

PRINCIPLES FOR OUTBREAK INVESTIGATION: COVID-19 AND FUTURE INFECTIOUS DISEASES

HEARING BEFORE THE SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHT OF THE COMMITTEE ON SCIENCE, SPACE, AND TECHNOLOGY HOUSE OF REPRESENTATIVES ONE HUNDRED SEVENTEENTH CONGRESS

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**PRINCIPLES FOR OUTBREAK
INVESTIGATION: COVID-19 AND
FUTURE INFECTIOUS DISEASES**

WEDNESDAY, JULY 14, 2021

HOUSE OF REPRESENTATIVES,
SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHT,
COMMITTEE ON SCIENCE, SPACE, AND TECHNOLOGY,
Washington, D.C.

The Subcommittee met, pursuant to notice, at 12:05 p.m., via Zoom, Hon. Bill Foster [Chairman of the Subcommittee] presiding.

**U.S. HOUSE OF REPRESENTATIVES
COMMITTEE ON SCIENCE, SPACE, AND TECHNOLOGY
SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHT**

HEARING CHARTER

*Principles for Outbreak Investigation:
COVID-19 and Future Infectious Diseases*

Wednesday, July 14, 2021
12:00 p.m. EDT
Zoom

PURPOSE

The purpose of this hearing is to discuss the principles for investigating infectious disease outbreaks in the context of public interest in the origins of COVID-19. The hearing will consider how infectious diseases normally emerge; how understanding disease origins helps protect public health; methods for tracing outbreaks to a discrete origin; the scientific datasets and access that investigators need; and the principles for transparency, scientific integrity, objectivity, and public communication that accompany an ideal outbreak investigation.

WITNESSES

- **Dr. David Relman**, Thomas C. and Joan M. Merigan Professor, Stanford University School of Medicine, and Senior Fellow, Center for International Security and Cooperation, Stanford University
- **Dr. Stanley Perlman**, Professor of Microbiology and Immunology, University of Iowa
- **Dr. Connie Price**, Chief Medical Officer, Denver Health, and Professor of Medicine, Division of Infectious Diseases, University of Colorado School of Medicine
- **Dr. Suzan Murray**, Program Director, Smithsonian Global Health Program, Smithsonian National Zoo & Conservation Biology Institute.

OVERARCHING QUESTIONS

- What are the scientific questions used to determine the origins of a virus?
- What are the data sets, interviews, samples, and visual inspections that inform an outbreak investigation?
- What are the principles for transparency and data access in the investigation of an outbreak, particularly one with international consequences? What should they be?
- How do our international health treaties and global governance structures provide for information/data sharing around infectious disease outbreaks? Where do they fall short?

BACKGROUND

World Health Organization Report on Origins

In July 2020, the WHO sent an initial team of two experts to China to investigate the origins of SARS-CoV-2. However, this inquiry did not include any visit to the city of Wuhan.¹ On November 5, 2020, the World Health Organization (WHO) announced the Terms of Reference for a more fulsome Global Study of Origins of SARS-CoV-2. The charge established through negotiations between the WHO and China was:

- (i) explore how the circulation of SARS-CoV-2 might have started; and
- (ii) gather evidence from the cluster of cases identified in December 2019 for potential links and clues as to its origin.²

A team of 17 Chinese and 17 international subject matter experts were selected through a joint negotiation between WHO and China. The team established working groups on epidemiology, animals and the environment, and molecular epidemiology and bioinformatics. It convened in Wuhan and conducted its investigation from January 14 through February 10, 2021.³ Data reviewed included national surveillance data, pharmacy records of medication purchases, and mortality and pneumonia-specific mortality rates.

On March 30, 2021, the World Health Organization (WHO) released its Global Study of Origins of SARS-CoV-2. It considered four possible Pathways of Emergence:

- Direct zoonotic transmission
- Introduction through intermediate host followed by zoonotic transmission
- Introduction through the cold/food chain
- Introduction through a laboratory accident

The WHO team found that the most likely origin of SARS-CoV-2 is a natural origin through an intermediate host. The closest related viruses in assessed wildlife were found in bats, but the evolutionary characteristics suggest that the virus may have evolved in an intermediate host before jumping to humans. The animals and environment group were not able to identify an intermediate species or detect SARS-CoV-2 in samples of bats and other wildlife across China. The WHO team opined that SARS-CoV-2 “preceded the initial detection of cases [in early December 2019] by several weeks.”

The cold/food chain hypothesis, while assigned a “very low” probability by the WHO Study, was controversial in the broader research community for even being considered. A theory about viral transmission through imported frozen food was first reported in Chinese media outlets last summer and gained traction after China’s CDC released a statement October 2020.⁴ Critics of the WHO process allege that the theory’s inclusion is a sign that the Chinese government had

¹ <https://www.ft.com/content/f9dea077-66fb-4734-9d1d-076dc93568e1>

² <https://www.who.int/publications/m/item/who-convened-global-study-of-the-origins-of-sars-cov-2>

³ <https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-sars-cov-2-china-part>

⁴ <https://www.cnet.com/news/a-frozen-food-fallacy-the-coronavirus-origin-story-that-doesnt-stack-up/>

undue influence over the report and may be a strategy to distract from China's handling of early coronavirus cases and shift the blame for the pandemic. Critics also note that the WHO report called the laboratory incident theory the "least likely," but failed to review all data and records from the Wuhan Institute of Virology (WIV).

Overall, according to subject matter experts⁵ and the U.S. government,⁶ the WHO report provided some new information on the origins of SARS-CoV-2, but did not provide sufficient evidence to justify the report's conclusions on the four considered hypotheses. Scientists and world leaders continue to call for additional data-driven inquiry, led by experts.⁷

Timeline of the SARS-CoV-2 Debate

At the start of the pandemic, SARS-CoV-2 was widely assumed to have a zoonotic origin due to its similarity to the SARS and MERS viruses. At the time, this assumption was relatively noncontroversial in the public discourse. On February 3, 2020, the White House Office of Science and Technology Policy requested the National Academies to "examine information and identify data requirements that would help determine the origins of 2019-nCoV, specifically from an evolutionary/structural biology standpoint."⁸ This request was posed as part of an effort to "better understand animal/human and environmental transmission aspects of coronaviruses." There was no suggestion in the request of anything but zoonotic origin.

Alternate theories regarding a lab leak did arise in January and February of 2020, but those theories were advanced primarily by non-experts. On March 7, 2020, *The Lancet* published a statement from prominent public health scientists condemning "conspiracy theories suggesting that COVID-19 does not have a natural origin,"⁹ which helped cement the appearance of a scientific consensus around natural origin. On April 30, 2020, the Office of the Director for National Intelligence issued a press release that both concurred with "the wide scientific consensus that the COVID-19 virus was not manmade or genetically modified" and acknowledged that the intelligence community was still investigating the possibility of an accidental release from a laboratory.¹⁰ Researchers from Lawrence Livermore National Lab issued a classified report in May of 2020 that found that a lab leak was possible.¹¹

When the WHO Study was published on March 30, 2021, the WHO's own Director General issued a concurrent statement saying that the lab leak hypothesis should remain under

⁵ <https://science.sciencemag.org/content/372/6543/694.1>

⁶ http://downloads.vanityfair.com/lab-leak-theory/USG_Analysis_of_COVID-19_Origins%20Report_April_5_2021.pdf?_ga=2.180968805.399198319.1625757827-1804681798.1623079155

⁷ [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(21\)01419-7/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)01419-7/fulltext)

⁸ https://www.nationalacademies.org/documents/link/LD006CF17B5004C7B41F63CD7E0A0F4EDF738C451F32/fi/leview/D20D1390AB906330493E8A40B27E965A6761F4117EA2/OSTP%20Coronavirus%20Request%20to%20NASEM_02.06.2020.pdf

⁹ [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(20\)30418-9/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30418-9/fulltext)

¹⁰ <https://www.dni.gov/index.php/newsroom/press-releases/item/2112-intelligence-community-statement-on-origins-of-covid-19>

¹¹ <https://www.cnn.com/2021/06/07/politics/covid-lab-leak-theory-classified-report/index.html>

consideration.¹² That same day, 14 governments, including the U.S., issued a joint statement calling for greater transparency in the next phase of the investigation.¹³

On May 13, 2021, a second letter signed by 18 renowned scientists was published in *Science* magazine, calling for an origins investigation that seriously considered “both natural and laboratory spillovers.”¹⁴ The high profile of the authors and the publication empowered a more frank discussion in the scientific community about the need for further inquiry. On May 26, 2021, President Biden tasked the Intelligence Community “to collect and analyze information that could bring us closer to a definitive conclusion” on the origins of COVID-19 and report back in 90 days.¹⁵ On June 15, 2020, the Presidents of the National Academies of Sciences, Engineering and Medicine (NASEM) jointly called for further investigation of the origins of COVID to be conducted with a non-partisan lens and with scientific integrity.¹⁶ On July 5, 2021, the authors of the March 2020 letter that discounted the lab leak hypothesis published a new letter in *The Lancet*. It affirmed their continued belief that COVID-19 was zoonotic in origin, but acknowledged that more information was required regardless of whether COVID-19 arose from nature or “might somehow have reached the community via an alternative route.”¹⁷

State of the Science

The origin of COVID-19 is still uncertain. To conclusively verify a zoonotic origin for COVID-19 would require finding the animal which serves as a natural reservoir for the virus and, if applicable, identify the animal intermediary(s) through which the virus passed from the original animal to humans. In the case of the 2003 SARS virus, this effort took 15 years.¹⁸

A known bat coronavirus called RaTG13 was found to have 96% similarity to the genomic sequence of SARS-CoV-2.¹⁹ Given the plethora of un-sequenced coronaviruses in the wild, the discovery of RaTG13 indicates that a predecessor of SARS-CoV-2 could still be found. Researchers continue to survey animal populations to search for an intermediate host or a coronavirus that shares the distinctive features of SARS-CoV-2.^{20,21}

No direct evidence of a lab leak is known to the public at this time. The WIV’s lead coronavirus researcher has denied having samples of SARS-CoV-2 or a predecessor virus in the laboratory prior to the pandemic.²² Some proponents of the lab leak theory point to certain distinctive

¹² <https://www.who.int/news/item/30-03-2021-who-calls-for-further-studies-data-on-origin-of-sars-cov-2-virus-reiterates-that-all-hypotheses-remain-open>

¹³ <https://www.state.gov/joint-statement-on-the-who-convened-covid-19-origins-study/>

¹⁴ <https://science.sciencemag.org/content/372/6543/694.1>

¹⁵ <https://www.whitehouse.gov/briefing-room/statements-releases/2021/05/26/statement-by-president-joe-biden-on-the-investigation-into-the-origins-of-covid-19/>. Ninety days will expire on August 24, 2021.

¹⁶ <https://www.nationalacademies.org/news/2021/06/let-scientific-evidence-determine-origin-of-sars-cov-2-urge-presidents-of-the-national-academies>

¹⁷ [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(21\)01419-7/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)01419-7/fulltext)

¹⁸ <https://www.nature.com/articles/d41586-017-07766-9>

¹⁹ <https://www.nature.com/articles/s41586-020-2012-7>

²⁰ <https://onlinelibrary.wiley.com/doi/full/10.1002/jmv.25976>

²¹ <https://www.sciencedirect.com/science/article/pii/S096098222030662X>

²² <https://www.nytimes.com/2021/06/14/world/asia/china-covid-wuhan-lab-leak.html>

features in the genomic sequence of SARS-CoV-2.²³ Expert virologists differ in their interpretation of the genomic sequence, with some suspecting laboratory intervention and others believing the distinctive features are not anomalous or unexpected. Non-circumstantial evidence would be needed to affirm a lab leak theory.

Researchers continue to weigh the available evidence and identify what new data needs to be collected to resolve COVID-19's origins.²⁴ There seems to be a general consensus among experts that a zoonotic origin remains the most likely scenario, and further study of animal coronaviruses is needed to both understand this pandemic and to prepare for the next one. However, few scientific experts who have addressed the subject publicly in 2021 have argued that a lab leak origin can be ruled out entirely. Many have suggested that it may never be possible to conclusively rule it out.

Investigations of Previous Epidemics

While the world is anxious to know as much as possible about the virus that has killed over four million people across the globe, it is important to contextualize the search for an answer. Ebola, which first emerged in 1976 and most significantly spread between 2014-2016, still evades our full understanding. It is widely understood to be a zoonotic disease, but after years of investigation, researchers have still not found the natural reservoir.²⁵ Investigations into SARS and MERS, both coronaviruses, can provide perspective on how outbreak investigations have been conducted in recent memory.

