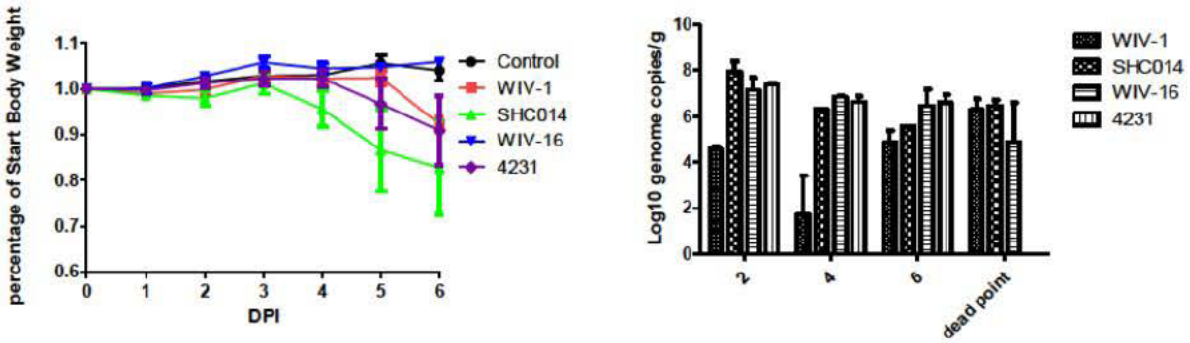


Figure 35. *In vivo infection of SARSr-CoVs in hACE2-expressing mice. (a, left) Body weight change after infection; (b, right) Viral load in lung tissues*

Additional Year 4 Results for Specific Aim 3:

Identification of a HKU2-related Coronavirus of Bat Origin that Caused Fatal Acute Diarrhea in Piglets

From October 2016, a series of fatal swine diarrhea disease outbreaks occurred in Guangdong province. By May 2017, it had resulted in death of 24,693 piglets across four farms. We identified a novel coronavirus as the etiological agent of the disease by metagenomic analysis, viral isolation and experimental infection, and named this "Swine Acute Diarrhea Syndrome coronavirus (SADS-CoV). During Year 4, we submitted and published a paper on this finding to *Nature* (Zhou *et al.*, 2018). The full-length genome of SADS-CoV shares 95% sequence identity to bat CoV HKU2. However, the S gene sequence identity is only 86%, suggesting that the previously reported HKU2-CoV is not the direct progenitor of SADS-CoV, but that they may have originated from a common ancestor.

Using a SADS-CoV specific qPCR assay based on its RdRp gene, SADS-related coronaviruses (SADSr-CoVs) were detected in rectal swabs of *Rhinolophus* bats collected from 2013 to 2016 in Guangdong. Full-length genome sequencing of 4 bat SADSr-CoVs revealed 96% to 98% overall genome sequence identity between SADSr-CoVs and SADS-CoV. Most importantly, the S protein of SADS-CoV shared more than 98% sequence identity with those of the two SADSr-CoVs (162149 and 141388), compared to 86% with HKU2-CoV (**Fig. *36a***). The phylogeny of S1 protein sequence showed strong co-evolutionary relationships with bat alphacoronavirus and their hosts, with swine SADS-CoV more closely related to SADSr-CoVs from *Rhinolophus affinis* than strains from *Rhinolophus sinicus* in which HKU2-CoV was found **(Fig. 36b)**. Analysis of the 33 SADS-CoV full genome sequences we were able to characterize from pigs suggests that viruses from the four farms may have been transmitted from their reservoir hosts independently. These findings highlight the importance of identifying coronavirus diversity and distribution in bats to mitigate future outbreaks that threaten livestock and public health.

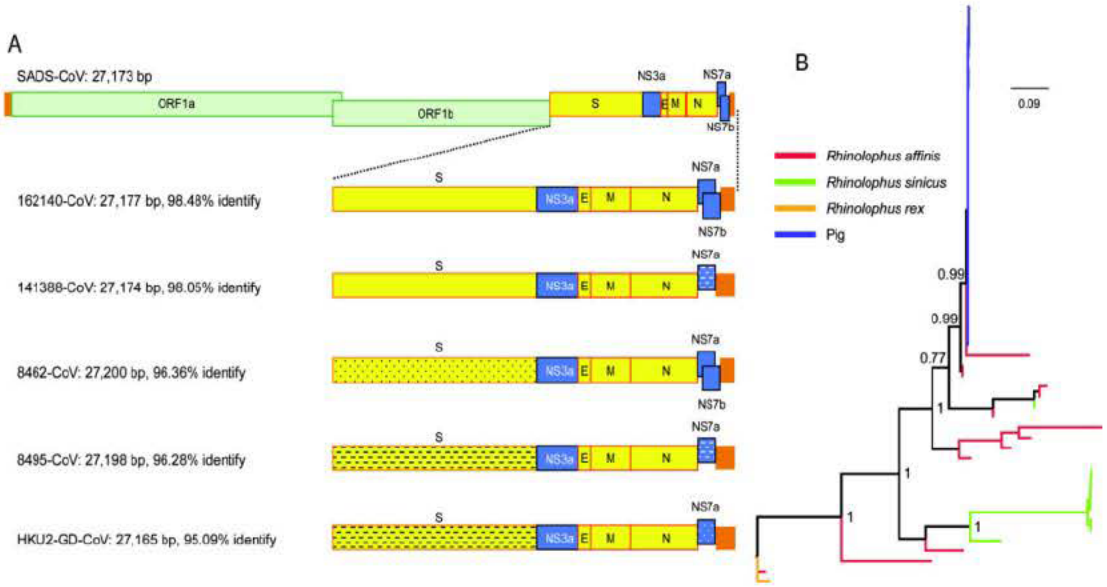
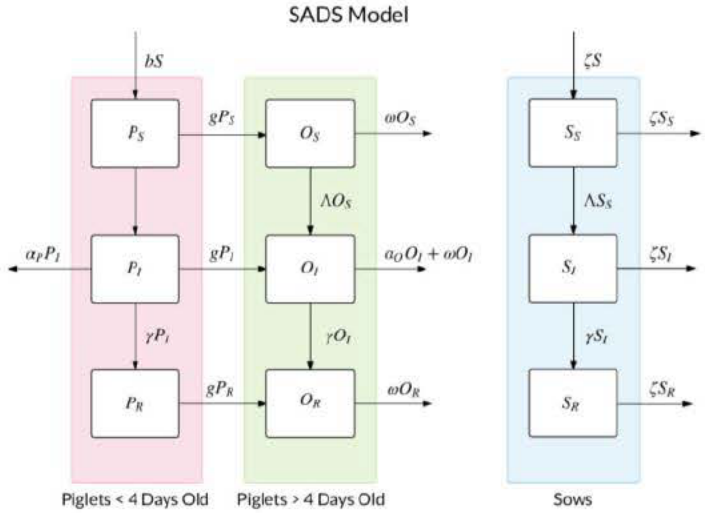


Figure 36. *Genome organization and comparison (a) and Phylogenetic analysis of S1 protein (b) of SADS-CoV and bat SADSr-CoVs*

Intra-Farm Transmission Model to Understand to Predict Future Transmission and Outbreak

To better understand amplification dynamics and assess the potential for future transmission resulting in large outbreaks, we developed an intra-farm, age-structured, stochastic transmission model for SADS-CoV **(Fig. 37)**. We developed multiple versions of this model to represent different hypotheses of disease transmission mechanisms and fit them to time-series data of reported deaths on multiple SADS-infected farms.



$$N = P_S + P_I + P_R + O_S + O_I + O_R + S_S + S_I + S_R$$

$$\Lambda = \beta \frac{(P_I + O_I + S_I)}{N^p}$$

Figure 37: *Schematic of intra-farm transmission mode.*

Our first model structure, which assumed equal mixing of animals across farms **(Fig. 38)** showed that age structure alone was insufficient to generate the temporal pattern of reported deaths on SADS-infected farms. Our second model structure **(Fig. 39)** represented individual barns on a farm as a series of pig-virus meta-populations. This structure was sufficient to re-create the dynamics of the series of rapid "mini-epidemics" that progressed in SADS-infected farms.

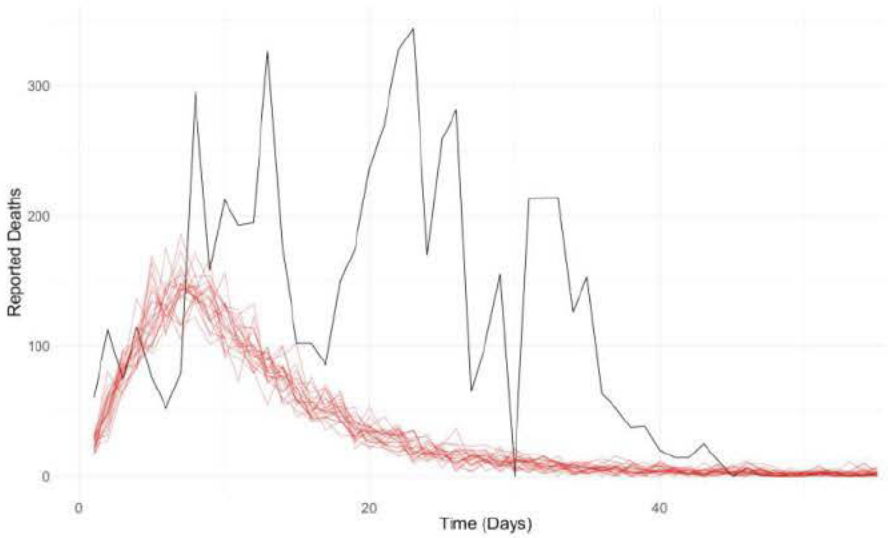


Figure 38: *Best-fit simulations (red) from an equal-mixing transmission model and actual reported death time series (black) on a SADS-infected farm.*

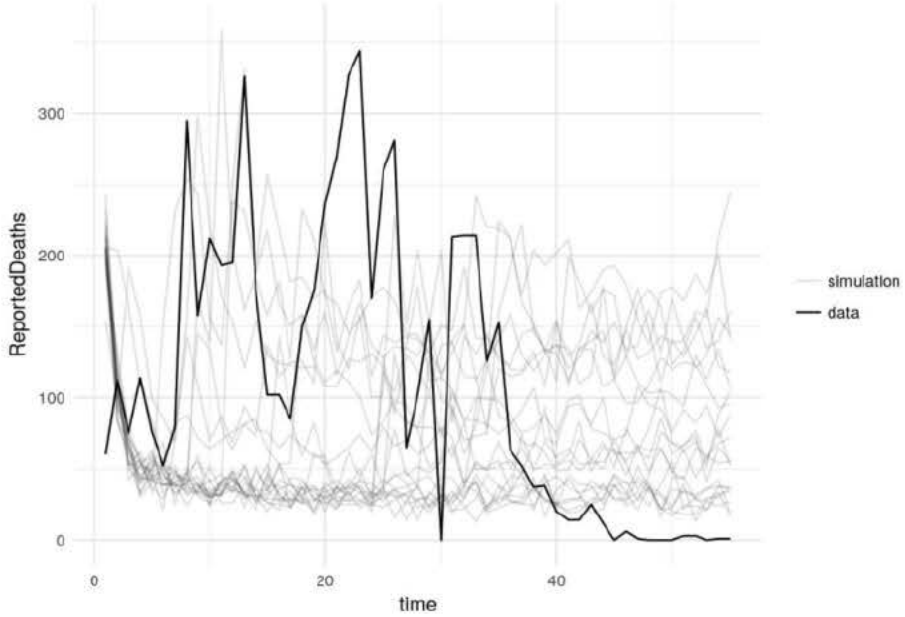


Figure 39: *Best-fit simulations (grey) from an metapopulation transmission model and actual reported death time series (black) on a SADS-infected farm.*

Specific Goals Not Meet

- The wild animal farm survey was piloted in early Y4, with data collected from seven wild animal farms, it was postponed due to the emergence of SADS-CoV where our group had focused on instead in Y4, but will be resumed in Y5 to continue collecting and analyzing data.
- The passive hospital surveillance has been piloted will continue in Year 4 to collect and test for CoVs.

B. 4 What opportunities for training and professional development has the project provided?

1. Conference and University lectures: We provided human subject research trainings to chief physicians and nurses at local clinics, staff from Yunnan Institute of Endemic Diseases Control and Prevention, students from Dali College and Wuhan University for both qualitative and quantitative research.

2. Agency and other briefing: Dr. Guangjian Zhu was invited by the Guangdong Institute of Applied Nature Resources, Guangdong Academy of Sciences to provide training to 8 field team members regarding biosafety and PPE use, bats and rodents sampling. Dr. Zhengli Shi participated in the US National Science Foundation-funded EcoHealthNet (grant to EcoHealth Alliance – Epstein PI) that provides research exchange opportunities to undergraduate and graduate-level students.

3. Public outreach: PI Daszak, and Co-investigators Shi, Epstein, and Olival presented the results of this project to the public via interviews with national central and local television, social media, newspaper and journals in China and the US.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Olival KJ, Hosseini PR, Zambrana-Torrelio C, Ross N, Bogich TL, Daszak P. Host and viral traits predict zoonotic spillover from mammals. Nature. 2017 June 29;546(7660):646-650. PubMed PMID: 28636590; PubMed Central PMCID: PMC5570460.
Complete	Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, Xie JZ, Shen XR, Zhang YZ, Wang N, Luo DS, Zheng XS, Wang MN, Daszak P, Wang LF, Cui J, Shi ZL. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS pathogens. 2017 November;13(11):e1006698. PubMed PMID: 29190287; PubMed Central PMCID: PMC5708621.
In Process at NIHMS	Luo Y, Li B, Jiang RD, Hu BJ, Luo DS, Zhu GJ, Hu B, Liu HZ, Zhang YZ, Yang XL, Shi ZL. Longitudinal Surveillance of Betacoronaviruses in Fruit Bats in Yunnan Province, China During 2009-2016. Virologica Sinica. 2018 February;33(1):87-95. PubMed PMID: 29500692.
In Process at NIHMS	Wang N, Li SY, Yang XL, Huang HM, Zhang YJ, Guo H, Luo CM, Miller M, Zhu G, Chmura AA, Hagan E, Zhou JH, Zhang YZ, Wang LF, Daszak P, Shi ZL. Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. Virologica Sinica. 2018 February;33(1):104-107. PubMed PMID: 29500691.
In Process at NIHMS	Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S, Zheng XS, Li B, Li JM, Guo H, Pei GQ, An XP, Chen JW, Zhou L, Mai KJ, Wu ZX, Li D, Anderson DE, Zhang LB, Li SY, Mi ZQ, He TT, Cong F, Guo PJ, Huang R, Luo Y, Liu XL, Chen J, Huang Y, Sun Q, Zhang XL, Wang YY, Xing SZ, Chen YS, Sun Y, Li J, Daszak P, Wang LF, Shi ZL, Tong YG, Ma JY. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. Nature. 2018 April;556(7700):255-258. PubMed PMID: 29618817.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b) (6)	Y	DASZAK, PETER	BS,PHD	PD/PI			(b) (4), (b) (6)			NA
(b) (6)	N	Chmura, Aleksei	BS,PHD	Non-Student Research Assistant						NA
(b) (6)	N	Ross, Noam Martin	PhD	Co-Investigator						NA
(b) (6)	Y	Olival, Kevin J.	PHD	Co-Investigator						NA
(b) (6)	Y	Zhang, Shu-yi	PHD	Co-Investigator				East China Normal University	CHINA	NA
	N	ZHU, GUANGJIAN	PHD	Co-Investigator				East China Normal University	CHINA	NA
	N	GE, XINGYI	PHD	Co-Investigator				Wuhan Institute of Virology	CHINA	NA
	N	KE, CHANGWEN	PHD	Co-Investigator				Center for Disease Control and Prevention of Guangdong Province	CHINA	NA
	Y	ZHANG, YUNZHI	PHD	Co-Investigator				Yunnan Provincial Institute of Endemic Diseases Control & Prevention	CHINA	NA
(b) (6)	N	EPSTEIN, JONATHAN H	MPH,DVM,BA,PHD	Co-Investigator						NA
(b) (6)	N	SHI, ZHENGLI	PhD	Co-Investigator				Wuhan Institute of Virology	CHINA	NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
$201,422	CHINA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional	Address

		District	
Primary: EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan
East China Normal University	420945495		3663 Zhongshan Beilu Shanghai

G.9 FOREIGN COMPONENT

Organization Name: Wuhan Institute of Virology
Country: CHINA
Description of Foreign Component:
Principal Laboratory for all Research in China as per section G8 (above) and detailed in our Specific Aims

Organization Name: Wuhan School of Public Health
Country: CHINA
Description of Foreign Component:
Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 166195

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

Planned Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0		0	0					0
Asian	1230	1230		0	0					2460
Native Hawaiian or Other Pacific Islander	0	0		0	0					0
Black or African American	0	0		0	0					0
White	0	0		0	0					0
More than One Race	0	0		0	0					0
Unknown or Not Reported										
Total	**1230**	**1230**		**0**	**0**					**2460**

Cumulative Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	980	616	0	0	0	0	0	0	0	1596
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	**980**	**616**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1596**

To: Selgrade, Sara (NIH/NIAID) [E] (b) (6); Arms, Erin (NIH/NIAID) [E] (b) (6) NIAID DEA DART <dart@mail.nih.gov>
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: RE: Action ASAP: follow-up Q's on Year 4 RPPR - FLIPs for Nov 4th Senate HELP hearing

I did get the background of what happened, so I can answer that question to the best of my ability. I will ask eRA if the RPPR regeneration would have pulled in more recent publications and will try to have that answer by Friday as well.

Thank you.

From: Selgrade, Sara (NIH/NIAID) [E] (b) (6)
Sent: Wednesday, October 20, 2021 2:38 PM
To: Linde, Emily (NIH/NIAID) [E] (b) (6) Arms, Erin (NIH/NIAID) [E] (b) (6)>; NIAID DEA DART <dart@mail.nih.gov>
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: RE: Action ASAP: follow-up Q's on Year 4 RPPR - FLIPs for Nov 4th Senate HELP hearing

Hi Emily,

At the meeting on Friday with Dr. Fauci, would you be the right person to explain to him what may have happened with eRA when the report was regenerated (i.e., would this have pulled a current version of the publication list into the report?)

Thanks,
Sara

From: Linde, Emily (NIH/NIAID) [E (b) (6)
Sent: Wednesday, October 20, 2021 2:35 PM
To: Arms, Erin (NIH/NIAID) [E] (b) (6); NIAID DEA DART <dart@mail.nih.gov>
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: RE: Action ASAP: follow-up Q's on Year 4 RPPR - FLIPs for Nov 4th Senate HELP hearing

There are instructions in the NIH RPPR Instructions – NIH and Other PHS Agency Research Performance Progress Report (RPPR) Instruction Guide: see 6.3 Section C – Products, C.1 Publications (bottom of page 76)

From: Arms, Erin (NIH/NIAID) [E] (b) (6)
Sent: Wednesday, October 20, 2021 2:22 PM
To: NIAID DEA DART <dart@mail.nih.gov>
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Action ASAP: follow-up Q's on Year 4 RPPR - FLIPs for Nov 4th Senate HELP hearing

Hi Mark,

Thanks for providing the additional information on the report regeneration issue. It would be helpful for us to have some additional information on how the publication section of an RPPR is generated so that we can respond to questions on why the regenerated Year 4 RPPR contains references published after the RPPR was originally submitted in 2018.

Can you please provide us with the following information related to this point?

- How is the publication section of the report generated? Is this automatically generated, or is it part of a PDF that the grantee uploads to the system?
    - The following link from the NIH website makes it appear to be the latter, so it would be helpful to have confirmation of how this is done: https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/general/g.400-phs-398-research-plan-form.htm#4

- Are copies of the original (2018) and the regenerated (2020) RPPR available for us to review? If so, please send us copies so that we can do a direct compare.

I am cc'ing DMID so they are aware of these requests. We would appreciate having this information as soon as possible.

Please let us know if we need to set up a call to discuss.

Thanks,
Erin

From: Helfman, Mark (NIH/NIAID) [E] (b) (6)
Sent: Wednesday, October 20, 2021 10:43 AM
To: Arms, Erin (NIH/NIAID) [E] (b) (6)
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>; NIAID DEA DART <dart@mail.nih.gov>
Subject: Re: Action by 2 pm today (10/19): FLIPs for Nov 4th Senate HELP hearing

Erin, see attached.

Please note, regarding your question about whether we have other progress reports for year -05, please note the Interim RPPR is the progress report that reports on progress in year -05. Year 4 does include information on what they plan to do in year -05, but these are separate reports. There are no other RPPRs.

--
Mark Helfman
Division of Extramural Activities (DEA), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)
Phone (b) (6) | Email: (b) (6) | Office: 4G29

From: "Arms, Erin (NIH/NIAID) [E]" <(b) (6)
Date: Wednesday, October 20, 2021 at 8:57 AM
To: "Helfman, Mark (NIH/NIAID) [E]" <(b) (6)
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>, NIAID DEA DART <dart@mail.nih.gov>
Subject: RE: Action by 2 pm today (10/19): FLIPs for Nov 4th Senate HELP hearing

Hi Mark,

I just wanted to check on the status of the Year 4 FLIP. We are working to clear FLIPs through the FO and would appreciate having this as soon as possible.

Thanks,
Erin

From: Helfman, Mark (NIH/NIAID) [E] (b) (6)
Sent: Tuesday, October 19, 2021 2:09 PM
To: Arms, Erin (NIH/NIAID) [E] (b) (6)
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>; NIAID DEA DART <dart@mail.nih.gov>
Subject: Re: Action by 2 pm today (10/19): FLIPs for Nov 4th Senate HELP hearing

Hi Erin. We reviewed one of the FLIPs, EcoHealth Alliance CoV Grant (2014-2018) Year 5. See our comments, attached. Stand by for our response to the other.

--
Mark Helfman
Division of Extramural Activities (DEA), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)
Phone: (b) (6) | Email: (b) (6)v | Office: 4G29

From: "Arms, Erin (NIH/NIAID) [E]" (b) (6)
(b) (6) October 19, 2021 at 11:26 AM
To: NIAID DEA DART <dart@mail.nih.gov>
Cc: Matthew Fenton (b) (6)>, "Linde, Emily (NIH/NIAID) [E]"
(b) (6)>, "Billet, Courtney (NIH/NIAID) [E]" (b) (6), NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>
Subject: Action by 2 pm today (10/19): FLIPs for Nov 4th Senate HELP hearing

Hello DEA,

Background

As you are aware, Dr. Fauci has been invited to testify before the Senate HELP Committee on November 4th at 10 a.m. to discuss the ongoing Federal response to COVID-19. In preparation for this hearing, we are working to prepare FLIPs and other background materials. OCGR-Leg has prepared the attached FLIPS. We would appreciate if you would review these FLIPs and respond to any specific comments directed to DEA.

- EcoHealth Alliance CoV Grant (2014-2018) Year 4 [Previously reviewed by DMID]
- EcoHealth Alliance CoV Grant (2014-2018) Year 5 [Also going to DMID for review]

Action

This is an expedited request for FLIP review. Please send completed FLIPs to OCGR-Leg by **2 p.m. today, October 19th.** As these are for Dr. Fauci's use during the upcoming hearing, we do request that Dr. Fenton or Emily Linde clear DEA's updates as appropriate.

Please let us know if you have any questions.

Thanks,
Erin

Erin Arms, Ph.D.
(she, her, hers)
Public Health Analyst
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
31 Center Drive
Bldg. 31, Room 7A17H, MSC 2520
Bethesda, MD 20892-2080
(b) (6)

From:	Hauguel, Teresa (NIH/NIAID) [E]
To:	Ford, Andrew (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]
Subject:	RE: For review again: Response to GoF inquiry from FactCheck.org
Date:	Thursday, July 22, 2021 6:18:30 PM
Attachments:	Response to FactCheck JR ed ae cb AOF clean ss ae AOF TH.docx

Forgot to include my comment about the (b) (5) – see attached

Teresa M. Hauguel, Ph.D.
Acting Chief, Respiratory Diseases Branch
COR, Collaborative Influenza Vaccine Innovation Centers (CIVICs)
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
5601 Fishers Lane, Room 8E19
Rockville, MD 20852
Phone (b) (6)
Email:

From: Hauguel, Teresa (NIH/NIAID) [E]
Sent: Thursday, July 22, 2021 6:16 PM
To: Ford, Andrew (NIH/NIAID) [E] (b) (6) Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Subject: RE: For review again: Response to GoF inquiry from FactCheck.org

Andrew and Erik – see attached comment.

Not sure if Erik will have a chance to review but we really need his opinion on the language.

Teresa M. Hauguel, Ph.D.
Acting Chief, Respiratory Diseases Branch
COR, Collaborative Influenza Vaccine Innovation Centers (CIVICs)
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
5601 Fishers Lane, Room 8E19
Rockville, MD 20852
Phone (b) (6)
Email:

From: Ford, Andrew (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 5:46 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6); Hauguel, Teresa (NIH/NIAID) [E] (b) (6)
Cc: NIAID BUGS <BUGS@niaid.nih.gov>; Embry, Alan (NIH/NIAID) [E] (b) (6)
Subject: FW: For review again: Response to GoF inquiry from FactCheck.org
Importance: High

(b) (5)

(b) (5)

Hey Erik and Teresa,

Attached for another round of review is the response to FactChecker. Note, in response to Alan's comment, I attempted to provide alternative language; (b) (5) Please do send your thoughts as soon as possible.

Thanks and apologies,
Andrew

From: "Embry, Alan (NIH/NIAID) [E]" (b) (6)
Date: Thursday, July 22, 2021 at 5:16 PM
To: "Billet, Courtney (NIH/NIAID) [E]" (b) (6) "Routh, Jennifer (NIH/NIAID) [E]" (b) (6) Sara Selgrade (b) (6) "Deatrick, Elizabeth (NIH/NIAID) [E]' (b) (6) "Ford, Andrew (NIH/NIAID) [E]" (b) (6), NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>, NIAID Media Inquiries <mediainquiries@niaid.nih.gov>, "Haskins, Melinda (NIH/NIAID) [E]" (b) (6)
Subject: RE: For review again: Response to GoF inquiry from FactCheck.org

This language needs revision. Please work with DMID to fix.

From: Billet, Courtney (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 5:05 PM
To: Routh, Jennifer (NIH/NIAID) [E] (b) (6) Selgrade, Sara (NIH/NIAID) [E] (b) (6)>; Deatrick, Elizabeth (NIH/NIAID) [E] (b) (6)>; Embry, Alan (NIH/NIAID) [E] (b) (6)Ford, Andrew (NIH/NIAID) [E] (b) (6) NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>; NIAID Media Inquiries <mediainquiries@niaid.nih.gov>; Haskins, Melinda (NIH/NIAID) [E] (b) (6)
Subject: RE: For review again: Response to GoF inquiry from FactCheck.org

It's ok with me, with the caveat that I defer to Alan's expertise. Alan, OK with you?

From: Routh, Jennifer (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 4:55 PM
To: Selgrade, Sara (NIH/NIAID) [E] (b) (6) Deatrick, Elizabeth (NIH/NIAID) [E] (b) (6) Embry, Alan (NIH/NIAID) [E] (b) (6); Ford, Andrew (NIH/NIAID) [E] (b) (6); Billet, Courtney (NIH/NIAID) [E] (b) (6); NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>; NIAID Media Inquiries

<mediainquiries@niaid.nih.gov>; Haskins, Melinda (NIH/NIAID) [E] (b) (6)
Subject: RE: For review again: Response to GoF inquiry from FactCheck.org
Importance: High

I am adding BUGS, Andrew and Courtney to this thread so we are all on the same thread. The original response pulled from cleared language. I'd like to get this finalized ASAP. We really need to move this forward.

Jennifer Routh [E]
News and Science Writing Branch
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
NIH/HHS
31 Center Drive Room 7A17C
Bethesda, MD 20892
Direct: (b) (6)
(b) (6)

From: Selgrade, Sara (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 4:49 PM
To: Deatrick, Elizabeth (NIH/NIAID) [E] (b) (6) Embry, Alan (NIH/NIAID) [E] (b) (6)
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>; NIAID Media Inquiries <mediainquiries@niaid.nih.gov>; Haskins, Melinda (NIH/NIAID) [E] (b) (6)
Subject: RE: For review again: Response to GoF inquiry from FactCheck.org

Thanks Elizabeth. Some edits for your consideration in the attached. I'm copying Melinda as well for her review.

Defer to Alan on whether DMID should review again.

From: Deatrick, Elizabeth (NIH/NIAID) [E] (b) (6)
Sent: Thursday, July 22, 2021 4:33 PM
To: Embry, Alan (NIH/NIAID) [E] (b) (6) Selgrade, Sara (NIH/NIAID) [E] (b) (6)
Cc: NIAID OCGR NSWB <NIAIDOCGRNSWB@mail.nih.gov>; NIAID Media Inquiries <mediainquiries@niaid.nih.gov>
Subject: For review again: Response to GoF inquiry from FactCheck.org

Good afternoon,

DMID has reviewed our response to FactCheck.org on GoF research, but recommended that we run the copy past you one more time before moving it forward in the clearance process. Please let me know if you have any notes on the attached clean document.

Best,

Elizabeth Deatrick
Technical Writer-Editor
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
(b) (6)

Please note that I am not a spokesperson for NIAID and should not be quoted as such.

From: Hauguel, Teresa (NIH/NIAID) [E]
To: Ford, Andrew (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Miers, Sarah (NIH/NIAID) [E]
Subject: RE: Urgent Action: Additional FLIPs for review
Date: Tuesday, October 19, 2021 1:51:22 PM

Nothing additional from me

Teresa M. Hauguel, Ph.D.
Acting Chief, Respiratory Diseases Branch
COR, Collaborative Influenza Vaccine Innovation Centers (CIVICs)
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
5601 Fishers Lane, Room 8E19
Rockville, MD 20852
Phone (b) (6)
Email:

From: Ford, Andrew (NIH/NIAID) [E] (b) (6)
Sent: Tuesday, October 19, 2021 1:15 PM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)>; Miers, Sarah (NIH/NIAID) [E] (b) (6) Hauguel, Teresa (NIH/NIAID) [E (b) (6)
Subject: RE: Urgent Action: Additional FLIPs for review

I added one comment to Erik's (b) (5)

Andrew Q. Ford, Ph.D.
Office of Scientific Coordination and Program Operations
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
5601 Fishers Lane Room 7G64
Rockville, MD 20892
(b) (6)

From: Stemmy, Erik (NIH/NIAID) [E] (b) (6)
Sent: Tuesday, October 19, 2021 1:00 PM

To: Miers, Sarah (NIH/NIAID) [E] (b) (6); Hauguel, Teresa (NIH/NIAID) [E] (b) (6)>; Beigel, John (NIH) [E] (b) (6)>; Cardemil, Cristina (NIH/NIAID) [E] (b) (6) Ford, Andrew (NIH/NIAID) [E] (b) (6)>
Subject: RE: Urgent Action: Additional FLIPs for review

Hi Sarah,
Comments from me in the attached. I didn't have comments for the others.

Erik

From: Miers, Sarah (NIH/NIAID) [E] (b) (6)
Sent: Tuesday, October 19, 2021 11:42 AM
To: Stemmy, Erik (NIH/NIAID) [E] (b) (6)>; Hauguel, Teresa (NIH/NIAID) [E] (b) (6) Beigel, John (NIH) [E] (b) (6) Cardemil, Cristina (NIH/NIAID) [E] (b) (6) Ford, Andrew (NIH/NIAID) [E] (b) (6)
Subject: Urgent Action: Additional FLIPs for review
Importance: High

Hello –

We have received additional flip cards to review for Dr. Fauci for the upcoming Senate HELP hearing. Some of these cards have been reviewed before and OCGR-Leg has added questions or edits for us to review on them. Here is the list of cards:

- 2016 – Baric Nature Microbiology Paper
- _Covid – Vax Trials in Children
- 2017 PLOS Pathogens Paper
- Benefits of GOF research
- Furin Cleavage Site_CGG Repeats
- EcoHealth Alliance CoV Grant (2014-2018) Year 5 [Also going to DEA for review]
- _COVID – Pregnant Women and Vax Trials [Also going to DAIDS for review]
- Natural Immunity to SARS-CoV [Going to all Divisions for review]

Unfortunately we have a very short turnaround time for these and Emily needs to review them. Please respond to me by 2:00 PM today. My apologies for the rapid turnaround.

Thanks
Sarah

From: "Arms, Erin (NIH/NIAID) [E]" (b) (6)>
Date: Tuesday, October 19, 2021 at 11:14 AM
To: NIAID BUGS <BUGS@niaid.nih.gov>
Cc: NIAID OCGR Leg <NIAIDOCGRLeg@mail.nih.gov>

Subject: Action by 3 pm today (10/19): Additional FLIPs for review

Hello Bugs,

Background

Thank you for your ongoing assistance to prepare FLIPs for the November 4th Senate HELP Committee hearing to discuss the ongoing Federal response to COVID-19. Attached are additional FLIPs for your review. Please note – some of the FLIPs have already been reviewed. The versions of these FLIPs included here have additional edits or questions for your review.

- 2016 – Baric Nature Microbiology Paper
- _Covid – Vax Trials in Children
- 2017 PLOS Pathogens Paper
- Benefits of GOF research
- Furin Cleavage Site_CGG Repeats
- EcoHealth Alliance CoV Grant (2014-2018) Year 5 [Also going to DEA for review]
- _COVID – Pregnant Women and Vax Trials [Also going to DAIDS for review]
- Natural Immunity to SARS-CoV [Going to all Divisions for review]

Action

This is an expedited request for FLIP review. Please send completed FLIPs to OCGR-Leg by **3 PM today, October 19th.** As these are for Dr. Fauci's use during the upcoming hearing, we do request that Dr. Erbelding clear DMID's updated FLIPs.

Please let us know if you have any questions or if any of the above FLIPs should be reviewed by additional Divisions.

Thanks,
Erin

Erin Arms, Ph.D.
(she, her, hers)
Public Health Analyst
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
31 Center Drive
Bldg. 31, Room 7A17H, MSC 2520
Bethesda, MD 20892-2080
(b) (6)

From:	Zendt, Mackenzie (NIH/NIAID) [E]
To:	Harper, Jill (NIH/NIAID) [E]; Billet, Courtney (NIH/NIAID) [E]; Embry, Alan (NIH/NIAID) [E]; Stemmy, Erik (NIH/NIAID) [E]; Hauguel, Teresa (NIH/NIAID) [E]; Ford, Andrew (NIH/NIAID) [E]
Cc:	NIAID OCGR Leg
Subject:	Urgent ASAP: 4 additional flip cards
Date:	Tuesday, July 13, 2021 3:48:11 PM
Attachments:	Jesse Bloom Preprint and Early SARS-CoV-2 Sequences_toGroup.docx FLIP 2017 PLOS Paper_toGroup.docx Holmes Preprint - Origins of SARS-CoV-2_toGroup.docx FLIP_P3CO Requirements for Researchers_toGroup.docx

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Hello,

Background

As you may be aware, Dr. Fauci is testifying before the Senate Health, Education, Labor, and Pensions (HELP) Committee this month. OCGR-Leg would appreciate your review of the attached new flip cards.

- Jesse Bloom Preprint and Early SARS-CoV-2 Sequences
- PLOS Paper
- Holmes Preprint
- P3CO Requirements for Researchers

Action

As soon as possible and no later than 4p.m. Tuesday, July 13th, please review the attached flip card and provide any edits or comments using tracked changes.