SARS first emerged in November 2002, detected as an atypical case of pneumonia in southern China. Over the next two years, 8,096 probable cases were detected, killing 774 people worldwide. By May 2003, a WHO consensus document discussed what was known at the time about whether SARS could be considered a zoonotic infection, and concluded that no evidence existed that wild animal species that tested positive for the virus were the source of human infection.²⁶ About a year after the initial infections were detected, researchers determined that the likely intermediate host of the SARS virus was the civet, sold in live animal markets in Guangdong Province, China.²⁷ While it was suspected early on that bats were the original source of the virus—their immune systems carry and tolerate a very high number of viruses²⁸—it wasn't until 2017 that researchers identified the strains of coronavirus that likely mixed and transferred to humans. The strains were identified in samples taken from a cave 1,000 kilometers from the original outbreak.²⁹

The Middle East Respiratory Syndrome (MERS) outbreak was first identified in 2012. An international team of experts was assembled and sent to Saudi Arabia to investigate the

²³ <https://www.wsj.com/articles/the-science-suggests-a-wuhan-lab-leak-11622995184>

²⁴ https://zenodo.org/record/5075888#_YOY3-xMzaDX

²⁵ <https://www.gov.uk/government/publications/ebola-origins-reservoirs-transmission-and-guidelines/ebola-overview-history-origins-and-transmission#natural-reservoir>

²⁶ <https://www.who.int/csr/sars/en/WHOconsensus.pdf>

²⁷ <https://science.sciencemag.org/content/302/5643/276>

²⁸ https://www.washingtonpost.com/science/why-do-bats-have-so-many-viruses/2020/07/10/0327f584-b65e-11ea-a8da-693df3d7674a_story.html

²⁹ <https://www.nature.com/articles/d41586-017-07766-9>

outbreak's spread in Saudi hospitals. In cooperation with the Saudi Ministry of Health, researchers from the U.S., Canada, and the United Kingdom reviewed medical records, determined exposure pathways, estimated incubation periods, and sequenced the viral RNA.³⁰ Their findings were published in the *New England Journal of Medicine* in August 2013. The ability of the team to review the medical records and collect additional samples, with the cooperation of the Saudi Arabian government, was crucial in understanding how to stop the spread of a disease with a 35 percent mortality rate.³¹ The importance of international collaboration and scientific transparency in the MERS investigation was noted in a 2015 National Academies workshop summary.³²

The global scale and rate of infection SARS-CoV-2 is orders of magnitude greater than any infectious disease we have seen in recent history. However, prior investigations of smaller outbreaks help illustrate the importance of patience, scientific transparency, objectivity, and international collaboration.

China's Record on Transparency

China's record on information sharing during COVID-19 has been poor. In accordance with the International Health Regulations, China did notify the WHO of the initial outbreak on December 31, 2019. By January 11, the genomic sequence of SARS-CoV-2 had been made available, but only because it was leaked by a Chinese scientist to an Australian scientist and published online.^{33,34} China agreed to participate in the WHO investigative team, but maintained veto power in the selection of experts, limited the WHO team to brief Q&A sessions, and withheld access to some of the raw data from the WIV, Huanan Market, and area hospitals with early cases. Researchers with experience working with Chinese scientists told Committee staff that they believe the information China has shared with the WHO team is as good as the international community is likely to get. One researcher suggested that a wet market origin of COVID-19 could be more consequential to China than an accidental lab leak because of the cultural significance of wet markets.

It is apparent that the Chinese government has withheld key data sets and interviews from investigators, that other important data sets were never collected, and that other evidence was destroyed, whether incidentally or intentionally. Significant details on the WIV's operations have been withheld from the WHO and other investigators. Some proponents of the lab leak theory have conflated obfuscation by China with direct evidence, but the mere "withholding of information" would not itself be a particularly applicable to the process of determining origins according to the best practices for outbreak investigations established in the CDC Field Epidemiology Manual.³⁵ The absence of data is not data. However, the CDC Manual does note that data inadequacy – irrespective of *why* it is inadequate – can lead to inconclusive results.

³⁰ <https://www.nejm.org/doi/full/10.1056/nejmoa1306742>

³¹ [https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-\(mers-cov\)](https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-(mers-cov))

³² <https://www.nap.edu/read/18975/chapter/9>

³³ <https://www.who.int/news/item/29-06-2020-covid-timeline>

³⁴ <https://virological.org/t/novel-2019-coronavirus-genome/319>

³⁵ <https://www.cdc.gov/eis/field-epi-manual/index.html>

International Data Sharing Obligations

The WHO's International Health Regulations (IHR) of 2005 are the primary global governance framework that require data sharing at the beginning of a potential international health emergency. The language of this mandate is broad:

*Following a notification, a State Party shall continue to communicate to WHO timely, accurate and sufficiently detailed public health information available to it on the notified event, where possible including case definitions, laboratory results, source and type of the risk, number of cases and deaths, conditions affecting the spread of the disease and the health measures employed; and report, when necessary, the difficulties faced and support needed in responding to the potential public health emergency of international concern.*³⁶

WHO's Review Committee on Functioning of IHR (2005) during the COVID-19 Response has called for new technology to allow for the automatic sharing of real-time emergency information and the sharing of genomic sequencing.³⁷ In addition, researchers have called for new protocols standardizing the type and format of data around international health events, as well as whom they can contact in order to gain access.³⁸

The cooperation of the nation where an infectious disease outbreak is first identified is indispensable to conducting any complete outbreak investigation. The United States and the World Health Organization will not resolve the details of the origins of COVID-19 without the partnership of the scientific community in China.³⁹

Laboratory Biosafety

Regardless of what conclusions the scientific community may reach regarding the origins of COVID-19, the question has inspired a conversation about laboratory biosafety. The WHO defines four biosafety levels (BSLs) for laboratories according to the risk posed by organisms being handled and the types of experiments performed.⁴⁰ Based on these stratified levels of risk, WHO specifies the equipment, procedures, and design features of the lab required to conduct experiments safely. The CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL), known as the "Bible" of lab safety in the United States, articulates more detailed requirements for each BSL within its borders.⁴¹

- BSL-1 (lowest): Basic safety procedures and no special design features are needed.
- BSL-2: Moderate-risk agents require equipment that can decontaminate lab waste.

³⁶ [International health regulations \(who.int\)](https://www.who.int/publications/i/item/9789240011311)

³⁷ [Report of the Review Committee on the Functioning of the International Health Regulations \(2005\) during the COVID-19 response \(who.int\)](https://www.who.int/publications/i/item/9789240011311)

³⁸ [Wanted: rules for pandemic data access that everyone can trust \(nature.com\)](https://www.nature.com/news/04/blinken-blasts-china-failure-transparency-covid.html)

³⁹ <https://slate.com/news-and-politics/2021/04/blinken-blasts-china-failure-transparency-covid.html>

⁴⁰ <https://www.who.int/publications/i/item/9789240011311>

⁴¹ <https://www.cdc.gov/labs/BMBL.html>

- BSL-3: Infectious agents with some risk of airborne transmission require biosafety cabinets, controlled air flow, and filtered ventilation system.
- BSL-4 (highest): Deadly and infectious agents with high risk of aerosol transmission require maximum containment and full-body, air-supplied suits.

The U.S. has eleven BSL-4 facilities. Each are regularly inspected with oversight from the CDC and other federal agencies as appropriate. Globally, 23 countries maintain 59 BSL-4 facilities, with further proliferation of BSL-4 facilities expected. Countries must report the existence of the BSL-4 facilities under the Biological Weapons Convention, and organizations such as International Experts Group of Biosafety and Biosecurity Regulators (IEGBBR) enable limited international coordination around best practices with BSL-4 labs. However, there is no international authority with oversight of the maintenance of BSL-4 facilities and safe practice of research involving deadly agents. As a result, laboratory biosafety practices are neither consistent nor transparent from country to country.

The March 2021 WHO Study suggested that “although rare, laboratory accidents do happen.”⁴² But a review conducted by the Government Accountability Office (GAO) in 2014 found that laboratory accidents are not particularly rare.⁴³ Broad concerns over the public health risk posed by improper laboratory handling of infectious agents are not necessarily unfounded. The last documented death from smallpox was the result of a 1978 laboratory accident in the United Kingdom.⁴⁴ The last documented outbreak of SARS before it was contained in 2004 was deemed a result of biosecurity failures at a virology laboratory in Beijing.⁴⁵ While the lab-leak theory is not supported by any direct evidence known to the public at this time, the lack of consistency and transparency around BSL-3 and BSL-4 facilities abroad has enabled global speculation that the WIV could be culpable.

Infectious Disease Outbreak Monitoring Infrastructure

In addition to analytical teams that can be dispatched to investigate after an infectious disease outbreak is recorded, standing public health surveillance systems provide information on the onset, location, and severity of outbreaks. Traditionally, surveillance systems relied primarily on syndromic surveillance, in which local health facilities track standardized lists of symptoms among their patients and provide information on potential outbreaks to public health officials.⁴⁶ However, the COVID-19 pandemic demonstrated that many patients can be infected prior to detection of an outbreak via syndromic surveillance. More proactive surveillance system would allow detection of pre-pandemic viruses before they become highly harmful to humans.

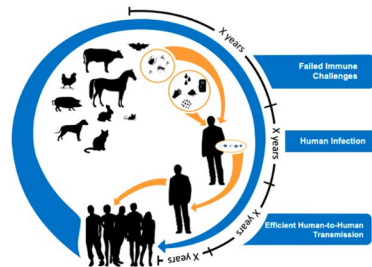
⁴² <https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-sars-cov-2-china-part> Page 119

⁴³ <https://www.gao.gov/assets/gao-14-785t.pdf>

⁴⁴ <https://www.bbc.com/news/uk-england-birmingham-45101091>

⁴⁵ <https://www.sciencemag.org/news/2004/07/sars-crisis-topples-china-lab-chief>

⁴⁶ <https://www.cdc.gov/nssp/overview.html>



Viral evolutionary theory indicates that animal viruses can become dangerous human viruses over time. It is rare for animal viruses to have the ability to successfully infect an individual human, much less have the ability for human-to-human transmission. The process of an animal virus becoming a human virus usually requires not just one but several “intermediate hosts” to enable a variant with the ability to infect a human. Moreover, the final host that allows a variant to become fully adapted to humans may be human themselves. This

process of selecting for highly transmissible variants among human hosts is playing out in real time with the spread of the delta COVID-19 variant (B.1.617.2), which the CDC now estimates accounts for over 51% of cases in the U.S.⁴⁷

Under evolutionary processes, it requires many years for an animal virus variant to have the ability to successfully infect humans, much less for it to be able to efficiently transmit among humans and to become more harmful.⁴⁸ With good surveillance in place, this lengthy process gives virologists time to detect viruses before they become dangerous to humans. For example, assays collected of Liberians in 1982 identified Ebola antibodies in over 5% of people sampled, decades prior to the West Africa Ebola outbreak in 2014.⁴⁹

Epidemiologists have recommended integrated biological and behavioral surveillance among healthy communities to help identify potential zoonotic disease spillover at known hotspots. Assuming natural origins, this strategy may have helped detect COVID-19 virus in its earlier stages. Southern China is home to the bats known to carry SARS-like coronaviruses, such as the one that caused the 2003 SARS pandemic and the one most closely associated with SARS-CoV-2.⁵⁰ Long-term, regular surveillance at hotspots for emerging disease is the backbone of a pandemic threats warning system.

PREDICT, a project of the U.S. Agency for International Development (USAID) Emerging Pandemic Threats Program founded in 2009, works as an early pandemic prediction system.⁵¹ Other relevant efforts include the Global Virome Project, STOP Spillover, and DEEP VZN.⁴⁸ During this century, the frequency of outbreaks are expected to increase, driven by trends of globalization, urbanization, and climate change. A more complete pandemic threats warning system could help control future infectious disease outbreaks.

⁴⁷ <https://www.npr.org/sections/coronavirus-live-updates/2021/07/06/1013582342/delta-is-now-the-dominant-coronavirus-variant-in-the-u-s>

⁴⁸ <https://www.mdpi.com/1999-4915/13/4/637>

⁴⁹ <https://theconversation.com/the-next-pandemic-is-already-happening-targeted-disease-surveillance-can-help-prevent-it-160429>

⁵⁰ <https://www.nature.com/articles/s41586-020-2012-7>

⁵¹ <https://www.usaid.gov/sites/default/files/documents/1864/predict-global-flyer-508.pdf>

Chairman FOSTER. OK. Well, this hearing will now come to order. And, without objection, the Chair is authorized to declare recess at any time, and before I deliver my opening remarks, I wanted to note that today the Committee is meeting virtually, and I want to announce a couple of reminders to the Members about the conduct of this hearing. First, Members should keep their video feed on as long as they are present at the hearing. Members are responsible for their own microphones. Please also keep your microphones muted, unless you are speaking. Finally, if Members have documents that they wish to submit for the record, please e-mail them to the Committee Clerk, whose e-mail address was circulated prior to the hearing.

Well, good morning again, and welcome, and thanks to our Members and panelists. I guess I don't have to remind people about the importance of the topic that we are discussing today, the origins of COVID-19. For the past year and a half the Science Committee and Congress have been appropriately focused on optimizing our response to the pandemic, and making sure that the full power of our country's scientific apparatus is engaged in that battle. But now that the vaccines are succeeding in easing—in crushing the national caseload of COVID-19, it is time to turn some of our attention to how that international catastrophe started, and what can be done to lessen the toll of future outbreaks. We are convening this first hearing to look at the scientific underpinnings of that investigation.

There is one profoundly correct reason to pursue this rigorous investigation: to help protect public health and our national security in the future. If we don't learn everything that we can about how infectious diseases like COVID-19 get started, we will be less safe moving forward. Now, as many of you know, I spent most of my career in science before spending the last decade in Congress, and I'd like to thank our witnesses for their very thoughtful written testimony that allowed me to spend a couple hours last night reading it over, and remembering what it was like to think like a scientist.

In science, if you see an interesting data set, you look at the logically possible hypotheses that could explain that data. You then look at the statistical likelihood of each hypothesis with a given set of prior assumptions, and then you ask what additional data or measurements are needed to confirm or refute each hypothesis. Then, armed with that new information, including the uncertainties, you enter the engineering phase of the problem, using the—that information to make our systems work better. And that is one reason why I feel so fortunate to have an engineer, Ranking Member Obernolte, as my partner on this Subcommittee, because in politics it's often very different. If you see something interesting, the first question is not—is often not what is true, but what can you convince people is true? Sort of like a lawyer convincing a jury. Instead of looking at the full set of facts, we look to cherry pick facts to support your political position. And when this happens, it often leads to bad policy that, in the case of COVID-19, has killed hundreds of thousands of people around the world. And that's why I'm proud of the way that the House Science Committee conducts its business, by letting facts, logic, and science lead the way.

Unfortunately, we do not at present have the full set of facts. Right now American citizens and senior government officials have suspicions about China's role, and there is no disputing that the Chinese government has withheld information and obfuscated efforts to understand the origins. The Chinese scientists were very quick to make sure that the genome sequence of SARS (Severe Acute Respiratory Syndrome)-CoV-2 was available to the public back in January of 2020, which is to their eternal credit. This is the master key that NIH (National Institutes of Health) and drug makers needed to start work on a vaccine, and those Chinese scientists are heroes for publishing it. But they took this bold step in spite of the Chinese government, and not with its blessing.

Let the record show that Members of Congress understand that the lack of transparency from the Chinese government about health emergencies of international consequence is a very serious geopolitical and science concern. It is a threat to our Nation, and it predates the COVID-19 crisis, and it is not entirely restricted to China. At the same time, China's lack of transparency is distinct from the body of epidemiological data around the origins of COVID-19. The absence of data is not itself evidence of a lab leak, or something more sinister. Our witnesses today will help us set expectations, and understand what real evidence is, and what other ingredients comprise a credible inquiry on disease origins. Patience may be one of those ingredients. We all remember the SARS virus of 2003. Well, it took most of 15 years to identify the original source of that virus. It was ultimately found in a bat cave 1,000 kilometers from the site of the original outbreak.

President Biden has asked the U.S. Intelligence Community to report back in late August on any new information that could bring us closer to a conclusion about the origins of COVID-19. This is a constructive step, but I would caution that there is a very good chance that we still won't have all the answers after that report comes back. Like so many important topics in science, the deliberate endeavor of establishing origins of COVID is likely nowhere near as simple as we'd like it to be. If epidemiology were easy, we'd have the answer by now. If the Intelligence Community were in possession of a smoking gun about COVID origins, I believe that we had heard of—we had—we would have heard about it.

At today's hearing I intend today to establish some expectations of Federal and global leaders in their efforts to investigate the origins of COVID-19 and future diseases. We will not tolerate equivocation and innuendo from public officials. Where the facts aren't clear enough to support a conclusion, please do not offer one just to humor the news media. I ask all Members to take care to remember the distinction between the theories of a journalist or blogger and the observations of an experienced science professional with firsthand knowledge of the virus and similar outbreaks.

The Science Committee has a long history of playing the long game, of seeing the 10,000 foot view on topics that push the boundaries of human understanding, and of transcending political noise. Our witnesses are going to help steer the origins conversation to the high road, where scientific expertise, transparency, objectivity, and data are paramount. We have the four corners of outbreak investigative—represented at today's panel, microbiology, virology,

epidemiology, and zoonotics. To the four of you—too many of your peers have experienced rancor, and even threats, for speaking out publicly about this politically hot topic, and that is really, really appalling, and your participation today is a service to the republic, so thank you again.

[The prepared statement of Chairman Foster follows:]

Good morning, and welcome and thanks to our members and panelists.

I guess I do not have to remind people of the importance of the topic we are discussing today: the Origins of COVID-19.

For the past year and a half, the Science Committee and Congress have been appropriately focused on optimizing our response to the pandemic and making sure that the full power of our country's scientific apparatus is engaged in that battle. But now that vaccines are succeeding at easing the crushing national caseload of COVID-19, it is time to turn some of our attention to how that international catastrophe started, and what can be done to lessen the toll of future outbreaks. We are convening this first hearing to look at the scientific underpinnings of that investigation.

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In Science, if you see an interesting dataset, you ask what logically possible hypotheses could explain that data, you then look at the statistical likelihood of each hypothesis with a given set of prior assumptions, then you ask what additional data or measurements needed to confirm or refute each hypothesis. Then, armed with that new information, including the uncertainties, you enter the engineering phase of the problem, using that information to make our systems work better. That is why I feel so fortunate to have an engineer, Ranking Member Obernolte, as my partner on this subcommittee.

Because in politics it is often very different. If you see something interesting, the first question is often not what is true, but what can you convince people is true, like a lawyer convincing a jury. Instead of looking at the full set of facts, we look to cherry-pick facts to support your political position. When this happens, it leads to bad policy that, in the case of COVID-19, has killed millions of people around the world. That is why I am proud of the way the House Science Committee conducts its business, by letting facts, logic and science lead the way.

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Let the record show that Members of Congress understand that the lack of transparency from the Chinese government about health emergencies of international consequence is a very serious geopolitical and science diplomacy challenge. It is a threat to our nation, and it pre-dates the COVID-19 crisis.

At the same time, China's lack of transparency is distinct from the body of epidemiological data around the origins of COVID-19. The absence of data is not itself evidence of a lab leak or something more sinister. Our witnesses today will help us set expectations and understand what real evidence is and what other ingredients comprise a credible inquiry on disease origins.

Patience may be one of those ingredients. We all remember the SARS virus of 2003. It took 15 years to identify the original source of that virus. It was ultimately found in a bat cave, 1,000 kilometers from the site of the original outbreak.

President Biden has asked the U.S. intelligence community to report back by late August on any new information that could bring us closer to a conclusion about the origins of COVID-19. This is a constructive step, but I would caution that there is a very good chance that we still won't have all the answers after that report comes back. Like so many important topics in science, the deliberate endeavor of establishing origins of COVID is likely nowhere near as simple as we'd like it to be. If

epidemiology were easy, we'd have the answer by now. If the intelligence community were in possession of a smoking gun about COVID origins, I believe we'd know about it.

At today's hearing, I intend today to establish some expectations of federal and global leaders in their efforts to investigate the origins of COVID-19 and future diseases. We will not tolerate equivocation and innuendo from public officials. Where the facts aren't clear enough to support a conclusion, do not offer one just to humor the news media. Take care to remember the distinction between the theories of a journalist or a blogger with the observations of an experienced science professional with first-hand knowledge of the virus.

The Science Committee has a long history of playing the long game, of seeing the 10,000 foot view on topics that push the boundaries of human understanding, and of transcending the political noise. Our witnesses are going to help steer the origins conversation to the high road, where scientific expertise, transparency, objectivity and data are paramount. We have the four corners of outbreak investigation represented today's panel: microbiology, virology, epidemiology and zoonotics. To the four of you: Too many of your peers have experienced rancor and even threats for speaking publicly about this politically hot topic, and that really is appalling. Your participation today is a service to the public.

I yield to Ranking Member Obernolte.

Chairman FOSTER. And I now yield to Ranking Member Obernolte.

Mr. OBERNOLTE. Well, thank you very much, Mr. Chairman, and thank you for holding today's hearings on the principles for investigating the origins of COVID-19. Before I begin, I'd actually like to thank—take a moment to commend Chairman Foster for his bipartisanship, and his willingness to hold this first official House Committee related to the origins of the epidemic. I'm very hopeful that this hearing can serve as an example of how Congress can work together to move forward on investigating the origins of what happened last year. This is not a partisan issue, and I think it's something that we definitely all should work together on.

It's also an opportunity for us to begin looking into the principles and standards by which an effective investigation into the origins of COVID-19 should be conducted, and, perhaps more importantly, to reflect on some of the lessons that we've learned over the past 18 months about scientific communication, the importance of fair and open public discourse about—discourse about scientific hypotheses, and the impact that media censorship, the politicization of science, and a possible lack of scientific integrity has had on efforts thus far to investigate the origins of this terrible pandemic.

Since the early days of COVID-19 discussion regarding its origins have, unfortunately, been hampered by the politicization of this entire topic. Renowned, reputable scientists have, at critical times during the pandemic, presented their opinions unfortunately as if they were scientific fact, and sometimes avoidable conflict of interests have called into question the independence and impartiality of efforts that have been undertaken to examine the origins of COVID-19, including, unfortunately, some of those at the World Health Organization (WHO). These shortcomings have made it much more difficult to have fair and open discourse about this issue.

Also equally troubling to me have been the actions of media, Big Tech, and their respective fact-checkers, which have compounded this difficulty. During the onset of the pandemic some media outlets latched onto a preordained narrative about the origins of COVID-19, dismissing competing theories as false, and even

xenophobic conspiracy theories. Social media companies then censored information about COVID's origins, labeling posts as misinformation despite there being no conclusive evidence for one theory over another. The unfortunate effect of these actions was to shut down legitimate scientific discourse and inquiry, which I think is important to everyone on this Committee. Those actions made it more difficult for scientists and the public at large to challenge what had been held out by only a few as scientific consensus, even where the science and facts supported alternative theories and hypotheses.

Fear of retaliation, of being labeled as xenophobic conspiracy theorists, and of being ostracized by the scientific community led some scientists to self-censor and remain silent instead of searching publicly and transparently for the truth, which I think is our goal as scientists. To me, censorship in any form is very troubling, but even the more so when it prevents scientific inquiry, so we're seeking to understand the origins of COVID-19. And that matter is not so that we can point fingers, or assign blame to a specific country or specific leaders, but more importantly that we can better prevent future outbreaks. That's certainly my intention today, as we consider these principles that should inform how we investigate disease outbreaks in the future, and that includes acknowledging the shortcomings that have complicated those inquiries into the origins issue last year.

Certainly we've made a lot of progress. Many scientists, scholars, and journalists are now publicly calling for renewed investigation, and it is my fervent hope that we can move forward with a more open discourse and a productive dialog. And although I agree with the Chairman, we may never definitively know the origin of COVID-19, thanks in no small part to a lack of cooperation by the Chinese government, that certainly does not mean that investigation into its origins will not bear fruit. And, to the contrary, I think the facts and evidence that are uncovered during that—such an investigation will help us prepare for future pandemics, irrespective of its conclusions, so I'm looking very much forward today to learning more about what that investigation should look for, as well as reflecting on the principles and standards that should form the framework of investigations in the future.

Again, I want to thank Chairman Foster for holding the hearing today, and thank our witnesses for appearing before us to discuss this very important issue. Thank you, Mr. Chair. I yield back the balance of my time.

[The prepared statement of Mr. Obernolte follows:]

Thank you, Chairman Foster, for holding today's hearing on principles for investigating the origins of COVID-19. And thank you to our witnesses for appearing before us today.

Before I begin, I'd like to take a moment to commend Chairman Foster for his bipartisanship and his willingness to hold the first official House Committee hearing related to the origins of COVID-19. I'm hopeful that this hearing will serve as an example of how Congress can work together to move forward on investigating the origins of this terrible pandemic.

Today's hearing is an opportunity for this Committee to begin looking into the origins of COVID-19 and to discuss principles and standards by which an effective investigation into its origins can and should be conducted. It is also an opportunity to reflect on some of the lessons learned over the past 18 months about science communication, the importance of fair and open public discourse about scientific

hypotheses, and the impact that media censorship, the politicization of science, and a lack of scientific integrity has had on efforts thus far to investigate the origins of this pandemic.

Since the early days of the pandemic, discourse and discussion regarding the origins of COVID-19 have unfortunately been hampered by the politicization of science.

Renowned, reputable scientists have at critical times during the pandemic presented their opinions as if they were scientific fact. And avoidable conflicts of interest have called into question the independence and impartiality of efforts that have been undertaken to examine the origins of COVID-19, including those at the World Health Organization. These shortcomings have made it more difficult to have fair and open discourse about the origins issue.

Also, the actions of media, big tech, and their respective fact checkers have compounded this difficulty. During the onset of the pandemic, some media outlets latched on to a preordained narrative about the origins of COVID-19 and dismissed competing theories as false and even xenophobic conspiracy theories.

Social media companies even censored information about COVID's origins, labeling posts as misinformation, despite there being no conclusive evidence for one theory over another.

The effect of these actions was to shut down legitimate scientific discourse and inquiry. They made it more difficult for scientists and the public at large to challenge what had been held out by only a few as scientific consensus, even where the science and facts supported alternative theories and hypotheses.