Thanks,
Mackenzie

Mackenzie Zendt, MPH
Health Specialist | Presidential Management Fellow
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Email: (b) (6)
Phone

(b) (5)

(b) (5)

(b) (5)

(b) (5)

(b) (6) **[E]**

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Monday, September 27, 2021 3:45 PM
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: asf - those two ajph papers / i pulled out the table
Attachments: ajph.2021.306310.pdf; ajph.2021.306326.pdf

Comparing pandemics: 1918 influenza and 2019 COVID-19

Variable	1918 Influenza	2019 COVID-19
Infectious Agent	Novel respiratory virus	Novel respiratory virus
Mechanism of emergence	Host switching	Host switching
Source of emergence	Wild waterfowl (*Anseriformes*)	**Wild *Rhinolophus* bat**
Cell receptor	**Sialic acids on respiratory epithelia**	**ACE2 receptor on multiple cells, multiple organs**
Viral preadaptation	Virus preadapted or quickly adapted to human spread	Virus preadapted or quickly adapted to human spread
Clinical & Pathological Disease		
Clinical	Upper respiratory disease, pneumonia	Upper respiratory disease, pneumonia
	No viremia, no systemic disease	**Viremia with systemic disease, vascular damage**
Complications	Secondary bacterial pneumonia, empyema	**Secondary bacterial pneumonia less frequent; Multisystem disease**
Pulmonary pathology	Viral pneumonia, DAD, edema	Viral pneumonia, DAD, edema
	Microthrombi, variable hemorrhage in some	Microthrombi, variable hemorrhage in some
	Aberrant immune response	Aberrant immune response
	Massive neutrophilic infiltrates in some	Neutrophilic infiltrate **less frequent**
Epidemiology		
Preexisting immunity	Possible immunity in older persons	**Prior immunity status not yet certain**
Mortality	Case-fatality ratio about 1% in United States	Case-fatality ratio estimated around 1% in United States
	Higher mortality in infants, elderly, chronically ill	**Children and young adults: lower incidence & severity**
	Pregnant women/fetuses	**No extreme mortality in pregnant women/fetuses?**
	Mortality peak in adults aged 20–40 years	**No mortality peak in adults aged 20–40 years**
Morbidity	Morbidity peak in school-aged children	**Low morbidity in children & young adults**
Origin & spread	Spread by travel, from big cities, spread outward	Spread by travel, from big cities, spread outward
	R_0 estimated to be about 1–2	R_0 about 1–2, but varies greatly
	Spread by droplet, aerosol, hands and fomites	Spread by droplet, aerosol, hands, and fomites
	Asymptomatic carriers	Asymptomatic carriers
	Super spreaders probable	Super spreaders
	Induces full or partial protective immunity	Induction of full or partial protective immunity not established
	Persisted by means of viral evolution	Persistence potential not yet established
Public Health Responses	Closures, isolation, social distancing, masks	Closures, isolation, social distancing, masks
	Bacterial vaccines	Bacterial vaccines, SARS-CoV-2 **viral vaccines**
Treatment	Supportive care, plasma therapy, no ICUs	Supportive care, plasma therapy, **ICUs**
	No antibiotics or antivirals	**Antibiotic, antivirals, glucocorticoids**
	Quack and untried remedies	Quack and untried remedies
Psychosocial Reactions	Widespread disease fear	Widespread disease fear
	Common defiance of public health recommendations	Common defiance of public health recommendations
	Altruism and helping others was common	Altruism and helping others was common

A Centenary Tale of Two Pandemics: The 1918 Influenza Pandemic and COVID-19, Part II

David M. Morens, MD, Jeffery K. Taubenberger, MD, PhD, and Anthony S. Fauci, MD

Both the 1918 influenza pandemic and the 2019–2021 COVID-19 pandemic are among the most disastrous infectious disease emergences of modern times. In addition to similarities in their clinical, pathological, and epidemiological features, the two pandemics, separated by more than a century, were each met with essentially the same, or very similar, public health responses, and elicited research efforts to control them with vaccines, therapeutics, and other medical approaches. Both pandemics had lasting, if at times invisible, psychosocial effects related to loss and hardship. In considering these two deadly pandemics, we ask: what lessons have we learned over the span of a century, and how are we applying those lessons to the challenges of COVID-19? (*Am J Public Health*. 2021;111(7):1267–1272. https://doi.org/10.2105/AJPH.2021.306326)

There are many similarities, and some differences, between the influenza pandemic of 1918–1919 and the COVID-19 pandemic of 2019–2021. Epidemiological and clinical similarities, including viral origin, transmission, and disease morbidity and mortality, were discussed in Part I of this article.[1]

PUBLIC HEALTH RESPONSES

In 1918, fundamental knowledge of sanitation, hygiene, and principles of disease transmission were almost as well understood as they are today.[2] Mechanisms of respiratory spread and means of preventing respiratory transmission were particularly well understood (Figure 1). The dangerous effects of crowding in public places and closed airflow in buildings and the need to socially distance were likewise fully appreciated. This knowledge had been accumulating since the beginning of the sanitary movement in the 1840s, was greatly advanced by acceptance of a "germ theory" in the 1870s, and had been publicly visible since the 1880s in international public health efforts to control the spread of tuberculosis, then a major killer.

Masks, coughing etiquette, use of clean handkerchiefs, proscription of spitting, placement of spittoons in saloons, isolation of the ill, avoidance of congregation, and closing of sports events, theaters, schools, and churches were all employed in 1918 (Figure 1). In the pandemics of both 1918 and 2019–2021, public health officials recommended wearing face masks. As neither N95 nor modern surgical masks were available in 1918, newspapers printed illustrated instructions on making homemade masks using cloth handkerchiefs and string. Both pandemics prompted fanciful improvisations, including morbid art that seemed to mock death; others made masks for domestic pets (Figure 1). In 1918, some professional, collegiate, and other sports events were closed,[3] but in other cases athletes went on playing with or without masks (Figure 1). Public refusal to wear masks was nearly as common as it is today, even though in 1918 scofflaws often faced stiff fines.

Church gatherings and even court proceedings in 1918 were held outdoors, even in the streets. Forced and self-isolation were common. Just as Boccaccio and friends had done more than five centuries earlier, during the 1348 pandemic of bubonic and pneumonic plague, in 1918 citizens took their own public health actions, such as isolating themselves away from crowds, work, and school. After he was rejected for US military service, future novelist William Faulkner fled to Canada for air force training; the Royal Canadian Air Force locked down (i.e., isolated) Faulkner and the other trainees for a period of time during the pandemic, preventing them from being infected. In the

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NIH -001338

FIGURE 1— Wearing of Face Masks, 1918 and 2020

Note. In the pandemics of both 1918 (influenza) and 2019–2021 (COVID-19), public health officials recommended wearing face masks for both casual outings and at sports events, and at other large gatherings. Parts a and b: Masked pet owners and pets, circa 1918 (a) and 2020 (b). Parts c and d: Fanciful masks seem to mock the pandemic's "grim reaper" circa 1918 (c) and 2020 (d). Parts e and f: In 1918, some sports events were canceled but others went on, often with masked players or spectators, or both (e). In 2019–2021, many live sports events have been canceled or played without live spectators (f; Photo by Mike Kireev/NurPhoto via Getty images; published with permission).

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COVID-19 pandemic, many more people are able to self-isolate at home because of teleworking and better-organized food-delivery services.

Public health programs in the United States in 1918 were largely state- and city-based. The key pandemic decision-makers were governors, mayors, local health departments, businessmen, and community leaders, and sometimes nurses and volunteers. Because the pandemic spread so rapidly across the United States,[4] there was little time for planning or coordination. In smaller towns, the pandemic abruptly emerged, peaked, and was often receding or gone within three or four weeks. Different public health response plans were improvised on the spot. Some were more effective than others; mortality varied greatly from one place to the next. Many citizens defied public health recommendations.

Associations between strictness of public health measures and low mortality were immediately noted and much discussed in 1918, especially in cities such as Pittsburgh and Philadelphia, Pennsylvania, where overcrowding, lockdown resistance, and tolerance of social gatherings were associated with increased mortality. Modern analyses are consistent with the beneficial effects of stricter measures.[2] Inactivated bacterial vaccines, intended to prevent death from influenza-associated secondary bacterial pneumonia, which caused the vast majority of pandemic deaths, were often used in 1918, and seem to have been moderately effective in preventing death.[5,6]

Similarities between the public health responses in 1918–1919 and 2019–2021 are many. National and international public health approaches to both pandemics varied widely, with predictable and unpredictable successes and failures. COVID-19 public health responses rely on the basic strategies of 1918: public "lock down," social distancing, hygiene, and self-isolation. During the COVID-19 pandemic, we have also had polymerase chain reaction and serologic testing to identify the virus and its immune fingerprints, as well as contact tracing, well understood in 1918 but not widely used, probably in part because pandemic explosivity led quickly to an overwhelming number of unmanaged cases. In 2019–2021, we have had bacterial vaccines for two of the bacteria (*Streptococcus pneumoniae* and *Haemophilus*

influenzae type b) associated with fatal secondary pneumonias in 1918.[7] Deployment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines (ongoing as of May 1, 2021) is expected to offer the most realistic hope of ending or at least slowing down the pandemic in the immediate future, although many months of scale-up and vaccine distribution and uptake, prioritizing who gets vaccinated, overcoming vaccine hesitancy, and conceivably dealing with vaccine complications, remain as challenges, especially in countries such as India.

An ominous turn of events now unfolding (as of May 2021) is the emergence of multiple SARS-CoV-2 genetic variants apparently associated with increased transmissibility and possible immune escape, potentially affecting vaccine efficacy and diagnostic test sensitivity.[8] Though some of these variants have been suspected of causing more severe disease, this has not been scientifically established at the time of this writing. In 1918–1919, high influenza mortality was associated with viral genetic stability, but over the decade of the 1920s, as population immunity rose, mortality and case-fatality declined. Because viruses have not been recovered from the period of 1920–1932, it is unclear whether and when viral attenuation occurred and what were its genetic determinants. In contrast to early suspicions about SARS-CoV-2, there are no data to support that the 1918 influenza virus became more transmissible or more deadly after its emergence.

DIAGNOSIS AND TREATMENT

As the viral cause of the 1918 pandemic was unknown, diagnosis was clinical and treatment largely supportive. This was the first major disease emergence in which the new technique of diagnostic radiology was used, particularly in the US military.[9] Although most physicians did not have access to diagnostic x-rays, they were often remarkably skilled at using auscultation, percussion, elicitation of tactile fremitus, and observation of respiration, among other diagnostic skills. Oxygen was often available and used. Appearance of so-called "heliotrope cyanosis" of the prominent facial parts,[4,10] although not unique to the 1918 pandemic, was recognized as a terminal event associated with profound hypoxia attributable to loss of gas exchange together with metabolic acidosis.

Those who survived bacterial pneumonias often developed life-threatening empyemas, requiring difficult clinical and surgical management.[11] In an era when therapeutic successes for various other diseases had been achieved with immune plasmas obtained from hyperimmunized horses, goats, or other animals, some influenza patients were treated, with apparent success, by using human convalescent plasmas,[12] as is now the case with COVID-19.[13] Then, as now, the pandemics brought out wishfully repurposed drugs that had little chance of success (e.g., quinine in 1918, hydroxychloroquine in 2020). Then, as now, irrational and often harmful remedies enticed the hopeful (enemas and laxatives in 1918; bleach, disinfectants, and colloidal silver in 2020), in addition to known therapeutics such as immune plasmas and monoclonal antibodies, dexamethasone, and the antiviral remdesivir (used in the United States under Food and Drug Administration emergency use authorization), but the efficacy and appropriate therapeutic indications of the latter remain uncertain. Early data, for example, suggest that in certain patients remdesivir may be of some benefit in shortening illness, although reduction in overall mortality has yet to be fully established.[14]

Lacking antivirals and antibiotics in 1918, supportive care was the mainstay of treatment, with an emphasis on attentive nursing care, and was considered the most effective way to save lives. Nurses from the Red Cross and other agencies, as well as volunteer nurses, mostly women with little or no previous nursing training, went into homes, especially in poorer neighborhoods, to tend to the sick; they were widely regarded as pandemic heroes, as are frontline health care workers in 2019–2021. It is of note that deployment of physicians in the war opened leadership positions for women physicians and scientists on the home front at a time when the women's suffrage movement was at its peak.

The COVID-19 pandemic arrives at a time when remarkable medical advances create a diagnostic and therapeutic world unimagined in 1918: rapid viral diagnostics, x-rays and magnetic resonance imaging, blood gasses and chemistries, antibiotics, antivirals, intensive care units with ventilators and monitors, and extracorporeal membrane oxygenation. However, even with the very best care, many patients who survive the period of SARS-CoV-2 replication and cellular damage still do not survive, or survive with serious long-term complications. Lack of complete understanding of the natural history and pathogenesis of COVID-19 stands in counterpoint to the high level of understanding of the mechanisms of secondary bacterial pneumonia in 1918, even though, ironically, treatment options were far fewer in that era. COVID-19 causes pneumonia; however, unlike influenza, it also damages a wide

FIGURE 2— The 1918 Pandemic Inspired Many Artists

Source. Part b used with permission of the artist, Pete Ryan (https://www.peterthomasryan.com).
Note. Part a: Dying in his Vienna, Austria, apartment of influenza pneumonia (1918), painter Egon Schiele produced his last artistic work, a drawing of his wife, Edith Harms, 6 months pregnant and suffering from the same disease. She died hours after the drawing. Part b: A 2020 illustration captures the anxieties of COVID-19 spread; design by Pete Ryan for *Vox*, printed with permission.

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range of organ systems, causing vascular[15] and neurologic symptoms,[16] and may be associated with aberrant immune responses[17] that may differ from those of influenza, often complicated by microthrombi in lungs and other organs associated with thromboembolic phenomena.[18] Our understanding of the natural history and pathogenesis of COVID-19 is just beginning.

RESEARCH

The 1918 pandemic occurred at the dawn of the era of virology. Viruses as we know them today had been characterized only as "filter-passing agents," submicroscopic entities of some sort that were able to cause diseases after passage through porcelain filters that trapped bacteria.[19] Although a descendant of the 1918 human influenza virus was not officially isolated until 15 years after the pandemic, it seems likely that in 1918 two research groups, one in Tunisia and the other in Japan, actually did isolate the virus, but had no way to maintain the agent via continuous passage in humans or animals, or via freezing.[20,21] Human challenge studies were conducted with human secretions; however, results were problematic. The 1918 pandemic came and went so quickly that comprehensive research programs could not be set up in time, and isolates of virus-containing infectious material could not be easily propagated or maintained.

In contrast, complete genome sequences of SARS-CoV-2 were made public in early January 2020, and, as of May 2021, many tens of thousands of genome sequences have already been published in online databases. In vitro culture and initiation of in vivo experimental animal modeling have occurred rapidly, followed by extensive basic and clinical testing of diagnostic assays, therapeutics, and vaccines leading to studies on natural history and pathogenesis. The rapidity with which important scientific knowledge about COVID-19 has accrued in just a few months would have astonished scientists in 1918.

RESPONSES TO PSYCHOLOGICAL AND PSYCHOSOCIAL EFFECTS

Then, as now, contemporary photographs show images of horror: stacked bodies, rows of grave markers, and open pits into which bodies are thrown (see Figure 1 in Part I[1]). People were dying alone, in their own homes, with no one to comfort them in their final hours. Mercifully, the horrors of the 1918 pandemic were brief, as the pandemic passed through most towns and cities like lightening and was suddenly gone. In 2019–2021, many months of ever-climbing COVID-19 deaths, lockdown, dread, and uncertainty, have added to the tragedy and led to outbreaks of depression, suicide, anger, hopelessness, and even anomie.

It has often been said that the 1918 pandemic was quickly forgotten, reflecting a global exercise in intentional amnesia; however, a closer look at the legacy of 1918 suggests otherwise. For example, the pandemic inspired many artists. In a Vienna, Austria, apartment, a brilliant painter who always aimed to shock and infuriate, Egon Schiele (1890–1918), lay dying of influenza pneumonia in late October 1918. Schiele's last work was a drawing of his wife, Edith Harms (1893–1918), six months pregnant and dying beside him, also of influenza pneumonia (Figure 2). She died hours after the drawing; Schiele survived another two days. Before dying, he arranged with friend Marta Fein-Spraider (1894–c. 1941) to take a photograph at the moment of his death.[22] There are also the self-portraits of painter Edvard Munch suffering from influenza in his own bedroom; Thomas Wolfe's wrenching account of the death of his beloved brother Benjamin, written down in exacting autobiographical detail (*Look Homeward, Angel* and *O Lost: A Story of the Buried Life*); Katherine Anne Porter's haunting tale of her own survival (*Pale Horse, Pale Rider*), unfolding dream-like to its tragic ending; the surge in the Dada art movement in response to the horrors of the war and the pandemic; the hedonistic escapism of the Roaring Twenties; and the exhaustingly comprehensive files of millions of photos, letters, diaries, and recollections that still survive today, and that still speak to us. The 1918 pandemic was never really forgotten. We just forgot that we had never forgotten it.

It will probably be a long time until we can fairly look back to compare and contrast all of the effects of these centenary pandemics. Both came at times of upheaval, periods in which dramatic changes seemed inevitable, but in what direction could not be predicted. In 1918, the world had been stunned by the carnage of the Great War (around 40 million deaths), including the senseless deaths of a whole generation of young men, leaving widows, orphans, and broken, grieving families. Shock, disbelief, anhedonia, and dark cynicism prevailed. Then, just at the war's end, the pandemic came, lightening-like, killing tens of millions more.

The year 1918 marked the last year of the deadliest war, and the first year of the deadliest pandemic in human history, up to that time. Looking back across the last century, we can see that the "War to End All Wars" did not, in fact, end wars, and that the deadliest pandemic did not end deadly pandemics. A century later, tragic wars and tragic pandemics are still occurring, and we are still struggling to deal with them. We retain a hope that we can one day end wars, pandemic diseases, and many other human ills, but, in May 2021, as the COVID-19 pandemic still spreads, it is hard for many to be optimistic.

Like global wars, pandemics are clearly existential threats. Even in the midst of fear and loss, such deadly challenges can bring out the best in us. If 2021 seems the worst of times, we can still look back, as did centenarian Marilee Shapiro Asher,[1] down a path that is dark and long, but still seeded with hope. Asher, the artist who survived both the 1918 and the 2020 pandemics, died at home on September 11, 2020. Through two pandemics, four major wars, a Great Depression, and a Great Recession, Asher saw more than a century of progress and struggle and was able to find a life-long joy and fulfillment. Near the end, she confronted humankind's latest existential challenge, COVID-19, and survived it. If we can remember the best in Asher and in humankind, so will most of the rest of us. AJPH

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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A Centenary Tale of Two Pandemics: The 1918 Influenza Pandemic and COVID-19, Part I

David M. Morens, MD, Jeffery K. Taubenberger, MD, PhD, and Anthony S. Fauci, MD

See also Leavitt, p. 996.

Separated by a century, the influenza pandemic of 1918 and the COVID-19 pandemic of 2019–2021 are among the most disastrous infectious disease emergences of modern times. Although caused by unrelated viruses, the two pandemics are nevertheless similar in their clinical, pathological, and epidemiological features, and in the civic, public health, and medical responses to combat them. Comparing and contrasting the two pandemics, we consider what lessons we have learned over the span of a century and how we are applying those lessons to the challenges of COVID-19. (*Am J Public Health*. 2021;111:1086–1094. https://doi.org/10.2105/AJPH.2021.306310)

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It was the best of times when renowned artist Marilee Shapiro Asher finally left the hospital, in April 2020, after five days of struggling with COVID-19 (caused by severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]).[1] We know that Asher was glad to be home, looking forward to returning to her studio and her exhibitions, because she had already written about what it was like surviving an eerily similar respiratory illness as a 6-year-old girl: her most vivid memory was not the days in bed, but finally being allowed to get up one morning and join her family at the breakfast table, a joyous event signaling recovery.

That joyous breakfast was in 1918, when Marilee survived the so-called "Spanish" influenza, estimated to have killed at least 50 million people worldwide, one of the deadliest single events in all of human history.[2] Recovering from COVID-19 last year, Asher, then in her 108th year, was among a dwindling cohort of 1918 pandemic survivors who not only still remembered it but who also were now facing another lethal pandemic: COVID-19. Childhood memories like Asher's were supplemented by an enormous body of medical, scientific, public heath, and societal information concerning that earlier pandemic. It is worth reflecting on this body of collective memory as we travel through the dark uncertainty of another pandemic that threatens and impacts millions of lives. From the vantage point of an additional century of medical and social progress, it is hoped that we are mastering history's lessons.

THE 1918 INFLUENZA PANDEMIC

Before 1918, influenza was a poorly understood disease of unknown cause. The 1918 pandemic appeared suddenly in a few populous cities including in China in June[3] and in Northern Europe in July–August 1918.[4] It rebounded over most of the world (in both the Northern and Southern Hemispheres) in September–November 1918, featuring from one to several additional recurrences beginning in late 1918–early 1919.[2,5,6] In the United States, an estimated 675 000 people died in the first year, equivalent to about 2.16 million deaths in today's much larger population, an approximate 1% case–fatality ratio.[2] The explosivity of the pandemic was staggering. Bodies were sometimes "stacked like cord wood" in hospitals, or by roads outside of cemeteries; coffins had to be mass produced on a large scale (Figure 1).

Over a few years, the 1918 pandemic settled into a pattern of less fatal annual seasonality. Human influenza A viruses were first isolated in 1933.[7] At that time, isolation materials from the 1918 pandemic were thought not to exist; however, decades later (1996–2005) the viral genome was fully sequenced from RNA fragments in pathological materials of 1918–1919 pandemic victims; soon thereafter, it was reconstructed as a fully infectious virus and studied experimentally.[7] Viral descendants of the 1918 "founder" virus are still circulating today as seasonal influenza A viruses; subsequent pandemics in 1957, 1968, and 2009 all resulted from genetic

FIGURE 1— Both the (a) 1918 and the (b) 2020 Pandemics Featured Hastily Assembled Cemeteries, Mass Graves, and Collections of Unburied Bodies

Note. Photo by Willy Kurniawan, courtesy of Reuters. Printed with permission.

updating of the 1918 virus via a mutational mechanism called gene segment reassortment.[8] Over the period of a century, viral descendants of this single emergent virus have caused tens of millions of additional deaths, adding to the tragic losses of 1918. Fortunately, to date, there is evidence that public health restrictions to control COVID-19 (e.g., social distancing, mask wearing, business closures) are controlling influenza as well. As we are in the early second year of the COVID-19 pandemic, we cannot predict with certainty whether the virus will persist as the 1918 influenza virus did, or die out in the face of growing population immunity associated with natural infection and new COVID-19 vaccines.

CLINICAL AND PATHOLOGICAL COMPARISONS

Although caused by unrelated viruses, the two diseases are similar in their clinical features (Figure 2). Both are respiratory viruses transmitted and acquired via respiratory inoculation, and both emerged in global populations with little or no preexisting immunity. Typical signs and symptoms of both full-blown diseases include fever, chills, fatigue, muscle aches, nasal congestion or rhinorrhea, headache, and cough, with variable sore throat, dyspnea, and nausea, vomiting, or diarrhea. Both diseases feature many mild, atypical, and asymptomatic

Comparing pandemics: 1918 influenza and 2019 COVID-19

Variable	1918 Influenza	2019 COVID-19
Infectious Agent	Novel respiratory virus	Novel respiratory virus
Mechanism of emergence	Host switching	Host switching
Source of emergence	Wild waterfowl (*Anseriformes*)	**Wild *Rhinolophus* bat**
Cell receptor	**Sialic acids on respiratory epithelia**	**ACE2 receptor on multiple cells, multiple organs**
Viral preadaptation	Virus preadapted or quickly adapted to human spread	Virus preadapted or quickly adapted to human spread
Clinical & Pathological Disease		
Clinical	Upper respiratory disease, pneumonia	Upper respiratory disease, pneumonia
	No viremia, no systemic disease	**Viremia with systemic disease, vascular damage**
Complications	Secondary bacterial pneumonia, empyema	**Secondary bacterial pneumonia less frequent; Multisystem disease**
Pulmonary pathology	Viral pneumonia, DAD, edema	Viral pneumonia, DAD, edema
	Microthrombi, variable hemorrhage in some	Microthrombi, variable hemorrhage in some
	Aberrant immune response	Aberrant immune response
	Massive neutrophilic infiltrates in some	Neutrophilic infiltrate **less frequent**
Epidemiology		
Preexisting immunity	Possible immunity in older persons	**Prior immunity status not yet certain**
Mortality	Case–fatality ratio about 1% in United States	Case–fatality ratio estimated around 1% in United States
	Higher mortality in infants, elderly, chronically ill	**Children and young adults: lower incidence & severity**
	Pregnant women/fetuses	**No extreme mortality in pregnant women/fetuses?**
	Mortality peak in adults aged 20–40 years	**No mortality peak in adults aged 20–40 years**
Morbidity	Morbidity peak in school-aged children	**Low morbidity in children & young adults**
Origin & spread	Spread by travel, from big cities, spread outward	Spread by travel, from big cities, spread outward
	R_0 estimated to be about 1–2	R_0 about 1–2, but varies greatly
	Spread by droplet, aerosol, hands and fomites	Spread by droplet, aerosol, hands, and fomites
	Asymptomatic carriers	Asymptomatic carriers
	Super spreaders probable	Super spreaders
	Induces full or partial protective immunity	Induction of full or partial protective immunity not established
	Persisted by means of viral evolution	Persistence potential not yet established
Public Health Responses	Closures, isolation, social distancing, masks	Closures, isolation, social distancing, masks
	Bacterial vaccines	Bacterial vaccines, SARS-CoV-2 **viral vaccines**
Treatment	Supportive care, plasma therapy, no ICUs	Supportive care, plasma therapy, **ICUs**
	No antibiotics or antivirals	**Antibiotic, antivirals, glucocorticoids**
	Quack and untried remedies	Quack and untried remedies
Psychosocial Reactions	Widespread disease fear	Widespread disease fear
	Common defiance of public health recommendations	Common defiance of public health recommendations
	Altruism and helping others was common	Altruism and helping others was common

FIGURE 2— Comparing Pandemics: 1918 Influenza and 2019 COVID-19

Note. DAD = diffuse alveolar damage; ICU = intensive care unit; SARS-CoV-2 = severe acute respiratory syndrome-2.

infections, but also complicating, sometimes fatal, pneumonias in about 2% of those clinically ill. In the case of COVID-19, unusual as well as late complications are being noted with increasing frequency, including tissue and organ damage, neurological complications, and inflammatory syndromes. It is not clear to what extent, if any, such complications occurred with 1918 influenza, although, curiously, neurological complications were said to be prominent in the 1889 influenza pandemic.

Typical influenza pneumonia in 1918 occurred in a bronchopneumonic pattern associated with secondary bacterial pneumonias caused by pathogens carried silently in the upper respiratory tract, including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and

Staphylococcus aureus.[9,10] Initial autopsy data from COVID-19 patients suggest a similar histologic picture of viral pneumonic damage with, however, fewer secondary bacterial pneumonias,[11] perhaps in part reflecting widespread use of broad-spectrum antibiotics not available in 1918.

In both diseases, severe pneumonias have been associated histologically with diffuse alveolar damage, hyaline membrane formation, pulmonary edema, and, often, neutrophilic infiltrates[11,12] (Figure 3). Autopsy studies of COVID-19 patients reveal widespread medium- and small-vessel thromboses[13]; pulmonary small-vessel thrombosis was prominent in 1918 influenza as well[14,15]; however, it has been less frequently observed in more recent influenza autopsies (e.g., during the 2009 H1N1 pandemic[16,17]). In contrast to 1918, in which tissue damage was mostly pulmonary, in COVID-19, tissue damage has been observed in tissues and organs systemically.[18]

Important pathological differences between the two infections (Figure 2) include the following: influenza infects primarily by binding to sialic acid receptors found on respiratory epithelial cells, whereas SARS-CoV-2 infects various cells of the respiratory tract, gastrointestinal enterocytes, and arterial and venous endothelial cells, as well as arterial smooth-muscle cells, presumably by binding to ACE2 receptors.[19] As influenza caused by human-adapted influenza viruses is not associated with viremia, live influenza virus has little direct interaction with the systemic immune system, explaining in part why natural and vaccine-induced protective immunity against influenza is often imperfect. Preliminary data from COVID-19, however, suggest systemic infection of multiple organs,[20–22] which can potentially elicit protective immunity more durable than that of influenza, although duration of COVID-19 protection remains to be determined, and reinfections have been documented.[23]

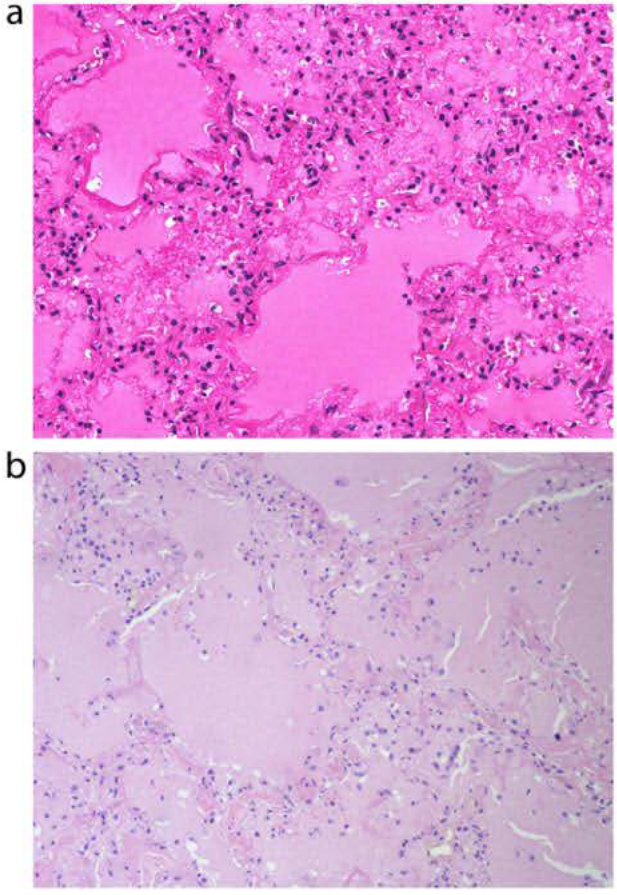

FIGURE 3— Representative Pulmonary Histopathology of (a) Fatal 1918 Influenza and (b) Fatal SARS-CoV-2 Infection Showing Acute Diffuse Alveolar Damage With Pulmonary Edema and Hyaline Membranes

Source. Sauter et al.[11] and Sheng et al.[12]
Note. SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. The histologic patterns of acute diffuse alveolar damage are virtually indistinguishable.

EPIDEMIOLOGICAL COMPARISONS

It is extremely difficult to know the exact origin of any pandemic disease, because emerging infectious agents arise via host switching from an animal to a human, after which successful adaptation associated with human-to-human transmission occurs.[24–26] This process necessarily takes time: by the time the new disease is eventually recognized, its occult beginnings are unlikely to be discovered. In this regard, it is noteworthy that over many centuries, from the 1500s until the modern era, almost all influenza pandemics were first recognized in Asia or Southeast Asia, and then spread westward to Europe and, at some point after the 16th century, from Europe or Asia to the Western Hemisphere.[27] As some of the earliest evidence of the existence of the 1918 pandemic came from China,[28] this same historical pattern remains plausible, although the geographic

origin of the 1918 pandemic remains unknown, with hypotheses ranging from China to Europe to the United States.[28–30]

When the 1918 pandemic was first recognized clinically and epidemiologically in July 1918, and again in September–November 1918, it was robustly emerging almost simultaneously in large populous cities all over the globe, in both the Northern and Southern Hemispheres. This pattern indicates that rather than spreading from city to city along travel routes at the time of such explosive emergence, many regions of the world must have been seeded by the virus previously.[2] Presumably, the relatively slow global spread of infections by ship, rail, and other means of human travel went undetected until international metropolitan mortality data began to show excess respiratory mortality increases. From these large cities, the disease spread outward to smaller towns and to rural areas, and also caused additional rounds of global spread by ships.

Because of modern international air travel, COVID-19 spread slightly more rapidly than 1918 pandemic influenza; however, the patterns of spread were probably very similar: (1) local emergences and initial spread that went undetected because of low case–fatality, followed by (2) local, national, and eventually international movement of infectious persons, leading to seeding of cases in crowded metropolitan areas, followed by (3) clusters of respiratory disease mortality that were eventually detected in sensitive metropolitan mortality data, followed quickly by (4) massive global emergence.[31]

SARS-CoV-2 was first detected in Wuhan, Hubei Province, China, and spread simultaneously outward within China and via international air routes. It is highly likely that SARS-CoV-2 emerged from within a tight phylogenetic cluster of *Sarbecoviruses* infecting *Rhinolophus* (horseshoe) bats found mostly in Southwest China and contiguous areas of Cambodia, the Lao PDR, Myanmar, and Vietnam.[26,32,33] How the virus got to the place of its initial detection, at least 850 miles away in Wuhan, remains unknown; possible explanations include the mobility and long-distance ranges of various bat species, undetected cross-infection from *Rhinolophus* to other bat species, or infection and movement of secondarily infected animal hosts or of humans.

EMERGENCE VIA ANIMAL-TO-HUMAN VIRAL HOST SWITCHING

The 1918 pandemic "founder" virus was genetically and functionally very similar, in sequences of all eight genes, to avian viruses that then existed, and that still exist, in the global reservoir of wild waterfowl (*Anseriformes*).[34] It is unknown whether an avian virus host-switched directly into humans or first switched into a different host, perhaps another mammal, and from there to humans.[35] However, phylogenetic analysis of the human virus suggests that emergence must have occurred in or shortly before 1918.[2]

SARS-CoV-2 is very close genetically to numerous enzootic *Sarbecoviruses* of *Rhinolophus* bats found in Southwest China and contiguous areas, suggesting one of three possibilities[32,36–38]: (1) an as-yet-undiscovered enzootic *Sarbecovirus* identical to SARS-CoV-2 emerged into humans directly; (2) a different but closely related *Sarbecovirus* emerged directly into humans and spread silently for some period of time, accumulating new mutations as it adapted to human transmission; or (3) humans were infected via an intermediate animal host that had originally been infected by a *Rhinolophus*-transmitted *Sarbecovirus*.[36,37,39] Thus, 1918 influenza and SARS-CoV-2 share the same origin mysteries of direct versus indirect emergence from a natural animal host, and of extent of postemergence genetic adaptation to humans.

Both 1918 influenza and COVID-19 are among the deadliest examples of viral emergences from the animal–human interface.[33] How this happens and what we can do to prevent it from happening are among the most important areas of research in the study of emerging infections.[33,40] The host-switching ability of both viruses may be an established evolutionary mechanism: both 1918 influenza and SARS-CoV-2 are promiscuous in their ability to infect mammals, facilitating broad epidemicity and epizooticity. In 1918, the human virus was quickly transmitted to pigs,[41] while housecats were sometimes infected by their owners (as seen in previous influenza pandemics). A century later, humans and pigs are still frequently exchanging their influenza viruses.[42] Unexpected deaths of chimpanzees and gorillas in 1918 were thought to be attributable to influenza. Horses, dogs, seals, and other animals have also been involved in influenza virus exchanges.[43] SARS-CoV-2 has infected not only *Rhinolophus* bats, their reservoir host, but also cats, dogs, minks, and other animals[44]; closely related SARS-like viruses have infected pangolins (*Manis javanica*, a species of anteater).[32] Such efficient intra- and interspecies exchanges may have enhanced evolution and survival of both viruses.

VIRAL TRANSMISSION

Both viruses are transmitted by the respiratory route via large droplets,

fine-particle (< 5 μm) aerosols, or by hands or fomites contaminated with respiratory secretions. Both viruses spread by silent transmission—that is, transmission by presymptomatic (incubating) people, by asymptomatic infected people, by people with mild or atypical symptoms who are not recognized as being potentially infectious, and, less commonly, by people who have recovered from illness but may still be excreting virus.[39,45] Unlike influenza, SARS-CoV-2 infects enteric cells, but gastrointestinal transmission has not yet been shown to be important.

Preliminary evidence suggests roughly equivalent effects of environmental variables on spread of both viruses (e.g., effects of airflow, temperature, and humidity). This has important implications for COVID-19 public health control measures such as social distancing and controlling airflow in hospitals, nursing homes, workplaces, and recreational venues, such as restaurants and bars.

Regarding seasonality, 1918 pandemic influenza was first detected in the early summer of the Northern Hemisphere and did not spread globally until September–October 1918. When it did so, it aggressively spread not only in the Northern Hemisphere but also in the Southern Hemisphere's spring season (e.g., in South Africa[46] and in New Zealand[47]). Five hundred years of observation[48] suggest that influenza pandemics can appear at any time of year, but when they arrive in summer they are likely to be somewhat blunted until they rebound more forcefully in the fall; when pandemics arrive at other times of year, summers seem to temporarily slow viral spread.[48] This pattern was seen in both the 1957 and 2009 influenza pandemics; in the United States, both pandemics arrived in the spring, slowed down in the summer, and then picked up in the fall. The presumed reasons for this pattern include physical effects of temperature and humidity on viral spread and more summer hours spent outdoors where airflow is optimal and crowding usually less extreme. To date, seasonal effects on COVID-19 spread have not been fully documented because few regions have been in the throes of COVID-19 for much more than a full calendar year. Moreover, the effects of season and of often-intermittent and incomplete public health control efforts are hard to disentangle.