Fear of retaliation, of being labeled as a xenophobic conspiracy theorist, and of being ostracized by the scientific community led some scientists to self-censor and remain silent instead of searching publicly and transparently for the truth.

Censorship in any form is troubling, but all the more so when it prevents scientific inquiry. Understanding the origins of COVID matters not so that we can assign blame to a specific country or politician, but so we can better prevent future outbreaks.

That's my intention here today as we consider the principles that should inform how we investigate disease outbreaks. We must acknowledge the shortcomings that have complicated inquiries into the origins issue. I'd also like to acknowledge the progress that has been made, as many scientists, scholars, and journalists alike are now publicly calling for a renewed investigation into the origins of COVID-19.

It is my hope that we can move forward with a more open discourse and a productive dialogue about the principles and standards by which an effective origins investigation should be undertaken. Although we may never definitively know the origin of COVID-19—thanks in no small part to the Chinese Communist Party's lack of transparency and cooperation—that does not mean that an investigation into its origins will not bear fruit. To the contrary, the facts and evidence that are uncovered during such an investigation will almost certainly help us prepare for future pandemics, irrespective of its conclusion.

I am looking forward to learning more today about what a proper investigation should look like, as well as the principles and standards that should form the framework of such an investigation. I also hope we learn how to effectively screen for conflicts of interest and reinforce the scientific principles of impartiality, transparency, and openness in this endeavor.

I again want to thank Chairman Foster for holding today's hearing, and to thank our witnesses for appearing before us today to discuss this important issue.

I yield back the balance of my time.

Chairman FOSTER. Thank you. And we are pleased to have the Full Committee Ranking Member, Mr. Lucas, with us today. The Chair now recognizes the Ranking Member for an opening statement.

Mr. LUCAS. Thank you, Chairman Foster, for holding today's hearing, and thank you to our witnesses for your participation. While this Committee has held a handful of hearings over the last 18 months examining the role of the scientific community in combatting COVID-19, I would be remiss if I did not mention that today's hearing is the first official effort in the U.S. House of Representatives to explore the origins of this pandemic. I hope we can use today's hearing as an important first step toward a full congressional inquiry into the origins of COVID-19.

In the last 18 months the United States has been battling this pandemic, and over 600,000 American lives have been claimed by the virus. COVID-19 is unlike anything we've seen in the United States since the deadly 1918 Spanish Flu, but here we are, 18 months later, and only just now discussing the principles of investigating the origins of the virus. This conversation is long overdue, and over the past several months Members of House Democratic leadership had made comments that this Committee is investigating the origins of COVID-19. This Subcommittee hearing is a good start to determining how this outbreak started, but this is not an investigation. In order to get answers on COVID-19 origins Congress needs to step up and get involved. Relevant Committees of jurisdiction, including this Committee, need to come together in a bipartisan manner and take this inquiry seriously.

Understanding the origins of COVID-19 isn't about scoring political points, or detracting from ongoing efforts to fight the virus. It's about preventing and mitigating future pandemics, and ensuring that we can responsibly fund public health research. We shouldn't have a preferred outbreak origin theory because we prefer one narrative over another, and we shouldn't bury our heads in the sand and refuse to investigate COVID's origins because we're concerned about what might happen if we get answers we don't like. The job of science isn't to prop up one argument or another. The job of science is to determine the facts. And, as policymakers, our job is to take those facts, weigh them alongside other information, and then make decisions. We can't do that without a better understanding of this outbreak. I urge congressional leadership to allow a transparent and thorough investigation of COVID's origins.

The Chinese Communist Party (CCP) has not been forthcoming or trustworthy in sharing information about the origins of the virus, so U.S. Federal agencies must work closely with the intelligence community to find answers. I hope we can work together on this Committee to continue to support American scientists and researchers at Federal agencies, National Labs, and academia who have been working tirelessly since COVID-19 reached our shores to continue to learn more about where this virus came from, and to eradicate it for good.

We also need to work together to improve trust in science. During the COVID-19 pandemic, the way science has been communicated to the general public has not been handled well. Information has been presented in ways that are confusing, misleading, and occasionally polarizing. That's created tremendous problems. The CDC (Centers for Disease Control and Prevention) reports that roughly 40 percent of adults over the age of 18 still remain unvaccinated against COVID-19. The only way COVID-19 will stop spreading in the United States and around the world is if vaccination rates increase, and herd immunity is achieved. This message needs to be communicated urgently to the public, and we need to ensure the public has reason to trust that information.

For more than a year people were told there was only one possible explanation for the pandemic, and that any other theories were not only wrong, but dangerous. But now there's an acknowledgement that we don't have conclusive evidence for or against any specific origin theory. That creates mistrust. Communicating

science during a global pandemic can be a matter of life or death. We need to do so thoughtfully and accurately. We cannot treat the assumptions and the opinions of scientists and officials as established fact if their claims are not supported by evidence. In order to have a credible investigation moving forward, all available information and theories must be considered fairly, and the process must be transparent, free of conflicts of interest, and protected from political interference. While we may never know the true origins of COVID-19 virus, we owe it to the American people, and the rest of the world, to give it our best shot at investigating where it came from to better prepare for pandemics to come.

I'd like to thank the witnesses again for their participation today, and I look forward to this discussion. Thank you, Mr. Chairman. [The prepared statement of Mr. Lucas follows:]

Thank you, Chairman Foster for holding today's hearing. And thank you to our witnesses for your participation.

While this Committee has held a handful of hearings over the last 18 months examining the role of the scientific community in combatting COVID-19, I would be remiss if I did not mention that today's hearing is the first official effort in the U.S. House of Representatives to explore the origins of this pandemic. I hope we can use today's hearing as an important first step towards a full Congressional inquiry into the origins of COVID-19.

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The job of science isn't to prop up one argument or another. The job of science is to determine the facts. As policymakers, our job is to take those facts, weigh them alongside other information, and then make decisions. We can't do that without a better understanding of this outbreak. So I urge Congressional leadership to allow a transparent and thorough investigation of COVID's origins.

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The CDC reports that roughly 40 percent of adults over the age of 18 still remain unvaccinated against COVID-19. The only way COVID-19 will stop spreading in the United States and around the world is if vaccination rates increase and herd immunity is achieved. This message needs to be communicated urgently to the public. And we need to ensure the public has reason to trust that information.

For more than a year, people were told there was only one possible explanation for this pandemic and that any other theories were not only wrong but dangerous.

But now there is an acknowledgement that we don't have conclusive evidence for or against any specific origin theory. That creates mistrust. Communicating science during a global pandemic can be a matter of life or death. We need to do so thoughtfully and accurately and we cannot treat the assumptions and opinions of scientists and officials as established fact if their claims are not supported by evidence.

In order to have a credible investigation moving forward, all available evidence and theories must be considered fairly, and the process must be transparent, free of conflicts of interest, and protected from political interference. While we may never know the true origins of the COVID-19 virus, we owe it to the American people, and the rest of the world to give it our best shot at investigating where it came from to better prepare for pandemics to come.

I would like to thank the witnesses again for their participation here today and I look forward to this discussion.

I yield back.

Chairman FOSTER. And thank you, Mr. Ranking Member. I should say that if you ever got tired of politics, you may have a good career waiting for you as a scientist. And if there are any Members who wish to submit additional opening statements, your statements will be added to the record at this point.

[The prepared statement of Chairwoman Johnson follows:]

Good afternoon to our witnesses and thank you for joining us here today.

As we all know, the global pandemic has ravaged our communities and demanded the attention of this country for the past 18 months. We have all dedicated our efforts to weathering this storm. Thanks to the tireless work of the medical community, and the vaccines they have developed, we can finally see the light at the end of the tunnel, although it is too soon to relax our vigilance given the emergence of variants.

We now have the opportunity to examine the lessons to be learned from this terrible experience. In particular, by developing principles for how best to investigate the origins of COVID-19, we can prepare for and possibly prevent the next pandemic.

Debate over COVID-19's origins has spread far beyond the sphere of science into the realms of national politics and international geopolitics. People without scientific expertise or firsthand knowledge have dominated the discussion. Scientists on all sides of the debate have hesitated to speak out in fear of threats or other negative public reactions. Let me say right now how harmful and destructive this kind of behavior is to the scientific process specifically. If anything, it is making it harder for scientists to get to the truth.

Science communicators have also struggled. The nuances of this scientific debate have not been communicated to the public in a particularly clear way. New scientific evidence that emerged was quickly cited as an argument in favor of one theory or the other, without being put into proper context. Public understanding of the origins of COVID-19 is cloudier today than it was a year ago.

The question of the origins of COVID-19 will not be settled here today, and that is not the purpose of today's hearing. The famous Texan epidemiologist Patricia Buffler once said: "The work of epidemiology is related to unanswered questions, but also to unquestioned answers." By asking questions about how an investigation of the origins of COVID-19 should be conducted, we will improve our approach to outbreak investigation in the future. We will affirm stronger principles for international health data sharing and transparency. And we can take a fresh approach to addressing safety concerns in laboratories that conduct genomic engineering and gain-of-function research of concern.

The possibility of an animal origin for COVID-19 tells us that we must cast a wider net with our animal disease surveillance. Similarly, the possibility of a laboratory leak means that there is work to be done to improve laboratory biosafety.

With this hearing, the Science Committee intends to recenter the discussion around data-driven investigations and the expertise of scientists. We will rely on our expert panel of witness to ground the discussion within the realm of reason. And, most importantly, we will seek to learn about how best to investigate the origins of the disease so we can prepare for the inevitable disease outbreaks of the future.

Thank you and I yield back.

Chairman FOSTER. So at this time I'd like to introduce our witnesses. Our first witness is Dr. David Relman. Dr. Relman is the Thomas C. and Joan M. Merigan Professor in Medicine, and a Pro-

fessor of Microbiology and Immunology at Stanford University, and Chief of Infectious Diseases at the Veterans' Affairs Palo Alto Healthcare System. He was an early pioneer in the modern study of the human indigenous microbiome. He currently serves on the Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats at the U.S. National Academies.

After Dr. Relman is Dr. Stanley Perlman. Dr. Perlman is a Professor of Microbiology and Immunology at the University of Iowa. His current research efforts are focused on coronavirus pathogenesis, including Severe Acute Respiratory Syndrome, or SARS, the Middle East Respiratory Syndrome, MERS, and COVID-19. His laboratory has developed several novel animal models useful for studying pathogenesis, and evaluating vaccines and antiviral therapies. He and his colleagues provided the first rodent model for—useful for studying MERS, and similar approaches have been used to develop several mouse models for COVID-19.

Our third witness is Dr. Connie Price. Dr. Price is the Chief Medical Officer at Denver Health, and has—a Professor of Medicine in the Division of Infectious Diseases at the University of Colorado School of Medicine. Dr. Price's research focuses on healthcare, epidemiology, and methods to prevent and rapidly detect emerging and anti-microbial resistant infections. She has expertise in outbreak management, and has served as a consultant for public health authorities around the world on controlling of emergency—emerging infections, especially MERS and SARS, as well as Ebola preparedness.

Our final witness is Dr. Suzan Murray. Dr. Murray is Board—a Board certified zoo veterinarian at the Smithsonian Conservation Biology Institute (SCBI), and serves as both the Program Director of the Global Health Program, and as the SCBI's Chief Wildlife Veterinary Medical Officer. She leads an interdisciplinary team engaged in worldwide efforts to address health issues in endangered wildlife and combat emerging infection—infectious diseases of global significance, including zoonotic diseases. Dr. Murray's work focuses on pathogen detection, advanced diagnostics, capacity building, and collaboration in infectious disease research at the human/wildlife domestic animal interface.

Now, as our witnesses should know, you will each have five minutes to—for your spoken testimony. Your written testimony will be included in its entirety in the record for the hearing. And when you have completed your spoken testimony, we will begin with questions. Each Member will have five minutes to question the panel. And we will start now with Dr. Relman. You're now recognized for five minutes.

**TESTIMONY OF DR. DAVID RELMAN,
THOMAS C. AND JOAN M. MERIGAN PROFESSOR,
STANFORD UNIVERSITY SCHOOL
OF MEDICINE, AND SENIOR FELLOW,
CENTER FOR INTERNATIONAL SECURITY
AND COOPERATION, STANFORD UNIVERSITY**

Dr. RELMAN. Chairman Foster, Ranking Members Lucas and Obernolte, and Members of the Committee, I'm grateful for the opportunity to join you at today's hearing. My name is David Relman.

I'm a Professor of Medicine, and of Microbiology and Immunology, at Stanford University. Over the past several decades the rate, distribution, and diversity of recognized emerging infections has accelerated and expanded, especially those involving animal-associated viruses. Among the many reasons, the intrusion by humans into the natural habitats of animals and their viruses, as well as urbanization. In response to these events, scientists have doubled down on efforts to understand these viruses, creating both benefits and risks.

Soon after disease first appeared in Wuhan, China, the causative agent was identified, a coronavirus called SARS-CoV-2, whose closest known relatives are found in bats in Southern China. The key question became how, when, and where did SARS-CoV-2 first encounter and infect a human? There are two major hypotheses: First, a natural spillover, in which the virus jumped directly from a bat, or indirectly from another animal, to a human, and second, a laboratory-associated mechanism. Both hypotheses involve human activities, but suggest different opportunities for risk reduction going forward.

What is the evidence in support of these hypotheses? First, despite much analysis and discussion, the sequence of SARS-CoV-2 tells us only so much. The problem is that the immediate ancestors of this virus are missing. The most closely related known virus is not that closely related. Meanwhile, SARS-CoV-2 has not been found in nature, and it had not been reported from a laboratory prior to 2020. So, where does this leave us? We have only circumstantial evidence. In support of a natural spillover: First, the fact that nearly all previous outbreaks involving animal viruses are believed to have had a natural origin. Second, the immense diversity of coronaviruses in nature, and the possibility that SARS-CoV-2 is out there, but we simply haven't found it yet. Third, the extensive wildlife trade, and the risk that it creates. And fourth, the fact that natural spillovers happen much more often than we had thought.

In support of a laboratory associated origin, first, geography. The closest known virus relatives of SARS-CoV-2 had been found more than 1,000 miles from Wuhan, while the laboratory with one of the largest collections of bat samples and bat-associated coronaviruses in the world is located in Wuhan, where disease first emerged. Second, the risky work at laboratories in Wuhan. Third, the fact that laboratory accidents happen much more often than we had thought. And fourth, the lack of transparency and incomplete information about samples and sequences at laboratories.

Again, all of this is just circumstantial. Neither hypothesis can be ruled in, nor ruled out. Both are plausible. The goals of an outbreak investigation are to provide greater clarity about the circumstances and factors that contributed to the event. A definitive answer is often not achieved, nor is it necessary for an investigation to yield important benefits. Some of the key elements of a credible investigation concern process, such as objectivity, transparency, representativeness, breadth in expertise, independence, and responsible management of conflicts of interest. Other key elements concern data, such as archived animal samples and derivative data, archived clinical samples and derivative data, and mul-

tiple kinds of laboratory information. Importantly, the veracity and providence of samples and data must be assured.

I will close with some observations and suggested next steps. It is unfortunate that the origin issue has become grossly politicized, and opinion valued more than rigorous analysis. Many scientists are hesitant about addressing this issue because of fear that their comments will be misinterpreted. All of this undermines the credibility of scientists, and of the scientific enterprise. However, there are signs of progress, including proposals for roles to be played by National Academies of Science under the oversight, and with authorities, granted by national and international political leadership. Meanwhile, effective international governance is still a work in progress. I applaud your efforts, and urge you to articulate a plan now for an effective investigation of this pandemic, and of pandemics that will inevitably follow. Thank you.

[The prepared statement of Dr. Relman follows:]

Testimony of David A. Relman, MD

Thomas C. and Joan M. Merigan Professor, Departments of Medicine, and of Microbiology & Immunology, Stanford University School of Medicine; Senior Fellow, Center for International Security and Cooperation, Stanford University, Stanford, CA

Before the U.S. House of Representatives
Committee on Science, Space, and Technology
Subcommittee on Investigations & Oversight
Hearing on “Principles for Outbreak Investigation: COVID-19 and Future Infectious Diseases”
Wednesday, July 14, 2021

Chairman Foster, Ranking Member Obernolte, and members of the Subcommittee, thank you for the opportunity to join you today for this important hearing and discussion about principles for outbreak investigation. My name is Dr. David Relman. I am a professor of Medicine, and of Microbiology & Immunology at Stanford University School of Medicine, and a senior fellow at the Center for International Security and Cooperation, also at Stanford University. I am also Chief of Infectious Diseases at the Veterans Affairs Palo Alto Health Care System, but am appearing here today on my own time. Today’s hearing takes place at a critical and consequential moment for this nation and the world, as we struggle to address one of the most calamitous public events of the past century, COVID-19.

I will begin with some brief remarks about the emergence of infectious diseases and the changing factors that underlie recent outbreaks, as well as create risks for future ones. I will present some of the hypotheses that could explain the origins of the current pandemic, including the evidence that supports these hypotheses, and summarize where we stand with respect to an understanding of pandemic and virus origins. I will then discuss the key elements of pandemic investigations, present and future, offer thoughts about why it is so important that we seek greater clarity about the origins of outbreaks and pandemics, lessons learned so far in assessing COVID-19 origins, and suggest some next steps. Some of my thoughts on this topic have been published elsewhere¹.

The opinions expressed in my testimony are my own and do not represent the views of my employers, Stanford University and the U.S. Department of Veterans Affairs.

Emergence

Ever since the time that humans first congregated in close proximity, domesticated animals, and cultivated plants as food, microbes have had increasingly important impacts on

¹ Relman DA. Opinion: To stop the next pandemic we need to unravel the origins of COVID-19. Proc Natl Acad Sci USA 117:29246-48, 2020. <https://www.pnas.org/content/117/47/29246>

morbidity and mortality. Human history was regularly punctuated by plagues and large-scale die-offs until the advent of public health measures such as isolation and quarantine in the 17th century, sanitation in the 18th century, and vaccines in the 19th century. In the second half of the 20th century, methods for microbial discovery and diagnosis became more powerful, and revealed microbes that were new causes of human, animal, and plant disease, along with others that had caused disease for some time but were unrecognized. By the early part of this century, a rapidly growing number of infectious disease outbreaks suggested that microbial disease “emergence” deserved special attention². In 1992, a study at the Institute of Medicine (now, National Academy of Medicine) organized the factors responsible for emerging infections according to three categories: those related to microbe, host, and environment. Two environmental factors appeared to be unusually important, both of them anthropogenic: climate change and changing land use leading to more frequent human encounters with animals harboring zoonotic pathogens. In 2003, a re-assessment of emergence by the same group at the Institute of Medicine (on which I later served for a dozen years) identified the previously described factors, as well as social, political and economic factors, and the growing human capabilities for genetically engineering microbes and hosts³. This work serves as an important backdrop to current thinking about the origins of the ongoing pandemic.

Hypotheses about the origins of COVID-19

The initial characterization of the causative agent of COVID-19, SARS-CoV-2 revealed it to be a beta-coronavirus whose closest known virus relatives had been found in bats in China^{4,5}. Based on this finding and the known capacity of bats to serve as reservoirs for coronaviruses, it is assumed that at some point in the past, SARS-CoV-2 colonized and evolved in bats. How, when, and where SARS-CoV-2 first encountered and infected a human, and then started a chain of uncontrolled human-to-human transmission, is the key question.

Hypotheses about the origins of COVID-19 and the circumstances of that first encounter can be grouped into two major categories: 1) those involving a natural “spill-over” (from natural host to accidental host) either directly from a bat to a human, or indirectly through an intermediate host animal; and 2) those involving a spill-over facilitated by research activities at a laboratory. This second category includes collection of animal samples by researchers and the handling and study of these samples and their viruses in a laboratory. In both cases, the initial fateful encounter may have taken place in the natural habitat of the animal host when a human intruded into this environment, e.g., a cave, or it may have taken place closer to the places where humans live and work, when humans deliberately relocated the animal hosts to these sites, e.g., a city market or a laboratory. In both of these hypothetical categories, the actions of humans may be responsible to varying degrees for precipitating the pandemic. The difference between the two

² Institute of Medicine. Emerging Infections: Microbial Threats to Health in the United States, 1992.

<https://www.nap.edu/catalog/2008/emerging-infections-microbial-threats-to-health-in-the-united-states>

³ Institute of Medicine. Microbial Threats to Health: Emergence, Detection, and Response, 2003.

<https://www.nap.edu/catalog/10636/microbial-threats-to-health-emergence-detection-and-response>

⁴ Zhu N, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*. 2020 Feb 20;382(8):727-733. doi: 10.1056/NEJMoa2001017.

⁵ Zhou P, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020 Mar;579(7798):270-273. doi: 10.1038/s41586-020-2012-7.

sets of hypotheses concerns the kinds of human activities implicated in the origin of the pandemic: in the first case, violation of natural habitat boundaries and the promulgation of wildlife trade; in the second case, the manipulation of potential pathogens and the strategies used to understand microbial threats.

Evidence in support of COVID-19 origin hypotheses

Many have gazed at the genome sequence of SARS-CoV-2 and from this sequence, tried to infer the origin of the virus. Despite much analysis and discussion, the sequence yields only limited insight. The basic problem is that the immediate, most recent viral ancestors are missing (are not available in the public domain). Therefore, the most recent evolutionary history of SARS-CoV-2, and the circumstances under which this most recent evolution took place, is unknown.

It is reasonably clear from available coronavirus sequences that ancestors of SARS-CoV-2 resided in bats. The most closely related known virus, RaTG13 was apparently detected and recovered from a sample collected from a bat in a mine in Yunnan province in southwestern China⁶. Yet, RaTG13 is not that closely related; it is estimated to have shared a most recent common ancestor with SARS-CoV-2 several decades ago. RaTG13 is an unlikely progenitor of a hypothetical laboratory-based genome engineering effort that would have produced SARS-CoV-2. Analyses based on all available genome sequences of clinical SARS-CoV-2 isolates have produced estimates of the timing of the first human cases of COVID-19 within the period beginning mid-October and ending late November 2019⁷.

Controversy has arisen over the seemingly unusual sequence-based and functional features of SARS-CoV-2, such as its furin cleavage site or its apparent high degree of adaptation to human cells and receptors, which to some observers, indicate a laboratory origin of the virus. To others, these features are best explained by natural origins and suggest to them that the virus could not possibly have originated in a laboratory⁸. However, both conclusions rely on unfounded assumptions, and lack direct evidentiary support. The missing, most recent viral ancestors of SARS-CoV-2 may well turn out to be found somewhere in the natural world, especially because efforts to sample the immense diversity of coronaviruses, e.g., in bats, so far are woefully incomplete. On the other hand, these ancestors may turn out to be found in the large collections of uncharacterized samples located in laboratories that have dedicated themselves to the study of these viruses. They may have accidentally infected unknowing laboratory personnel either at the time of sample collection or back in the laboratory during subsequent efforts to cultivate or manipulate viruses, or during disposal of samples afterwards. Many of the steps undertaken in laboratories to study animal samples and associated viruses are not closely monitored using deep sequencing methods. Importantly, without direct detection of SARS-CoV-2, either in a natural environment or in a laboratory, all we have is speculation and hunches.

⁶ Zhou P, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020 Mar;579(7798):270-273. doi: 10.1038/s41586-020-2012-7.

⁷ Pekar J, Worobey M, Moshiri N, Scheffler K, Wertheim JO. Timing the SARS-CoV-2 index case in Hubei province. *Science*. 2021 Apr 23;372(6540):412-417. doi: 10.1126/science.abf8003.

⁸ Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. *Nat Med*. 2020 Apr;26(4):450-452. doi: 10.1038/s41591-020-0820-9.

So, where does this leave us?

There are four kinds of circumstantial evidence that favor the first set of hypotheses (natural origin):

1. History. Because nearly all previous infectious diseases outbreaks involving a pathogen associated with animals are presumed to have begun with a natural spillover event^{9,10,11}, one might assume that the same kind of event caused this pandemic.
2. Immense viral diversity in nature. Recent efforts to catalog viral diversity in bats in China indicates that much of this diversity has yet to be discovered¹². One might then logically assume that SARS-CoV-2 resides in nature but that surveillance studies have been so far insufficient to detect it.
3. Extensive wildlife trade. Despite the known risks, humans continue to collect, raise, and distribute live animals that are potential sources of viral pathogens, or their derivative parts, to markets where encounters with humans are likely¹³.
4. Natural spillovers happen more often than we had thought. Surveys of humans living near potential animal hosts of zoonotic pathogens have revealed evidence of past asymptomatic and therefore unrecognized infection¹⁴. Such evidence was published by investigators at the Wuhan Institute of Virology (WIV) after a blood-based survey for antibodies indicating past exposure to bat-associated coronaviruses in four villages near the mine where RaTG13 was found¹⁵.

There are also four kinds of circumstantial evidence that favor the second set of hypotheses (laboratory-associated origin):

1. Geography. The closest known virus relatives of SARS-CoV-2 were found in bats in Yunnan province more than 1000 miles from the city where COVID-19 appears to have first emerged, Wuhan. Surveys of bats across China suggest that beta-coronaviruses are found in greatest number and diversity in Yunnan and other locations in southern China, rather than further north¹⁶. The survey for exposure to bat-associated coronaviruses mentioned above included 1000

⁹ Institute of Medicine. Emerging Infections: Microbial Threats to Health in the United States, 1992.

<https://www.nap.edu/catalog/2008/emerging-infections-microbial-threats-to-health-in-the-united-states>

¹⁰ Institute of Medicine. Microbial Threats to Health: Emergence, Detection, and Response, 2003.

<https://www.nap.edu/catalog/10636/microbial-threats-to-health-emergence-detection-and-response>

¹¹ Morens DM, Fauci AS. Emerging infectious diseases: threats to human health and global stability. PLoS Pathog. 2013;9(7):e1003467. doi: 10.1371/journal.ppat.1003467.