PATTERNS OF MORBIDITY AND MORTALITY

In all circumstances studied over the past 130 years, except in 1918–1920, patterns of age-specific morbidity, mortality, and case–fatality for pandemic influenza have been similar. Because influenza pandemics emerge when all or most of the global population lacks immunity to the new pandemic virus, moderately high attack rates within the first year, usually between 30% and 60% of the population, are common. Age-specific morbidity patterns have been highly similar for known influenza pandemics, featuring peak morbidity rates in school-aged children and young adults, slightly lower rates in both very young children and in adults aged 30 to 55 years, and much lower rates at older ages (Figure 4). This pattern presumably reflects exposure risks related to school, work, and other congregating activities, as well as the possibility of prior exposure to related influenza viruses within the older age group.

Overall influenza mortality varies significantly, with some pandemic viruses being highly pathogenic (approximate 1% case–fatality in the United States in the 1918 pandemic vs less than 0.05% case–fatality in the 2009 pandemic). The elderly; people with serious respiratory, cardiac, metabolic, and other diseases; and pregnant women are always at elevated mortality risk from influenza.

With the exception of 1918–1920, pandemic and seasonal influenza exhibit a characteristic mortality pattern. Age-specific influenza mortality is classically U-shaped, with elevated mortality in infants and young toddlers and the elderly, but with very low mortality at all ages in between. A different pattern was seen in 1918–1920: a W-shaped pattern (Figure 4) featured a third mortality peak in those aged 20 to 40 years. This pattern, never seen before or since, disappeared entirely in the early 1920s.[50,51] It remains unexplained, and, while likely not a signature of the 1918 virus, it may be related to preexisting age cohort–specific, cross-protective immunity.

In the early stages of the COVID-19 pandemic, morbidity and mortality patterns are still not fully established, in part because of the relatively high percentage of asymptomatic infections coupled with underdiagnosis of cases. Overall case– and infection–fatality ratios, which are population structure–dependent, have been estimated from as high as 3% to well below 1%.[52] Speculative theories to explain low morbidity and mortality in the young include (1) protection afforded by prior and recent exposure to circulating endemic coronaviruses, two of which—HCoV-HKU1 and HCoV-OC43—are β-coronaviruses, albeit not closely related to SARS-CoV-2; (2) increased exposures to other infectious agents that stimulate generic innate immune responses; or (3) immune enhancement mechanisms.[39]

In contrast to influenza, which causes high mortality and high fetal loss, significant COVID-19 mortality in pregnant

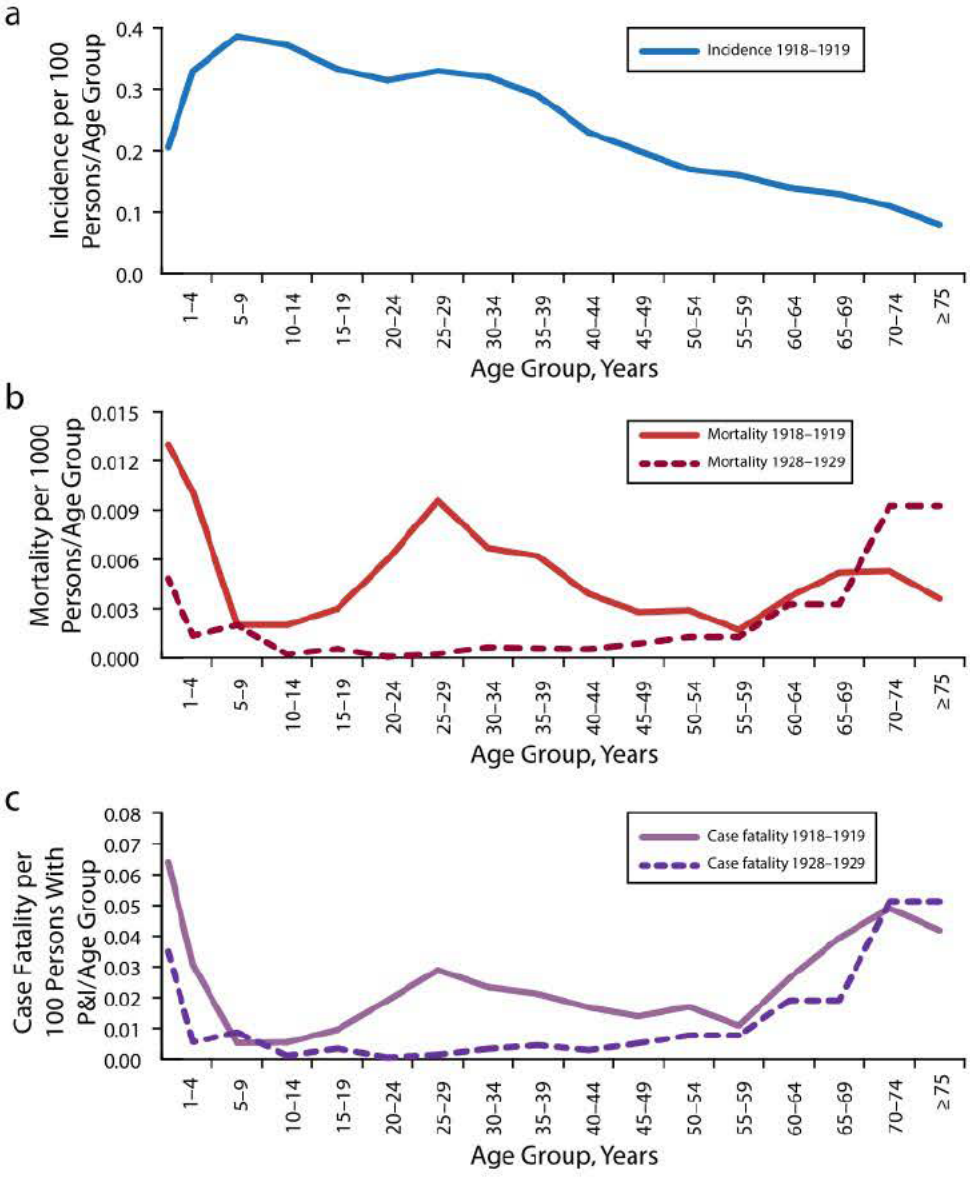

FIGURE 4— Age-Specific Morbidity and Mortality of Influenza in 1918–1919 and, for Comparison, in 1928–1929, as Determined by US "P and I" Data by (a) Incidence per 100 Persons Ill With Pneumonia and Influenza per Age Group; (b) Mortality per 1000 Persons per Age Group; and (c) Case–Fatality

Source. Morens and Taubenberger.[49]
Note. P and I = pneumonia and influenza. Parts b and c compare the W-shaped curves of age-specific mortality and case–fatality seen in 1918–1919 with more typical U-shaped curves from 1928 to 1929. Between 1889 and the present time, U-shaped curves have been seen in all pandemics and seasonal epidemics except for 1918 and the several years thereafter. Morbidity and mortality data reflecting diagnoses of pneumonia and influenza (so-called "P and I") are still widely used today for epidemiological purposes (e.g., for estimating total influenza deaths during periods of influenza prevalence) because incomplete morbidity reporting and imperfect death certificate accuracy greatly underestimate infections and deaths from influenza and its secondary bacterial complications. National or large-population data permitting similar calculations for COVID-19 are not yet available, although preliminary data suggest that age-specific mortality is very low in infants and children, rising regularly with age thereafter.

women and their fetuses is only now beginning to become better appreciated, although the extent of maternal and fetal risks remains to be fully established.[53–56] In 1918, as in 2020,[57] mortality was higher in the poor, in African Americans and Native Americans, in health care workers, and in workers in crowded occupations.[50,58–60] These patterns, observed for most infectious diseases, reflect societal inequalities and inadequate occupational safety measures.

As descendants of the 1918 influenza virus persist to this day,[8] a question

arises about whether SARS-CoV-2 will do the same. Furthermore, a possibility to be considered is whether, similar to influenza, it will elicit a weakly protective immune response and then circumvent that response with further viral evolution by antigenic drift or other mechanisms such as viral genetic recombination. The recent (in late 2020) emergences of SARS-CoV-2 genetic variants, some apparently associated with increased transmissibility and immune escape,[61] may be an early answer to this question, auguring future COVID-19 reemergences caused by antigenically drifting strains, in a manner analogous to the genetic drift of influenza A viruses. Descendants of the 1918 virus still circulate; we can only speculate whether SARS-CoV-2 or its descendants will still be circulating in 2120. (Continued in Part II.[62]) AJPH

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CONTRIBUTORS

All authors contributed equally to the content and writing.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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From: Embry, Alan (NIH/NIAID) [E]
Sent: Fri, 18 Jun 2021 02:03:35 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Article/papers
Attachments: 1 Civets in markets.pdf, 2 Bats are natural reservoirs of SARS-like coronaviruses.pdf, 3 Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor.pdf, 4 Discovery of a rich gene pool of bat SARSrelated coronaviruses.pdf

Tony,

The article you referenced is here: https://www.nature.com/articles/d41586-021-01383-3

If you follow the link at the end of the article, it will lead you to the 4th paper attached.

Also attached are other key papers in chronological order.

Thanks,
Alan

Isolation and Characterization of Viruses Related to the SARS Coronavirus from Animals in Southern China

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A novel coronavirus (SCoV) is the etiological agent of severe acute respiratory syndrome (SARS). SCoV-like viruses were isolated from Himalayan palm civets found in a live-animal market in Guangdong, China. Evidence of virus infection was also detected in other animals (including a raccoon dog, *Nyctereutes procyonoides*) and in humans working at the same market. All the animal isolates retain a 29-nucleotide sequence that is not found in most human isolates. The detection of SCoV-like viruses in small, live wild mammals in a retail market indicates a route of interspecies transmission, although the natural reservoir is not known.

Severe acute respiratory syndrome (SARS) recently emerged as a human disease associated with pneumonia (*1*). This disease was first recognized in Guangdong Province, China, in November 2002. Subsequent to its introduction to Hong Kong in mid-February 2003, the virus spread to more than 30 countries and caused disease in more than 7900 patients across five continents (*2*). A novel coronavirus (SCoV) was identified as the etiological agent of SARS (*3*, *4*), and the virus causes a similar disease in cynomolgous macaques (*5*). Human SCoV appears to be an animal virus that crossed to humans relatively recently. Thus, identifying animals carrying the virus is of major scientific interest and public health importance. This prompted us to examine a range of domestic and wild mammals in Guangdong Province.

Because the early cases of SARS in Guangdong reportedly occurred in restaurant workers handling wild mammals as exotic food (*6*), our attention focused on wild animals recently captured and marketed for culinary purposes. We investigated a live-animal retail market in Shenzhen. Animals were held, one per cage, in small wire cages. The animals sampled included seven wild, and one domestic, animal species (Table 1). They originated from different regions of southern China and had been kept in separate storehouses before arrival to the market. The animals remained in the markets for a variable period of time, and each stall holder had only a few animals of a given species. Animals from different stalls within the market were sampled. Nasal and fecal samples were collected with swabs and stored in medium 199 with bovine serum albumin and antibiotics. Where possible, blood samples were collected for serology. Before sampling, all animals were examined by a veterinary surgeon and confirmed to be free of overt disease. Serum samples were also obtained, after informed consent, from traders in animals (n = 35) and vegetables (n = 20) within the market. Sera (n = 60) submitted for routine laboratory tests from patients hospitalized for nonrespiratory disease in Guangdong were made anonymous and used for comparison.

Nasal and fecal swabs from 25 animals were tested for SCoV viral nucleic acid by using reverse transcription–polymerase chain reaction (RT-PCR) for the N gene of the human SCoV. Swabs from four of six Himalayan palm civets were positive in the RT-PCR assay (Table 1). All specimens were inoculated into FRhK-4 cells as previously described for virus isolation (*3*). A cytopathic effect was observed in cells inoculated with specimens from four Himalayan palm civets (*Paguma larvata*), two of which also positive for coronavirus in the original specimen by RT-PCR. A virus was also detected by virus isolation and direct RT-PCR from the fecal swab of a raccoon dog (*Nyctereutes procyonoides*). No virus was detectable in six other species sampled. Electron microscopy of one infected cell supernatant (SZ16) showed viral particles with a morphology compatible with coronavirus (fig. S1). Sera from five animals had neutralizing antibody to the animal coronavirus; these were from three palm civets, a raccoon dog, and a Chinese ferret badger, respectively (Table 1).

To further validate the results from the neutralization test, a Western blot assay was used to detect SCoV-specific antibodies from these animal serum samples (Fig. 1). Indications of positive antibodies were observed from samples SZ2, SZ3, SZ11, and SZ17 (which were also positive in the neutralization assay) and from the positive control human serum. No positive signal was observed from those serum samples that were negative in the neutralization test. There was insufficient serum left over from the raccoon dog (SZ13) to be analyzed by this assay.

Table 1. Animal species tested for coronavirus detection. Abbreviations of animal species: B, beaver (*Castor fiber*); CFB, Chinese ferret-badger (*Melogale moschata*); CH, Chinese hare (*Lepus sinensis*); CM, Chinese muntjac (*Muntiacus reevesi*); DC, domestic cat (*Felis catus*); HB, hog-badger (*Arctonyx collaris*); HPC, Himalayan palm civet (*P. larvata*); RD, raccoon dog (*N. procyonoides*) (*9*). N, nasal sample; F, fecal sample; titer to SZ16, neutralizing antibody titer to SZ16; + denotes positive by RT-PCR or virus isolation; * denotes the PCR product or virus isolates sequenced in the study. ND, not done.

Sample number	Animal	Virus detection				Titer to SZ16
		RT-PCR		Isolation		
		N	F	N	F	
SZ1	HPC	+*	+			ND
SZ2	HPC	+	+			40
SZ3	HPC	+	+	+*		40
SZ4	HB					<20
SZ5	B					<20
SZ6	DC					ND
SZ7	DC					<20
SZ8	CH					ND
SZ9	CH					<20
SZ10	CM					<20
SZ11	CFB					160
SZ12	CFB					<20
SZ13	RD		+		+*	≥640
SZ14	CM					<20
SZ15	B					<20
SZ16	HPC	+	+	+*	+	<20
SZ17	HPC			+		≥640
SZ18	B					<20
SZ19	CH					<20
SZ20	CH					<20
SZ21	DC					<20
SZ22	DC					<20
SZ23	HB					ND
SZ24	HB					ND
SZ25	HPC			+		ND

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Sera from humans working in the market were tested for antibody to SZ16 virus by neutralization and indirect immunofluorescence assays. Although 8 out of 20 (40%) of the wild-animal traders and 3 of 15 (20%) of those who slaughter these animals had evidence of antibody, only 1 (5%) of 20 vegetable traders was seropositive. None of these workers reported SARS-like symptoms in the past 6 months. In comparison, none of 60 control sera from patients admitted to a Guangdong hospital for nonrespiratory diseases was seropositive (Table 2).

Two of the virus isolates (SZ3 and SZ16) isolated from the nasal swabs of palm civets were completely sequenced, and the amino acid sequence was deduced. Two other viruses were partially sequenced, from the S gene to the 3′ end of the virus (GenBank accession numbers AY304486 to AY304489). Viral RNA sequences from these original swab samples from animal were confirmed in an independent laboratory (*7*). The full-length genome sequences had 99.8% homology to the human SCoV, which indicates that the human and animal SCoV-like viruses were closely related. Phylogenetic analysis of the S gene of both human and animal SCoV-like viruses indicated that the animal viruses are separate from the human virus cluster (Fig. 2 and fig. S2). However, the viruses SZ1, SZ3, and SZ16 from palm civets were phylogenetically distinct. The viruses SZ3 and SZ16 had 18 nucleotide differences between them over the 29,709–base pair (bp) genome, whereas the human SCoV isolated from five geographically separate sites (GZ50, CUHK-W1, Tor-2, HKU-39848, and Urbani) differed by only 14 nucleotides (nt). Nevertheless, animal virus SZ13 (rac-

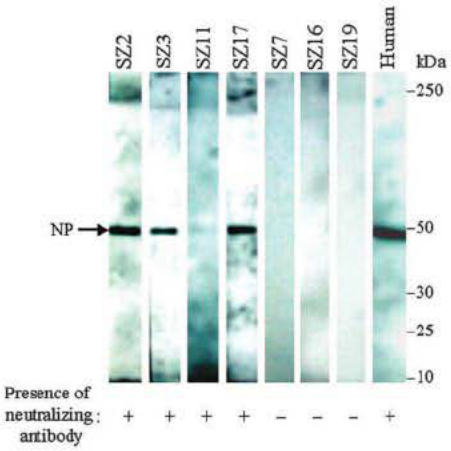


Fig. 1. Detection of antibodies against recombinant nucleocapsid protein of SCoV in animal sera by Western blot assay. Recombinant nucleocapsid protein (NP, 49.6 kD) was used as an antigen to detect anti-SCoV antibodies in animal sera. Protein A-HRP was used as a secondary antibody, and reactive bands were visualized by the enhanced chemiluminesence Western blotting system. A serum sample from a convalescent SARS patient was used as a positive control. Blots reacted with animal (SZ2, SZ3, SZ11, SZ17, SZ7, SZ16, or SZ19) or human sera are indicated. Results from the neutralization test for SCoV-specific antibodies in these serum samples are also shown.

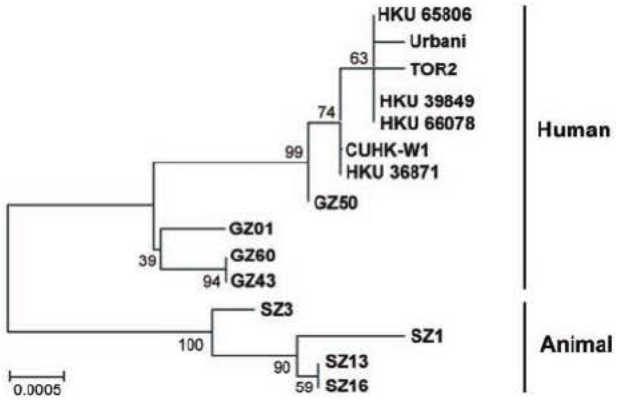
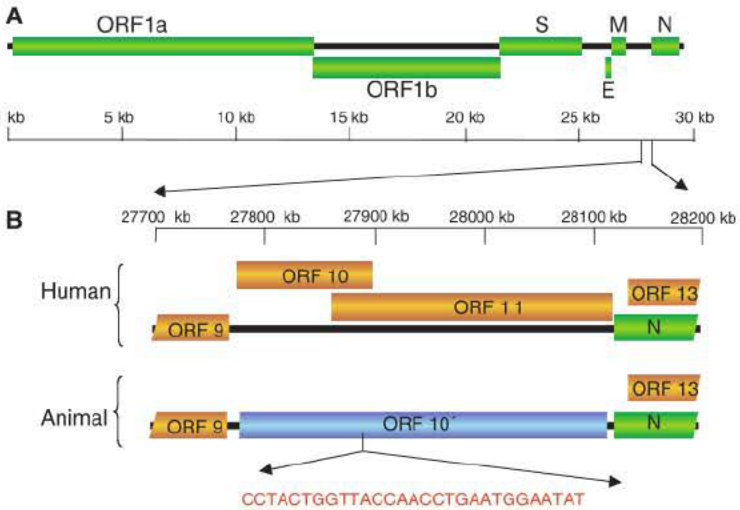


Fig. 2. Phylogenetic analysis of the nucleotide acid sequence of the spike gene of SCoV-like viruses. Nucleotide sequences of representative SCoV S genes (S gene coding region 21477 to 25244, 3768 bp) were analyzed. The phylogenetic tree was constructed by the neighbor-joining method with bootstrap analysis (1000 replicates) using MEGA 2 (*10*). Number at the nodes indicates bootstrap values in percentage. The scale bar shows genetic distance estimated using Kimura's two-parameter substitution model (*11*). In addition to viruses sequenced in the present study, the other sequences used in the analysis could be found in GenBank with accession number: from AY304490 to AY304495, AY278741, AY278554, AY278491, AY274119, and AY278489.

Table 2. Prevalence of antibody to animal SCoV SZ16 in humans. Controls are serum specimens from patients hospitalized for nonrespiratory diseases in Guangdong made anonymous.

Occupation	Sample numbers	Antibody positive (%)
Wild-animal trader	20	8 (40)
Slaughterer of animals	15	3 (20)
Vegetable trader	20	1 (5)
Control	60	0 (0)



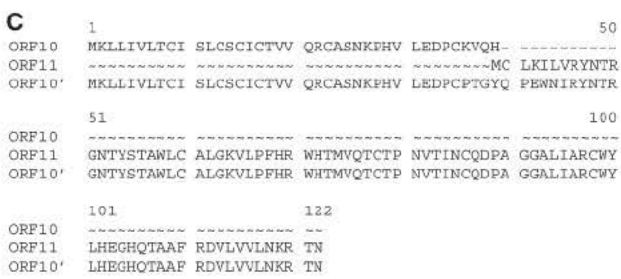


Fig. 3. A 29-nt deletion in the human SCoV genome. (**A**) Genetic organization of SCoV-like viruses found in humans and animals. ORFs 1a and 1b, encoding the nonstructural polyproteins, and those encoding the S, E, M, and N structural proteins are indicated (green boxes). (**B**) Expanded view of the SCoV genomic sequence (27700 nt to 28200 nt, based on AY278554 numbering). ORFs for putative proteins and for N in human isolates are indicated as brown and green boxes, respectively (*8*). An extra 29-nt sequence is present downstream of the nucleotide of 27868 of the animal SCoV (based on AY278554 numbering). The presence of this 29-nt sequence in animals isolates results in fusing the ORFs 10 and 11 (top) into a new ORF (bottom; ORF10′, light blue box). (**C**) Protein sequence alignment of ORF10 and 11 from human isolates and ORF 10′ from animal isolates.

Table 3. Nucleotide sequence variation of the S gene of animal and human SCoV. The nucleotide residues are based on AY278554 numbering. Nonsilent mutations are highlighted in red. Dash indicates a nucleotide deletion.

	Nucleotide residue																																							
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	5			
	6	6	6	6	7	7	1	1	1	2	2	2	4	5	5	5	6	9	9	9	0	1	2	2	4	5	6	7	7	8	8	1	1	3	5	8	9	0		
	2	9	9	9	0	0	3	5	9	0	0	5	0	0	0	5	4	1	3	7	0	4	0	9	7	7	1	0	3	0	5	5	9	6	5	5	6	1		
Virus	2	0	1	2	0	6	0	7	2	5	7	8	7	2	7	5	6	3	6	8	3	8	5	5	0	8	7	3	7	8	6	6	7	8	1	7	3	2		
SZ3	C	A	T	T	C	A	T	A	T	T	C	A	G	G	G	C	A	A	G	T	G	T	T	C	C	T	C	G	T	G	C	G	C	G	C	T	G	T		
SZ16	C	A	T	T	C	A	T	A	T	C	C	A	G	G	G	C	G	A	G	T	G	A	T	C	C	T	C	G	T	G	C	G	C	T	C	T	G	T		
SZ1	C	A	T	T	C	A	T	A	T	T	C	A	G	G	G	C	G	A	G	T	T	A	T	C	C	T	T	G	T	G	C	G	T	T	T	T	G	T		
SZ13	C	A	T	T	C	A	T	A	T	T	C	A	G	G	G	C	G	A	G	T	G	A	T	C	C	T	C	G	T	G	C	G	C	T	C	T	G	T		
GZ01	C	A	T	T	C	A	C	C	T	C	C	C	A	G	G	T	G	T	C	A	G	T	T	T	T	C	C	A	C	G	T	A	C	G	C	T	A	T		
GZ43	C	–	–	–	G	A	T	C	T	C	C	C	A	G	G	T	G	T	C	T	G	T	T	T	T	C	C	C	A	C	G	C	A	C	G	C	T	A	C	
GZ60	C	–	–	–	G	A	T	C	T	C	C	C	A	G	G	T	G	T	C	T	G	T	T	T	T	C	C	C	A	C	G	C	A	C	G	C	T	A	C	
GZ50	T	A	T	T	C	A	T	C	C	C	C	C	G	A	A	T	G	T	C	T	G	T	T	T	T	C	C	A	C	G	C	A	C	G	T	T	A	T		
CUHK-W1	C	A	T	T	C	A	T	C	C	C	C	C	G	A	A	T	G	T	C	T	G	T	T	T	T	C	C	A	C	T	C	A	C	G	T	T	A	T		
HKU-36871	C	A	T	T	C	A	T	C	C	C	C	C	G	A	A	T	G	T	C	T	G	T	T	T	T	C	C	A	C	T	C	A	C	G	T	T	A	T		
HKU-39848	C	A	T	T	C	G	T	C	C	C	T	C	G	A	A	T	G	T	C	T	G	T	T	T	T	C	C	A	C	T	C	A	C	G	T	T	A	T		
HKU-66078	C	A	T	T	C	G	T	C	C	C	T	C	G	A	A	T	G	T	C	T	G	T	T	T	T	C	C	A	C	T	C	A	C	G	T	T	A	T		
HKU-65806	C	A	T	T	C	G	T	C	C	C	T	C	G	A	A	T	G	T	C	T	G	T	T	T	T	C	C	A	C	T	C	A	C	G	T	T	A	T		
Urbani	C	A	T	T	C	G	T	C	C	C	T	C	G	A	A	T	G	T	C	T	G	T	T	T	T	C	C	A	C	T	C	A	C	G	T	C	A	T		
Tor2	C	A	T	T	C	G	T	C	C	C	T	C	G	A	A	T	G	T	C	T	G	T	G	T	T	C	C	A	C	T	C	A	C	G	T	T	A	T		

coon dog) and SZ16 (palm civet) were genetically almost identical, and transmission or contamination from one host to the other within the market cannot be excluded.

When the full genome of the animal ($n = 2$) and human ($n = 5$, see above) virus groups were compared, the most striking difference was that these human viruses have a 29-nt deletion (5′-CCTACTGGTTACCAACCTGAATGGAATAT-3′, residue 27869 to 27897) that is 246 nt upstream of the start codon of the N gene (Fig. 3). Of human SCoV sequences currently available in GenBank, there was only one (GZ01) with this additional 29-nt sequence. In addition to that, there were 43 to 57 nucleotide differences observed over the rest of the genome. Most of these differences were found in the S gene coding region. The existence of the additional 29-nt sequence in the animal viruses results in demolishing the open reading frames (ORFs) 10 and 11 (*8*) and merging these two ORFs into a new ORF encoding a putative protein of 122 amino acids (Fig. 3). This putative peptide has a high homology to the putative proteins encoded by ORF10 and ORF11. Because ORF11 does not have a typical transcription regulatory sequence for SCoV (*8*), the putative ORF11 reported by others may just be the direct result of the deletion of the 29-nt sequence. BLAST search of this peptide yields no significant match to any other known peptide. Further investigation is required to elucidate the biological significance of this finding.

When the S-gene sequences of the four animal viruses were compared with 11 human SCoV viruses, 38 nucleotide polymorphisms were noted, and 26 of them were nonsynonymous changes (Table 3). The S genes among the four animal viruses had eight nucleotide differences, whereas there were 20 nucleotide differences among 11 human viruses. Thus, the animal viruses, although isolated from one market, are no less divergent than the human viruses isolated from Hong Kong, Guangdong, Canada, and Vietnam. However, whereas 14 (70%) of the 20 polymorphisms among the human viruses were nonsynonymous mutations, only two (25%) of the eight nucleotide substitutions within the animal viruses were. An amino acid deletion (nucleotide positions 21690 to 21692) was observed in two of the human viruses (GZ43 and GZ60). Of the 38 polymorphisms, there were 11 consistent nucleotide signatures that appeared to distinguish animal and human viruses. The observation that the human and animal viruses are phylogenetically distinct (Fig. 2) makes it highly unlikely that the SCoV-like viruses isolated in these wild animals is due to the transmission of SCoV from human to animals.

Our findings suggest that the markets provide a venue for the animal SCoV-like viruses to amplify and to be transmitted to new hosts, including humans, and this is critically important from the point of view of public health. However, it is not clear whether any one or more of these animals are the natural reservoir in the wild. It is conceivable that civets, raccoon dog, and ferret badgers were all infected from another, as yet unknown, animal source, which is in fact the true reservoir in nature. However, because of the culinary practices of southern China, these market animals may be intermediate hosts that increase the opportunity for transmission of infection to humans. Further extensive surveillance on animals will help to better understand the animal reservoir in nature and the interspecies transmission events that led to the origin of the SARS outbreak.

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Supporting Online Material

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Figs. S1 and S2
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Isolation and Characterization of Viruses Related to the SARS Coronavirus from Animals in Southern China

Y. Guan, B. J. Zheng, Y. Q. He, X. L. Liu, Z. X. Zhuang, C. L. Cheung, S. W. Luo, P. H. Li, L. J. Zhang, Y. J. Guan, K. M. Butt, K. L. Wong, K. W. Chan, W. Lim, K. F. Shortridge, K. Y. Yuen, J. S. M. Peiris and L. L. M. Poon

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14. *Yersinia* builds injectisomes when the temperature reaches 37°C, the host's body temperature. Yop secretion is triggered by contact with a target cell or artificially by chelation of Ca^{2+} ions (*15*).
15. Materials and methods are available as supporting material on *Science* Online.
16. Removal of *lcrV* leads to reduced synthesis of YopB and YopD because of a regulatory effect of LcrV on their expression. This undesired effect can be compensated by deleting *yopQ* (*5*).
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Figs. S1 to S8
Tables S1 and S2
References and Notes

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Bats Are Natural Reservoirs of SARS-Like Coronaviruses

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Severe acute respiratory syndrome (SARS) emerged in 2002 to 2003 in southern China. The origin of its etiological agent, the SARS coronavirus (SARS-CoV), remains elusive. Here we report that species of bats are a natural host of coronaviruses closely related to those responsible for the SARS outbreak. These viruses, termed SARS-like coronaviruses (SL-CoVs), display greater genetic variation than SARS-CoV isolated from humans or from civets. The human and civet isolates of SARS-CoV nestle phylogenetically within the spectrum of SL-CoVs, indicating that the virus responsible for the SARS outbreak was a member of this coronavirus group.

Severe acute respiratory syndrome (SARS) was caused by a newly emerged coronavirus, now known as SARS coronavirus (SARS-CoV) (*1*, *2*). In spite of the early success of etiological studies and molecular characterization of this virus (*3*, *4*), efforts to identify the origin of SARS-CoV have been less successful. Without knowledge of the reservoir host distribution and transmission routes of SARS-CoV, it will be difficult to prevent and control future outbreaks of SARS.

Studies conducted previously on animals sampled from live animal markets in Guangdong, China, indicated that masked palm civets (*Paguma larvata*) and two other species had been infected by SARS-CoV (*5*). This led to a large-scale culling of civets to prevent further SARS outbreaks. However, subsequent studies have revealed no widespread infection in wild or farmed civets (*6*, *7*). Experimental infection of civets with two different human isolates of SARS-CoV resulted in overt clinical symptoms, rendering them unlikely to be the natural reservoir hosts (*8*). These data suggest that although *P. larvata* may have been the source of the human infection that precipitated the SARS outbreak, infection in this and other common species in animal markets was more likely a reflection of an "artificial" market cycle in naïve species than an indication of the natural reservoir of the virus.

Bats are reservoir hosts of several zoonotic viruses, including the Hendra and Nipah viruses, which have recently emerged in Australia and East Asia, respectively (*9*–*11*). Bats may be persistently infected with many viruses but rarely display clinical symptoms (*12*). These characteristics and the increasing presence of bats and bat products in food and traditional medicine markets in southern China and elsewhere in Asia (*13*) led us to survey bats in the search for the natural reservoir of SARS-CoV.

In this study, conducted from March to December of 2004, we sampled 408 bats representing nine species, six genera, and three families from four locations in China (Guangdong, Guangxi, Hubei, and Tianjin) after trapping them in their native habitat (Table 1). Blood, fecal, and throat swabs were collected; serum samples and cDNA from fecal or throat samples were independently analyzed, double-blind, with different methods in our laboratories in Wuhan and Geelong (*14*).

Among six genera of bat species surveyed (*Rousettus*, *Cynopterus*, *Myotis*, *Rhinolophus*, *Nyctalus*, and *Miniopterus*), three communal, cave-dwelling species from the genus *Rhinolophus* (horseshoe bats) in the family *Rhinolophidae* demonstrated a high SARS-CoV antibody prevalence: 13 out of 46 bats (28%) in *R. pearsoni* from Guangxi, 2 out of 6 bats (33%) in *R. pussilus* from Guangxi, and 5 out of 7 bats (71%) in *R. macrotis* from Hubei. The high seroprevalence and wide distribution of seropositive bats is expected for a wildlife reservoir host for a pathogen (*15*).

The serological findings were corroborated by poylmerase chain reaction (PCR) analyses with primer pairs derived from the nucleocapsid (N) and polymerase (P) genes (table S1). Five fecal samples tested positive, all of them from the genus *Rhinolophus*: three in *R. pearsoni* from Guangxi and one each in *R. macrotis* and *R. ferrumequinum*, respectively, from Hubei. No virus was isolated from an inoculation of Vero E6 cells with fecal swabs of PCR-positive samples.

A complete genome sequence was determined directly from PCR products from one of the fecal samples (sample Rp3) that contained relatively high levels of genetic material. The genome organization of this virus (Fig. 1), tentatively named SARS-like coronavirus isolate Rp3 (SL-CoV Rp3), was essentially identical to that of SARS-CoV, with the exception of three regions (Fig. 1, shaded boxes). The overall nucleotide sequence identity between SL-CoV Rp3 and SARS-CoV Tor2 was 92% and increased to ~94% when the three variable regions were excluded. The variable regions are located at the 5′ end of the S gene (equivalent to the S1 coding region of coronavirus S protein) and the region immediately upstream of the N gene. These regions have been identified as "high mutation" regions among different SARS-CoVs (*5*, *16*, *17*). The region upstream of the N gene is known to be prone to deletions of various sizes (*5*, *16*, *18*).

Predicted protein products from each gene or putative open reading frame (ORF) of SL-CoV Rp3 and SARS-CoV Tor2 were com-

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Table 1. Detection of antibodies to SARS-CoV and PCR amplification of N and P gene fragments with SARS-CoV–specific primers. ND, not determined because of poor sample quality or unavailability of specimens from individual animals.

Sampling		Bat species	Antibody test: positive/total (%)	PCR analysis: positive/total (%)	
Time	Location			Fecal swabs	Respiratory swabs
Mar 04	Nanning, Guangxi	*Rousettus leschenaulti*	1/84 (1.2%)	0/110	ND
	Maoming, Guangdong	*Rousettus leschenaulti*	0/42	0/45	ND
		Cynopterus sphinx	0/17	0/27	ND
July 04	Nanning, Guangxi	*Rousettus leschenaulti*	ND	0/55	0/55
	Tianjin	*Myotis ricketti*	ND	0/21	0/21
Nov 04	Yichang, Hubei	*Rhinolophus pusillus*	ND	0/15	ND
		Rhinolophus ferrumequinum	0/4	1/8 (12.5%)*	ND
		Rhinolophus macrotis	5/7 (71%)	1/8 (12.5%)†	0/3
		Nyctalus plancyi	0/1	0/1	ND
		Miniopterus schreibersi	0/1	0/1	ND
		Myotis altarium	0/1	0/1	ND
Dec 04	Nanning, Guangxi	*Rousettus leschenaulti*	1/58 (1.8)	ND	ND
		Rhinolophus pearsoni	13/46 (28.3%)	3/30 (10%)‡	0/11
		Rhinolophus pussilus	2/6 (33.3%)	0/6	0/2

*Positive fecal sample designated Rf1 †Positive fecal sample designated Rm1 ‡Positive fecal samples designated Rp1, Rp2, and Rp3, respectively.