¹² Latinne A, et al. Origin and cross-species transmission of bat coronaviruses in China. Nat Commun. 2020 Aug 25;11(1):4235. doi: 10.1038/s41467-020-17687-3.

¹³ Xiao X, Newman C, Buesching CD, Macdonald DW, Zhou ZM. Animal sales from Wuhan wet markets immediately prior to the COVID-19 pandemic. Sci Rep. 2021 Jun 7;11(1):11898. doi: 10.1038/s41598-021-91470-2.

¹⁴ Pernet O, Schneider BS, Beaty SM, LeBreton M, Yun TE, Park A, Zachariah TT, Bowden TA, Hitchens P, Ramirez CM, Daszak P, Mazet J, Freiberg AN, Wolfe ND, Lee B. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342. doi: 10.1038/ncomms6342.

¹⁵ 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. Virol Sin. 2018 Feb;33(1):104-107. doi: 10.1007/s12250-018-0012-7.

¹⁶ Latinne A, et al. Origin and cross-species transmission of bat coronaviruses in China. Nat Commun. 2020 Aug 25;11(1):4235. doi: 10.1038/s41467-020-17687-3.

blood samples from Wuhan; all were negative¹⁷. At the same time, the laboratory with one of the largest known collections of bat samples and bat-associated coronaviruses (WIV) is located within the city of Wuhan.

2. Known work at laboratories in Wuhan. WIV not only contains one of the largest collections of bat samples and bat-associated coronaviruses (the WHO Report from March 2021¹⁸ mentions more than 19,000 bat samples, 13% of which were said to be positive for coronaviruses), but their published work indicates efforts to cultivate viruses from bat samples, as well as recombinant engineering work that created hybrid coronaviruses from a variety of poorly characterized bat-associated viruses¹⁹. It is not necessarily always clear to researchers when experiments produce limited growth of a virus or limited growth of a recombinant that arises from multiple co-existing viruses in an animal sample. Risk of inadvertent infection of a researcher is especially high when growth is not recognized. With respect to the virus engineering work, none of the published hybrid viruses nor their source genomes are plausible recent ancestors of SARS-CoV-2; however, this kind of work does pose risks. WIV and other laboratories around the world working with coronaviruses have not provided a comprehensive description of recombinant engineering experiments that have been undertaken in recent years, nor a comprehensive list of virus sequences generated at their facilities.

3. Laboratory accidents happen more often than we had thought. A surprisingly large number of accidents involving dangerous pathogens (so called, Select Agents) has occurred in the United States during the past decade, even at some laboratories with the most stringent safety conditions²⁰. In 2003-2004, three separate laboratory accidents in three countries resulted in infection of personnel with SARS, including the death of a family member²¹.

4. Lack of transparency and incomplete data. A failure to alert the world about six cases of SARS-like illness in 2012 and to provide a full report of the laboratory investigation of them²², as well as the incomplete or delayed information about work that has been undertaken on bat-associated coronavirus diversity²³, suggest that the research community has not yet provided all of the information available that might contribute to a more complete understanding of the origins of this virus and pandemic.

Again, all four kinds of evidence for each of these two sets of hypotheses are just circumstantial. SARS-CoV-2 has not been found anywhere other than in infected humans, and in

¹⁷ 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. *Virology*. 2018 Feb;33(1):104-107. doi: 10.1007/s12250-018-0012-7.

¹⁸ World Health Organization. "WHO-convened global study of origins of SARS-CoV-2". March 30, 2021. <https://www.who.int/health-topics/coronavirus/origins-of-the-virus>

¹⁹ Hu B, et al. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog*. 2017 Nov 30;13(11):e1006698. doi: 10.1371/journal.ppat.1006698.

²⁰ From 2006-2013, laboratories notified US federal regulators of approximately 1500 incidents with Select Agent pathogens, resulting in medical treatment of workers in more than 800 cases. Young A and Penzenstadler N. "Inside America's secretive biolabs", *USA Today*, May 28, 2015.

<https://www.usatoday.com/story/news/2015/05/28/biolabs-pathogens-location-incidents/26587505/>

²¹ Normile D. Mounting lab accidents raise SARS fears. *Science* 304:659, 2004.

²² Zhou P, et al. Addendum: A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020 Dec;588(7836):E6. doi: 10.1038/s41586-020-2951-z.

²³ Bloom JD. Recovery of deleted deep sequencing data sheds more light on the early Wuhan SARS-CoV-2 epidemic. *bioRxiv* 2021.06.18.449051; doi: <https://doi.org/10.1101/2021.06.18.449051>

animals that have been infected by humans or on fomites contaminated by infected humans. Neither set of hypotheses can be ruled in, nor ruled out.

I have not and will not offer a personal opinion about which hypothesis I believe is more likely, because I do not think that opinions at this point, in the absence of additional primary, verifiable data, are useful, especially in the present political climate.

Goals and key elements of outbreak investigations

The goals of an outbreak investigation are to provide greater clarity about the circumstances and factors that contributed to the event. A definitive answer is often not achieved, nor is it necessary for an investigation to yield important benefits. First and foremost, greater clarity about the circumstances and contributory factors that underlie the origin of an outbreak will enable us to anticipate and prevent future outbreaks; for example, targeted interventions and early warning strategies can be designed and tested based on an understanding of mechanisms and risks. Second, every investigation, if undertaken thoughtfully, provides an opportunity to learn how to undertake the next investigation in a more effective manner. Third, any reduction in uncertainty about the origins of a pandemic can help minimize further recriminations, speculation, and conflict.

Some have argued that further clarity about the origins of the current pandemic is not needed, because we already know enough to motivate efforts to address both sets of hypotheses. I disagree. As I have written elsewhere²⁴, if scientists are not forced to confront the issues of laboratory safety and risky research in a serious manner, history suggests that we will not do so. I will set aside the semantic issue of what defines “gain-of-function” research. More importantly, we need to consider whether there is life sciences research that is so risky that it should not be undertaken. I view the work performed at WIV and described in Hu et al.²⁵ to be unusually risky. The strategy they chose to characterize novel coronaviruses detected in bat samples relied on creating hybrid viruses in the laboratory starting with a previously characterized bat-associated coronavirus, called WIV1, that had been viewed by experts as already “poised for human emergence”²⁶ and then inserting the all-important spike protein from the novel viruses. Although the purpose was not to make a virus with greater capabilities for human infection, this could easily have been the outcome. If we learn nothing more about the work undertaken around the world to explore the properties of novel coronaviruses, life scientists and funding agencies are likely to forego the difficult discussions that are needed about risk assessment, risk mitigation, and ‘red lines’.

Some of the key elements of a credible investigation concern process, others concern data. On the issue of process, there are at least six elements worthy of consideration. 1) Objectivity--A priori assumptions should be specified and challenged. Findings should be based

²⁴ Relman DA. “Lab leaks happen, and not just in China. We need to take them seriously”. Washington Post, June 2, 2021. <https://www.washingtonpost.com/outlook/2021/06/02/lab-leak-investigate-risky-research/>

²⁵ Hu B, et al. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS Pathog. 2017 Nov 30;13(11):e1006698. doi: 10.1371/journal.ppat.1006698.

²⁶ Menachery VD, et al. SARS-like WIV1-CoV poised for human emergence. Proc Natl Acad Sci U S A. 2016 Mar 15;113(11):3048-53. doi: 10.1073/pnas.1517719113.

on verifiable data and should be reproducible. The integrity of any scientific process must be assured. 2) Transparency--The process of creating the terms of the investigation should be shared with the public in an ongoing manner. Primary data, methods, and the basis for any conclusions should all be placed in the public domain. 3) Representativeness--The interests of all relevant key international constituencies should be represented in the planning and execution of the investigation. The latter should be international, and include nations with diverse levels of resource, and differing kinds of impact by the pandemic. 4) Expertise--Relevant scientific expertise is crucial but should not be defined too narrowly. In the case of COVID-19, expertise must extend beyond virology, infectious diseases, and public health, to include ecology, evolutionary biology, molecular biology, comparative medicine, sociology, political science and security, forensic science, and international law. 5) Independence--The conduct of the investigation must be independent of political interference. In addition, the investigation must be subject to independent oversight and review. 6) Conflicts of interest--Any nation suspected of serving as the place of origin of an international outbreak, any organization with responsibilities for preventing or managing an outbreak, and any scientist whose work is suspected of playing a role in the origins of an outbreak, will all have self-interests that may impede an effective and objective investigation. These conflicts must be articulated and responsibly managed.

With respect to data and materials needed for an effective investigation of a zoonotic outbreak, there are several categories to be considered. 1) Archived animal samples and derivative data--Such samples will be derived from animals in their natural habitats, from various points along the path of wildlife trafficking, and at the places of suspected human encounter where infection was first detected. 2) Archived clinical samples and derivative data--These samples and data will include those from suspected early cases, public health surveillance, wastewater, and social media. 3) Laboratory data--From any laboratory that may have played a role in the origins of an outbreak, a variety of records, data, and samples will be important. These should include records of field activities, animal sample inventories, virus isolate collections, sequence databases, experiment logs and methods, records of all genetic constructions, safety records, and personnel health records and clinical specimens.

Controls are critical for any investigation and should include populations and hosts clinically unaffected early in the outbreak that share features with those affected. The veracity and provenance of samples and data must be addressed. For example, widespread dissemination of a pandemic virus will lead to contamination of a wide variety of materials and spill-back into previously uninfected animals. Thus, efforts to assure the integrity and provenance of any archived sample are essential.

Observations about the current state of affairs regarding the study of COVID-19 origins, and next steps

While it is unrealistic to believe that the origins of a pandemic should be entirely understood one and a half years later, we should expect by this point a more coherent, balanced, coordinated, and international effort to investigate COVID-19, and a more substantial body of verified data in the public domain. Instead, the origin issue has become grossly politicized, opinion valued more than rigorous analysis, and a balanced scientific discourse hard to find. Too many good scientists have failed to challenge their assumptions or provide proper scientific

support for their conclusions. Some have formulated their opinions based on subjective assessments of likelihoods from prior outbreaks. There has been ‘group-think’ on all sides of this issue. Conflicts of interest have not been properly addressed. Many scientists are hesitant about addressing this issue because of fear that their comments will be misinterpreted. All of this threatens to harm the credibility of scientists and of the scientific enterprise.

In the midst of this situation, there are positive signs of useful engagement and discussion, a willingness by some scientists and policy-makers to deliberate on paths forward, and some first steps. I helped to organize a letter by a group of 17 other scientists directed at our colleagues and published in a scientific journal that called for a sober and dispassionate assessment of what we know and don’t know about COVID-19 origins, and for a renewed process that relies on sound principles of scientific investigation.²⁷ We did not express an opinion about the source of the pandemic, and suggested that scientists help lead in this effort relying on principles that we know well. The presidents of the U.S. National Academies of Science, Engineering, and Medicine have also issued a similar call²⁸. I believe that national academies of science and other credible organizations that represent the global science community have an opportunity and some responsibility to participate in investigations of pandemic origins. The US NASEM has a productive history of bilateral ‘track 2’ engagements with their counterparts in China, Russia, and many other countries; it is this kind of partnership that will be most effective now. However, groups of scientists will need to proceed under the auspices and authorities of those that represent other global communities, including national governments.

Discussion is underway in many quarters to examine whether there is an effective international governance mechanism for leading an investigation of COVID-19 origins. The World Health Organization would have been an obvious candidate, but during the past year, it has created some doubt as to its ability to operate without undue degrees of external influence and with sufficient degrees of impartiality. Alternatives are well-worth exploring. And although I have some bias and conflict, given my participation in these efforts, I find useful proposals that have arisen from the ongoing work of the COVID Commission Planning Group (Miller Center Foundation, University of Virginia)²⁹ and work by the Nuclear Threat Initiative and World Economic Forum who have proposed a new global entity³⁰ to fill gaps in the current global biosecurity governance scheme and to provide a mechanism for international investigation of outbreaks.

²⁷ Bloom JD, et al. Investigate the origins of COVID-19. *Science*. 2021 May 14;372(6543):694. doi: 10.1126/science.abj0016.

²⁸ McNutt M, Anderson JL, Dzau VJ. “Let scientific evidence determine origin of SARS-CoV-2 urge presidents of the National Academies”. June 15, 2021. <https://www.nationalacademies.org/news/2021/06/let-scientific-evidence-determine-origin-of-sars-cov-2-urge-presidents-of-the-national-academies>

²⁹ UVA Miller Center. “COVID Commission Planning Group”. <https://millercenter.org/covidcpg>

³⁰ Hamburg MA, Yassif J. “We must take action to guard against future global biological risks”. *The Hill*, June 8, 2021. <https://thehill.com/opinion/healthcare/557296-we-must-take-action-to-guard-against-future-global-biological-risks>

“NTI / bio Convenes Experts to Establish a Global Entity Dedicated to Reducing Biotechnology Risks”. <https://www.nti.org/newsroom/news/nti-bio-convenes-experts-establish-global-entity-dedicated-reducing-biotechnology-risks/>

In closing, as I have written previously³¹, a more complete understanding of the origins of COVID-19 and any other international health emergency clearly serves the interests of every person in every country on this planet. Despite the challenges, we must remember that delineating the pandemic origin story will help elucidate the very nature of our precarious coexistence within the biosphere.