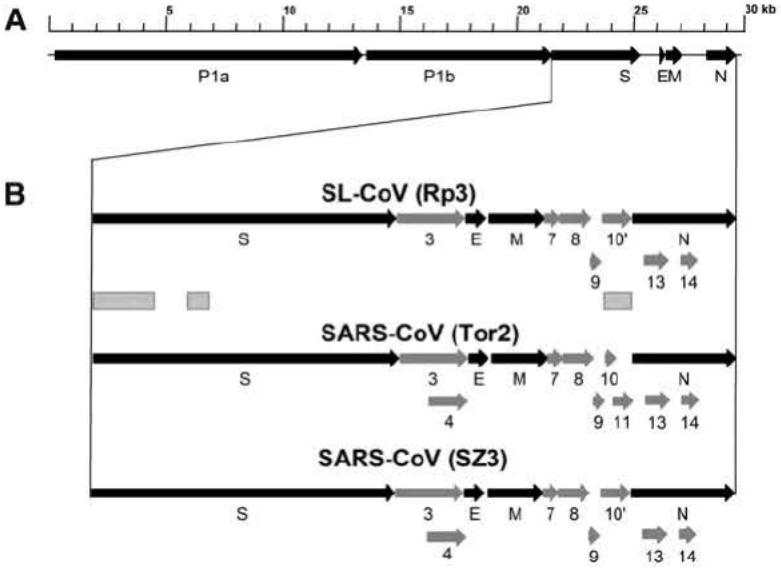

Fig. 1. Genome organization of, and comparison between, SL-CoV and SARS-CoV. (**A**) Overall genome organization of SL-CoV Rp3. (**B**) Expanded diagram of the 3′ region of the genome in comparison with SARS-CoV strains Tor2 and SZ3, following the same nomenclature used by Marra *et al.* (*4*). The genes (named by letters P, S, E, M, and N) present in all coronaviruses are shown in dark-colored arrows, whereas the SARS-CoV–specific ORFs are numbered and illustrated in light-colored arrows. ORF10′ follows the nomenclature by Guan *et al.* (*5*) to indicate that the single ORF present between ORF9 and N in SL-CoV is equivalent to the fusion of ORF10 and ORF11 in the same region in SARS-CoV Tor2. The shaded boxes mark the only three regions displaying significant sequence difference between the two viruses (table S2).

pared (table S2). The P, S, E, M, and N proteins, which are present in all coronaviruses, were similarly sized in the two viruses, with sequence identities ranging from 96% to 100%. The only exception was the S1 domain of the S protein, where sequence identity fell to 64%. The S1 domain is involved in receptor binding, whereas the S2 domain is responsible for the fusion of virus and host cell membranes (*19*). The sequence divergence in the S1 domain corroborated our serum neutralization studies, which indicated that although bat sera have a high level of cross-reactive antibodies (with enzyme-linked immunosorbent assay titers ranging from 1:100 to 1:6400), they failed to neutralize SARS-CoV when tested on Vero E6 cells. This finding suggests that S1 is the main target for antibody-mediated neutralization of this group of viruses, which is consistent with previous reports indicating that major SARS-CoV neutralization epitopes are located in the S1 region (*20*, *21*).

In addition to the five genes present in all coronavirus genomes, coronaviruses also have several ORFs between the P gene and the 3′ end of the genome that code for nonstructural proteins. The function of these nonstructural proteins is largely unknown. The location and sequence of ORFs are group- or virus-specific and hence can serve as important molecular markers for studying virus evolution and classification (*19*, *22*). SARS-CoV has a unique set of ORFs not shared by any of the known coronaviruses (*3*, *4*). Most of these ORFs were also present in SL-CoV, confirming the extremely close genetic relationship between SARS-CoV and SL-CoV (Fig. 1 and table S2).

Coronaviruses produce subgenomic mRNAs through a discontinuous transcription process not fully characterized (*19*). Conserved nucleotide sequences functioning as transcription regulatory sequences (TRSs) are required for the production of the subgenomic mRNAs. In SARS-CoV, such TRSs were identified at each of the predicted gene start sites (*3*, *4*). All of these TRSs were absolutely conserved between SARS-CoV Tor2 and SL-CoV Rp3 (table S3), further demonstrating that these two viruses are very closely related.

SL-CoV is completely different from a bat coronavirus (bat-CoV) recently identified by Poon *et al.* (*7*) from species of bats in the genus *Miniopterus* during a wildlife surveillance study in Hong Kong (Fig. 2). Because the complete genome sequence was not available for bat-CoV, only the trees covering the common sequences (i.e., parts of the P1b and S2 proteins) are shown. The phylogenetic analysis demonstrated that SL-CoV Rp3 and SARS-CoVs are clustered together but that bat-CoV is placed among the relatively distant group 1 viruses. Hereafter, SARS-CoVs and SL-CoVs will be collectively called the SARS cluster of coronaviruses.

In addition to the complete genome sequence of SL-CoV Rp3, partial genome sequences for the other four PCR-positive bat samples were also determined. Phylogenetic analysis based on the N protein sequences (Fig. 3A) revealed that the genetic variation among the SL-CoV sequences was much greater than that exhibited by SARS-CoVs (for simplicity, only three human and civet SARS-CoV isolates were used; the remainder are almost identical to those shown). This was especially obvious when SL-CoVs isolated from different bat species were compared. Moreover, the results suggested that SARS-CoVs nestle phylogenetically within the spectrum of SL-CoVs.

We also compared the "high mutation" regions in samples Rf1, Rm1, and Rp3. For the region upstream of the N gene, SL-CoVs from all three bat species contained a single ORF (ORF10′), similar to that found in SARS-CoV isolates from civets (*5*) and patients in the early phase of the outbreaks (*16*, *18*) but different from that in most human isolates, which

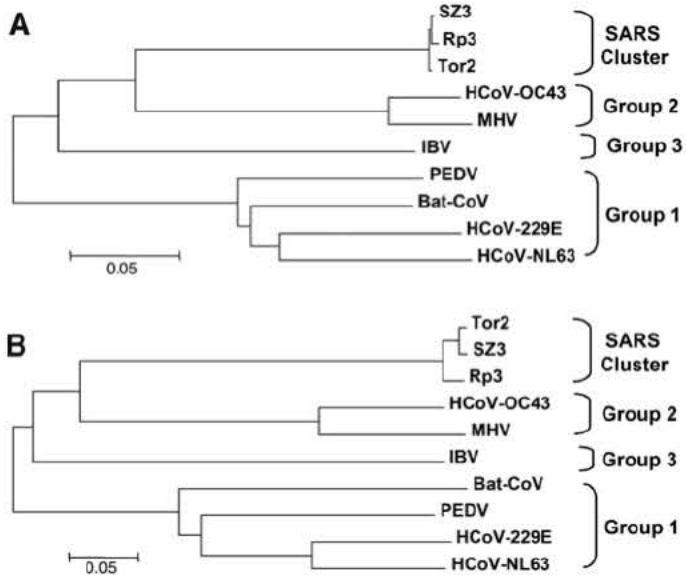

Fig. 2. Phylogenetic trees. (A) and (B) are trees based on deduced amino acid sequences of the same regions in P1b and S, respectively, as used by Poon *et al.* (*7*) for bat-CoV. Tor2 and SZ3, SARS-CoV strains Tor2 and SZ3; Rp3, SL-CoV Rp3; HCoV, human coronavirus; MHV, mouse hepatitis virus; PEDV, porcine epidemic diarrhea virus; IBV, avian infectious bronchitis virus.

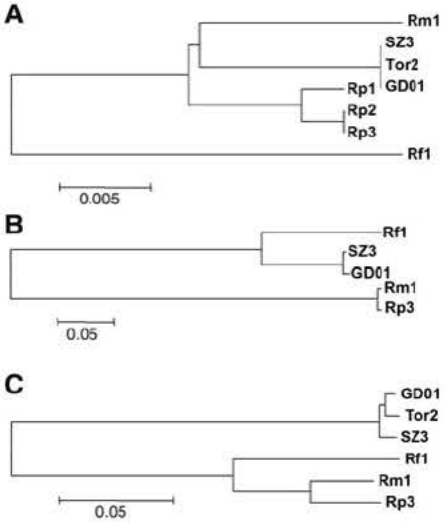

Fig. 3. Phylogenetic trees based on deduced amino acid sequences of (A) N, (B) ORF10′, and (C) S1 proteins. Tor2, SZ3, and GD01, different SARS-CoV strains; Rf1, Rm1, and Rp1-3, different SL-CoV sequences. The genetic distance scale shown for (A) is different from those for (B) and (C).

have a 29-nucleotide deletion in this region (*3*, *4*, *16*). ORF10′ in Rf1 codes for a protein having the same size (122 amino acids) as and more than 80% sequence identity to ORF10′ proteins of SARS-CoVs, but those in Rm1 and Rp3 code for a 121–amino acid protein with only 35% sequence identity (Fig. 3B and fig. S2). By contrast, analysis of the S1 protein regions (Fig. 3C and fig. S3) indicated that Rf1 was more closely related to SL-CoVs from two other bat species than to SARS-CoVs, suggesting that the SARS cluster of coronaviruses could recombine to increase genetic diversity and fitness, as is well documented for other coronaviruses (*19*). We were unable to sequence these regions for Rp1 or Rp2, owing to the poor quality of the fecal materials from these two animals. The limited amount of cDNA available was used up for N gene analysis and in initial sequencing trials with SARS-CoV–derived primers, which were largely unsuccessful. Judging from the close relationship of the N genes between Rp1, Rp2, and Rp3 (fig. S1), it is unlikely that Rp1 or Rp2 will have major sequence differences from Rp3 in the S1 or ORF10′ regions. This is not unexpected, considering that these three positive samples were obtained from the same bat species in the same location.

The genetic diversity of bat-derived sequences supports the notion that bats are a natural reservoir host of the SARS cluster of coronaviruses. A similar observation has been made for henipaviruses, another important group of emerging zoonotic viruses of bat origin, which show greater genetic diversity in bats than was observed among viruses isolated during the initial Nipah outbreaks in Malaysia (*23*–*26*). The overall nucleotide sequence identity of 92% between SL-CoVs and SARS-CoVs is very similar to that observed between Nipah viruses isolated from Malaysia and Bangladesh in 1999 and 2004, respectively (*25*) (fig. S4). SL-CoVs present a new challenge to the diagnosis and treatment of future disease outbreaks. The current tests and therapeutic strategies may not work effectively against all viruses in this group, owing to their great genetic variability in the S1 domain region of the S gene.

The genus *Rhinolophus* contains 69 species and has a wide distribution from Australia to Europe (*27*). They roost primarily in caves and feed mainly on moths and beetles. However, notwithstanding the predominant *Rhinolophus* findings in this study, it is highly likely that there are more SARS-related coronaviruses to be discovered in bats. Indeed, our positive serological findings in the cave-dwelling fruit bat *Rousettus leschenaulti* indicate that infection by a related virus could occur in fruit bats as well, albeit at a much lower frequency. A plausible mechanism for emergence from a natural bat reservoir can be readily envisaged. Fruit bats including *R. leschenaulti*, and less frequently insectivorous bats, are found in markets in southern China. An infectious consignment of bats serendipitously juxtaposed with a susceptible amplifying species, such as *P. larvata*, at some point in the wildlife supply chain could result in spillover and establishment of a market cycle while susceptible animals are available to maintain infection. Further studies in field epidemiology, laboratory infection, and receptor distribution and usage are being conducted to assess potential roles played by different bat species in SARS emergence.

These findings on coronaviruses, together with data on henipaviruses (*23*–*25*, *28*), suggest that genetic diversity exists among zoonotic viruses in bats, increasing the possibility of variants crossing the species barrier and causing outbreaks of disease in human populations. It is therefore essential that we enhance our knowledge and understanding of reservoir host distribution, animal-animal and human-animal interaction (particularly within the wet-market system), and the genetic diversity of bat-borne viruses to prevent future outbreaks.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/1118391/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S3
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Neurogenesis in the Hypothalamus of Adult Mice: Potential Role in Energy Balance

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Ciliary neurotrophic factor (CNTF) induces weight loss in obese rodents and humans, and for reasons that are not understood, its effects persist after the cessation of treatment. Here we demonstrate that centrally administered CNTF induces cell proliferation in feeding centers of the murine hypothalamus. Many of the newborn cells express neuronal markers and show functional phenotypes relevant for energy-balance control, including a capacity for leptin-induced phosphorylation of signal transducer and activator of transcription 3 (STAT3). Co-administration of the mitotic blocker cytosine-β-D-arabinofuranoside (Ara-C) eliminates the proliferation of neural cells and abrogates the long-term, but not the short-term, effect of CNTF on body weight. These findings link the sustained effect of CNTF on energy balance to hypothalamic neurogenesis and suggest that regulated hypothalamic neurogenesis in adult mice may play a previously unappreciated role in physiology and disease.

The obesity epidemic has prompted major efforts to develop safe and effective therapies (*1*, *2*). However, approved drugs for obesity have limited efficacy and act only acutely, with patients rapidly regaining weight after terminating treatment (*3*). Only the neurocytokine ciliary neurotrophic factor (CNTF) and Axokine, an analog of CNTF developed as a drug candidate for the treatment of obesity, appear to deviate from this paradigm. Rodents and patients treated with Axokine were reported to maintain lowered body weights weeks to months after the cessation of treatment (*4*, *5*). This feature of Axokine/CNTF action is unexplained and suggests that CNTF induces long-lasting changes in one or more elements of the energy-balance circuitry.

In rodents, CNTF is most potent when administered directly into the cerebrospinal fluid (*6*) and activates signaling cascades in hypothalamic nuclei involved in feeding control (*5*, *7*, *8*). For instance, CNTF activates phosphorylation of signal transducer and activator of transcription 3 (STAT3) in a population of hypothalamic neurons that substantially overlaps with those activated by leptin (*5*). However, in contrast to CNTF, leptin-treated animals do not maintain their lowered body weight after the cessation of treatment. We thus sought a CNTF-specific mechanism to explain this long-term effect.

CNTF supports the survival of neurons in vitro and in vivo (*9*) and has also been implicated in the maintenance of adult neural stem cells (*10*). Furthermore, other trophic factors, such as epidermal growth factor and fibroblast growth factor 2, are known to act as mitogens on adult neuronal progenitors (*11*, *12*), and they promote the functional regeneration of hippocampal pyramidal neurons (*13*). Neurogenesis in the adult brain is most clearly defined in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal formation (*14*). However, recent reports indicate that the neuroproliferative potency in the adult extends to other brain structures, including the hypothalamus (*15*–*17*). On the basis of these findings, we hypothesized that the long-term effect of CNTF on body-weight regulation might involve neurogenesis in the hypothalamus, which is the brain region most relevant for energy-balance regulation.

To assess the mitogenic potency of CNTF in the adult nervous system in vivo, we delivered the cell-proliferation marker bromodeoxyuridine (BrdU) alone (vehicle treatment) or together with CNTF directly into the cerebrospinal fluid of mouse brains (*18*). CNTF and BrdU were continuously infused for 7 days into the right lateral ventricle using osmotic minipumps. Mice were switched to a high-fat diet two months before surgery and were kept on this diet throughout the experiments. In accordance with previous results (*5*), CNTF-treated mice showed a marked reduction in body weights (Fig. 1A), which persisted after termination of CNTF delivery. Mice were killed 22 days after surgery, and brain sections were immunostained with an antibody against BrdU. Because BrdU incorporates into DNA of dividing cells, BrdU-positive ($BrdU^+$) cells are thought to represent newborn cells. Figure 1B shows coronal sections of vehicle- and CNTF-infused animals at the level of the arcuate, ventromedial, and dorsomedial nuclei, well-known hypothalamic centers for energy-balance regulation (*19*). In vehicle-infused animals, few $BrdU^+$ cells were detected in the parenchyma surrounding the third ventricle (Fig. 1B, left). Administration with CNTF led to a dramatic increase of BrdU+ cells (Fig. 1B, right). Note the higher density of $BrdU^+$ cells at the base of the third ventricle, which is part of the arcuate nucleus/median eminence.

The pattern of CNTF receptor (CNTFR) mRNA expression is consistent with this observation. In situ hybridization using a riboprobe against CNTFR mRNA revealed strong staining in the walls of the basal third ventricle and surrounding arcuate nucleus parenchyma (Fig. 1C). Because this section originated from an animal treated with both CNTF and BrdU, we colabeled with antibodies to BrdU. Many $BrdU^+$ cells were positive for CNTFR expression, indicating that CNTF, at least in part, directly promotes cell division by binding to CNTFR on putative neural progenitor cells (Fig. 1D, inset). By counting all newly generated cells in the caudal hypothalamus, CNTF treatment led to a marked increase of $BrdU^+$ cells over vehicle-infused animals (Fig. 1E). The total number of $BrdU^+$ cells in CNTF-treated animals remained constant for at least 2 weeks after the infusion period. Subsequently, the numbers decreased but plateaued at a high level. Vehicle-infused animals showed a similar fractional decrease over time. Thus it appears that the majority of hypothalamic $BrdU^+$ cells do not die or migrate to distant areas as reported for newborn neurons of the SVZ, which follow the rostral migratory stream toward the olfactory bulb (*20*).

To investigate the origin of adult-born cells in the hypothalamus, we examined CNTF and vehicle-infused brains every 12 hours starting 48 hours after surgery, a time when the infused CNTF/BrdU should just reach the ventricular system (*18*). Hypothalamic BrdU incorporation was first detected 60 to 72 hours

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Bats Are Natural Reservoirs of SARS-Like Coronaviruses

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LETTER

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Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor

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The 2002–3 pandemic caused by severe acute respiratory syndrome coronavirus (SARS-CoV) was one of the most significant public health events in recent history[1]. An ongoing outbreak of Middle East respiratory syndrome coronavirus[2] suggests that this group of viruses remains a key threat and that their distribution is wider than previously recognized. Although bats have been suggested to be the natural reservoirs of both viruses[3–5], attempts to isolate the progenitor virus of SARS-CoV from bats have been unsuccessful. Diverse SARS-like coronaviruses (SL-CoVs) have now been reported from bats in China, Europe and Africa[5–8], but none is considered a direct progenitor of SARS-CoV because of their phylogenetic disparity from this virus and the inability of their spike proteins to use the SARS-CoV cellular receptor molecule, the human angiotensin converting enzyme II (ACE2)[9,10]. Here we report whole-genome sequences of two novel bat coronaviruses from Chinese horseshoe bats (family: Rhinolophidae) in Yunnan, China: RsSHC014 and Rs3367. These viruses are far more closely related to SARS-CoV than any previously identified bat coronaviruses, particularly in the receptor binding domain of the spike protein. Most importantly, we report the first recorded isolation of a live SL-CoV (bat SL-CoV-WIV1) from bat faecal samples in Vero E6 cells, which has typical coronavirus morphology, 99.9% sequence identity to Rs3367 and uses ACE2 from humans, civets and Chinese horseshoe bats for cell entry. Preliminary *in vitro* testing indicates that WIV1 also has a broad species tropism. Our results provide the strongest evidence to date that Chinese horseshoe bats are natural reservoirs of SARS-CoV, and that intermediate hosts may not be necessary for direct human infection by some bat SL-CoVs. They also highlight the importance of pathogen-discovery programs targeting high-risk wildlife groups in emerging disease hotspots as a strategy for pandemic preparedness.

The 2002–3 pandemic of SARS[1] and the ongoing emergence of the Middle East respiratory syndrome coronavirus (MERS-CoV)[2] demonstrate that CoVs are a significant public health threat. SARS-CoV was shown to use the human ACE2 molecule as its entry receptor, and this is considered a hallmark of its cross-species transmissibility[11]. The receptor binding domain (RBD) located in the amino-terminal region (amino acids 318–510) of the SARS-CoV spike (S) protein is directly involved in binding to ACE2 (ref. 12). However, despite phylogenetic evidence that SARS-CoV evolved from bat SL-CoVs, all previously identified SL-CoVs have major sequence differences from SARS-CoV in the RBD of their S proteins, including one or two deletions[6,9]. Replacing the RBD of one SL-CoV S protein with SARS-CoV S conferred the ability to use human ACE2 and replicate efficiently in mice[9,13]. However, to date, no SL-CoVs have been isolated from bats, and no wild-type SL-CoV of bat origin has been shown to use ACE2.

We conducted a 12-month longitudinal survey (April 2011–September 2012) of SL-CoVs in a colony of *Rhinolophus sinicus* at a single location in Kunming, Yunnan Province, China (Extended Data Table 1). A total of 117 anal swabs or faecal samples were collected from individual bats using a previously published method[5,14]. A one-step reverse transcription (RT)-nested PCR was conducted to amplify the RNA-dependent RNA polymerase (RdRP) motifs A and C, which are conserved among alphacoronaviruses and betacoronaviruses[15].

Twenty-seven of the 117 samples (23%) were classed as positive by PCR and subsequently confirmed by sequencing. The species origin of all positive samples was confirmed to be *R. sinicus* by cytochrome b sequence analysis, as described previously[16]. A higher prevalence was observed in samples collected in October (30% in 2011 and 48.7% in 2012) than those in April (7.1% in 2011) or May (7.4% in 2012) (Extended Data Table 1). Analysis of the S protein RBD sequences indicated the presence of seven different strains of SL-CoVs (Fig. 1a and Extended Data Figs 1 and 2). In addition to RBD sequences, which closely matched previously described SL-CoVs (Rs672, Rf1 and HKU3)[5,8,17,18], two novel strains (designated SL-CoV RsSHC014 and Rs3367) were discovered. Their full-length genome sequences were determined, and both were found to be 29,787 base pairs in size (excluding the poly(A) tail). The overall nucleotide sequence identity of these two genomes with human SARS-CoV (Tor2 strain) is 95%, higher than that observed previously for bat SL-CoVs in China (88–92%)[5,8,17,18] or Europe (76%)[6] (Extended Data Table 2 and Extended Data Figs 3 and 4). Higher sequence identities were observed at the protein level between these new SL-CoVs and SARS-CoVs (Extended Data Tables 3 and 4). To understand the evolutionary origin of these two novel SL-CoV strains, we conducted recombination analysis with the Recombination Detection Program 4.0 package[19] using available genome sequences of bat SL-CoV strains (Rf1, Rp3, Rs672, Rm1, HKU3 and BM48-31) and human and civet representative SARS-CoV strains (BJ01, SZ3, Tor2 and GZ02). Three breakpoints were detected with strong P values ($<10^{-20}$) and supported by similarity plot and bootscan analysis (Extended Data Fig. 5a, b). Breakpoints were located at nucleotides 20,827, 26,553 and 28,685 in the Rs3367 (and RsSHC014) genome, and generated recombination fragments covering nucleotides 20,827–26,533 (5,727 nucleotides) (including partial open reading frame (ORF) 1b, full-length S, ORF3, E and partial M gene) and nucleotides 26,534–28,685 (2,133 nucleotides) (including partial ORF M, full-length ORF6, ORF7, ORF8 and partial N gene). Phylogenetic analysis using the major and minor parental regions suggested that Rs3367, or RsSHC014, is the descendent of a recombination of lineages that ultimately lead to SARS-CoV and SL-CoV Rs672 (Fig. 1b).

The most notable sequence differences between these two new SL-CoVs and previously identified SL-CoVs is in the RBD regions of their S proteins. First, they have higher amino acid sequence identity to SARS-CoV (85% and 96% for RsSHC014 and Rs3367, respectively). Second, there are no deletions and they have perfect sequence alignment with the SARS-CoV RBD region (Extended Data Figs 1 and 2). Structural

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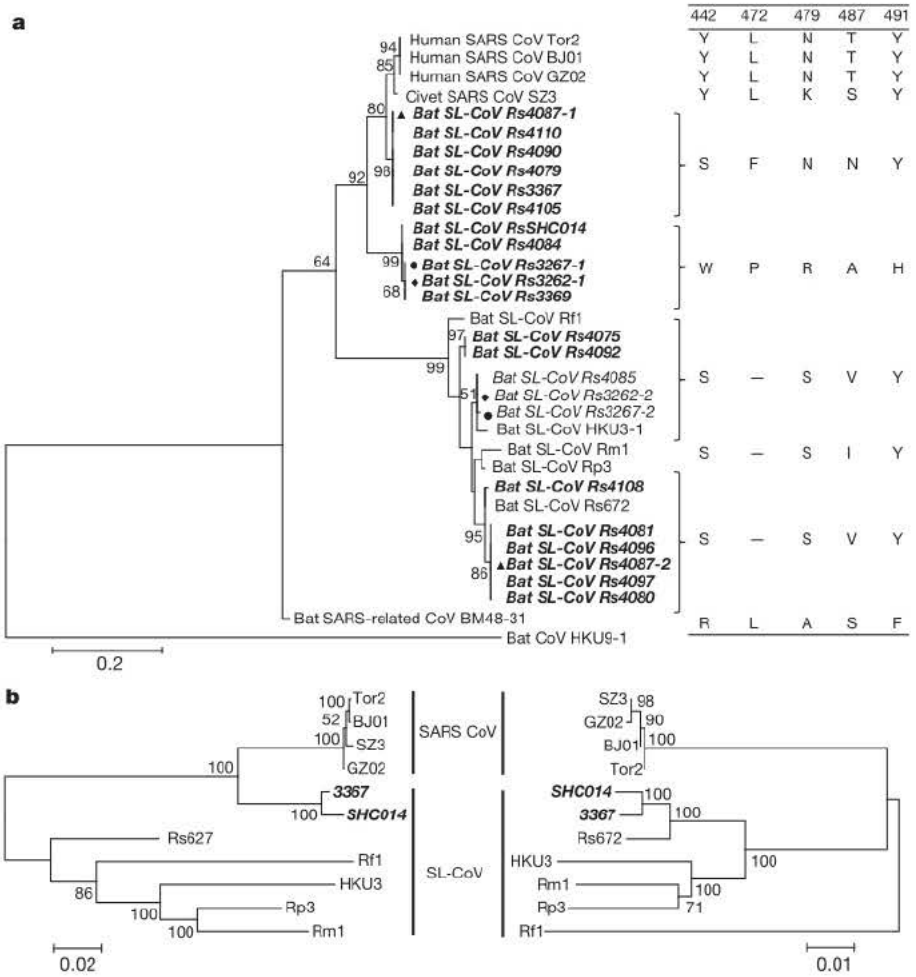

Figure 1 | Phylogenetic tree based on amino acid sequences of the S RBD region and the two parental regions of bat SL-CoV Rs3367 or RsSHC014. **a**, SARS-CoV S protein amino acid residues 310–520 were aligned with homologous regions of bat SL-CoVs using the ClustalW software. A maximum-likelihood phylogenetic tree was constructed using a Poisson model with bootstrap values determined by 1,000 replicates in the MEGA5 software package. The RBD sequences identified in this study are in bold and named by the sample numbers. The key amino acid residues involved in interacting with the human ACE2 molecule are indicated on the right of the tree. SARS-CoV GZ02, BJ01 and Tor2 were isolated from patients in the early, middle and late phase, respectively, of the SARS outbreak in 2003. SARS-CoV SZ3 was identified from *Paguma larvata* in 2003 collected in Guangdong, China. SL-CoV Rp3, Rs672 and HKU3-1 were identified from *R. sinicus* collected in China (respectively: Guangxi, 2004; Guizhou, 2006; Hong Kong, 2005). Rf1 and Rm1 were identified from *R. ferrumequinum* and *R. macrotis*, respectively, collected in Hubei, China, in 2004. Bat SARS-related CoV BM48-31 was identified from *R. blasii* collected in Bulgaria in 2008. Bat CoV HKU9-1 was identified from *Rousettus leschenaultii* collected in Guangdong, China in 2005/2006 and used as an outgroup. All sequences in bold and italics were identified in the current study. Filled triangles, circles and diamonds indicate samples with co-infection by two different SL-CoVs. '–' indicates the amino acid deletion. **b**, Phylogenetic origins of the two parental regions of Rs3367 or RsSHC014. Maximum likelihood phylogenetic trees were constructed from alignments of two fragments covering nucleotides 20,827–26,533 (5,727 nucleotides) and 26,534 –28,685 (2,133 nucleotides) of the Rs3367 genome, respectively. For display purposes, the trees were midpoint rooted. The taxa were annotated according to strain names: SARS-CoV, SARS coronavirus; SARS-like CoV, bat SARS-like coronavirus. The two novel SL-CoVs, Rs3367 and RsSHC014, are in bold and italics.

and mutagenesis studies have previously identified five key residues (amino acids 442, 472, 479, 487 and 491) in the RBD of the SARS-CoV S protein that have a pivotal role in receptor binding[20,21]. Although all five residues in the RsSHC014 S protein were found to be different from those of SARS-CoV, two of the five residues in the Rs3367 RBD were conserved (Fig. 1 and Extended Data Fig. 1).

Despite the rapid accumulation of bat CoV sequences in the last decade, there has been no report of successful virus isolation[6,22,23]. We attempted isolation from SL-CoV PCR-positive samples. Using an optimized protocol and Vero E6 cells, we obtained one isolate which caused cytopathic effect during the second blind passage. Purified virions displayed typical coronavirus morphology under electron microscopy (Fig. 2). Sequence analysis using a sequence-independent amplification method[14] to avoid PCR-introduced contamination indicated that the isolate was almost identical to Rs3367, with 99.9% nucleotide genome sequence identity and 100% amino acid sequence identity for the S1 region. The new isolate was named SL-CoV-WIV1.

To determine whether WIV1 can use ACE2 as a cellular entry receptor, we conducted virus infectivity studies using HeLa cells expressing or not expressing ACE2 from humans, civets or Chinese horseshoe bats. We found that WIV1 is able to use ACE2 of different origins as an entry receptor and replicated efficiently in the ACE2-expressing cells (Fig. 3). This is, to our knowledge, the first identification of a wild-type bat SL-CoV capable of using ACE2 as an entry receptor.

To assess its cross-species transmission potential, we conducted infectivity assays in cell lines from a range of species. Our results (Fig. 4 and Extended Data Table 5) indicate that bat SL-CoV-WIV1 can grow in human alveolar basal epithelial (A549), pig kidney 15 (PK-15) and *Rhinolophus sinicus* kidney (RSKT) cell lines, but not in human cervix (HeLa), Syrian golden hamster kidney (BHK21), *Myotis davidii* kidney (BK), *Myotis chinensis* kidney (MCKT), *Rousettus leschenaulti* kidney (RLK) or *Pteropus alecto* kidney (PaKi) cell lines. Real-time RT–PCR indicated that WIV1 replicated much less efficiently in A549, PK-15 and RSKT cells than in Vero E6 cells (Fig. 4).

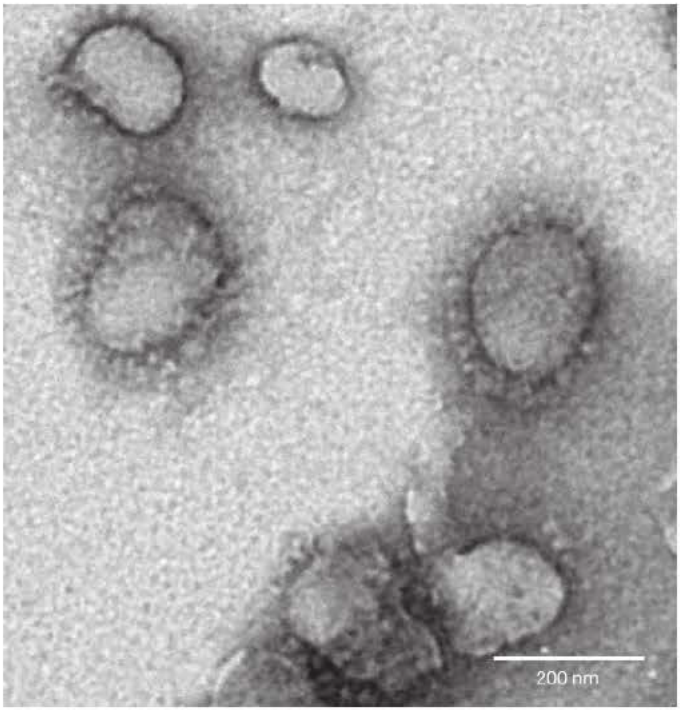

Figure 2 | Electron micrograph of purified virions. Virions from a 10-ml culture were collected, fixed and concentrated/purified by sucrose gradient centrifugation. The pelleted viral particles were suspended in 100 µl PBS, stained with 2% phosphotungstic acid (pH 7.0) and examined directly using a Tecnai transmission electron microscope (FEI) at 200 kV.

To assess the cross-neutralization activity of human SARS-CoV sera against WIV1, we conducted serum-neutralization assays using nine convalescent sera from SARS patients collected in 2003. The results showed that seven of these were able to completely neutralize 100 tissue culture infectious dose 50 ($TCID_{50}$) WIV1 at dilutions of 1:10 to 1:40, further confirming the close relationship between WIV1 and SARS-CoV.

Our findings have important implications for public health. First, they provide the clearest evidence yet that SARS-CoV originated in bats. Our previous work provided phylogenetic evidence of this[5], but the lack of an isolate or evidence that bat SL-CoVs can naturally infect human cells, until now, had cast doubt on this hypothesis. Second, the lack of capacity of SL-CoVs to use of ACE2 receptors has previously been considered as the key barrier for their direct spillover into humans, supporting the suggestion that civets were intermediate hosts for SARS-CoV adaptation to human transmission during the SARS outbreak[24]. However, the ability of SL-CoV-WIV1 to use human ACE2 argues against the necessity of this step for SL-CoV-WIV1 and suggests that direct bat-to-human infection is a plausible scenario for some bat SL-CoVs. This has implications for public health control measures in the face of potential spillover of a diverse and growing pool of recently discovered SARS-like CoVs with a wide geographic distribution.

Our findings suggest that the diversity of bat CoVs is substantially higher than that previously reported. In this study we were able to demonstrate the circulation of at least seven different strains of SL-CoVs within a single colony of *R. sinicus* during a 12-month period. The high genetic diversity of SL-CoVs within this colony was mirrored by high phenotypic diversity in the differential use of ACE2 by different strains. It would therefore not be surprising if further surveillance reveals a broad diversity of bat SL-CoVs that are able to use ACE2, some of which may have even closer homology to SARS-CoV than SL-CoV-WIV1. Our results—in addition to the recent demonstration of MERS-CoV in a Saudi Arabian bat[25], and of bat CoVs closely related to MERS-CoV in China, Africa, Europe and North America[3,26,27]—suggest that bat coronaviruses remain a substantial global threat to public health.

Finally, this study demonstrates the public health importance of pathogen discovery programs targeting wildlife that aim to identify the 'known unknowns'—previously unknown viral strains closely related to known pathogens. These programs, focused on specific high-risk wildlife groups and hotspots of disease emergence, may be a critical part of future global strategies to predict, prepare for, and prevent pandemic emergence[28].

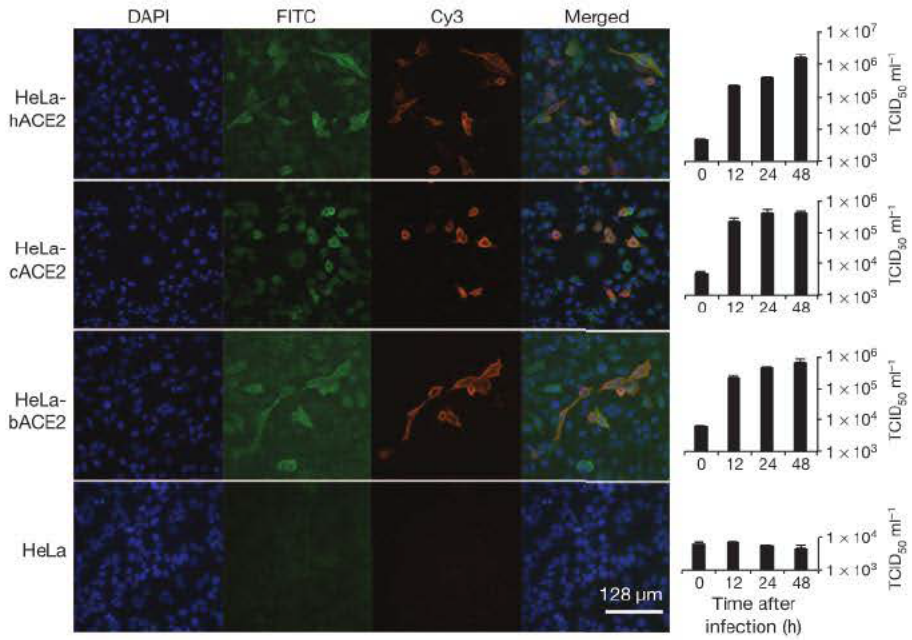

Figure 3 | Analysis of receptor usage of SL-CoV-WIV1 determined by immunofluorescence assay and real-time PCR. Determination of virus infectivity in HeLa cells with and without the expression of ACE2. b, bat; c, civet; h, human. ACE2 expression was detected with goat anti-humanACE2 antibody followed by fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat IgG. Virus replication was detected with rabbit antibody against the SL-CoV Rp3 nucleocapsid protein followed by cyanine 3 (Cy3)-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole). The columns (from left to right) show staining of nuclei (blue), ACE2 expression (green), virus replication (red), merged triple-stained images and real-time PCR results, respectively. ($n = 3$); error bars represent standard deviation.

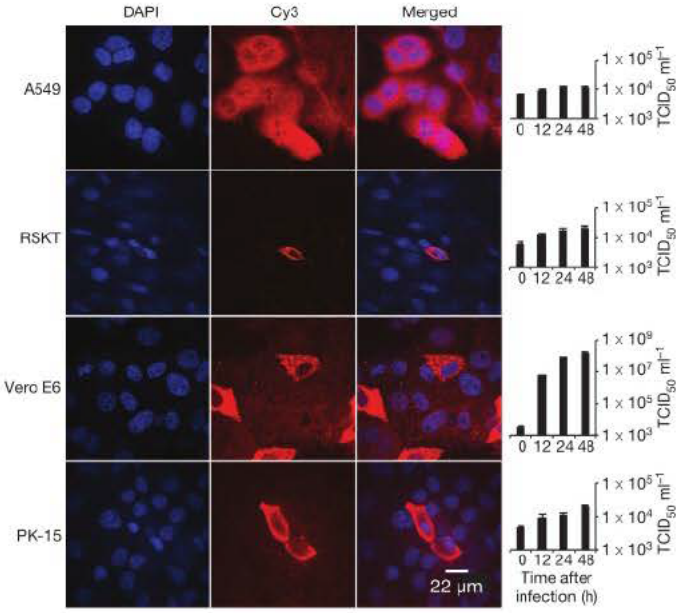

Figure 4 | Analysis of host range of SL-CoV-WIV1 determined by immunofluorescence assay and real-time PCR. Virus infection in A549, RSKT, Vero E6 and PK-15 cells. Virus replication was detected as described for Fig. 3. The columns (from left to right) show staining of nuclei (blue), virus replication (red), merged double-stained images and real-time PCR results, respectively. $n = 3$; error bars represent s.d.

METHODS SUMMARY

Throat and faecal swabs or fresh faecal samples were collected in viral transport medium as described previously[14]. All PCR was conducted with the One-Step RT–PCR kit (Invitrogen). Primers targeting the highly conserved regions of the RdRP gene were used for detection of all alphacoronaviruses and betacoronaviruses as described previously[15]. Degenerate primers were designed on the basis of all available genomic sequences of SARS-CoVs and SL-CoVs and used for amplification of the RBD sequences of S genes or full-length genomic sequences. Degenerate primers were used for amplification of the bat *ACE2* gene as described previously[29]. PCR products were gel purified and cloned into pGEM-T Easy Vector (Promega). At least four independent clones were sequenced to obtain a consensus sequence. PCR-positive faecal samples (in 200 μl buffer) were gradient centrifuged at 3,000–12,000*g* and supernatant diluted at 1:10 in DMEM before being added to Vero E6 cells. After incubation at 37 °C for 1 h, inocula were removed and replaced with fresh DMEM with 2% FCS. Cells were incubated at 37 °C and checked daily for cytopathic effect. Cell lines from different origins were grown on coverslips in 24-well plates and inoculated with the novel SL-CoV at a multiplicity of infection of 10. Virus replication was detected at 24 h after infection using rabbit antibodies against the SL-CoV Rp3 nucleocapsid protein followed by Cy3-conjugated goat anti-rabbit IgG.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions Z.-L.S. and P.D. designed and coordinated the study. X.-Y.G., J.-L. L. and X.-L.Y. conducted majority of experiments and contributed equally to the study. A.A.C., B.H., W.Z., C.P., Y.-J.Z., C.-M.L., B.T., N.W. and Y.Z. conducted parts of the experiments and analyses. J.H.E., J.K.M. and S.-Y.Z. coordinated the field study. X.-Y.G., J.-L.L, X.-L.Y., B.T. and G.-J.Z. collected the samples. G.C. and L.-F.W. designed and supervised part of the experiments. All authors contributed to the interpretations and conclusions presented. Z.-L.S. and X-Y.G. wrote the manuscript with significant contributions from P.D. and L-F.W. and input from all authors.

Author Information Sequences of three bat SL-CoV genomes, bat SL-CoV RBD and *R. sinicus ACE2* genes have been deposited in GenBank under accession numbers KC881005–KC881007 (genomes from SL-CoV RsSHC014, Rs3367 and W1V1, respectively), KC880984–KC881003 (bat SL-CoV RBD genes) and KC881004 (*R. sinicus ACE2*), respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to P.D. (daszak@ecohealthalliance.org) or Z.-L.S. (zlshi@wh.iov.cn).

METHODS

Sampling. Bats were trapped in their natural habitat as described previously[5]. Throat and faecal swab samples were collected in viral transport medium (VTM) composed of Hank's balanced salt solution, pH 7.4, containing BSA (1%), amphotericin (15 μg ml^{-1}), penicillin G (100 U ml^{-1}) and streptomycin (50 μg ml^{-1}). To collect fresh faecal samples, clean plastic sheets measuring 2.0 by 2.0 m were placed under known bat roosting sites at about 18:00 h each evening. Relatively fresh faecal samples were collected from sheets at approximately 05:30–06:00 the next morning and placed in VTM. Samples were transported to the laboratory and stored at −80 °C until use. All animals trapped for this study were released back to their habitat after sample collection. All sampling processes were performed by veterinarians with approval from Animal Ethics Committee of the Wuhan Institute of Virology (WIVH05210201) and EcoHealth Alliance under an inter-institutional agreement with University of California, Davis (UC Davis protocol no. 16048).

RNA extraction, PCR and sequencing. RNA was extracted from 140 μl of swab or faecal samples with a Viral RNA Mini Kit (Qiagen) following the manufacturer's instructions. RNA was eluted in 60 μl RNAse-free buffer (buffer AVE, Qiagen), then aliquoted and stored at −80 °C. One-step RT–PCR (Invitrogen) was used to detect coronavirus sequences as described previously[15]. First round PCR was conducted in a 25-μl reaction mix containing 12.5 μl PCR 2× reaction mix buffer, 10 pmol of each primer, 2.5 mM MgSO4, 20 U RNase inhibitor, 1 μl SuperScript III/ Platinum Taq Enzyme Mix and 5 μl RNA. Amplification of the RdRP-gene fragment was performed as follows: 50 °C for 30 min, 94 °C for 2 min, followed by 40 cycles consisting of 94 °C for 15 s, 62 °C for 15 s, 68 °C for 40 s, and a final extension of 68 °C for 5 min. Second round PCR was conducted in a 25-μl reaction mix containing 2.5 μl PCR reaction buffer, 5 pmol of each primer, 50 mM $MgCl_2$, 0.5 mM dNTP, 0.1 μl Platinum Taq Enzyme (Invitrogen) and 1 μl first round PCR product. The amplification of RdRP-gene fragment was performed as follows: 94 °C for 5 min followed by 35 cycles consisting of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 40 s, and a final extension of 72 °C for 5 min.

To amplify the RBD region, one-step RT–PCR was performed with primers designed based on available SARS-CoV or bat SL-CoVs (first round PCR primers; F, forward; R, reverse: CoVS931F-5′-VWGADGTTGTKAGRTTYCCT-3′ and CoVS1909R-5′-TAARACAVCCWGCYTGWGT-3′; second PCR primers: CoVS 951F-5′-TGTKAGRTTYCCTAAYATTAC-3′ and CoVS1805R-5′-ACATCYTG ATANARAACAGC-3′). First-round PCR was conducted in a 25-μl reaction mix as described above except primers specific for the S gene were used. The amplification of the RBD region of the S gene was performed as follows: 50 °C for 30 min, 94 °C for 2 min, followed by 35 cycles consisting of 94 °C for 15 s, 43 °C for 15 s, 68 °C for 90 s, and a final extension of 68 °C for 5 min. Second-round PCR was conducted in a 25-μl reaction mix containing 2.5 μl PCR reaction buffer, 5 pmol of each primer, 50 mM $MgCl_2$, 0.5 mM dNTP, 0.1 μl Platinum Taq Enzyme (Invitrogen) and 1 μl first round PCR product. Amplification was performed as follows: 94 °C for 5 min followed by 40 cycles consisting of 94 °C for 30 s, 41 °C for 30 s, 72 °C for 60 s, and a final extension of 72 °C for 5 min.

PCR products were gel purified and cloned into pGEM-T Easy Vector (Promega). At least four independent clones were sequenced to obtain a consensus sequence for each of the amplified regions.

Sequencing full-length genomes. Degenerate coronavirus primers were designed based on all available SARS-CoV and bat SL-CoV sequences in GenBank and specific primers were designed from genome sequences generated from previous rounds of sequencing in this study (primer sequences will be provided upon request). All PCRs were conducted using the One-Step RT–PCR kit (Invitrogen). The 5′ and 3′ genomic ends were determined using the 5′ or 3′ RACE kit (Roche), respectively. PCR products were gel purified and sequenced directly or following cloning into pGEM-T Easy Vector (Promega). At least four independent clones were sequenced to obtain a consensus sequence for each of the amplified regions and each region was sequenced at least twice.

Sequence analysis and databank accession numbers. Routine sequence management and analysis was carried out using DNAStar or Geneious. Sequence alignment and editing was conducted using ClustalW, BioEdit or GeneDoc. Maximum Likelihood phylogenetic trees based on the protein sequences were constructed using a Poisson model with bootstrap values determined by 1,000 replicates in the MEGA5 software package.

Sequences obtained in this study have been deposited in GenBank as follows (accession numbers given in parenthesis): full-length genome sequence of SL-CoV RsSHC014 and Rs3367 (KC881005, KC881006); full-length sequence of WIV1 S (KC881007); RBD (KC880984-KC881003); ACE2 (KC8810040). SARS-CoV sequences used in this study: human SARS-CoV strains Tor2 (AY274119), BJ01 (AY278488), GZ02 (AY390556) and civet SARS-CoV strain SZ3 (AY304486). Bat coronavirus sequences used in this study: Rs672 (FJ588686), Rp3 (DQ071615), Rf1 (DQ412042), Rm1 (DQ412043), HKU3-1 (DQ022305), BM48-31 (NC_014470), HKU9-1 (NC_009021), HKU4 (NC_009019), HKU5 (NC_009020), HKU8 (DQ249228), HKU2 (EF203067), BtCoV512 (NC_009657), 1A (NC_010437). Other coronavirus sequences used in this study: HCoV-229E (AF304460), HCoV-OC43 (AY391777), HCoV-NL63 (AY567487), HKU1 (NC_006577), EMC (JX869059), FIPV (NC_002306), PRCV (DQ811787), BWCoV (NC_010646), MHV (AY700211), IBV (AY851295).

Amplification, cloning and expression of the bat ACE2 gene. Construction of expression clones for human and civet ACE2 in pcDNA3.1 has been described previously[29]. Bat ACE2 was amplified from a *R. sinicus* (sample no. 3357). In brief, total RNA was extracted from bat rectal tissue using the RNeasy Mini Kit (Qiagen). First-strand complementary DNA was synthesized from total RNA by reverse transcription with random hexamers. Full-length bat *ACE2* fragments were amplified using forward primer bAF2 and reverse primer bAR2 (ref. 29). The *ACE2* gene was cloned into pCDNA3.1 with KpnI and XhoI, and verified by sequencing. Purified *ACE2* plasmids were transfected to HeLa cells. After 24 h, lysates of HeLa cells expressing human, civet, or bat ACE2 were confirmed by western blot or immunofluorescence assay.

Western blot analysis. Lysates of cells or filtered supernatants containing pseudoviruses were separated by SDS–PAGE, followed by transfer to a nitrocellulose membrane (Millipore). For detection of S protein, the membrane was incubated with rabbit anti-Rp3 S fragment (amino acids 561–666) polyantibodies (1:200), and the bound antibodies were detected by alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG (1:1,000). For detection of HIV-1 p24 in supernatants, monoclonal antibody against HIV p24 (p24 MAb) was used as the primary antibody at a dilution of 1:1,000, followed by incubation with AP-conjugated goat anti-mouse IgG at the same dilution. To detect the expression of ACE2 in HeLa cells, goat antibody against the human ACE2 ectodomain (1:500) was used as the first antibody, followed by incubation with horseradish peroxidase-conjugated donkey anti-goat IgG (1:1,000).

Virus isolation. Vero E6 cell monolayers were maintained in DMEM supplemented with 10% FCS. PCR-positive samples (in 200 μl buffer) were gradient centrifuged at 3,000–12,000*g*, and supernatant were diluted 1:10 in DMEM before being added to Vero E6 cells. After incubation at 37 °C for 1 h, inocula were removed and replaced with fresh DMEM with 2% FCS. Cells were incubated at 37 °C for 3 days and checked daily for cytopathic effect. Double-dose triple antibiotics penicillin/streptomycin/amphotericin (Gibco) were included in all tissue culture media (penicillin 200 IU ml^{-1}, streptomycin 0.2 mg ml^{-1}, amphotericin 0.5 μg ml^{-1}). Three blind passages were carried out for each sample. After each passage, both the culture supernatant and cell pellet were examined for presence of virus by RT–PCR using primers targeting the RdRP or S gene. Virions in supernatant (10 ml) were collected and fixed using 0.1% formaldehyde for 4 h, then concentrated by ultracentrifugation through a 20% sucrose cushion (5 ml) at 80,000*g* for 90 min using a Ty90 rotor (Beckman). The pelleted viral particles were suspended in 100 μl PBS, stained with 2% phosphotungstic acid (pH 7.0) and examined using a Tecnai transmission electron microscope (FEI) at 200 kV.

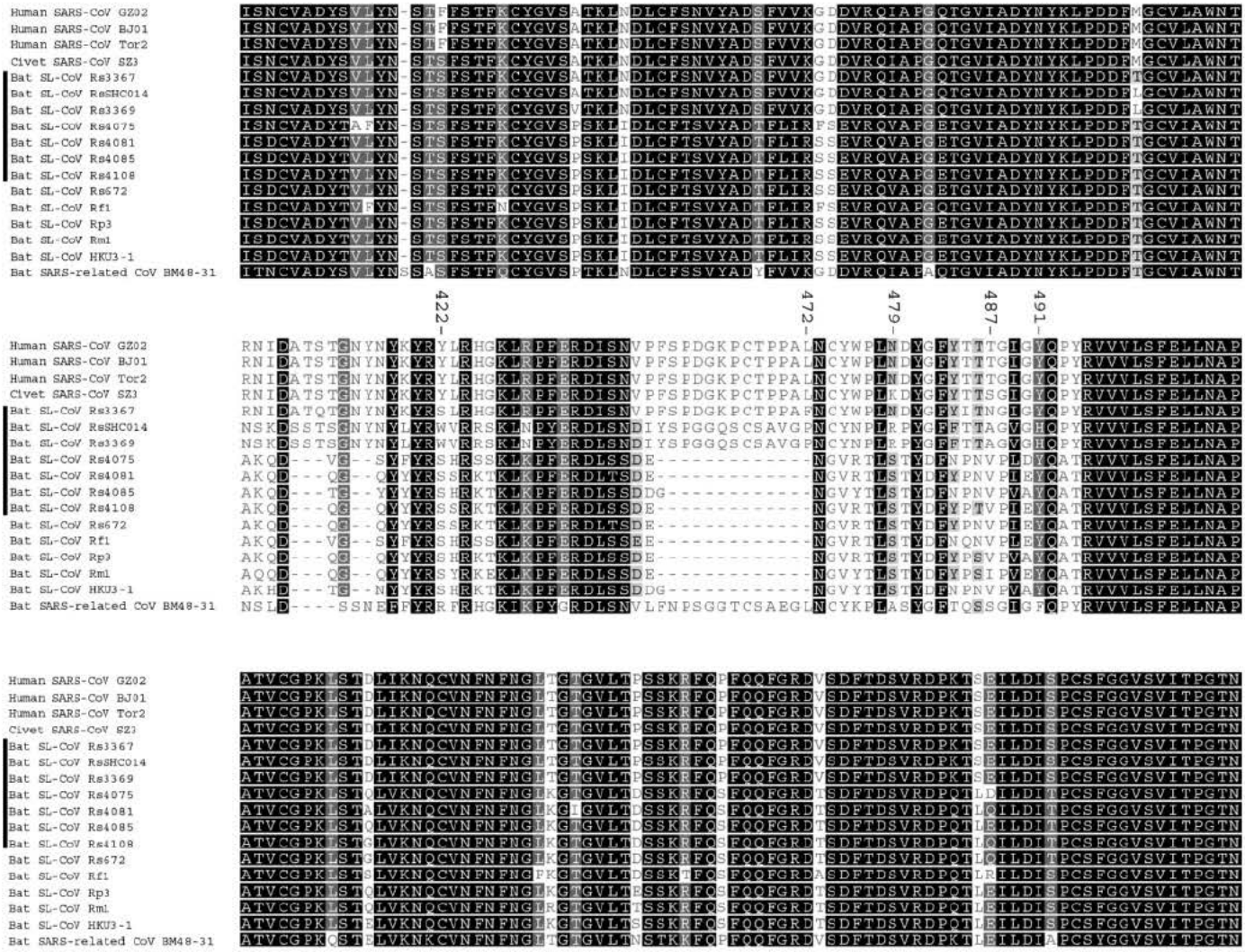
Virus infectivity detected by immunofluorescence assay. Cell lines used for this study and their culture conditions are summarized in Extended Data Table 5. Virus titre was determined in Vero E6 cells by cytopathic effect (CPE) counts. Cell lines from different origins and HeLa cells expressing ACE2 from human, civet or Chinese horseshoe bat were grown on coverslips in 24-well plates (Corning) incubated with bat SL-CoV-WIV1 at a multiplicity of infection = 10 for 1 h. The inoculum was removed and washed twice with PBS and supplemented with medium. HeLa cells without ACE2 expression and Vero E6 cells were used as negative and positive controls, respectively. At 24 h after infection, cells were washed with PBS and fixed with 4% formaldehyde in PBS (pH 7.4) for 20 min at 4 °C. ACE2 expression was detected using goat anti-human ACE2 immunoglobulin (R&D Systems) followed by FITC-labelled donkey anti-goat immunoglobulin (PTGLab). Virus replication was detected using rabbit antibody against the SL-CoV Rp3 nucleocapsid protein followed by Cy3-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI. Staining patterns were examined using a FV1200 confocal microscope (Olympus).

Virus infectivity detected by real-time RT–PCR. Vero E6, A549, PK15, RSKT and HeLa cells with or without expression of ACE2 of different origins were inoculated with 0.1 $TCID_{50}$ WIV-1 and incubated for 1 h at 37 °C. After removing the inoculum, the cells were cultured with medium containing 1% FBS. Supernatants were collected at 0, 12, 24 and 48 h. RNA from 140 μl of each supernatant was extracted with the Viral RNA Mini Kit (Qiagen) following manufacturer's instructions and eluted in 60 μl buffer AVE (Qiagen). RNA was quantified on the ABI StepOne system, with the TaqMan AgPath-ID One-Step RT–PCR Kit (Applied Biosystems) in a 25 μl reaction mix containing 4 μl RNA, 1 × RT–PCR enzyme mix, 1 × RT–PCR buffer, 40 pmol forward primer (5′-GTGGTGGTGACGGCA AAATG-3′), 40 pmol reverse primer (5′-AAGTGAAGCTTCTGGGCCAG-3′) and 12 pmol probe (5′-FAM-AAAGAGCTCAGCCCCAGATG-BHQ1-3′). Amplification parameters were 10 min at 50 °C, 10 min at 95 °C and 50 cycles of 15 s at 95 °C and 20 s at 60 °C. RNA dilutions from purified WIV-1 stock were used as a standard.

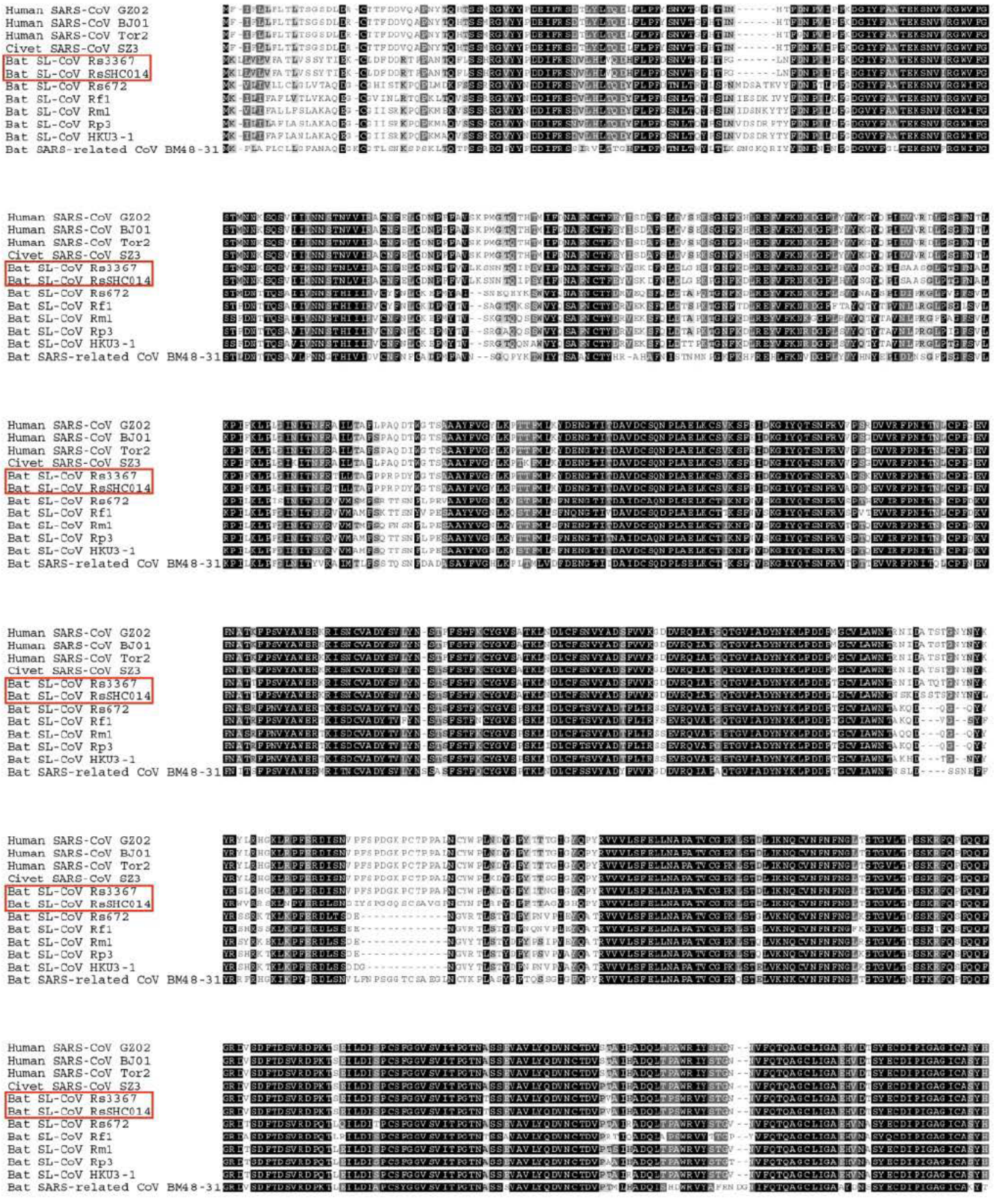
Serum neutralization test. SARS patient sera were inactivated at 56 °C for 30 min and then used for virus neutralization testing. Sera were diluted starting with 1:10

and then serially twofold diluted in 96-well cell plates to 1:40. Each 100 µl serum dilution was mixed with 100 µl viral supernatant containing 100 $TCID5_0$ of WIV1 and incubated at 37 °C for 1 h. The mixture was added in triplicate wells of 96-well cell plates with plated monolayers of Vero E6 cells and further incubated at 37 °C for 2 days. Serum from a healthy blood donor was used as a negative control in each experiment. CPE was observed using an inverted microscope 2 days after inoculation. The neutralizing antibody titre was read as the highest dilution of serum which completely suppressed CPE in infected wells. The neutralization test was repeated twice.

Recombination analysis. Full-length genomic sequences of SL-CoV Rs3367 or RsSHC014 were aligned with those of selected SARS-CoVs and bat SL-CoVs using Clustal X. The aligned sequences were preliminarily scanned for recombination events using Recombination Detection Program (RDP) 4.0 (ref. 19). The potential recombination events suggested by RDP owing to their strong *P* values (<10–20) were investigated further by similarity plot and bootscan analyses implemented in Simplot 3.5.1. Phylogenetic origin of the major and minor parental regions of Rs3367 or RsSHC014 were constructed from the concatenated sequences of the essential ORFs of the major and minor parental regions of selected SARS-CoV and SL-CoVs. Two genome regions between three estimated breakpoints (20,827–26,553 and 26,554–28,685) were aligned independently using ClustalX and generated two alignments of 5,727 base pairs and 2,133 base pairs. The two alignments were used to construct maximum likelihood trees to better infer the fragment parents. All nucleotide numberings in this study are based on Rs3367 genome position.



Extended Data Figure 1 | Sequence alignment of CoV S protein RBD. SARS-CoV S protein (amino acids 310–520) is aligned with homologous regions of bat SL-CoVs using ClustalW. The newly discovered bat SL-CoVs are indicated with a bold vertical line on the left. The key amino acid residues involved in the interaction with human ACE2 are numbered on the top of the aligned sequences.



Extended Data Figure 2 | Alignment of CoV S protein S1 sequences. Alignment of S1 sequences (amino acids 1–660) of the two novel bat SL-CoV S proteins with those of previously reported bat SL-CoVs and human and civet SARS-CoVs. The newly discovered bat SL-CoVs are boxed in red. SARS-CoV GZ02, BJ01 and Tor2 were isolated from patients in the early, middle and late phase, respectively, of the SARS outbreak in 2003. SARS-CoV SZ3 was identified from *P. larvata* in 2003 collected in Guangdong, China. SL-CoV Rp3, Rs 672 and HKU3-1 were identified from *R. sinicus* collected in Guangxi, Guizhou and Hong Kong, China, respectively. Rf1 and Rm1 were identified from *R. ferrumequinum* and *R. macrotis*, respectively, collected in Hubei Province, China. Bat SARS-related CoV BM48-31 was identified from *R. blasii* collected in Bulgaria.

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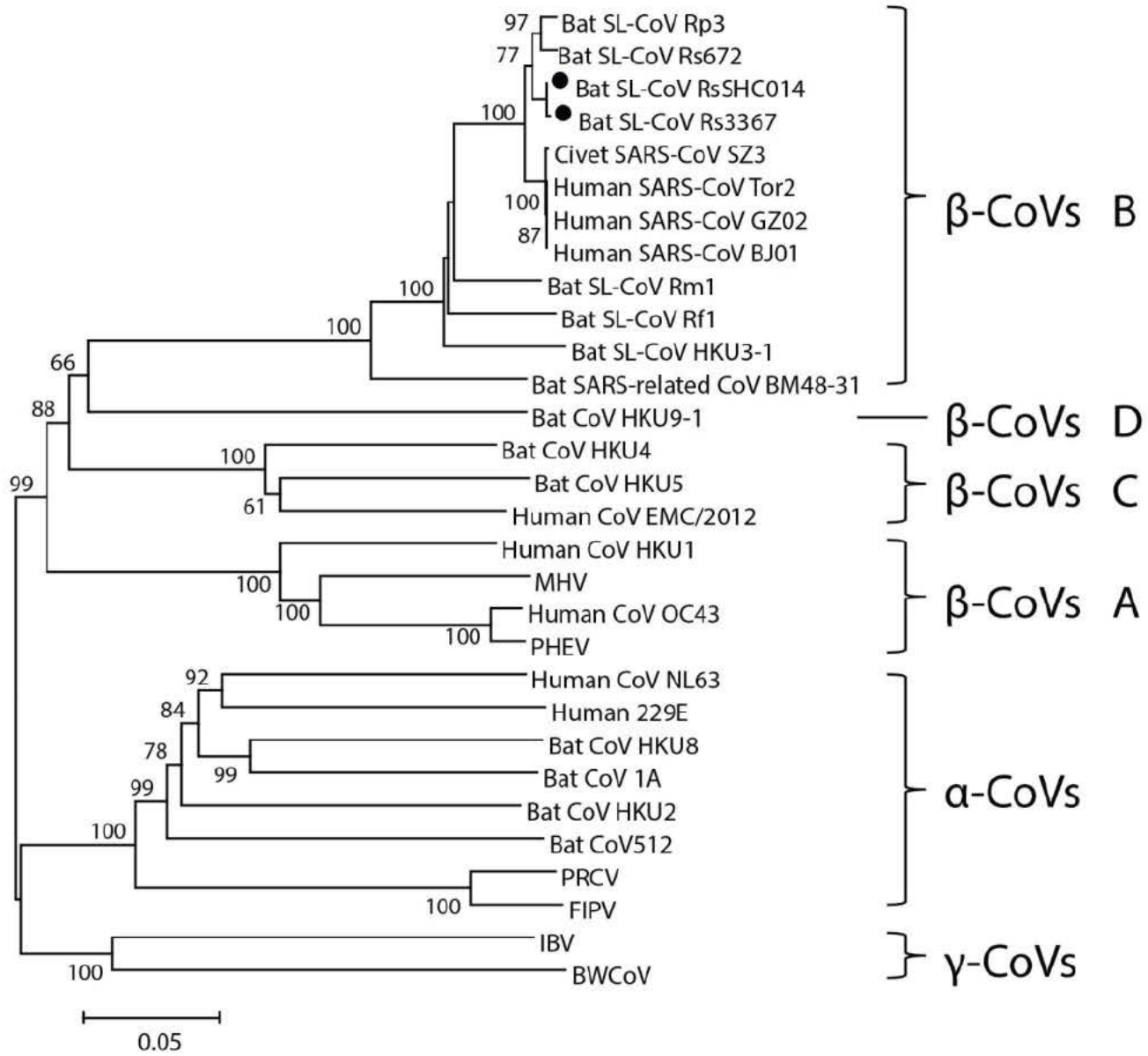
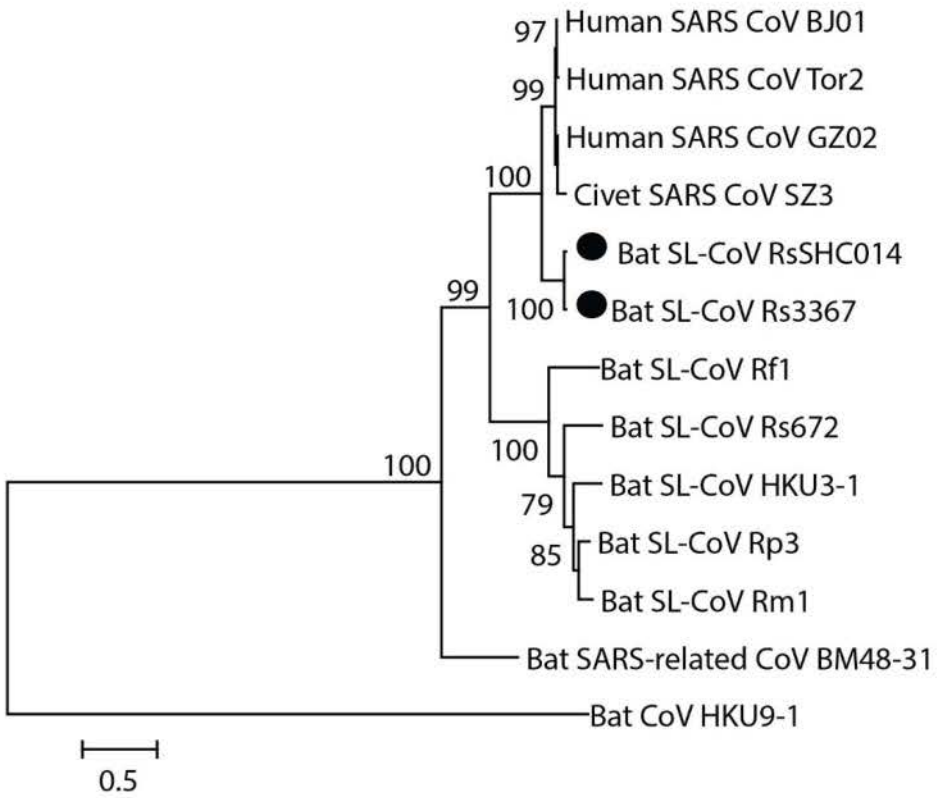
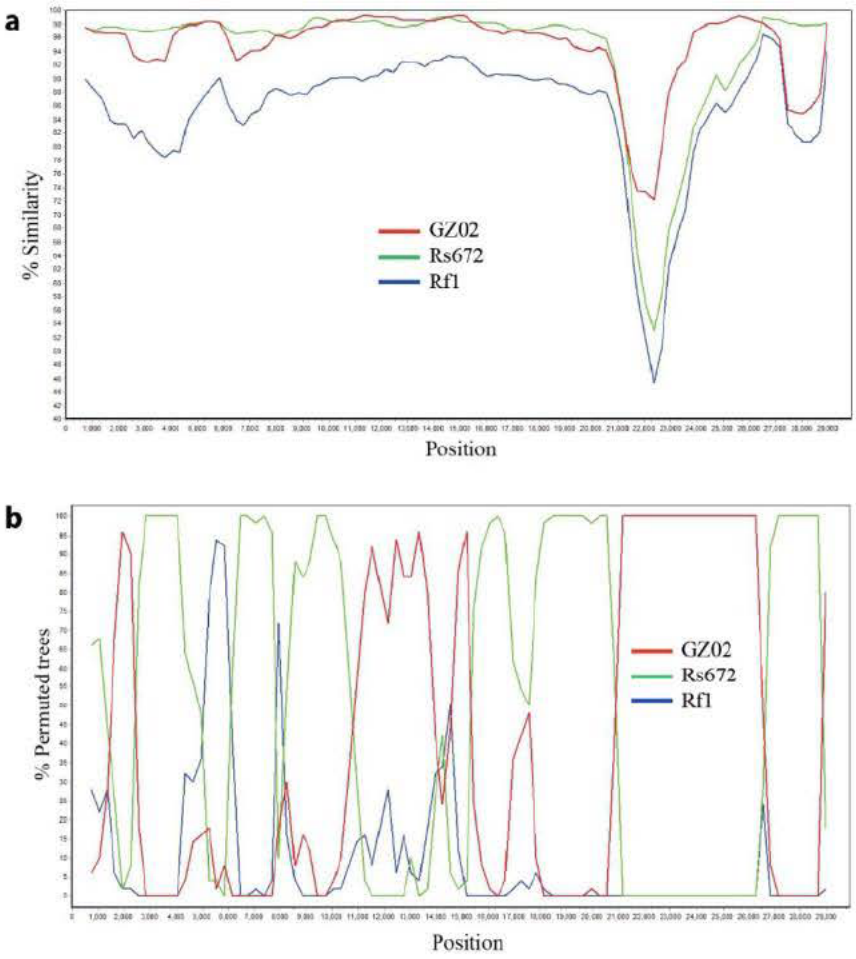


Extended Data Figure 3 | Complete RdRP sequence phylogeny. Phylogenetic tree of bat SL-CoVs and SARS-CoVs on the basis of complete RdRP sequences (2,796 nucleotides). Bat SL-CoVs RsSHC014 and Rs3367 are highlighted by filled circles. Three established coronaivirus genera, *Alphacoronavirus*, *Betacoronavirus* and *Gammacoronavirus* are marked as α, β and γ, respectively. Four CoV groups in the genus *Betacoronavirus* are indicated as A, B, C and D, respectively. MHV, murine hepatitis virus; PHEV, porcine haemagglutinating encephalomyelitis virus; PRCV, porcine respiratory coronavirus; FIPV, feline infectious peritonitis virus; IBV, infectious bronchitis coronavirus; BW, beluga whale coronavirus.



Extended Data Figure 4 | Sequence phylogeny of the complete S protein of SL-CoVs and SARS-CoV. Phylogenetic tree of bat SL-CoVs and SARS-CoVs on the basis of complete S protein sequences (1,256 amino acids). Bat SL-CoVs RsSHC014 and Rs3367 are highlighted by filled circles. Bat CoV HKU9 was used as an outgroup.



Extended Data Figure 5 | Detection of potential recombination events. **a**, **b**, Similarity plot (**a**) and bootscan analysis (**b**) detected three recombination breakpoints in the bat SL-CoV Rs3367 or SHC014 genome. The three breakpoints were located at the ORF1b (nt 20,827), M (nucleotides 26,553) and N (nucleotides 28,685) genes, respectively. Both analyses were performed with an F84 distance model, a window size of 1,500 base pairs and a step size of 300 base pairs.