I will be delighted to entertain any questions.

³¹ Relman DA. Opinion: To stop the next pandemic we need to unravel the origins of COVID-19. *Proc Natl Acad Sci USA* 117:29246-48, 2020. <https://www.pnas.org/content/117/47/29246>

David A. Relman is the Thomas C. and Joan M. Merigan Professor in Medicine, and a Professor of Microbiology & Immunology at Stanford University, and Chief of Infectious Diseases at the Veterans Affairs Palo Alto Health Care System. He is also Senior Fellow at the Center for International Security and Cooperation at Stanford, and served as the Center's Science Co-Director from 2013-2017. Relman trained at MIT and then Harvard Medical School, followed by clinical training in internal medicine and infectious diseases at the Massachusetts General Hospital in Boston, and a postdoctoral fellowship in microbiology at Stanford.

Relman was an early pioneer in the modern study of the human indigenous microbiota (microbiome). His current research work focuses on assembly, diversity, stability and resilience of human microbial communities. Previous work included pathogen discovery, and bacterial pathogenesis. He received an NIH Director's Pioneer Award in 2006 and an NIH Transformative Research Award in 2013. He has served as chair of the Board of Scientific Counselors at both NIDCR/NIH and NCBI/NIH. Among policy-relevant activities in biological security and emerging infections, Relman served as vice-chair of the National Research Council Committee that reviewed the science performed for the FBI 2001 Anthrax Letters investigation, chair of the Forum on Microbial Threats (2007-2017), chair of the Standing Committee that examined illnesses in U.S. State Department employees stationed in Cuba and China (2019-2020), and currently serves on the Intelligence Community Studies Board and the Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats, all at the U.S. National Academies of Science, Engineering, and Medicine. He was a founding member of the National Science Advisory Board on Biosecurity (2005-2014), a member of the Working Group on Biodefense for the President's Council of Advisors on Science and Technology (The White House) (2016), and President of the Infectious Diseases Society of America (2012-2013). He was elected to the National Academy of Medicine (NAM) in 2011, and received the Walsh McDermott Medal from NAM in 2020.

Chairman FOSTER. Thank you. And next is Dr. Perlman, who will be recognized for five minutes.

Mr. PERLMUTTER. You're still muted.

**TESTIMONY OF DR. STANLEY PERLMAN,
PROFESSOR OF MICROBIOLOGY AND IMMUNOLOGY,
UNIVERSITY OF IOWA**

Dr. PERLMAN. Chairman Foster, Ranking Member Obernolte, Ranking Member Lucas, and Members of the Subcommittee, thank you for the invitation to testify you—before you today about this incredibly important topic: outbreak investigation for COVID-19 and future infectious diseases. My name is Stanley Perlman, and I run a lab at the University of Iowa that has focused on coronaviruses for almost four decades. I am pleased to join this distinguished panel.

There are actually two issues to consider in addressing this question. The first is based on the actual origin of the virus, and the second is based on determining how the virus entered human populations. At the beginning of the pandemic it was postulated by some that that virus was engineered in a laboratory. However, this is not a viable possibility, in my opinion, because one would not know how to design a virus like SARS-CoV-2 from scratch. The next question is whether a naturally derived virus could have been manipulated in a laboratory. Certain characteristics of the virus have been considered as evidence for manipulation in a laboratory. In general, one would need to correctly identify or guess which coronavirus to manipulate, and then correctly determine or guess how to manipulate it.

Another question is whether naturally derived coronavirus-2 like virus could have been passaged in cells in a laboratory so that it adapted to growth in a new type of cell. This virus would have first had to have been present in the library—in the laboratory, and been growing in cell culture. Most bat SARS-CoV related viruses do not grow well in cell culture, and they exist in the laboratory only as non-viable viral RNA. The general experience is that only some viruses can be grown in new cross-species tissue culture cells and adapt to the new environment. Most of these will lose disease potential when passaged in cell culture, and this also explains how we obtain vaccines, like the poliovirus vaccine. This leaves a naturally derived virus not manipulated in the laboratory as the most likely source of SARS-CoV-2. Such a virus has not been found in nature, but viruses that are very similar to SARS-CoV-2 have been found in bats.

A second question is how the virus actually entered human populations. The possibilities that have been discussed in the public discourse and elsewhere are that the virus was transported to Wuhan by an infected human or infected wildlife, or it was released from a laboratory that had the virus in hand. The latter possibility has been raised because Wuhan is the home to several well-established virology laboratories. In support of the first possibility, there's increasing evidence that wildlife that is susceptible to infection with SARS-CoV-2, raccoon dogs, and minks, were, in fact, traded in the Wuhan Seafood Market. The question of whether release from a laboratory, a lab leak, is harder to address. For example, if the

virus was brought into a laboratory by someone working with relevant wildlife, and the virus then infected either that person or someone else in the laboratory, that person could then, in theory, infect others in the community. Therefore, since the—this possibility cannot be ruled out, it must be appropriately investigated.

Both data transparency and international collaboration are necessary and critical for investigations of the origin of an infectious disease. Both the WHO and the U.S. CDC, who have experts who have a long history of epidemiological investigations, and both institutions are natural candidates for working with these studies. It is evident from the COVID-19 pandemic that it is necessary to obtain as many samples as possible at the early stage of the pandemic from a broad group of wild, companion, and farmed animals, as well as from humans with unexplained respiratory or other disease to have the best chance, or perhaps the only chance, of determining how the virus crossed species to infect humans. COVID-19 has infected so many people around the world that it's impossible, virtually, to get samples now from the—from present populations.

In addition to surveillance, which needs to be done by these—both locally and internationally, we need to have risk assessment associated with the surveillance, otherwise we're just cataloguing viruses. All the zoonotic SARS and MERS coronavirus spillover events to date have occurred outside of the U.S. To reduce the risk of a pandemic like COVID-19 occurring again, we need to know as much as possible about viruses circulating in bats and other wildlife populations throughout the world. It should be noted that, while much of this sounds theoretically—most sounds seemingly theoretical, we know that there are MERS-like coronaviruses throughout Asia and Africa. MERS-COV does not spread efficiently between people, however, we learned in 2019 that even though SARS-CoV did not transmit readily between people, a fairly close relative, SARS-CoV-2, does. Similarly, a zoonotic MERS-like coronavirus could evolve, and potentially cause a pandemic. Like the SARS-CoV-2, such a virus would pose a great threat to human populations because, as was the case with SARS-CoV-2, we lack immunity to this virus. And with this I will conclude my remarks, and look forward to answering any questions that you have.

[The prepared statement of Dr. Perlman follows:]

**United States House of Representatives Committee on Science, Space and Technology
Subcommittee on Investigations and Oversight**

Principles for Outbreak Investigation: COVID-19 and Future Infectious Diseases.

July 14, 2021

Stanley Perlman, M.D., Ph.D.
University of Iowa Distinguished Chair
Professor, Department of Microbiology and Immunology
Professor, Department of Pediatrics

Questions presented by the Subcommittee

1. How should we understand the state of the science of the investigation into the origins of COVID-19? How can Congress and the American public contextualize the various theories circulating in the media, including the natural origin, lab leak, and genetic engineering theories and their relative likelihood?

There are actually two issues to consider in addressing this question. The first is based on the actual origin of the virus and the second issue is determining how the virus entered human populations. At the beginning of the pandemic and to a lesser extent now, it was postulated by some that the virus was engineered completely in the laboratory. However, this is not a viable possibility, in my opinion, because one would not know how to design a virus like SARS-CoV-2 from scratch. Given the similarity of SARS-CoV-2, the causative agent of COVID-19, to viruses isolated from bats, the data support that the virus has a natural origin.

The next question is whether a naturally derived virus could have been manipulated in a laboratory. Certain characteristics of the virus have been considered as evidence for manipulation in the laboratory. These include evidence of a 'furin-cleavage site' in the SARS-CoV-2 spike protein, which is important for entering human cells but is not found in other closely related viruses of the same group, as well as the use of certain codons to encode the amino acids of this site. The latter codons although rare in coronaviruses are not unique to SARS-CoV-2. Both of these possibilities have been discussed extensively recently in the scientific literature, the mainstream media, and public discourse.

I will discuss the furin cleavage site in a little more detail. Furin cleavage sites are present in some coronaviruses including the one that causes the Middle East respiratory syndrome (MERS) and other betacoronaviruses, but not in others. Past experience with furin cleavage sites indicates that when they are present in a coronavirus, their removal usually makes the virus less virulent (less able to cause severe disease). However, the reverse is not true. This was studied many years ago using mouse coronaviruses. The addition of a furin cleavage site to a mouse coronavirus that lacked this site actually weakened the virus (lessening its ability to cause severe disease in mice). So someone would first need to correctly identify or guess which coronavirus to manipulate and then correctly determine or guess that adding a furin cleavage site would make that specific coronavirus more, rather than less, virulent.

Another example of the complexity of coronavirus evolution is a recent report from Malaysia of a canine coronavirus that was found in eight children with respiratory disease (named 'CaCoV-HuPn-2018'). This virus was not a standard canine coronavirus but was a mixture of two different canine and one feline coronavirus, showing that recombination between viruses had occurred and is common. This report also indicates how difficult it is to isolate a virus that is the

initial source of an infection, since the recombinant canine coronavirus that infected the children has never been found in nature. Since recombination of CoVs is common in nature, it is also possible that SARS-CoV-2 may be a recombinant virus making the tracing of animal predecessors more challenging.

Another question is whether a naturally derived SARS-CoV-2-like virus could have been passaged in cells in a laboratory, so that it adapted to growth in a new type of cell. This virus would have had to first be present in the lab and been growing in cell culture. Most bat SARS-related CoVs do not grow in cell culture and they exist in the lab only as non-viable viral RNA. The general experience is that only some viruses can be grown in a new tissue culture cell and adapt to the new environment; most of these will become attenuated (lose disease potential) when passaged in cell culture. Examples are vaccines such as the poliovirus vaccine and the measles vaccine, both of which were grown in cells in the laboratory and both of which adapted to growing well in those cells. At the same time, these adapted viruses lost the ability to cause disease in people, which is why they are used as vaccines.

This leaves a naturally derived virus not manipulated in the laboratory as the most likely source of the SARS-CoV-2. Such a virus has not been found in nature, but viruses that are very similar to SARS-CoV-2 have been found in bats. These viruses are not close enough to SARS-CoV-2 to be the immediate ancestors of the virus but they support the notion that eventually a more closely related virus will be found. We know from the SARS epidemic that the original SARS-CoV was a bat virus and know that a putative bat progenitor virus needed to adapt to both intermediary animals and humans before it became an efficient at causing disease in humans. Of note, even after 18 years, SARS-CoV itself has not been found in bats. Similarly, it is very likely that the bat virus that was the original precursor to SARS-CoV-2 also had a period of adaptation in either an intermediary animal such as minks that are known to be infectable by SARS-CoV-2 or even in humans such as those living near to caves where bats infected with coronaviruses are known to reside. Further investigation is required to determine whether a virus that is very similar to SARS-CoV-2 was present in candidate wild or farmed animals in China or Southeast Asia or in humans living near to relevant caves, who could have asymptotically transmitted the virus to other humans.

My conclusion from the above discussion is that laboratory manipulation of a naturally derived virus is very implausible but there is still the question of how the virus actually entered human populations. The possibilities that are being discussed in the public discourse and elsewhere are that the virus was transported to Wuhan by an infected human or via infected wildlife for trade in a wet market, or was released from a laboratory that had the virus in hand for presumptive research purposes. The latter possibility has been raised because Wuhan, in addition to being the major site of amplification of the virus, is also the home to several well established and highly esteemed virology laboratories. However, there is increasing evidence that wildlife that are susceptible to infection with SARS-CoV-2 (raccoon dogs, mink) were in fact traded in the Wuhan seafood market, as well as evidence that the virus was circulating in Wuhan and perhaps elsewhere before December 2019. This evidence, which is continuing to accrue, supports the conclusion that the Wuhan seafood market and other wildlife markets in Wuhan and other wildlife markets in Wuhan were the sites of some of the earliest cases and were sites of amplification but not of origin. This is reminiscent of the early SARS cases, which were largely asymptomatic or had mild clinical signs of disease and were confirmed by finding specific antibody in animal vendors in wet markets in China.

The question of whether release from a laboratory ('lab leak') is involved is harder to address. So far, no investigations have supported a lab source for the virus and there is no epidemiological or other scientific evidence actually linking the virus to any of these laboratories. However, some scenarios are hard to prove or disprove. For example, if the virus was brought into a laboratory by someone working with relevant wildlife and the virus then infected either that person or someone else in the laboratory, that person could then in theory infect others in the community. There is no evidence that this occurred and at this point, it will be very hard to prove, whether or not these laboratories in Wuhan agree to share their data files. Further, it remains unclear if the sharing of these data files will be sufficiently convincing to end this discussion. Therefore, since this possibility cannot be ruled out, it must be appropriately investigated.