Extended Data Table 1 | Summary of sampling detail and CoV prevalence

Sampling time	Total number of swab or fecal samples collected	Number of CoV PCR positive samples (%)
April, 2011	14	1 (7.1)
October, 2011	10	3 (30)
May, 2012	54	4 (7.4)
September, 2012	39	19 (48.7)

Extended Data Table 2 | Genomic sequence identities of bat SL-CoVs with SARS-CoVs

		Pairwise genomic nucleotide acids identity (%)										
		Bat SARS-Like CoVs							Human and civet SARS-CoVs			
CoVs	Genome size (nt)	SHC014	Rs672	Rp3	Rf1	Rm1	HKU3-1	BM48-31	GZ02	BJ01	Tor2	SZ3
3367	29,787	98.8	92.5	93.2	87.3	88.0	87.8	76.9	95.4	95.3	95.4	95.3
SHC014	29,787	-	92.6	93.2	87.3	88.1	87.8	77.0	95.2	95.1	95.1	95.1
Rs672	29,059		-	92.4	86.2	87.4	87.0	75.2	90.9	90.9	90.8	91.0
Rp3	29,736			-	88.3	90.3	89.6	77.0	92.1	92.0	92.1	92.0
Rf1	29,709				-	89.4	88.4	76.6	87.2	87.1	87.2	87.1
Rm1	29,749					-	90.1	76.4	87.6	87.5	87.5	87.5
HKU3-1	29,728						-	76.8	87.4	87.3	87.4	87.3
BM48-31	29,276							-	76.9	77.1	77.0	76.9
GZ02	29,760								-	99.6	99.6	99.7
BJ01	29,725									-	99.8	99.6
Tor2	29,751										-	99.5
SZ3	29,741											-

Extended Data Table 3 | Genomic annotation and comparison of bat SL-CoV Rs3367 with human/civet SARS-CoVs and other bat SL-CoVs

					ORF Identity nt/aa (%)									
					Human and civet SARS-CoVs				Bat SARS-like CoVs					
ORFs	Start-End (nt.)	No. of Nt.	No. of Aa.	TRS	GZ02	BJ01	Tor2	SZ3	Rs672	Rp3	Rf1	Rm1	HKU3-1	BM48-31
P1a	265-13,398	13,134	4,377	$ACGAAC_{192}AUG$	96.7/97.9	96.6/97.9	96.8/97.9	96.8/98.1	93.3/94.2	95.5/96.9	88.1/94.0	87.9/93.3	87.9/94.2	76.3/80.8
P1b	13,398-21,485	8,088	2,695		96.3/99.2	96.3/99.2	96.3/99.2	96.3/99.2	97.2/99.2	97.2/99.2	90.6/98.4	91.0/98.7	90.7/98.5	83.4/93.7
S	21,492-25,262	3,771	1,256	ACGAACAUG	88.3/90.1	88.2/90.0	88.1/89.8	88.2/90.0	76.5/78.2	76.0/79.1	74.0/77.4	76.3/79.1	75.6/78.2	70.2/74.5
(S1) *	21,493-23,535	2,043	681		78.2/81.1	78.2/80.9	78.1/80.6	78.2/81.1	65.1/62.2	63.9/63.0	62.9/62.5	64.7/63.3	65.2/63.4	62.2/64.7
(S2) *	23,536-25,263	1,728	575		98.1/99.3	98.1/99.3	98.1/99.3	98.1/99.1	87.9/94.8	88.1/95.8	85.1/92.7	87.9/95.4	86/93.5	76.6/86.2
ORF3a	25,271-26,095	825	274	$ACGAAC_{2}AUG$	99.2/98.1	98.6/97.0	98.7/97.0	98.5/96.7	90.4/90.8	84.1/84.3	88.8/86.8	83.6/84.3	83.1/82.4	72.1/71.2
ORF3b	25,692-26,036	345	114		99.1/99.1	98.2/98.2	98.2/98.2	97.9/97.3	99.1/98.2	N/D	82.6/92.1	N/D	N/D	N/D
E	26,120-26,350	231	76	$ACGAAC_{3}AUG$	98.7/98.6	98.7/98.6	98.7/98.6	98.7/98.6	99.1/98.6	97.8/98.6	96.5/96.0	96.1/97.3	97.4/98.6	91.3/93.4
M	26,401-27,066	666	221	$ACGAAC_{44}AUG$	97.4/98.1	97.2/98.1	97.2/98.1	97.2/97.7	98.7/99.5	93.3/98.1	96.3/98.6	93.2/95.4	93.9/96.8	78.5/88.1
ORF6	27,077-27,268	192	63	$ACGAAC_{155}AUG$	97.3/95.2	96.8/93.6	97.3/95.2	97.3/95.2	97.3/96.8	95.8/92.0	94.2/92.0	95.3/92	94.7/90.4	63.5/49.2
ORF7a	27,276-27,644	369	122	ACGAACAUG	94.5/95.9	94.5/95.9	94.5/95.9	94.5/95.9	97.8/100	96.2/99.1	92.9/95.0	93.4/97.5	93.2/97.5	62.3/58.1
ORF7b	27,641-27,776	135	44		96.2/93.1	96.2/93.1	96.2/93.1	96.2/93.1	99.2/100	99.2/100	97.7/97.7	99.2/100	93.3/95.4	62.9/63.6
ORF8	27,782-28,147	366	121	ACGAACAUG	47.1/46.3	N/A	N/A	47.1/46.3	97.8/100	85.2/90.2	46.2/39.0	85.7/90.2	85.7/85.3	N/A
N	28,162-29,430	1,269	422	$ACGAAC_{4}AUG$	98.3/99.5	98.4/99.5	98.4/99.5	98.4/99.5	98/98.5	96.6/97.8	93.7/95.2	96.2/97.1	95.9/96.2	77.9/87.2
s2m	29,628-29,668	41			97.5	97.5	97.5	97.5	100	100	100	100	100	95.1

*S1, the N-terminal domain of the coronavirus S protein responsible for receptor binding. S2, the S protein C-terminal domain responsible for membrane fusion.

The ORFs in the genome were predicted and potential protein sequences were translated. The pairwise comparisons were conducted for all ORFs at nucleotide acids (nt) and amino acids (aa) levels. The s2m were compared at nt level. TRS: Transcription regulating-sequences, N/D, not done; N/A, not available.

Extended Data Table 4 | Genomic annotation and comparison of bat SL-CoV RsSHC014 with human/civet SARS-CoVs and other bat SL-CoVs

					ORF Identity nt/aa (%)								
					Human and civet SARS-CoVs				Bat SARS-like CoVs				
ORFs	Start-End (nt.)	No. of Nt.	No. of Aa.	TRS	GZ02	BJ01	Tor2	SZ3	Rs672	Rp3	Rf1	Rm1	HKU3-1	BM48-31
P1a	265-13,398	13,134	4,377	$ACGAAC_{192}AUG$	96.7/97.9	96.6/97.9	96.8/97.9	96.8/98.1	93.3/94.2	95.5/96.9	88.1/94.0	87.9/93.3	87.9/94.2	76.3/80.8
P1b	13,398-21,485	8,088	2,695		96.3/99.2	96.3/99.2	96.3/99.2	96.3/99.2	97.2/99.2	97.2/99.2	90.6/98.4	91.0/98.7	90.7/98.5	83.4/93.7
S	21,492-25,262	3,771	1,256	ACGAACAUG	88.3/90.1	88.2/90.0	88.1/89.8	88.2/90.0	76.5/78.2	76.0/79.1	74.0/77.4	76.3/79.1	75.6/78.2	70.2/74.5
(S1)*	21,493-23,535	2,043	681		78.2/81.1	78.2/80.9	78.1/80.6	78.2/81.1	65.1/62.2	63.9/63.0	62.9/62.5	64.7/63.3	65.2/63.4	62.2/64.7
(S2)*	23,536-25,263	1,728	575		98.1/99.3	98.1/99.3	98.1/99.3	98.1/99.1	87.9/94.8	88.1/95.8	85.1/92.7	87.8/95.4	86/93.5	76.6/88.2
ORF3a	25,271-26,095	825	274	$ACGAAC_{2}AUG$	99.2/98.1	98.6/97.0	98.7/97.0	98.5/96.7	90.4/90.8	84.1/84.3	88.8/86.8	83.6/84.3	83.1/82.4	72.1/71.2
ORF3b	25,692-26,036	345	114		99.1/99.1	98.2/98.2	98.2/98.2	97.9/97.3	99.1/98.2	N/D	82.6/92.1	N/D	N/D	N/D
E	26,120-26,350	231	76	$ACGAAC_{2}AUG$	98.7/98.6	98.7/98.6	98.7/98.6	98.7/98.6	99.1/98.6	97.8/98.6	96.5/96.0	96.1/97.3	97.4/98.6	91.3/93.4
M	26,401-27,066	666	221	$ACGAAC_{44}AUG$	97.4/98.1	97.2/98.1	97.2/98.1	97.2/97.7	98.7/99.5	93.3/98.1	96.3/98.6	93.2/95.4	93.9/98.8	78.5/88.1
ORF6	27,077-27,268	192	63	$ACGAAC_{155}AUG$	97.3/95.2	96.8/93.6	97.3/95.2	97.3/95.2	97.3/96.8	95.8/92.0	94.2/92.0	95.3/92	94.7/90.4	63.5/49.2
ORF7a	27,276-27,644	369	122	ACGAACAUG	94.5/95.9	94.5/95.9	94.5/95.9	94.5/95.9	97.8/100	96.2/99.1	92.9/95.0	93.4/97.5	93.2/97.5	62.3/58.1
ORF7b	27,641-27,776	135	44		96.2/93.1	96.2/93.1	96.2/93.1	96.2/93.1	99.2/100	99.2/100	97.7/97.7	99.2/100	93.3/95.4	62.9/63.6
ORF8	27,782-28,147	366	121	ACGAACAUG	47.1/46.3	N/A	N/A	47.1/46.3	97.8/100	85.2/90.2	46.2/39.0	85.7/90.2	85.7/85.3	N/A
N	28,162-29,430	1,269	422	$ACGAAC_{8}AUG$	98.3/99.5	98.4/99.5	98.4/99.5	98.4/99.5	98/98.5	96.6/97.6	93.7/95.2	96.2/97.1	95.9/96.2	77.9/87.2
s2m	29,628-29,668	41			97.5	97.5	97.5	97.5	100	100	100	100	100	95.1

*S1, the N-terminal domain of the coronavirus S protein responsible for receptor binding. S2, the S protein C-terminal domain responsible for membrane fusion.

The ORFs in the genome were predicted and potential protein sequences were translated. The pairwise comparisons were conducted for all ORFs at nucleotide acids (nt) and amino acids (aa) levels. The s2m were compared at nt level. TRS: Transcription regulating-sequences; N/D, not done; N/A, not available.

Extended Data Table 5 | Cell lines used for virus isolation and susceptibility tests

Cell lines	Species (organ) origin	Medium	Infectivity
293T	Human (kidney)	DMEM+10%FBS	-
Hela	Human (cervix)		-
VeroE6	Monkey (kidney)		+
PK15	Pig (kidney)		+
BHK21	Hamster (kidney)		-
A549	Human (alveolar basal epithelial)		+
BK	*Myotis davidii* (kidney)	RPMI1640+10%FBS	-
RSKT	*Rhinolophus sinicus* (kidney)	DMEM/F12+10%FBS	+
MCKT	*Myotis chinensis* (kidney)		-
PaKi	*Pteropus alecto* (kidney)		-
RLK	*Rousettus leschenaulti* (kidney)		-

* Infectivity was determined by the presence of viral antigen detected by immunofluorescence assay.

PLOS | PATHOGENS

RESEARCH ARTICLE

Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files. The complete genome sequences of the 11 bat SARS-related coronaviruses newly identified in this study have been deposited in the GenBank database and assigned accession numbers KY417142 to KY417152, respectively.

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Abstract

A large number of SARS-related coronaviruses (SARSr-CoV) have been detected in horseshoe bats since 2005 in different areas of China. However, these bat SARSr-CoVs show sequence differences from SARS coronavirus (SARS-CoV) in different genes (S, ORF8, ORF3, *etc*) and are considered unlikely to represent the direct progenitor of SARS-CoV. Herein, we report the findings of our 5-year surveillance of SARSr-CoVs in a cave inhabited by multiple species of horseshoe bats in Yunnan Province, China. The full-length genomes of 11 newly discovered SARSr-CoV strains, together with our previous findings, reveals that the SARSr-CoVs circulating in this single location are highly diverse in the S gene, ORF3 and ORF8. Importantly, strains with high genetic similarity to SARS-CoV in the hypervariable N-terminal domain (NTD) and receptor-binding domain (RBD) of the S1 gene, the ORF3 and ORF8 region, respectively, were all discovered in this cave. In addition, we report the first discovery of bat SARSr-CoVs highly similar to human SARS-CoV in ORF3b and in the split ORF8a and 8b. Moreover, SARSr-CoV strains from this cave were more closely related to SARS-CoV in the non-structural protein genes ORF1a and 1b compared with those detected elsewhere. Recombination analysis shows evidence of frequent recombination events within the S gene and around the ORF8 between these SARSr-CoVs. We hypothesize that the direct progenitor of SARS-CoV may have originated after sequential recombination events between the precursors of these SARSr-CoVs. Cell entry studies demonstrated that three newly identified SARSr-CoVs with different S protein sequences are all able to use human ACE2 as the receptor, further exhibiting the close relationship between strains in this cave and SARS-CoV. This work provides new insights into the origin and evolution of SARS-CoV and highlights the necessity of preparedness for future emergence of SARS-like diseases.

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Author summary

Increasing evidence has been gathered to support the bat origin of SARS coronavirus (SARS-CoV) in the past decade. However, none of the currently known bat SARSr-CoVs is thought to be the direct ancestor of SARS-CoV. Herein, we report the identification of a diverse group of bat SARSr-CoVs in a single cave in Yunnan, China. Importantly, all of the building blocks of SARS-CoV genome, including the highly variable S gene, ORF8 and ORF3, could be found in the genomes of different SARSr-CoV strains from this single location. Based on the analysis of full-length genome sequences of the newly identified bat SARSr-CoVs, we speculate that the direct ancestor of SARS-CoV may have arisen from sequential recombination events between the precursors of these bat SARSr-CoVs prior to spillover to an intermediate host. In addition, we found bat SARSr-CoV strains with different S proteins that can all use the receptor of SARS-CoV in humans (ACE2) for cell entry, suggesting diverse SARSr-CoVs capable of direct transmission to humans are circulating in bats in this cave. Our current study therefore offers a clearer picture on the evolutionary origin of SARS-CoV and highlights the risk of future emergence of SARS-like diseases.

Introduction

Severe Acute Respiratory Syndrome (SARS) is a severe emerging viral disease with high fatality characterized by fever, headache and severe respiratory symptoms including cough, dyspnea and pneumonia [1]. Due to its high transmissibility among humans, after its first emergence in southern China in late 2002, it rapidly led to a global pandemic in 2003 and was marked as one of the most significant public health threats in the 21st century [2,3]. The causative agent, SARS coronavirus (SARS-CoV), has been previously assigned to group 2b CoV and is now a member of the lineage B of genus *Betacoronavirus* in the family *Coronaviridae* [4]. It shares similar genome organization with other coronaviruses, but exhibits a unique genomic structure which includes a number of specific accessory genes, including ORF3a, 3b, ORF6, ORF7a, 7b, ORF8a, 8b and 9b [5,6].

Masked palm civets (*Paguma larvata*) were initially hypothesized to be the animal origin of SARS-CoV [7,8]. However, since a large number of genetically diverse SARS-related coronaviruses (SARSr-CoV) have been detected in multiple species of horseshoe bats (genus *Rhinolophus*) from different areas of China and Europe in the aftermath of SARS, it is prevailingly considered that SARS-CoV originated in horseshoe bats with civets acting as the intermediate amplifying and transmitting host [9–16]. Recently we have reported four novel SARSr-CoVs from Chinese horseshoe bats that shared much higher genomic sequence similarity to the epidemic strains, particularly in their S gene, of which two strains (termed WIV1 and WIV16) have been successfully cultured *in vitro* [17,18]. These newly identified SARSr-CoVs have been demonstrated to use the same cellular receptor (angiotensin converting enzyme-2 [ACE-2]) as SARS-CoV does and replicate efficiently in primary human airway cells [17–19].

Despite the cumulative evidence for the emergence of SARS-CoV from bats, all bat SARSr-CoVs described so far are clearly distinct from SARS-CoV in the S gene and/or one or more accessory genes such as ORF3 and ORF8, suggesting they are likely not the direct ancestor of SARS-CoV. Thus a critical gap remains in our understanding of how and where SARS-CoV originated from bat reservoirs. Previously, we reported a number of bat SARSr-CoVs with diverse S protein sequences from a single cave in Yunnan Province, including the four strains

mentioned above most closely related to SARS-CoV [17,18]. Here we report the latest results of our 5-year longitudinal surveillance of bat SARSr-CoVs in this single location and systematic evolutionary analysis using full-length genome sequences of 15 SARSr-CoV strains (11 novel ones and 4 from previous studies). Efficiency of human ACE2 usage and the functions of accessory genes ORF8 and 8a were also evaluated for some of the newly identified strains.

Results

Continued circulation of diverse SARSr-CoVs in bats from a single location

We have carried out a five-year longitudinal surveillance (April 2011 to October 2015) on SARSr-CoVs in bats from a single habitat in proximity to Kunming city, Yunnan province, China, which was mainly inhabited by horseshoe bats. A total of 602 alimentary specimens (anal swabs or feces) were collected and tested for the presence of CoVs by a Pan-CoV RT-PCR targeting the 440-nt RdRp fragment that is conserved among all known α- and β-CoVs [20]. In total, 84 samples tested positive for CoVs. Sequencing of the PCR amplicons revealed the presence of SARSr-CoVs in the majority (64/84) of the CoV-positive samples (Table 1). Host species identification by amplification of either *Cytb* or *ND1* gene suggested that most (57/64) of the SARSr-CoV positive samples were from *Rhinolophus sinicus*, while the remaining 7 samples were from *Rhinolophus ferrumequinum*, *Rhinolophus affinis* and from *Aselliscus stoliczkanus* which belongs to the family *Hipposideridae*.

Based on the preliminary analysis of the partial RdRp sequences, all of the 64 bat SARSr-CoV sequences showed high similarity among themselves and with other reported bat SARSr-CoVs and SARS-CoVs from humans and civets. To understand the genetic diversity of these bat SARSr-CoVs, the most variable region of the SARSr-CoV S gene, corresponding to the receptor-binding domain (RBD) of SARS-CoV, were amplified and sequenced. Due to low viral load in some samples, RBD sequences were successfully amplified only from 49 samples. These RBD sequences displayed high genetic diversity and could be divided into two large clades, both of which included multiple genotypes. Clade 1 strains shared an identical size and higher amino acid (aa) sequence identity with SARS-CoV RBD, while clade 2 had a shorter size than SARS-CoV S due to two deletions (5 and 12–13 aa, respectively) (S1 Fig). Co-infections by two strains of different clades were detected in two samples, Rs3262 and Rs4087 (S1 Fig).

Table 1. Summary of SARSr-CoV detection in bats from a single habitat in Kunming, Yunnan.

Sampling time	Sample type	Sample Numbers			SARSr-CoV + bat species (No.)
		Total	CoV +	SARSr-CoV +	
April, 2011	anal swab	14	1	1	*R. sinicus* (1)
October, 2011	anal swab	8	3	3	*R. sinicus* (3)
May, 2012	anal swab & feces	54	9	4	*R. sinicus* (4)
September, 2012	feces	39	20	19	*R. sinicus* (16)
					R. ferrumequinum (3)
April, 2013	feces	52	21	16	*R. sinicus* (16)
July, 2013	anal swab & feces	115	9	8	*R. sinicus* (8)
May, 2014	feces	131	8	4	*A. stoliczkamus* (3)
					R. affinis (1)
October, 2014	anal swab	19	4	4	*R. sinicus* (4)
May, 2015	feces	145	3	0	
October, 2015	anal swab	25	6	5	*R. sinicus* (5)
Total		***602***	***84***	***64***	***R (61) A (3)***

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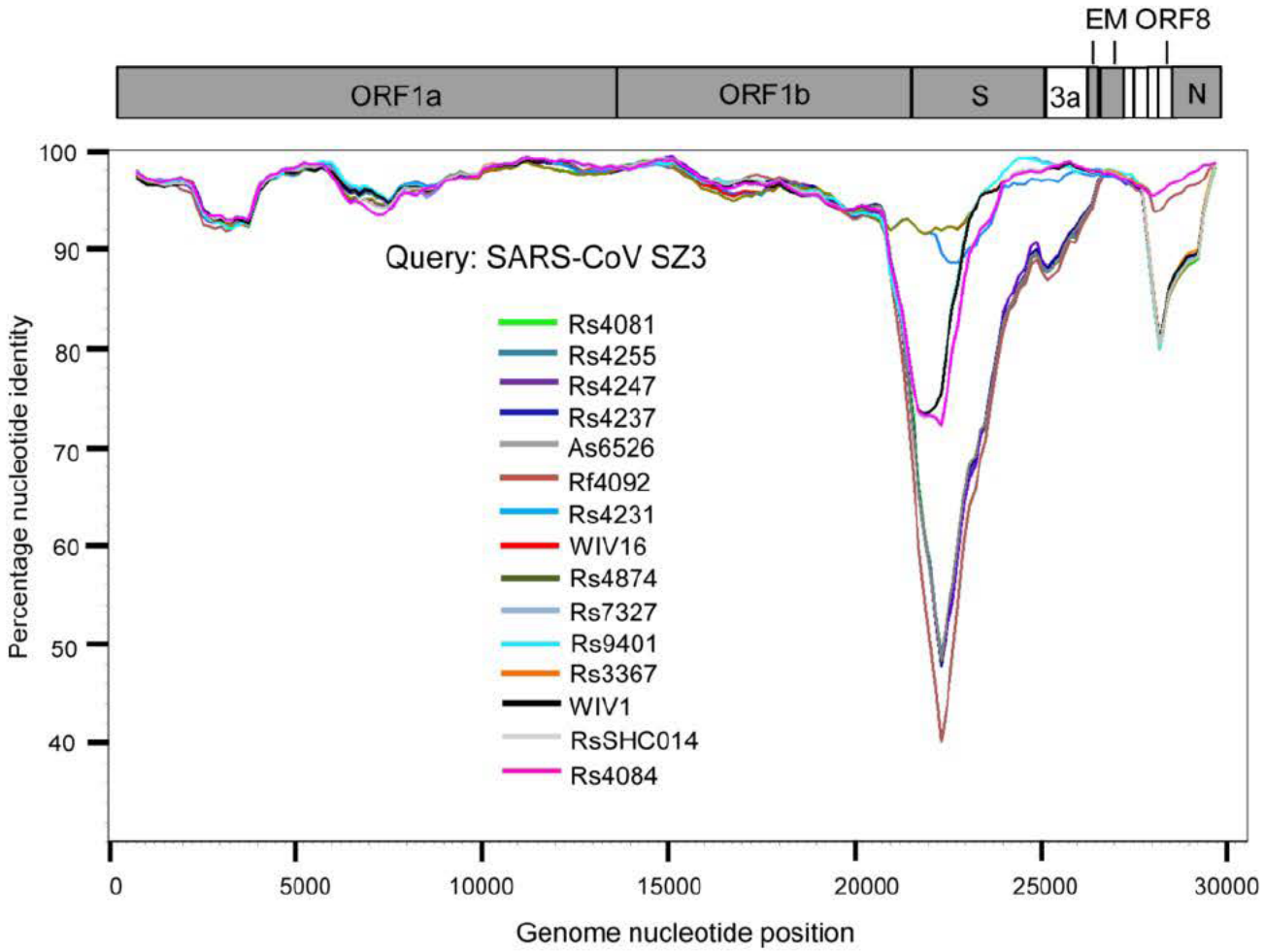

Fig 1. Similarity plot based on the full-length genome sequence of civet SARS CoV SZ3. Full-length genome sequences of all SARSr-CoV detected in bats from the cave investigated in this study were used as reference sequences. The analysis was performed with the Kimura model, a window size of 1500 base pairs and a step size of 150 base pairs.

https://doi.org/10.1371/journal.ppat.1006698.g001

Genomic characterization of the novel SARSr-CoVs

Based on the diversity of RBD sequences, 11 novel SARSr-CoV strains named by abbreviation of bat species and sample ID (Rs4081, Rs4084, Rs4231, Rs4237, Rs4247, Rs4255, Rs4874, Rs7327, Rs9401, Rf4092 and As6526) were selected for full-length genomic sequencing based on sample abundance, genotype of RBD as well as sampling time. For each RBD genotype and each time of sampling, at least one representative strain was selected. The genome size of these novel SARSr-CoVs ranged from 29694 to 30291 nucleotides (nt). This gave a total of 15 full-length genomes of bat SARSr-CoVs from this single location (13 from *R.sinicus*, and one each from *R. ferrumequinum* and *A. stoliczkanus*), including our previously reported strains, Rs3367, RsSHC014, WIV1 and WIV16 [17,18]. The genomes of all 15 SARSr-CoVs circulating in this single cave shared 92.0% to 99.9% nt sequence identity. The overall nt sequence identity between these SARSr-CoVs and human and civet SARS-CoVs is 93.2% to 96%, significantly higher than that observed for bat SARSr-CoVs reported from other locations in China (88–93%) [9,10,12,14,21,22]. The genome sequence similarity among the 15 SARSr-CoVs and SARS-CoV SZ3 strain was examined by Simplot analysis (Fig 1). The 15 SARSr-CoVs are

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highly conserved and share a uniformly high sequence similarity to SARS-CoV in the non-structural gene ORF1a (96.6% to 97.1% nt sequence identity, 98.0% to 98.3% aa sequence identity) and ORF1b (96.1% to 96.6% nt sequence identity, 99.0% to 99.4% aa sequence identity). In contrast, a considerable genetic diversity is shown in the S gene (corresponding to SZ3 genome position 21477 to 25244) and ORF8 (corresponding to SZ3 genome position 27764 to 28132) (Fig 1).

The 11 novel SARSr-CoVs identified from this single location generally shared similar genome organization with SARS-CoV and other bat SARSr-CoVs. In our previous study, we identified an additional ORF termed ORFx present between ORF6 and ORF7 in strain WIV1 and WIV16 [18,23]. In this study, ORFx was also found in the genomes of Rs7327 and Rs4874. Compared with that of WIV1 and WIV16, the length of ORFx in Rs7327 and Rs4874 was extended to 510 nt due to a deletion of 2 nt in a poly-T sequence that resulted in a shift of reading frame (Fig 2 and S2 Fig).

Co-circulation of different bat SARSr-CoVs with S, ORF8 and ORF3 sequences similar to those in SARS-CoV at a single location

The primary difference between SARS-CoV and most bat SARSr-CoVs is located in S gene. The S protein is functionally divided into two subunits, denoted S1 and S2, which is responsible for receptor binding and cellular membrane fusion, respectively. S1 consists of two domains, the N-terminal domain (NTD) and C-terminal domain (CTD) which is also known as the RBD in SARS-CoV [24]. SARS-CoV and bat SARSr-CoVs share high sequence identity in the S2 region in contrast to the S1 region. Among the 15 SARSr-CoVs identified from bats in the surveyed cave, six strains with deletions in their RBD regions (Rs4081, Rs4237, Rs4247, Rs4255, Rf4092 and As6526) showed 78.2% to 80.2% aa sequence identity to SARS-CoV in the S protein, while the other nine strains without deletions were much more closely related to SARS-CoV, with 90.0% (Rs4084) to 97.2% (Rs4874) aa sequence identity. These nine SARSr-CoVs can be further divided into four genotypes according to their S1 sequences (Fig 2): RsSHC014/Rs4084 showed more genetic differences from SARS-CoV in both NTD and RBD regions; The RBD sequences of SARSr-CoV Rs7327, Rs9401 and previously reported WIV1/Rs3367 closely resembled that of SARS-CoV. However, they were distinct from SARS-CoV but similar to RsSHC014 in NTD. In contrast, we found a novel SARSr-CoV, termed Rs4231, which shared highly similar NTD, but not RBD sequence with SARS-CoV (Figs 2 and 3). Its S protein showed 94.6% to 95% aa sequence identity to those of human and civet SARS-CoVs (S1 Table). Strains with both NTD and RBD highly homologous to those of SARS-CoV were also present in this cave. In addition to WIV16 which we described previously [18], Rs4874 was also found to have the S protein closest to SARS-CoV S (> 97% aa sequence identity) of all the bat SARSr-CoVs reported to date (Figs 2 and 3). In addition to the SARSr-CoVs subjected to full-length genome sequencing, we also obtained the RBD and NTD sequences from other samples collected in this cave. The sequences with high identity to SARS-CoV RBD were amplified from 10 more *R. sinicus* samples. SARSr-CoVs with this genotype of RBD were detected in different seasons throughout the five years. Strains containing the NTD similar to SARS-CoV were only found in 2013 (S2 Table).

ORF8 is another highly variable gene among different SARS-CoV and SARSr-CoV strains [25,26]. We aligned the ORF8 nt sequences of the representative SARSr-CoVs discovered in this surveillance with those of other SARSr-CoVs and SARS-CoVs (Fig 4). Though WIV16, WIV1, Rs4231 and RsSHC014 were genetically closer to SARS-CoV in S gene, they contained a single 366-nt ORF8 without the 29-nt deletion present in most human SARS-CoVs and showed only 47.1% to 51.0% nt sequence identity to human and civet SARS-CoVs. However,

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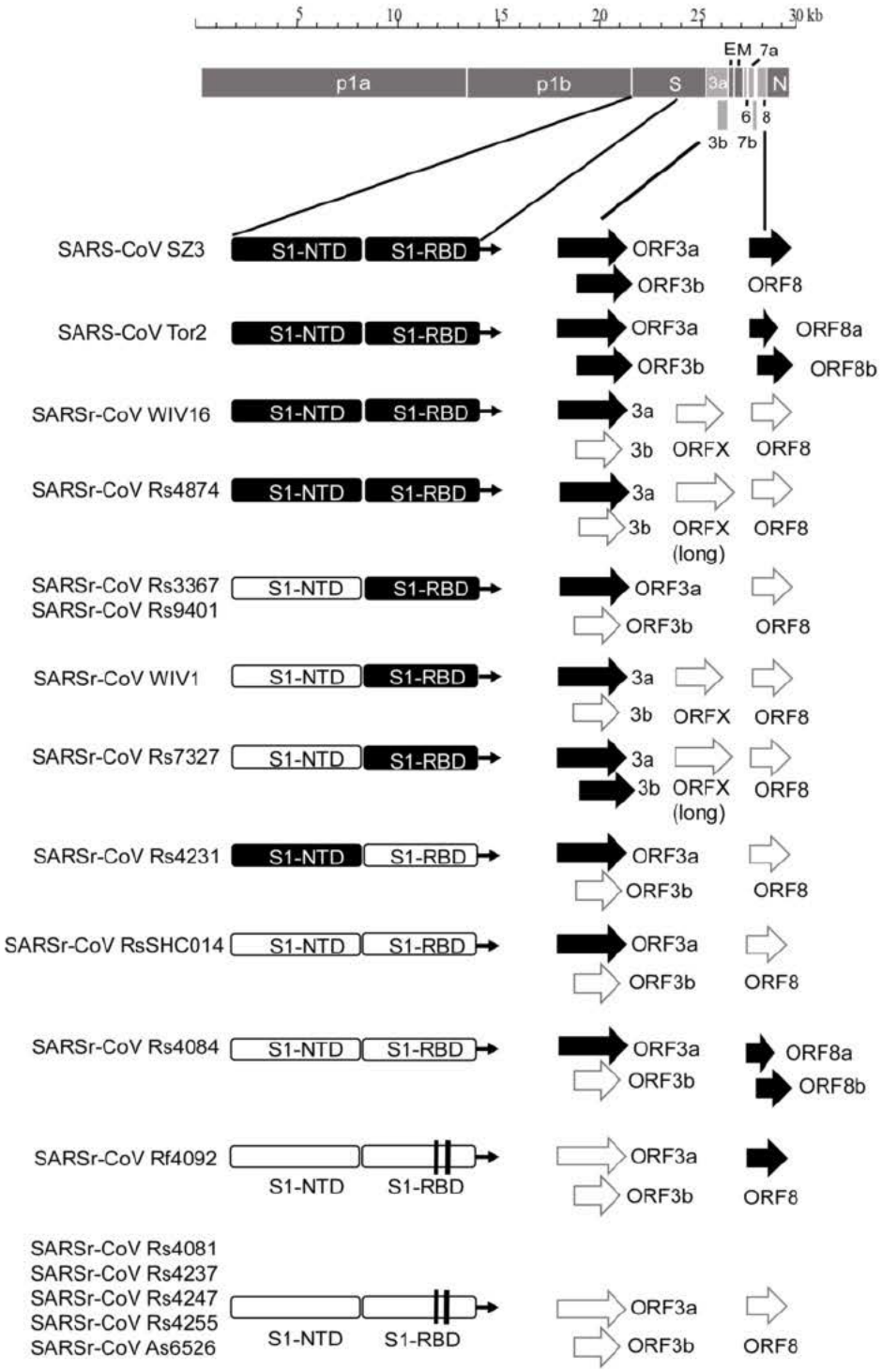

Fig 2. Schematic diagram illustrating the genomic regions or ORFs with most variation between different SARS-CoV and SARSr-CoV isolates. Coding regions of the N-terminal domain (NTD) and receptor-binding domain (RBD) of the spike protein, ORF3a/b and ORF8 (8a/b) in bat SARSr-CoV genomes highly similar to those in SARS CoV genome are indicated with black boxes or arrows while the hollow boxes or arrows represent corresponding regions with less sequence similarity to those of SARS-CoV. The deletions in the RBD of some SARSr-CoVs are indicated by two vertical lines.

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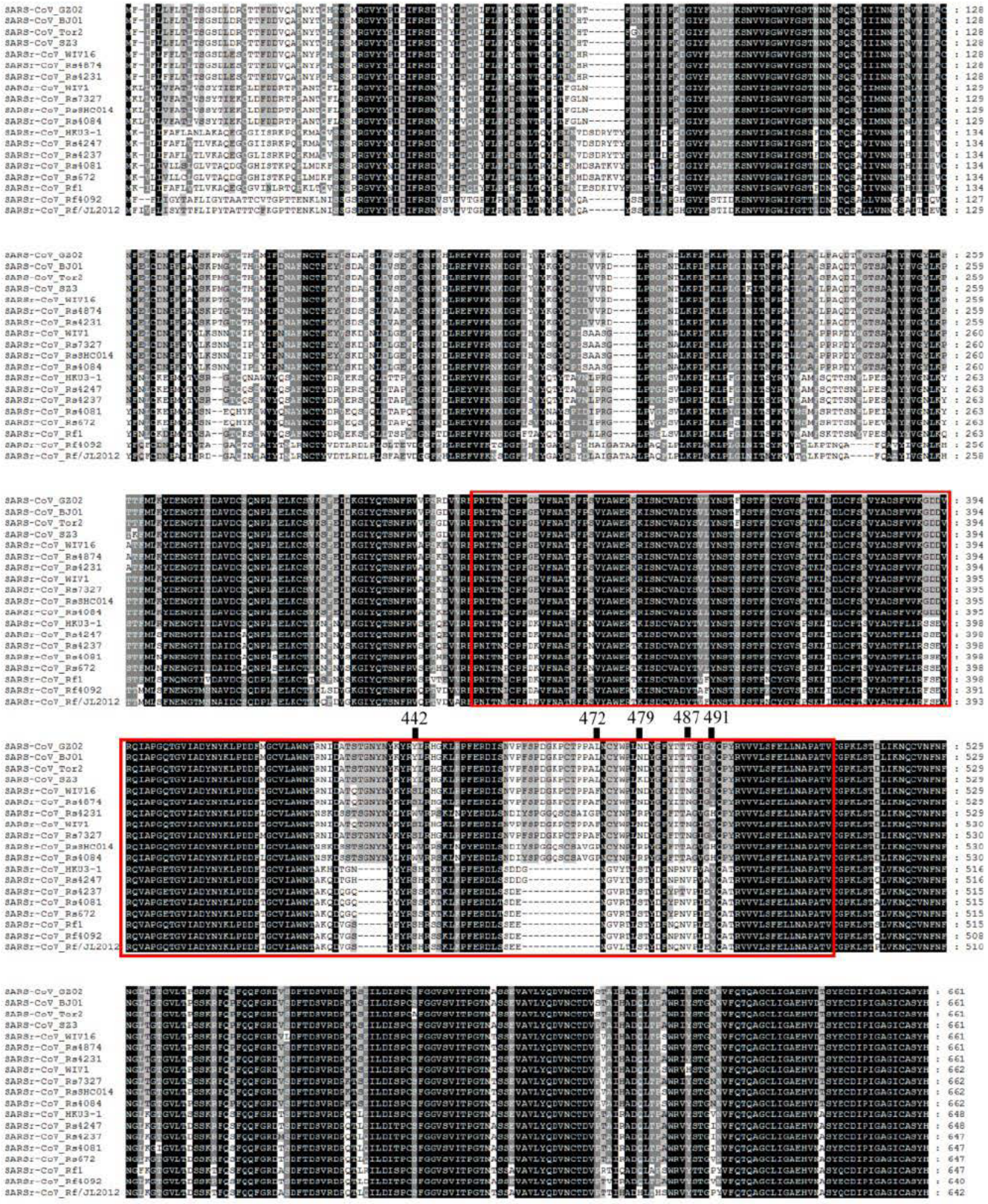

Fig 3. Amino acid sequence comparison of the S1 subunit (corresponding to aa 1–660 of the spike protein of SARS-CoV). The receptor-binding domain (aa 318–510) of SARS-CoV and the homologous region of bat SARSr-CoVs are indicated by the red box. The key aa

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residues involved in the interaction with human ACE2 are numbered on top of the aligned sequences. SARS-CoV GZ02, BJ01 and Tor2 were isolated from patients in the early, middle and late phase, respectively, of the SARS outbreak in 2003. SARS-CoV SZ3 was identified from civets in 2003. SARSr-CoV Rs 672 and YN2013 were identified from *R. sinicus* collected in Guizhou and Yunnan Province, respectively. SARSr-CoV Rf1 and JL2012 were identified from *R. ferrumequinum* collected in Hubei and Jilin Province, respectively. WIV1, WIV16, RsSHC014, Rs4081, Rs4084, Rs4231, Rs4237, Rs4247, Rs7327 and Rs4874 were identified from *R.sinicus*, and Rf4092 from *R. ferrumequinum* in the cave surveyed in this study.

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the ORF8 of strain Rf4092 from *R. ferrumequinum* exhibited high similarity to that of civet SARS-CoV. It possessed a single long ORF8 of the same length (369 nt) as that of civet SARS--CoV strain SZ3, with only 10 nt mutations and 3 aa mutations detected (Fig 4). Similar ORF8 sequences were also amplified from other 7 samples collected in the cave during 2011 to 2013, from both *R. ferrumequinum* and *R. sinicus* (S2 Table). The ORF8 of Rs4084 was highly similar to Rf4092's but was split into two overlapping ORFs, ORF8a and ORF8b, due to a short 5-nt deletion (Figs 2 and 4). The position of start codons and stop codons of the two ORFs were consistent with those in most human SARS-CoV strains. Excluding the 8-aa insertion, Rs4084 and SARS-CoV strain BJ01 displayed identical aa sequence of ORF8a, and only three different

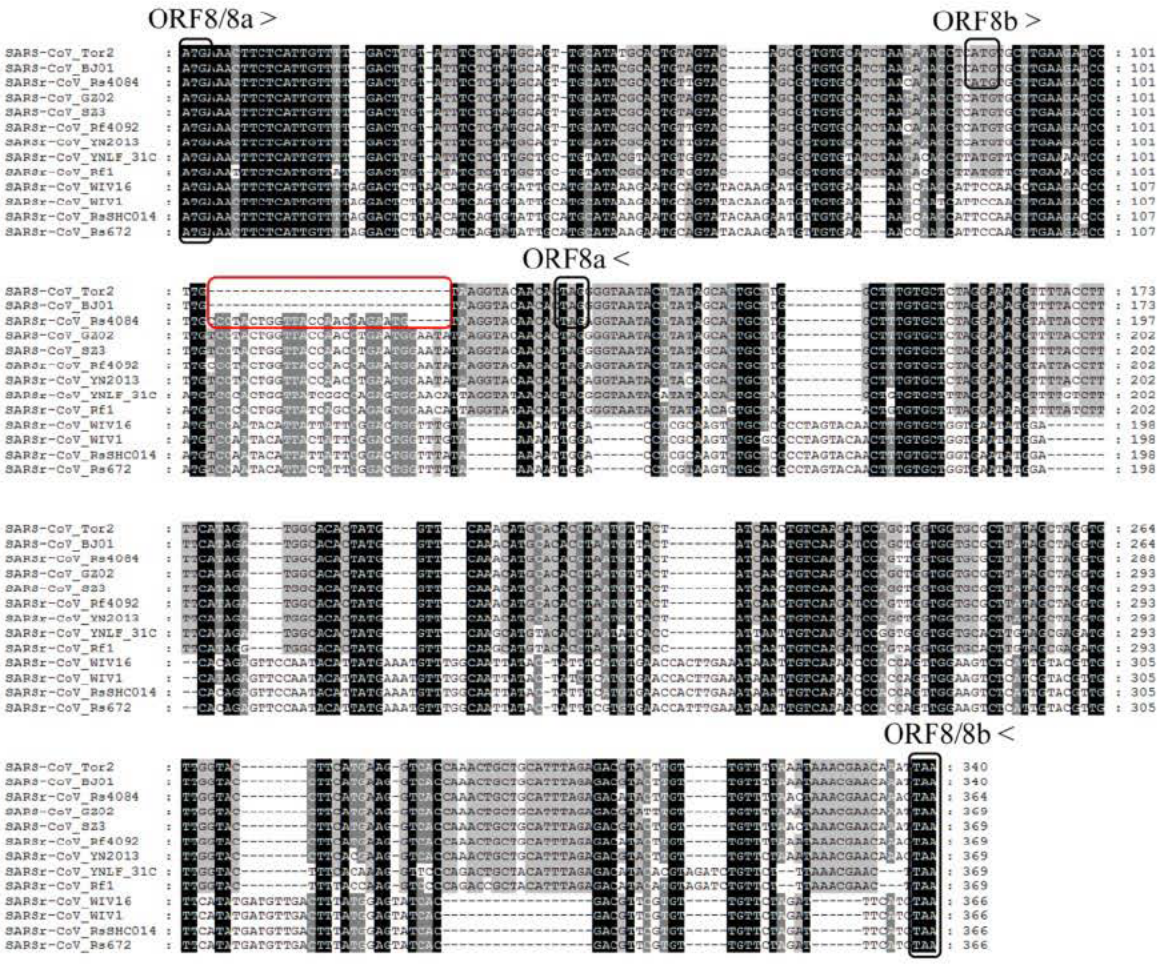

Fig 4. Alignment of nucleotide sequences of ORF8 or ORF8a/8b. The start codons and stop codons of ORF8, 8a and 8b are marked with black boxes and the forward and reverse arrows, respectively. The deletion responsible for the split ORF8a and 8b in human SARS-CoV BJ01, Tor2 and bat SARSr-CoV Rs4084 is marked with red boxes. See the legend for Fig 3 for the origin of various sequences used in this alignment.

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aa residues were observed between their ORF8b (Fig 4). To our knowledge, Rs4084 was the first bat SARSr-CoV reported that resembled the late human SARS-CoVs in both ORF8 gene organization and sequence.

Another key difference between SARS-CoV and bat SARSr-CoV genomes is the ORF3 coding region [10,17,21]. We analyzed the ORF3a sequences amplified from 42 samples and found that most of the SARSr-CoVs closely related to SARS-CoV in the S gene shared higher ORF3a sequence similarity (96.4% to 98.9% aa identity) with SARS-CoV (S3 Fig and S2 Table). The ORF3b of SARS CoV, sharing a large part of its coding sequence with the ORF3a, encodes a 154-aa protein [27], but it is truncated to different extents at the C-terminal in previously described bat SARSr-CoVs including WIV1 and WIV16 (S4 Fig). In the current study, we identified a non-truncated ORF3b for the first time (Rs7327), which maintained the nuclear localization signal at its C-terminal. Moreover, it shared 98.1% aa sequence identity with SARS-CoV strain Tor2 with only three aa substitutions (S4 Fig). Thus, Rs7327 is the bat SARSr-CoV most similar to SARS-CoV in the ORF3 region known to date.

Recombination analysis

The full-length genome sequences of all 15 SARSr-CoVs from the surveyed cave were screened for evidence of potential recombination events. Both similarity plot and bootscan analyses revealed frequent recombination events among these SARSr-CoV strains. It was suggested that WIV16, the closest progenitor of human SARS-CoV known to date [18], was likely to be a recombinant strain from three SARSr-CoVs harbored by bats in the same cave, namely WIV1, Rs4231 and Rs4081, with strong *P* value ($<10^{-30}$). Breakpoints were identified at genome positions nt 18391, 22615 and 28160 (Fig 5A). In the genomic region between nt 22615 and 28160, which contained the region encoding the RBD and the S2 subunit of the S protein, WIV16 was highly similar to WIV1, sharing 99% sequence identity. In contrast, in the region between nt 18391 and 22615, which covered a part of ORF1b and the region encoding the NTD of the S gene, WIV16 showed substantially closer relationship to Rs4231. Meanwhile, the ORF1ab sequences upstream from nt 18391 of WIV16 displayed the highest genetic similarity (99.8% nt sequence identity) to that of Rs4081.

Evidence of recombination event was also detected in the genome of the novel SARSr-CoV Rs4084, which had a unique genome organization with split ORF8a and 8b. The previously reported strain RsSHC014 and the newly identified strain Rf4092 were suggested to be the major and minor parent of Rs4084, respectively (P value $< 10^{-80}$). The breakpoint was located at nt 26796 (S5 Fig). In the region downstream of the breakpoint including ORF8, Rs4084 showed closet genetic relationship with Rf4092, sharing 98.9% nt sequence identity, while it shared the highest nt sequence identity (99.4%) with RsSHC014 in the majority of its genome upstream from the breakpoint.

When civet SARS-CoV SZ3 was used as the query sequence in similarity plot and bootscan analysis, evidence for recombination events was also detected (Fig 5B). In the region between the two breakpoints at the genome positions nt 21161 and nt 27766, including the S gene, closer genetic relationship between SZ3 and WIV16 was observed. However, from position nt 27766 towards the 3' end of its genome, a notably close genetic relationship was observed between SZ3 and Rf4092 instead. Throughout the non-structural gene, moreover, SZ3 shared a similarly high sequence identity with WIV16 and Rf4092. It indicates that civet SARS-CoV was likely to be the descendent from a recombinant of the precursors of WIV16 and Rf4092, or that the SARSr-CoVs found in this cave, like WIV16 or Rf4092, may have been the descendants of the SARS-CoV lineage.

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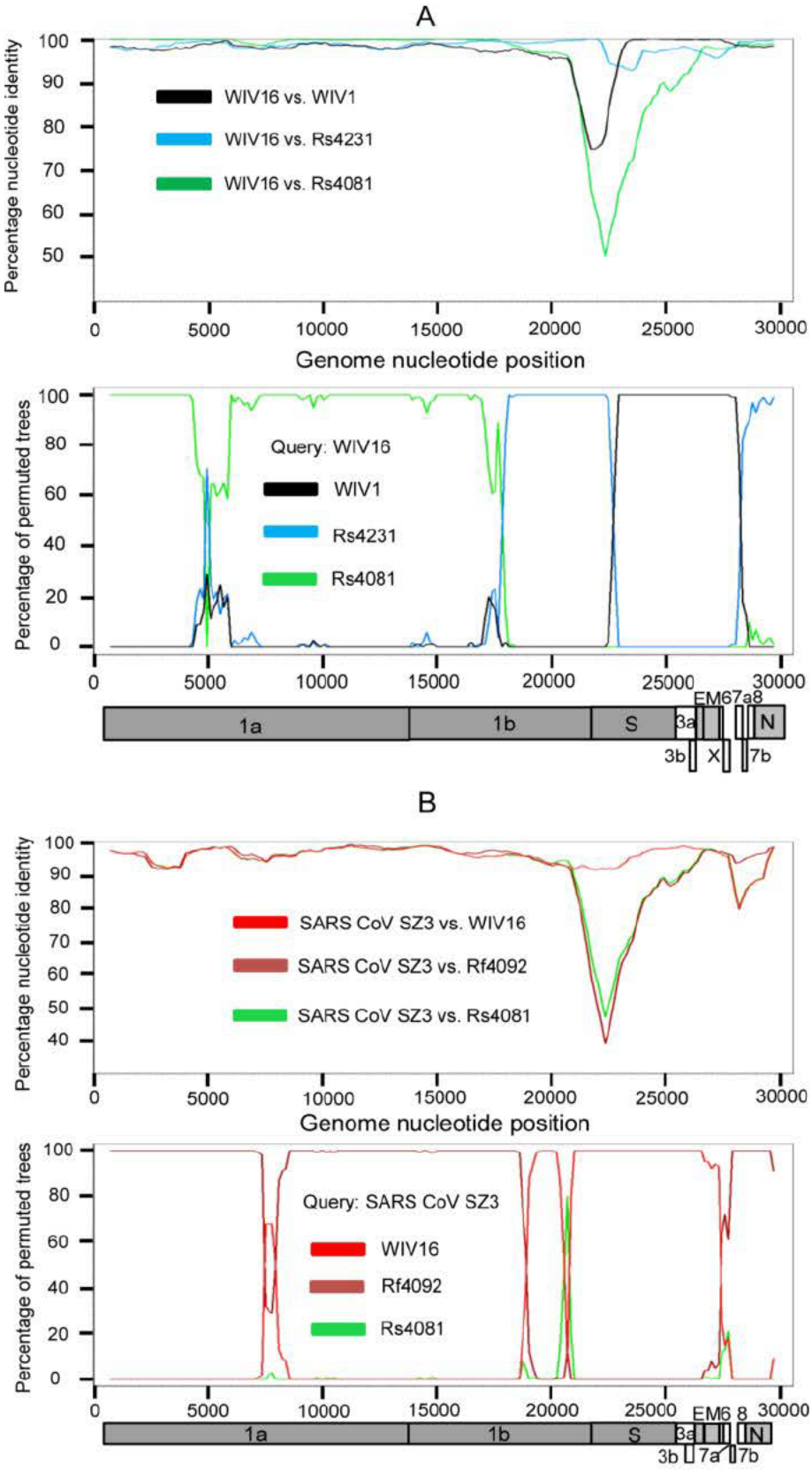
A
Percentage nucleotide identity
WIV16 vs. WIV1
WIV16 vs. Rs4231
WIV16 vs. Rs4081
Genome nucleotide position
Percentage of permuted trees
Query: WIV16
WIV1
Rs4231
Rs4081
1a
1b
S
3a
EM
6
7a
8
N
3b
X
7b
B
SARS CoV SZ3 vs. WIV16
SARS CoV SZ3 vs. Rf4092
SARS CoV SZ3 vs. Rs4081
Query: SARS CoV SZ3
WIV16
Rf4092
Rs4081

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Fig 5. Detection of potential recombination events by similarity plot and boot scan analysis. (A) Full-length genome sequence of SARSr-CoV WIV16 was used as query sequence and WIV1, Rs4231 and Rs4081 as reference sequences. (B) Full-length genome sequence of SARS-CoV SZ3 was used as query sequence and SARSr-CoV WIV16, Rf4092 and Rs4081 as reference sequences. All analyses were performed with a Kimura model, a window size of 1500 base pairs, and a step size of 150 base pairs. The gene map of query genome sequences are used to position breakpoints.

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Phylogenetic analysis

Phylogenetic trees were constructed using the nt sequences of nonstructural protein gene ORF1a and ORF1b. Unlike the high genetic diversity in the S gene, nearly all SARSr-CoVs from the bat cave we surveyed were closely clustered, and showed closer phylogenetic relationship to SARS-CoV than the majority of currently known bat SARSr-CoVs discovered from other locations, except YNLF_31C and 34C which were recently reported in greater horseshoe bats from another location in Yunnan [22] (Fig 6). The phylogeny of SARSr-CoVs in ORF1a and ORF1b appeared to be associated with their geographical distribution rather than with host species. Regardless of different host bat species, SARS-CoV and SARSr-CoVs detected in bats from southwestern China (Yunnan, Guizhou and Guangxi province) formed one clade, in which SARSr-CoV strains showing closer relationship to SARS-CoV were all from Yunnan. SARSr-CoVs detected in southeastern, central and northern provinces, such as Hong Kong, Hubei and Shaanxi, formed the other clade which was phylogenetically distant to human and civet SARS-CoVs (Fig 6 and S6 Fig).

Rescue of bat SARSr-CoVs and virus infectivity experiments

In the current study, we successfully cultured an additional novel SARSr-CoV Rs4874 from a single fecal sample using an optimized protocol and Vero E6 cells [17]. Its S protein shared 99.9% aa sequence identity with that of previously isolated WIV16 and it was identical to WIV16 in RBD. Using the reverse genetics technique we previously developed for WIV1 [23], we constructed a group of infectious bacterial artificial chromosome (BAC) clones with the backbone of WIV1 and variants of S genes from 8 different bat SARSr-CoVs. Only the infectious clones for Rs4231 and Rs7327 led to cytopathic effects in Vero E6 cells after transfection (S7 Fig). The other six strains with deletions in the RBD region, Rf4075, Rs4081, Rs4085, Rs4235, As6526 and Rp3 (S1 Fig) failed to be rescued, as no cytopathic effects was observed and viral replication cannot be detected by immunofluorescence assay in Vero E6 cells (S7 Fig). In contrast, when Vero E6 cells were respectively infected with the two successfully rescued chimeric SARSr-CoVs, WIV1-Rs4231S and WIV1-Rs7327S, and the newly isolated Rs4874, efficient virus replication was detected in all infections (Fig 7). To assess whether the three novel SARSr-CoVs can use human ACE2 as a cellular entry receptor, we conducted virus infectivity studies using HeLa cells with or without the expression of human ACE2. All viruses replicated efficiently in the human ACE2-expressing cells. The results were further confirmed by quantification of viral RNA using real-time RT-PCR (Fig 8).

Activation of activating transcription factor 6 (ATF6) by the ORF8 proteins of different bat SARSr-CoVs

The induction of the ATF6-dependent transcription by the ORF8s of SARS-CoV and bat SARSr-CoVs were investigated using a luciferase reporter, 5×ATF6-GL3. In HeLa cells transiently transfected with the expression plasmids of the ORF8s of bat SARSr-CoV Rf1, Rf4092 and WIV1, the relative luciferase activities of the 5×ATF6-GL3 reporter was enhanced by 5.56 to 9.26 folds compared with cells transfected with the pCAGGS empty vector, while it was

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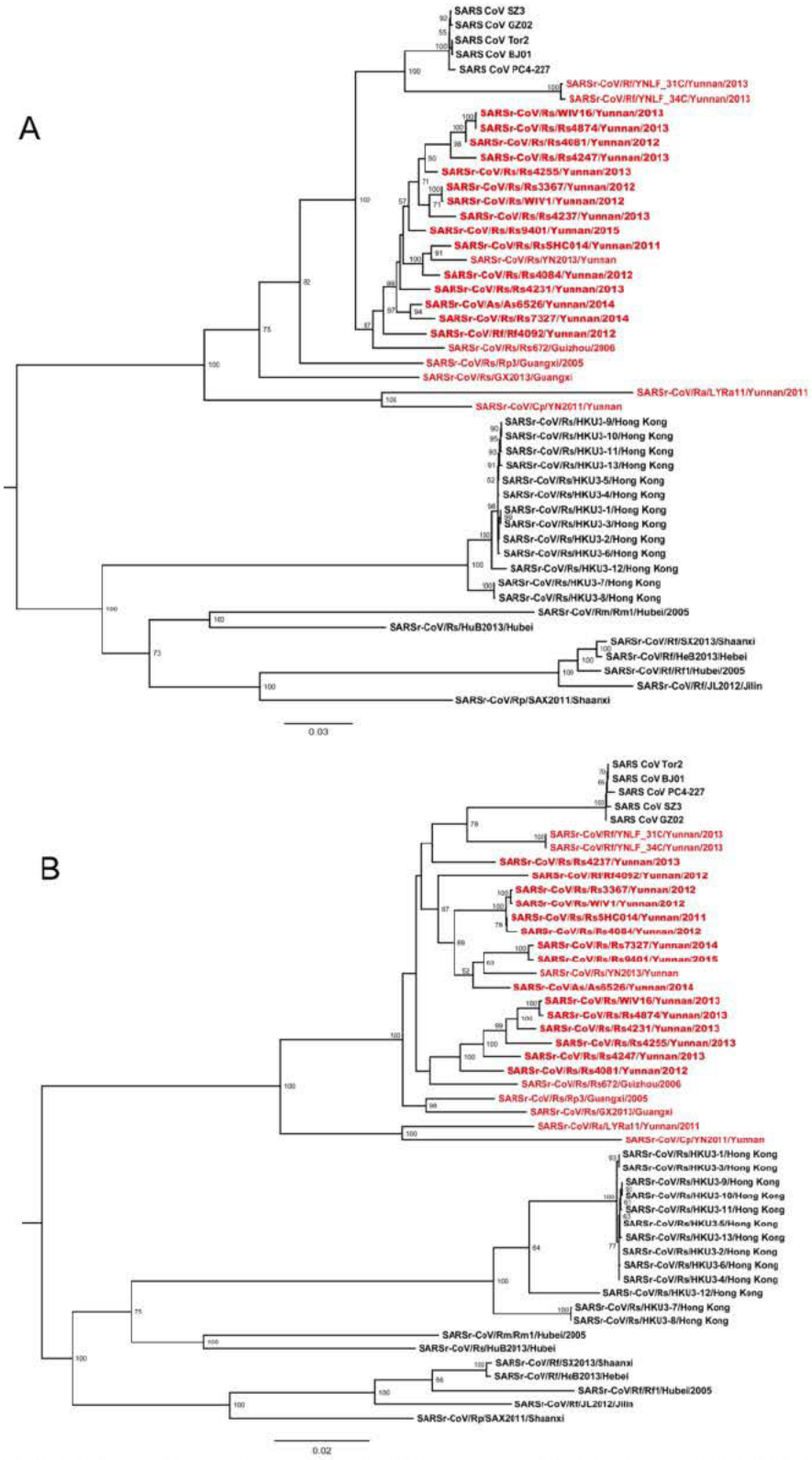

Fig 6. Phylogenetic trees based on nucleotide sequences of ORF1a (A) and ORF1b (B). The trees were constructed by the maximum likelihood method using the LG model with bootstrap values determined by 1000 replicates. Only bootstraps > 50% are shown. The scale bars represent 0.03 (A) and 0.02 (B) substitutions per

nucleotide position. Rs, *Rhinolophus sinicus*; Rf, *Rhinolophus ferremequinum*; Rm, *Rhinolophus macrotis*; Ra, *Rhinolophus affinis*; Rp, *Rhinolophus pusillus*; As, *Aselliscus stoliczkanus*; Cp, *Chaerephon plicata*. SARSr-CoVs detected in bats from the single cave surveyed in this study are in bold. Sequences detected in southwestern China are indicated in red.

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increased by 4.42 fold by the SARS-CoV GZ02 ORF8. As a control, the treatment with tunicamyxin (TM) stimulated the transcription by about 11 folds (Fig 9A). The results suggests that various ORF8 proteins of bat SARSr-CoVs can activate ATF6, and those of some strains have a stronger effect than the SARS-CoV ORF8.

Induction of apoptosis by the ORF8a of the newly identified bat SARSr-CoV

We conducted transient transfection to examine whether the ORF8a of SARSr-CoV Rs4084 triggered apoptosis. As shown in Fig 9B, 11.76% and 9.40% of the 293T cells transfected with the SARSr-CoV Rs4084-ORF8a and SARS-CoV Tor2-ORF8a expression plasmid underwent apoptosis, respectively. In contrast, transfection with the empty vector resulted in apoptosis in only 2.79% of the cells. The results indicate that Rs4084 ORF8a has an apoptosis induction activity similar to that of SARS-CoV [28].

Discussion

Genetically diverse SARSr-CoVs have been detected in various horseshoe bat species across a wide geographic range in China in the past decade [9–12,14,29]. However, most bat SARSr-CoVs show considerable genetic distance to SARS-CoV, particularly in the highly variable S1, ORF8 and ORF3 regions [10,25]. Recently, several novel SARSr-CoVs have been described to be more closely related to SARS-CoV, either in the S gene or in ORF8. The S proteins of RsSHC014, Rs3367, WIV1 and WIV16, which were reported in our previous studies, shared 90% to 97% aa sequence identities to those of human/civet SARS-CoVs [17,18]. Another strain from *Rhinolophus affinis* in Yunnan termed LYRa11 showed 90% aa sequence identity to SARS-CoV in the S gene [13]. In addition, two studies have described 4 novel SARSr-CoVs (YNLF_31C/34C and GX2013/YN2013) which possessed a full-length ORF8 with substantially higher similarity to that of SARS-CoV [22,30]. These findings provide strong genetic evidence for the bat origin of SARS-CoV with regard to the S gene or ORF8. However, all of these SARSr-CoVs were distinct from SARS-CoV in at least one other gene, suggesting that none of them was the immediate progenitor of SARS-CoV. Moreover, these SARSr-CoVs were discovered in bat populations from physically distinct locations. The site of origin of the true progenitor of SARS-CoV and the evolutionary origin of SARS-CoV have until now remained elusive. In the current study, we have identified a bat habitat potentially important for SARSr-CoV evolution where a series of recombination events have likely occurred among different SARSr-CoV strains, which provides new insights into the origin of SARS-CoV.

SARS first emerged in Guangdong province in late 2002 [7]. However, SARSr-CoVs discovered in bats from neighboring areas of Guangdong to date have shown phylogenetic disparity from SARS-CoV especially in the S gene [9,10,14], suggesting SARS-CoV may have originated from another region. Our analysis of the phylogeny of SARS-CoVs and all known bat SARSr-CoVs using the nt sequence of their non-structural ORF1a and ORF1b genes, which constitute the majority of the genome, shows that SARSr-CoV evolution is strongly correlated with their geographical origin, but not host species. It is noteworthy that SARSr-CoVs detected in Yunnan are more closely related to SARS-CoV than strains from other regions in China. This finding implies that Yunnan, or southwestern China, is more likely to be the geographical source

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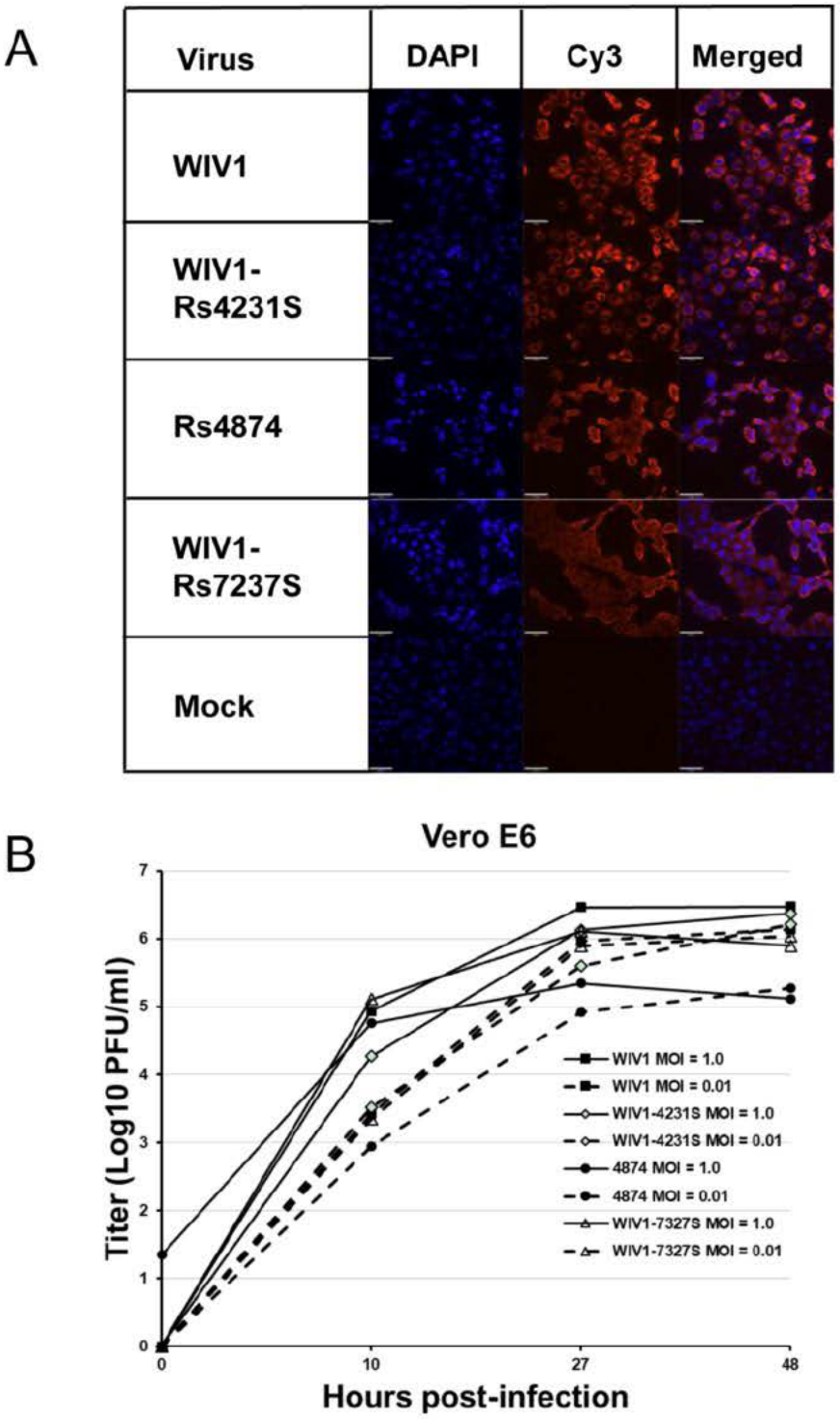

Fig 7. Infection of Vero E6 cells by bat SARSr-CoV WIV1, Rs4874, WIV1-Rs4231S and WIV1-Rs7327S. (A) The successful infection was confirmed by immunofluorescent antibody staining using rabbit antibody against the SARSr-CoV Rp3 nucleocapsid protein. The columns (from left to right) show staining of nuclei (blue), virus replication (red), and both nuclei and virus replication (merged double-stain images). (B) The growth curves in Vero E6 cells with a MOI of 1.0 and 0.01.

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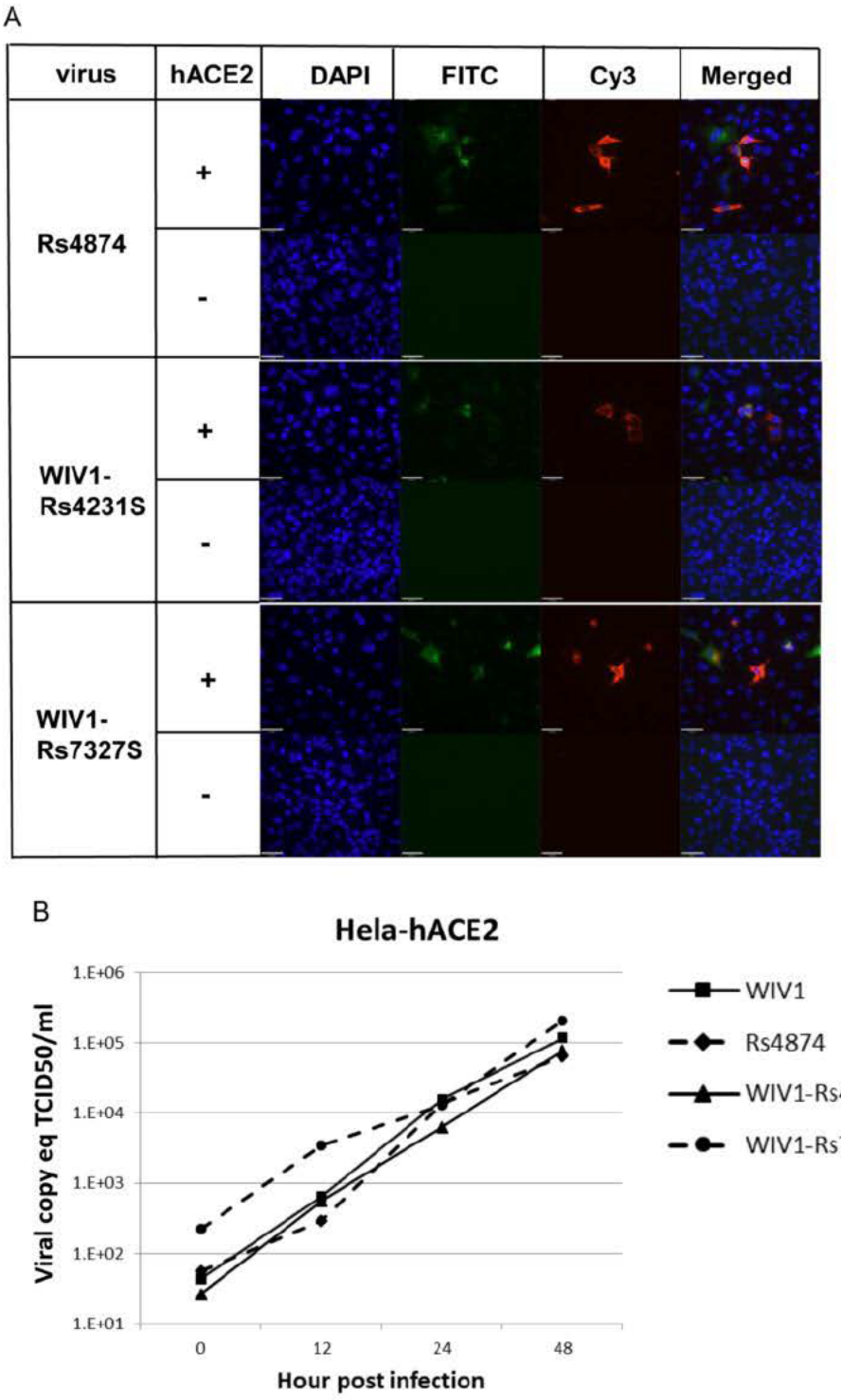

Fig 8. Analysis of receptor usage by immunofluorescence assay (A) and real-time PCR (B). Virus infectivity of Rs4874, WIV1-Rs4231S and WIV1-Rs7327S was determined in HeLa cells with and without the expression of human ACE2. ACE2 expression was detected with goat anti-human ACE2 antibody followed by fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat IgG. Virus replication was detected with rabbit antibody against the SARSr-CoV Rp3 nucleocapsid protein followed by cyanine 3 (Cy3)-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI (49,6-diamidino-2-phenylindole). The columns (from left to right) show staining of nuclei (blue), ACE2 expression (green), virus replication (red) and the merged triple-stained images, respectively.

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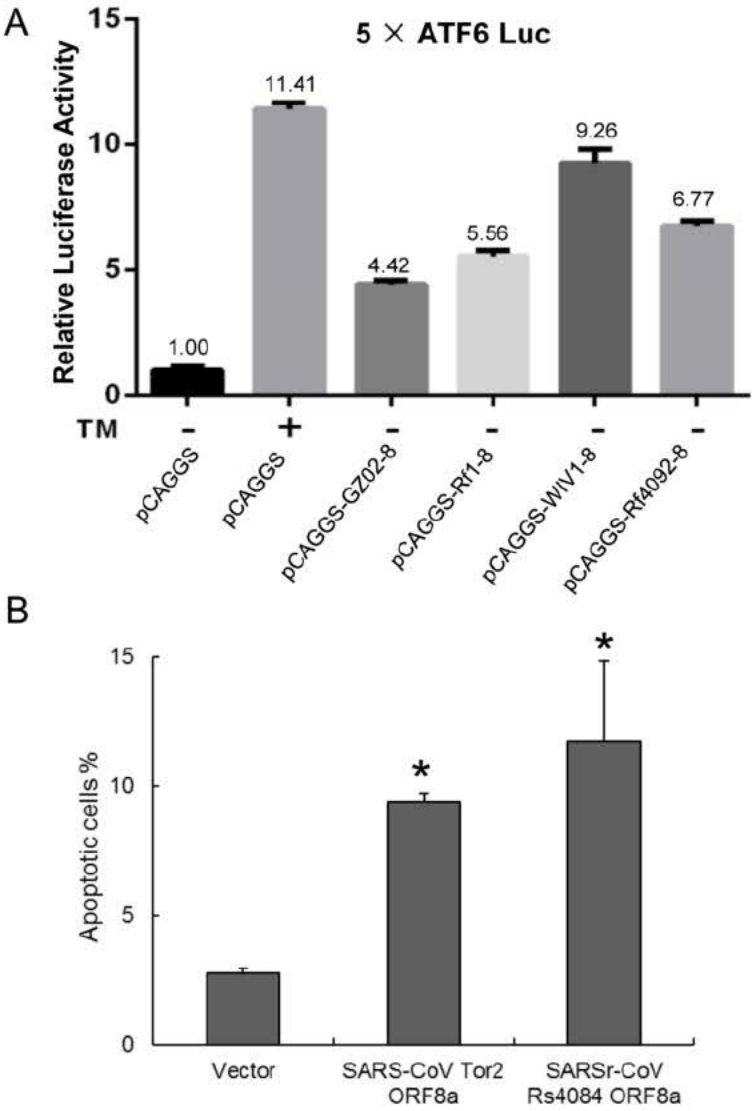

Fig 9. Functional characterization of diverse ORF8 and ORF8a proteins of bat SARSr-CoVs. (A) The ORF8 proteins of SARS-CoV and bat SARSr-CoVs induces the ATF6-dependent transcriptional activity. HeLa cells were transiently transfected with the pcAGGS expression plasmids of the ORF8 of SARS-CoV GZ02, bat SARSr-CoV Rf1, WIV1 and Rf4092 and the reporter plasmid 5×ATF6-GL3 for 40h. Control cells were co-transfected with the reporter plasmid and the empty pCAGGS vector for 24h, and treated with or without TM (2μg/ml) for an additional 16h. The cell lysates were harvested for dual luciferase assay and data are shown as the average values from triplicate wells. (B) The ORF8a proteins of SARS-CoV and bat SARSr-CoV triggered apoptosis. 293T cells were transfected with the expression plasmids of the ORF8a of SARS-CoV Tor2 and bat SARSr-CoV Rs4084 and a pcAGGS vector control for 24h. Apoptosis was analyzed by flow cytometry after annexin V staining and the percentage of apoptotic cells were calculated. Data are shown as the average values from triplicate cells. Error bars indicate SDs. * $P<0.05$.

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of SARS-CoV than other regions in China, but data from more extensive surveillance are yet needed to support this inference.

In our longitudinal surveillance of SARSr-CoVs in a single cave in Yunnan where we discovered Rs3367, RsSHC014, WIV1 and WIV16, the CoV prevalence in fecal samples varied among different sampling time. Generally, a higher prevalence was observed in autumn (September and October) than in spring and early summer (April and May). This may be due to

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the establishment of a susceptible subpopulation of newborn bats which had not developed their own immunity after the parturition period [31]. Another factor may be the changes in the composition of bat species in the cave at different sampling dates. For example, in September 2012 when the CoV prevalence reached 51.3%, the majority of samples were from *R. sinicus*, but in May 2015 when only 3 out of the 145 samples tested positive, *Aselliscus stoliczkanus* was the predominant bat species in the cave. We failed to amplify the RBD sequences from 15 of the 64 SARSr-CoV positive samples. Most of these samples had comparatively low viral concentration ($< 10^7$ copies/g) (S8 Fig), as revealed by our previous quantitative studies [32]. The unsuccessful amplification of RBD in some samples with high viral concentration was probably because of the more divergent sequences in this region of these SARSr-CoV genomes.

In this cave, we have now obtained full-length genome sequences of additional 11 novel SARSr-CoVs from bats. Our findings suggest the co-circulation of different bat SARSr-CoVs highly similar to SARS-CoV in the most variable S1 (NTD and RBD), ORF8 and ORF3 regions, respectively, in this single location. In the ORF1a, ORF1b, E, M and N genes, the SARSr-CoVs circulating in this cave also shared > 98% aa sequence identities with human/civet SARS-CoVs. Thus, all of the building blocks of the SARS-CoV genome were present in SARSr-CoVs from this single location in Yunnan during our sampling period. Furthermore, strains closely related to different representative bat SARSr-CoVs from other provinces (e.g. Rs672, HKU3 and Rf1) in the RBD region were also detected there. Therefore, this cave could be regarded as a rich gene pool of bat SARSr-CoVs, wherein concurrent circulation of a high diversity of SARSr-CoV strains has led to an unusually diverse assemblage of SARSr-CoVs.

During our 5-year surveillance in this single cave, we first reported Rs3367 and WIV1 in 2013, with RBD sequence closely resembling that of SARS-CoV [17]. More recently, we discovered WIV16 which had an RBD almost identical to WIV1's but shared much higher similarity with SARS-CoV than WIV1 in the NTD region of S1, making it the closest SARSr-CoV to the epidemic strains identified to date [18]. In this study, we found a novel strain Rs4231 from the same location sharing almost identical NTD sequence with WIV16 but distinct from it in the RBD, with evidence of a recombination event. Our recombination analysis indicated that a recombination event may have taken place at the junction between the coding region of NTD and RBD in the Rs4231 and WIV1 genomes and resulted in WIV16. Recombination at this genomic position also happened among other SARSr-CoVs relatively distant to SARS-CoV found in this location (e.g. Rs4081 and Rs4247, S5 Fig). The frequent recombination at this hotspot in the S gene increased the genetic diversity of SARSr-CoVs harbored in these bat populations and might have been responsible for the generation of the S gene of the direct progenitor strain of SARS-CoV.

The genomes of SARS-CoVs from patients during the early epidemic phase and civet SARS-CoVs all contained a single full-length ORF8 [3,7]. We have found that a number of bat SARSr-CoVs from this cave possessed a complete ORF8 highly similar to that of early human/civet SARS-CoV (>97% nt sequence identity), represented by strain Rf4092 (S3C Fig). This provided further evidence for the source of human SARS-CoV ORF8 in bats [22,30]. In contrast, the ORF8 was split into overlapping ORF8a and ORF8b in most human SARS-CoV strains from later-phase patients due to the acquisition of a 29-nt deletion [8,26]. In this study, we have discovered for the first time a bat SARSr-CoV with ORF8a and ORF8b highly similar to the later-phase human SARS-CoVs, though the split of ORF8 in the bat SARSr-CoV and that in human SARS-CoV were two independent events. Our recombination analysis suggests that this strain, Rs4084, likely acquired its ORF8 from Rf4092 through recombination, followed by the development of the 5-nt deletion which led to the splitting. It suggests that ORF8 region in bat SARSr-CoV genomes is prone to deletions as in human SARS-CoV [3,25]. Finally, the recombination analysis suggests that an ancestral strain of SARS-CoV SZ3 would have been generated if the recombination around ORF8 had occurred between the lineages that led to WIV16 and Rf4092.

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Taken together, the evidence of recombination events among SARSr-CoVs harbored by bats in this single location suggests that the direct progenitor of SARS-CoV may have originated as a result of a series of recombination within the S gene and around ORF8. This could have been followed by the spillover from bats to civets and people either in the region, or during movement of infected animals through the wildlife trade. However, given the paucity of data on animal trade prior to the SARS outbreak, the likely high geographical sampling bias in bat surveillance for SARSr-CoVs in southern China, and the possibility that other caves harbor similar bat species assemblages and a rich diversity of SARSr-CoVs, a definite conclusion about the geographical origin of SARS-CoV cannot be drawn at this point.

R. sinicus are regarded as the primary natural host of SARS-CoV, as all SARSr-CoVs highly homologous to SARS-CoV in the S gene were predominantly found in this species. However, it is noted that two SARSr-CoVs previously reported from *R. ferrumequinum* showed the closest phylogenetic position to SARS-CoV in the ORF1a/1b trees. These strains were discovered in another location in Yunnan 80 km from the cave surveyed in the current study [22]. This information also supports the speculation that SARS-CoV may have originated from this region. Nonetheless, since the correlation between the host species and the phylogeny of SARSr-CoV ORF1ab seems limited, more SARSr-CoV sequences need to be obtained from different *Rhinolophus* bat species in both locations in Yunnan, and from other locations in southern China. In particular, it will be important to assess whether *R. ferrumequinum* played a more important role in the evolution of SARS-CoV ORF1ab.

The cave we studied is located approximately 60 km from the city of Kunming. Beside a number of rhinolophid and hipposiderid species from which SARSr-CoVs have been detected, other bats like myotis were also present there. The temperature in the cave is around 22–25˚C and the humidity around 85%-90%. The physical nature of the cave is not unique, but it does appear to host a particularly dense population of bats in the reproductive season. Similar caves co-inhabited by bat populations of different species are not rare in other areas in Yunnan. We propose that efforts to study the ecology, host species diversity, and viral strain populations of these caves may provide critical information on what drives SARSr-CoV evolution.

Our previous studies demonstrated the capacity of both WIV1 and WIV16 to use ACE2 orthologs for cell entry and to efficiently replicate in human cells [17,18]. In this study, we confirmed the use of human ACE2 as receptor of two novel SARSr-CoVs by using chimeric viruses with the WIV1 backbone replaced with the S gene of the newly identified SARSr-CoVs. Rs7327's S protein varied from that of WIV1 and WIV16 at three aa residues in the receptor-binding motif, including one contact residue (aa 484) with human ACE2. This difference did not seem to affect its entry and replication efficiency in human ACE2-expressing cells. A previous study using the SARS-CoV infectious clone showed that the RsSHC014 S protein could efficiently utilize human ACE2 [33], despite being distinct from SARS-CoV and WIV1 in the RBD (S1 Fig). We examined the infectivity of Rs4231, which shared similar RBD sequence with RsSHC014 but had a distinct NTD sequence, and found the chimeric virus WIV1-Rs4231S also readily replicated in HeLa cells expressing human ACE2 molecule. The novel live SARSr-CoV we isolated in the current study (Rs4874) has an S gene almost identical to that of WIV16. As expected, it is also capable of utilizing human ACE2. These results indicate that diverse variants of SARSr-CoV S protein without deletions in their RBD are able to use human ACE2. In contrast, our previous study revealed that the S protein of a *R. sinicus* SARSr-CoV with deletions (Rp3) failed to use human, civet and bat ACE2 for cell entry [34]. In this study, in addition to Rs4231 and Rs7327, we also constructed infectious clones with the S gene of Rs4081, Rf4075, Rs4085, Rs4235 and As6526, which all contained the deletions in their RBD. These 7 strains, plus Rs4874 and the previously studied WIV1 and RsSHC014, could represent all types of S variants of SARSr-CoVs in this location (S3A Fig). However, none of the strains

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with deletions in the RBD could be rescued from Vero E6 cells. Therefore, the two distinct clades of SARSr-CoV S gene may represent the usage of different receptors in their bat hosts.

The full-length ORF8 protein of SARS-CoV is a luminal endoplasmic reticulum (ER) membrane-associated protein that induces the activation of ATF6, an ER stress-regulated transcription factor that activates the transcription of ER chaperones involved in protein folding [35]. We amplified the ORF8 genes of Rf1, Rf4092 and WIV1, which represent three different genotypes of bat SARSr-CoV ORF8 (S3C Fig), and constructed the expression plasmids. All of the three ORF8 proteins transiently expressed in HeLa cells can stimulate the ATF6-dependent transcription. Among them, the WIV1 ORF8, which is highly divergent from the SARS-CoV ORF8, exhibited the strongest activation. The results indicate that the variants of bat SARSr-CoV ORF8 proteins may play a role in modulating ER stress by activating the ATF6 pathway. In addition, the ORF8a protein of SARS-CoV from the later phase has been demonstrated to induce apoptosis [28]. In this study, we have found that the ORF8a protein of the newly identified SARSr-CoV Rs4084, which contained an 8-aa insertion compared with the SARS-CoV ORF8a, significantly triggered apoptosis in 293T cells as well.

Compared with the 154-aa ORF3b of SARS-CoV, the ORF3b proteins of all previously identified bat SARSr-CoVs were smaller in size due to the early translation termination. However, for the first time, we discovered an ORF3b without the C-terminal truncation in a bat SARSr-CoV, Rs7327, which differed from the ORF 3b of SARS-CoV GZ02 strain at only one aa residue. The SARS-CoV ORF3b antagonizes interferon function by modulating the activity of IFN regulatory factor 3 (IRF3) [27]. As previous studies suggested, the nuclear localization signal-containing C-terminal may not be required for the IFN antagonist activity of ORF3b [36]. Our previous studies also demonstrated that the ORF3b protein of a bat SARSr-CoV, termed Rm1, which was C-terminally truncated to 56 aa and shared 62% aa sequence identity with SARS-CoV, still displayed the IFN antagonist activity [37]. It is very interesting to investigate in further studies whether Rs7327's ORF3b and other versions of truncated ORF3b such as WIV1 and WIV16 also show IFN antagonism profiles.

As a whole, our findings from a 5-year longitudinal study conclusively demonstrate that all building blocks of the pandemic SARS-CoV genome are present in bat SARSr-CoVs from a single location in Yunnan. The data show that frequent recombination events have happened among those SARSr-CoVs in the same cave. While we cannot rule out the possibility that similar gene pools of SARSr-CoVs exist elsewhere, we have provided sufficient evidence to conclude that SARS-CoV most likely originated from horseshoe bats via recombination events among existing SARSr-CoVs. In addition, we have also revealed that various SARSr-CoVs capable of using human ACE2 are still circulating among bats in this region. Thus, the risk of spillover into people and emergence of a disease similar to SARS is possible. This is particularly important given that the nearest village to the bat cave we surveyed is only 1.1 km away, which indicates a potential risk of exposure to bats for the local residents. Thus, we propose that monitoring of SARSr-CoV evolution at this and other sites should continue, as well as examination of human behavioral risk for infection and serological surveys of people, to determine if spillover is already occurring at these sites and to design intervention strategies to avoid future disease emergence.

Materials and methods

Ethics statement

All sampling procedures were performed by veterinarians with approval from Animal Ethics Committee of the Wuhan Institute of Virology (WIVH05210201). The study was conducted in accordance with the Guide for the Care and Use of Wild Mammals in Research of the People's Republic of China.

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Sampling

Bat samplings were conducted ten times from April 2011 to October 2015 at different seasons in their natural habitat at a single location (cave) in Kunming, Yunnan Province, China. All members of field teams wore appropriate personal protective equipment, including N95 masks, tear-resistant gloves, disposable outerwear, and safety glasses. Bats were trapped and fecal swab samples were collected as described previously [9]. Clean plastic sheets measuring 2.0 by 2.0 m were placed under known bat roosting sites at about 18:00 h each evening for collection of fecal samples. Fresh fecal pellets were collected from sheets early in the next morning. Each sample (approximately 1 gram of fecal pellet) was collected in 1ml of viral transport medium composed of Hank's balanced salt solution at pH7.4 containing BSA (1%), amphotericin (15 μg/ml), penicillin G (100 units/ml), and streptomycin (50 μg/ml), and were stored at -80˚C until processing. Bats trapped for this study were released back into their habitat.

RNA extraction, PCR screening and sequencing

Fecal swab or pellet samples were vortexed for 1 min, and 140 μl of supernatant was collected from each sample after centrifuge at 3000 rpm under 4˚C for 1min. Viral RNA was extracted with Viral RNA Mini Kit (Qiagen) following the manufacturer's instructions. RNA was eluted in 60 μl of buffer AVE (RNase-free water with 0.04% sodium azide, Qiagen), aliquoted, and stored at -80˚C. One-step hemi-nested RT-PCR (Invitrogen) was employed to detect the presence of coronavirus sequences as described previously using a set of primers that target a 440-nt fragment in the RNA-dependent RNA polymerase gene (*RdRp*) of all known alpha- and betacoronaviruses [20]. For the first round PCR, the 25 μl reaction mix contained 12.5 μl PCR 2 × reaction mix buffer, 10 pmol of each primer, 2.5 mM $MgSO_4$, 20 U RNase inhibitor, 1 μl SuperScript III/Platinum Taq Enzyme Mix and 5 μl RNA template. The amplification was performed as follows: 50˚C for 30 min, 94˚C for 2 min, followed by 40 cycles consisting of 94˚C for 15 sec, 52˚C for 30 sec, 68˚C for 40 sec, and a final extension of 68˚C for 5 min. For the second round PCR, the 25 μl reaction mix contained 2.5 μl PCR reaction buffer, 5 pmol of each primer, 50 mM $MgCl_2$, 0.5mM dNTP, 0.1 μl Platinum Taq Enzyme (Invitrogen) and 1 μl product of the first round PCR. The amplification was performed as follows: 94˚C for 3 min followed by 35 cycles consisting of 94˚C for 30 sec, 52˚C for 30 sec, 72˚C for 40 sec, and a final extension of 72˚C for 7 min. The RBD region was amplified using the one-step nested RT-PCR method previously described [17].

PCR products were gel purified and sequenced with an ABI Prism 3730 DNA analyzer (Applied Biosystems, USA). PCR products with low concentration or generating heterogeneity in the sequencing chromatograms were cloned into pGEM-T Easy Vector (Promega) for sequencing. The positive samples in this study were termed using the abbreviated name of bat species plus the sample ID number (e.g. Rs4081). To confirm the bat species of individual sample, PCR amplification of cytochrome b (*Cytob*) or NADH dehydrogenase subunit 1 (*ND1*) gene was performed using DNA extracted from the feces or swabs [38,39].

Sequencing of full-length genomes

Full genomic sequences of 11 SARSr-CoVs were determined by One-step PCR (Invitrogen) amplification of overlapping genomic fragments with degenerate primers designed by multiple alignment of available SARS-CoV and bat SARSr- CoV sequences deposited in GenBank, and additional specific primers designed from the results of previous rounds of sequencing in this study. Primer sequences are available upon request. Sequences of the 5' and 3' genomic ends were obtained by 5' and 3' RACE (Roche), respectively. PCR products with expected size were gel-purified and subjected directly to sequencing. Each fragment was sequenced at least twice.

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The sequencing chromatogram of each product was thoroughly examined and sequence heterogeneity was not observed. For some fragments with low concentration of amplicons, the PCR products were cloned into pGEM-T Easy Vector (Promega) for sequencing. At least five independent clones were sequenced to obtain a consensus sequence. Co-presence of sequences of distinct SARSr-CoVs was not found in any of the amplicons. The sequences of overlapping genomic fragments were assembled to obtain the full-length genome sequences, with each overlapping sequence longer than 100 bp.

Evolution analysis

Full-length genome sequences of the 15 SARSr-CoVs detected from bats in the cave surveyed in this study were aligned with those of selected SARS-CoVs using MUSCLE [40]. The aligned sequences were scanned for recombination events by Recombination Detection Program (RDP) [41]. The potential recombination events suggested by strong *P* values ($<10^{-20}$) were further confirmed using similarity plot and bootscan analyses implemented in Simplot 3.5.1 [42]. Phylogenetic trees based on nucleotide sequences were constructed using the Maximum Likelihood algorithm under the LG model with bootstrap values determined by 1000 replicates in the PhyML (version 3.0) software package [43].

Virus isolation

The Vero E6 cell line was kindly provided by Australian Animal Health Laboratory, CSIRO (Geelong, Australia). Vero E6 monolayer was maintained in DMEM medium supplemented with 10% fetal calf serum (FCS). Fecal samples (in 200 μl buffer) were gradient centrifuged at 3,000–12,000 g, and the supernatant was diluted 1:10 in DMEM before being added to Vero E6 cells. After incubation at 37˚C for 1 h, the inoculum was removed and replaced with fresh DMEM medium with 2% FCS. The cells were incubated at 37˚C and checked daily for cytopathic effect. All tissue culture media were supplemented with triple antibiotics penicillin/streptomycin/amphotericin (Gibco) (penicillin 200 IU/ml, streptomycin 0.2 mg/ml, amphotericin 0.5 μg/ml). Three blind passages were carried out for each sample. After each passage, both the culture supernatant and cell pellet were examined for presence of SARSr-CoV by RT-PCR using specific primers targeting the RdRp or S gene. The viruses which caused obvious cytopathic effect and could be detected in three blind passages by RT-PCR were further confirmed by electron microscopy.

Construction of recombinant viruses

Recombinant viruses with the S gene of the novel bat SARSr-CoVs and the backbone of the infectious clone of SARSr-CoV WIV1 were constructed using the reverse genetic system described previously [23] (S9 Fig). The fragments E and F were re-amplified with primer pairs (FE, 5'-AGGGCCCACCTGGCACTGGTAAGAGTCATTTTGC-3', R-EsBsaI, 5'-ACTGGTCTCTTCGTTTAGTTATTAACTAAAATATCACTAGACACC-3') and (F-FsBsaI, 5'-TGAGGTCTCCGAACTTATGGATTTGTTTATGAG-3', RF, 5'-AGGTAGGCCTCTAGGGCAGCTAAC-3'), respectively. The products were named as fragment Es and Fs, which leave the spike gene coding region as an independent fragment. BsaI sites (5'-GGTCTCN|NNNN-3') were introduced into the 3' terminal of the Es fragment and the 5' terminal of the Fs fragment, respectively. The spike sequence of Rs4231 was amplified with the primer pair (F-Rs4231-BsmBI, 5'-AGTCGTCTCAACGAACATGTTTATTTTCTTATTCTTTCTCACTCTCAC-3' and R-Rs4231-BsmBI, 5'-TCACGTCTCAGTTCGTTTATGTGTAATGTAATTTGACACCCTTG-3'). The S gene sequence of Rs7327 was amplified with primer pair (F-Rs7327-BsaI, 5'-AGTGGTCTCAACGAACATGAAATTGTTAGTTTTAGTTTTTGCTAC-3' and R-

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Rs7327-BsaI, 5'- TCAGGTCTCAGTTCGTTTATGTGTAATGTAATTTAACACCCTTG-3'). The fragment Es and Fs were both digested with BglI (NEB) and BsaI (NEB). The Rs4231 S gene was digested with BsmBI. The Rs7327 S gene was digested with BsaI. The other fragments and bacterial artificial chromosome (BAC) were prepared as described previously. Then the two prepared spike DNA fragments were separately inserted into BAC with Es, Fs and other fragments. The correct infectious BAC clones were screened. The chimeric viruses were rescued as described previously [23].

Determination of virus infectivity by immunofluorescence assay

The HeLa cell line was kindly provided by Australian Animal Health Laboratory, CSIRO (Geelong, Australia). HeLa cells expressing human ACE2 were constructed as described previously [17]. HeLa cells expressing human ACE2 and Vero E6 cells were cultured on coverslips in 24-well plates (Corning) incubated with the newly isolated or recombinant bat SARSr-CoVs at a multiplicity of infection (MOI) = 1.0 for 1h. The inoculum was removed and the cells were washed twice with PBS and supplemented with medium. Vero E6 cells without virus inoculation and HeLa cells without ACE2 were used as negative control. Twenty-four hours after infection, cells were rinsed with PBS and fixed with 4% formaldehyde in PBS (pH7.4) at 4˚C for 20 min. ACE2 expression was detected by using goat anti-human ACE2 immunoglobulin followed by FITC-labelled donkey anti-goat immunoglobulin (PTGLab). Virus replication was detected by using rabbit antibody against the nucleocapsid protein of bat SARSr-CoV Rp3 followed by Cy3-conjugated mouse anti-rabbit IgG. Nuclei were stained with DAPI. Staining patterns were observed under an FV1200 confocal microscope (Olympus).

Determination of virus replication in Vero E6 cells by plaque assay

Vero E6 cells were infected with WIV1, Rs4874, WIV1-Rs4231S, and WIV1-Rs7327S at an MOI of 1.0 and 0.01. After incubation for an hour, the cells were washed with DHanks for three times and supplied with DMEM containing 2% FCS. Samples were collected at 0, 10, 27, and 48 h post infection. The viral titers were determined by plaque assay.

Determination of virus replication in HeLa cells expressing human ACE2 by quantitative RT-PCR

HeLa cells expressing human ACE2 were inoculated with WIV1, Rs4874, WIV1-Rs4231S, and WIV1-Rs7327S at an MOI of 1.0, and were incubated for 1h at 37˚C. After the inoculum was removed, the cells were supplemented with medium containing 1% FBS. Supernatants were collected at 0, 12, 24 and 48h. Virus titers were determined using quantitative RT-PCR targeting the partial N gene with a standard curve which expresses the correlation between Ct value and virus titer (shown as TCID50/ml). The standard curve was made using RNA dilutions from the purified Rs4874 virus stock (with a titer of 2.15×10^6 TCID50/ml). For qPCR, RNA was extracted from 140 μl of each supernatant with Viral RNA Mini Kit (Qiagen) following manufacturer's instructions and eluted in 60 μl AVE buffer. The PCR was performed with the TaqMan AgPath-ID One-Step RT–PCR Kit (Applied Biosystems) in a 25 μl reaction mix containing 4 μl RNA, 1 × RT–PCR enzyme mix, 1 × RT–PCR buffer, 40 pmol forward primer (5'-GTGGTGGTGACGGCA AAATG-3'), 40 pmol reverse primer (5'-AAGTGAAGCTTCTGG GCCAG-3') and 12 pmol probe (5'-FAM-AAAGAGCTCAGCCCCAGATG-BHQ1-3'). The amplification was performed as follows: 50˚C for 10 min, 95˚C for 10 min followed by 50 cycles consisting of 95˚C for 15 sec and 60˚C for 20 sec.

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Plasmids

The ORF8 genes of bat SARSr-CoV WIV1 and Rf4092 and the ORF8a gene of bat SARSr-CoV Rs4084 were amplified by PCR from the viral RNA extracted from the isolated virus or fecal samples. The ORF8 gene of SARS-CoV GZ02 and bat SARSr-CoV Rf1, and the ORF8a gene of SARS-CoV Tor2 were synthesized by Tsingke Biological Technology Co., Ltd (Wuhan, China). All genes were cloned into the pCAGGS vector constructed with a C-terminal HA tag. Expression of the proteins was confirmed by Western blotting using a mAb against the HA tag. Five tandem copies of the ATF6 consensus binding sites were synthesized and inserted into the pGL3-Basic vector to construct the luciferase reporter plasmid 5×ATF6-GL3, in which the luciferase gene is under the control of the c-*fos* minimal promoter and the ATF6 consensus binding sites.

Luciferase reporter assay

HeLa cells in 24-well plates were transfected using Lipofectamine 3000 reagent (Life Technologies) following the manufacturer's instruction. Cells per well were co-transfected with 600ng of the 5×ATF6-GL3 reporter plasmid, with 300ng of each expression plasmid of SARS-CoV and SARSr-CoV ORF8 or empty vector and 20ng of pRL-TK (Promega) which served as an internal control. The cells were incubated for 24h, and were treated with or without 2μg/ml tunicamycin for 16h. Cells were harvested and lysed. Luciferase activity was determined using a dual-luciferase assay system (Promega). The experiment was performed in triplicate wells.

Quantification of apoptotic cells

293T cells in 12-well plates were transfected using Lipofectamine 3000 reagent (Life Technologies) following the manufacturer's instruction. Cells per well were transfected with 3μg of the expression plasmid of SARS-CoV Tor2 or SARSr-CoV Rs4084 ORF8a, or the empty vector. 24h post transfection, apoptotic cells were quantified by using the Annexin V-fluorescein isothiocyanate (FITC)/PI Apoptosis Detection Kit (Yeasen Biotech, Shanghai) in accordance with the manufacturer's instruction. Apoptosis was analyzed by flow cytometry. The experiment was performed in triplicate wells.

Accession numbers

The complete genome sequences of bat SARS-related coronavirus strains As6526, Rs4081, Rs4084, Rf4092, Rs4231, Rs4237, Rs4247, Rs4255, Rs4874, Rs7327 and Rs9401 have been deposited in the GenBank database with the accession numbers from KY417142 to KY417152, respectively.

Supporting information

S1 Fig. Alignment of amino acid sequences of the receptor-binding motif (corresponding to aa 424–495 of SARS-CoV S protein). Two clades of the SARSr-CoVs identified from bats in the studied cave are indicated with vertical lines on the left.
(PPTX)

S2 Fig. Alignment of nucleotide sequences of a genomic region covering ORF6 to ORF7a. ORFX is located between ORF6 and ORF7a in the genomes of WIV1, WIV16, Rs7327 and Rs4874. The start codon and stop codon of ORFX are marked with red boxes. The deletion responsible for the long ORFX in Rs7327 and Rs4874 is marked with the blue box.
(PPTX)

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S3 Fig. Phylogenetic analyses based on nucleotide sequences of the S gene (A), ORF3a (B) and ORF8 (C). The trees were constructed by the maximum likelihood method using the LG model with bootstrap values determined by 1000 replicates. Only bootstraps > 50% are shown. Rs, *Rhinolophus sinicus*; Rf, *Rhinolophus ferremequinum*; Rm, *Rhinolophus macrotis*; Ra, *Rhinolophus affinis*; Rp, *Rhinolophus pusillus*; As, *Aselliscus stoliczkanus*; Cp, *Chaerephon plicata*. SARSr-CoVs detected in bats from the single cave surveyed in this study are in bold.
(PPTX)

S4 Fig. Alignment of amino acid sequences of ORF3b protein.
(PPTX)

S5 Fig. Detection of potential recombination events by similarity plot and boot scan analysis. (A) Full-length genome sequence of SARSr-CoV Rs4084 was used as query sequence and RsSHC014, Rf4092 and Rs4081 as reference sequences. (B) Full-length genome sequence of SARSr-CoV Rs4237 was used as query sequence and SARSr-CoV Rs4247, Rs4081 and Rs3367 as reference sequences. All analyses were performed with a Kimura model, a window size of 1500 base pairs, and a step size of 150 base pairs.
(PPTX)

S6 Fig. Chinese provinces where bat SARSr-CoVs have been detected.
(PPTX)

S7 Fig. The successful or failed rescue of the chimeric SARSr-CoVs. (A) Cytopathic effects in Vero E6 cells transfected with the infectious BAC clones constructed with the backbone of WIV1 and various S genes of different bat SARSr-CoV strains. Microphotographs were taken 24 hours post transfection. (B) The culture media supernatant collected from the cells transfected with the infectious BAC clones was used to infect Vero E6 cells. Immunofluorescent assay (IFA) was performed to detect infection and viral replication. Cells were fixed 24 hours post infection, and stained using rabbit antibody against the SARSr-CoV Rp3 nucleocapsid protein and a Cy3-conjugated anti-rabbit IgG.
(PPTX)

S8 Fig. Quantification of SARSr-CoV in individual bat fecal samples. The number of genome copies of SARSr-CoV per gram of bat feces was determined by quantitative real-time PCR targeting the RdRp gene. Samples from which the SARSr-CoV RBD sequences were successfully amplified are indicated in red.
(PPTX)

S9 Fig. Spike substitution strategy. The original fragments E and F were shortened to leave spike gene as an independent fragment. The new fragments were designated as Es and Fs. BsaI or BsmBI sites were introduced into the junctions of Es/Spike and Spike/Fs. Then any spike could be substituted into the genome of SARSr-CoV WIV1 through this strategy.
(TIF)

S1 Table. Comparison of the novel bat SARSr-CoVs identified in this study with human/civet SARS-CoVs and previously described bat SARSr-CoVs.
(DOCX)

S2 Table. Distribution of SARSr-CoVs highly similar to SARS-CoV in the variable S, ORF3 and ORF8 genes in the single cave.
(DOCX)

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S1 Dataset. Full-length genome sequences of bat SARSr-CoVs newly identified in this study.
(FAS)

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NIH -001405

From: Myles, Renate (NIH/OD) [E]
Sent: Mon, 17 May 2021 19:23:24 +0000
To: Collins, Francis (NIH/OD) [E];Fauci, Anthony (NIH/NIAID) [E];Tabak, Lawrence (NIH/OD) [E];Lauer, Michael (NIH/OD) [E];Jorgenson, Lyric (NIH/OD) [E];Tucker, Jessica (NIH/OD) [E];Hallett, Adrienne (NIH/OD) [E];Burklow, John (NIH/OD) [E];Lankford, David (NIH/OD) [E];Jacobs, Anna (NIH/OD) [E];Billet, Courtney (NIH/NIAID) [E];Embry, Alan (NIH/NIAID) [E]
Cc: Fine, Amanda (NIH/OD) [E];Wojtowicz, Emma (NIH/OD) [E]
Subject: RE: FOR YOUR REVIEW: Updated Backgrounder, TPs/QAs
Attachments: 2-Draft_Digital_Media_Kit_GOF_WIV_5.14.2021- Revised fsc_JT_rm - with Fauci tracked edits and comments_revised 5.17.docx, 2-Draft_Digital_Media_Kit_GOF_WIV_5.17.2021 Clean.docx, 3-Draft_TPs_GOF_WIV_05.14.2021_OGC 2 NIAID RM.docx, 3-Draft_TPs_GOF_WIV_05.17.2021 Clean.docx

Thank you for the feedback. Attached are tracked and clean versions of the backgrounder and TPs/QAs that address comments and changes. Please let us know if you have additional questions or concerns.

(b) (5)

(b) (5) Will let you know what I hear back.

Best,
Renate

From: Collins, Francis (NIH/OD) [E] (b) (6)
Sent: Monday, May 17, 2021 11:53 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b) (6) Myles, Renate (NIH/OD) [E] (b) (6); Tabak, Lawrence (NIH/OD) [E] (b) (6) Lauer, Michael (NIH/OD) [E] (b) (6); Jorgenson, Lyric (NIH/OD) [E] (b) (6) Tucker, Jessica (NIH/OD) [E] (b) (6)>; Hallett, Adrienne (NIH/OD) [E] (b) (6); Burklow, John (NIH/OD) [E] (b) (6) Lankford, David (NIH/OD) [E] (b) (6); Jacobs, Anna (NIH/OD) [E] (b) (6); Billet, Courtney (NIH/NIAID) [E] (b) (6)>; Embry, Alan (NIH/NIAID) [E] (b) (6)
Cc: Fine, Amanda (NIH/OD) [E] (b) (6); Wojtowicz, Emma (NIH/OD) [E] (b) (6)
Subject: RE: FOR YOUR REVIEW: Updated Backgrounder, TPs/QAs

Tony's comments are well taken.

From: Fauci, Anthony (NIH/NIAID) [E] (b) (6)
Sent: Monday, May 17, 2021 11:14 AM
To: Myles, Renate (NIH/OD) [E] (b) (6); Collins, Francis (NIH/OD) [E] (b) (6)>; Tabak, Lawrence (NIH/OD) [E] (b) (6) Lauer, Michael (NIH/OD) [E] (b) (6) Jorgenson, Lyric (NIH/OD) [E] (b) (6)

Tucker, Jessica (NIH/OD) [E] (b) (6); Hallett, Adrienne (NIH/OD) [E] (b) (6); Burklow, John (NIH/OD) [E] (b) (6) Lankford, David (NIH/OD) [E] (b) (6)>; Jacobs, Anna (NIH/OD) [E] (b) (6); Billet, Courtney (NIH/NIAID) [E] (b) (6); Embry, Alan (NIH/NIAID) [E] (b) (6)
Cc: Fine, Amanda (NIH/OD) [E] (b) (6); Wojtowicz, Emma (NIH/OD) [E] (b) (6)
Subject: RE: FOR YOUR REVIEW: Updated Backgrounder, TPs/QAs

(b) (5)

Thanks,
Tony

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From: Myles, Renate (NIH/OD) [E] (b) (6)>
Sent: Monday, May 17, 2021 10:44 AM
To: Collins, Francis (NIH/OD) [E] (b) (6) Tabak, Lawrence (NIH/OD) [E] (b) (6)>; Fauci, Anthony (NIH/NIAID) [E] (b) (6) Lauer, Michael (NIH/OD) [E] (b) (6) Jorgenson, Lyric (NIH/OD) [E] (b) (6); Tucker, Jessica (NIH/OD) [E] (b) (6); Hallett, Adrienne (NIH/OD) [E] (b) (6) Burklow, John (NIH/OD) [E] (b) (6)>; Lankford, David (NIH/OD) [E] (b) (6); Jacobs, Anna (NIH/OD) [E] (b) (6); Billet, Courtney (NIH/NIAID) [E] (b) (6) Embry, Alan (NIH/NIAID) [E] < (b) (6)
Cc: Fine, Amanda (NIH/OD) [E] (b) (6); Wojtowicz, Emma (NIH/OD) [E] (b) (6)
Subject: FOR YOUR REVIEW: Updated Backgrounder, TPs/QAs

Hi all:

As previously noted, [redacted] (b) (5) [redacted]. Attached is the latest version with a few tracked changes. I also updated the TPs/QAs based on the latest version of the statement. Please let me know if you have concerns with either of these documents.

Best,
Renate

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