2. What level of data transparency and international collaboration is expected and necessary for a complete investigation into the origins of an infectious disease? Please discuss your experience with the SARS and MERS investigations, as relevant.

Both data transparency and international collaboration are necessary and critical for investigations of both the origin of an infectious disease, and in the case of a zoonotic virus, to understand how it crosses animal species to infect humans. In the case of SARS-CoV-2, it is essential that all possible intermediary wildlife and companion and farmed animals be analyzed for evidence of prior infection, and that human samples be analyzed for evidence of infection worldwide prior to December 2019. These investigations will not be easy because the COVID-19 pandemic is so widespread. Thus, reliable analyses will be largely confined to samples obtained prior to the onset of the pandemic. International cooperation is also critical because some countries have expertise that will make investigations easier. For example, both the WHO (World Health Organization) and the U.S. Centers for Disease Control and Prevention (CDC) have experts who have a long history of epidemiological investigations and both institutions are natural candidates for helping with these studies, with the WHO taking the lead in deploying experts for international outbreak investigations. In addition, epidemiological studies require strong laboratory support, which would benefit from input from experts throughout the world through the WHO reference and collaborating virology labs as well as the United States CDC. It is evident from the COVID-19 pandemic that it is necessary to obtain as many samples as possible at the early stage of the pandemic from a broad group of wild, companion and farmed animals as well as from humans with unexplained respiratory or other disease to have the best chance of determining how the virus crossed species to infect humans.

I do not have personal experience with the SARS investigations although as a career coronavirologist, I followed these investigations as they were reported. In contrast, I worked and am still working with the Ministry of Health and others in Saudi Arabia, and in Africa, investigating MERS, with a focus on human-to-human and camel-to-human transmission. Within two years of the SARS epidemic, viruses very similar to SARS-CoV were found in animals (such as palm civet cats and raccoon dogs) present in wet markets in Guangdong province. These findings were reported in the scientific literature. However, evidence to prove more definitively that SARS-CoV was a bat virus was not published until 10 years after epidemic ended. In this case, it was evident fairly early that SARS-CoV entered human populations via these intermediary animals, but it took much longer to establish the origin of the virus. As discussed above, we still have not found a virus in bats that is identical to SARS-CoV. As part of these studies, farmed and wild animals such as palm civet cats, Chinese ferret badgers and raccoon dogs were studied for the presence of the virus. This widespread investigation in China, performed with international collaboration, coupled with the limited amount of human infection

(SARS-CoV is a more deadly but less contagious virus) allowed for a more complete picture of virus evolution and origin.

In the case of the Middle East respiratory syndrome (MERS), MERS-CoV was isolated in 2012. Virtually identical viruses were isolated from camels soon thereafter, although initial scientific publications describing MERS-CoV in camels did not appear until 2014. MERS-CoV, like SARS-CoV-2 is almost certainly derived from a virus that infects bats. MERS-like coronaviruses have been isolated from bats, but these are not very close to MERS-CoV, most likely because MERS has been in camels for decades. Thus, the virus has had time to evolve to adapt to camels and no longer resembles the ancestral virus that is present in bats.

3. What kind of ongoing genomic and surveillance work should the United States government prioritize to ensure we reduce the risk of zoonotic spillover and ensure we have as much information as possible on emerging pandemic risks?

All of these zoonotic SARS and MERS coronavirus spillover events to date have occurred outside of the U.S.A. To reduce the risk of a pandemic like COVID-19 occurring again, we need to know as much as possible about viruses circulating in bat and other wildlife populations in places such as southern China and Southeast Asia. We should not only support efforts that attempt to isolate specific coronaviruses and other viruses from bats to characterize them, but also perform large scale sequencing studies to obtain information about viruses that are present in these animals but cannot be grown in the laboratory using standard techniques. For the success of these studies, it will be important to work with laboratory, scientific and public health authorities in China and elsewhere in Southeast Asia. Of course, zoonotic spillover events could occur elsewhere in the world, so we should encourage surveillance in multiple locations. A model for this is influenza virus surveillance in humans and animals (One Health approach) conducted by WHO through designated international reference laboratories including at the US CDC. These labs provide the early data needed to formulate the annual seasonal influenza vaccines used worldwide. They also provide an early warning system to recognize influenza strains that could potentially cause a new pandemic. After 2 major recent human coronavirus outbreaks (SARS, MERS) and now the ongoing SARS-CoV-2 pandemic, a similar global alert system is essential for surveillance of emerging coronaviruses in animals and humans. Also, the NIH has funded large centers that have ties with investigators throughout Asia, Latin America and Africa. These centers make use of personnel in these places who are most familiar with the local physical and social environment and provide funding so that the surveillance activities can be conducted efficiently.

Importantly in case of outbreaks, WHO and US CDC teams can be deployed to work in collaboration with local teams to try to contain outbreaks (Ebola, etc) locally where they originate, before they can disseminate worldwide. Furthermore, the discovery of 2 new recent zoonotic coronavirus spillovers in hospitalized children (the canine coronavirus spillover in Malaysia described above) and in school children (a pig coronavirus spillover event in Haiti) has shown that it is critical to survey humans with undiagnosed diseases for animal CoVs. Such studies are best accomplished by a One Health approach employing teams with human and veterinary infectious disease expertise to investigate both the human infections and their animal origin. Notably, some of the most useful and relevant work on the surveillance of bats and other animals for potential viral zoonotic events occurs in Wuhan. It should also be noted that while much of this sounds seemingly theoretical, we know that there are MERS-like coronaviruses throughout Asia and Africa. MERS-CoV does not spread efficiently between people. However, we learned in 2019 that, even though SARS-CoV did not readily transmitted between people, a fairly close relative, SARS-CoV-2 does. Similarly, a zoonotic MERS-like coronavirus could

evolve and potentially cause a pandemic. Like SARS-CoV-2, such a virus would pose a grave threat to human populations, because, as was the case with SARS-CoV-2, we lack immunity to this virus. Learning as much as we can and investing significantly in research on MERS-like coronaviruses (and other viruses with potential to cross-species) would decrease this threat.

Dr. Stanley Perlman
The University of Iowa
Professor of Microbiology and Immunology, and of Pediatrics
USA

Dr. Perlman received his Ph.D. in Biophysics from M.I.T., Cambridge, Massachusetts and his M.D. from the University of Miami, Miami, Florida. He was trained in Pediatrics and Pediatric Infectious Diseases at Boston Children's Hospital, Boston, Massachusetts. His current research efforts are focused on coronavirus pathogenesis, including virus-induced demyelination and the Severe Acute Respiratory Syndrome (SARS), the Middle East Respiratory Syndrome (MERS) and COVID-19.

His laboratory has developed several novel animal models useful for studying pathogenesis and evaluating vaccines and anti-viral therapies. His studies are directed at understanding why aged patients and mice developed more severe disease than younger individuals after infection with SARS-CoV or SARS-CoV-2 and also on why there is a male predominance in patients with more severe disease after infection with SARS-CoV, MERS-CoV or SARS-CoV-2. He and his colleagues demonstrated that transduction of mice with an adenovirus expressing the human receptor for MERS-CoV, DPP4, rendered them sensitive to infection, providing the first rodent model useful for studying MERS. Similar approaches have been used to develop several mouse models for COVID-19. Among other topics, his research is now focusing on the loss of sense of smell (anosmia) and taste (ageusia) observed in patients with COVID-19.

Chairman FOSTER. Thank you. And after Dr. Perlman is Dr. Price, who is now recognized for five minutes. And whoops, you'll have to unmute, I'm afraid.

**TESTIMONY OF DR. CONNIE PRICE,
CHIEF MEDICAL OFFICER, DENVER HEALTH,
AND PROFESSOR OF MEDICINE,
DIVISION OF INFECTIOUS DISEASES,
UNIVERSITY OF COLORADO SCHOOL OF MEDICINE**

Dr. PRICE. Chairman Foster, Ranking Members Lucas and Obernolte, and Members of the Investigations and Oversight Subcommittee, my name is Connie Savor Price. I am a practicing infectious diseases physician and healthcare epidemiologist with experience and expertise in outbreak management. I have served as a consultant to public health authorities on control of emerging infections, including Middle East Respiratory Syndrome for the Kingdom of Saudi Arabia and Severe Acute Respiratory Syndrome for the Ontario Ministry of Health. I am the Executive Director for HHS (Health and Human Services) Region 8 Ebola and Special Pathogens Treatment Center and HHS Regional Disaster Health Response system. I'm a Professor of Medicine in the Division of Infectious Diseases at the University of Colorado School of Medicine. I'm honored to be here today to speak with you about the principles for investigating infectious diseases outbreak.

When a threat to the public's health occurs, epidemiologists and other relevant experts investigate the problem so they can identify sources and risk factors, implement prevention and control measures, and communicate with stakeholders. Whether the event occurs in a hospital setting or in a community, there's a well-described—defined process for performing a complete investigation into the origins of an infectious diseases outbreak. The process requires significant collaboration between public health officials, laboratory personnel, clinicians, and other stakeholders in areas affected by the outbreak.

The detailed review begins with the initial cases, but should also include a time period preceding the current problem to determine whether earlier cases existed, and if so, the occurrence rates between the period of the current problem and the comparator period. A high level of data transparency is required, including full and complete access to patient medical records and other pertinent clinical information to infected patients, and their contacts for interviews, and to laboratory specimens and microbiologic isolates for molecular epidemiology, and possibly to other environmental sources or animals, should the investigation lead that way.

In the United States there are data collection and preservation standards in place at hospitals to ensure they can track emerging infectious diseases. The Center for Medicare and Medicaid Services requires that a healthcare facility maintains an infection prevention and control program in order to prevent, recognize, and control, to the extent possible, the onset and spread of infection within a facility, including surveillance and investigation to prevent the spread of infection. It also requires that hospital complies with the reportable diseases requirements of the public health or local health authority. To comply with this regulation, accrediting agen-

cies, such as the Joint Commission, survey hospital infection prevention infrastructure and written plans for detecting and controlling infections. Public health authorities may also monitor required data received from hospitals to look for concerning trends.

As part of standard work, once a trend of concern is identified by the hospital infection control program, case ascertainment and maintaining an epidemiologic description of the outbreak will inform initial hypotheses for explaining a potential cause, source, and mode of spread of the outbreak's causative agents. A related step to confirming diagnoses is the need to proactively save clinical specimens for longer than conventional holding periods, and not discard them, so they are available for further analysis. Both the clinical data and the laboratory data together are essential for tracing an outbreak. These data should inform next steps, such as environmental sampling, obtaining specimens from suspect animals such as bats, or a suspected intermediary host.

When it comes to investigating the emergence and spread of infectious diseases, hospitals and local or State health departments will take the lead, but national and international health authorities are available upon invitation. The CDC, for instance, provides surveillance data and clinical guidance on the management of emerging infectious diseases, but they will also routinely provide consultation and laboratory assistance to healthcare facilities and health departments that are working to solve outbreaks and investigate infection control breaches and other adverse events, but only upon request.

Hospitals will reach out through their local and State public health, or vice versa, if a concerning reportable or trend is identified, and that health department in turn may extend a formal invitation to the CDC for help leading an onsite team. Public health will help gather additional information from community cases through interviews, case reviews, observations, and possibly environmental sampling. In the case of a multi-State outbreak, the CDC coordinates that investigation. The World Health Organization will investigate outbreaks if invited by the government, but currently it is more often looked to as an important and exclusive source for global surveillance information.

Thank you for the opportunity to testify before you today on data and access that investigators need in order to trace an outbreak to a discrete origin. Upholding the principles for transparency, scientific integrity and objectivity are critical to the understanding. Thank you.

[The prepared statement of Dr. Price follows:]



**Principles for Outbreak Investigation:
COVID-19 and Future Infectious Diseases**

Testimony of:

Connie Savor Price, M.D.
Chief Medical Officer
Denver Health
Professor of Medicine
Division of Infectious Diseases
University of Colorado School of Medicine

**United States House of Representatives
Committee on Science, Space, and Technology
Subcommittee on Investigations & Oversight
Wednesday, July 14, 2021
Washington, D.C.**



Chairman Foster, Ranking Member Norman, and Members of the Investigations and Oversight Subcommittee:

My name is Connie Savor Price. I am a practicing infectious diseases physician and healthcare epidemiologist with experience and expertise in outbreak management. I have served as a consultant to public health authorities on control of emerging infections, including Middle East Respiratory Syndrome (MERS) for the Kingdom of Saudi Arabia and Severe Acute Respiratory Syndrome (SARS) for the Ontario Ministry of Health. I am the Executive Director for HHS Region 8 Ebola and Special Pathogens Treatment Center and our Regional Disaster Health Response System. I am a Professor of Medicine in the Division of Infectious Diseases at the University of Colorado School of Medicine. I am honored to be here today to talk with you about the principles for investigating infectious disease outbreaks in the context of public interest in the origins of COVID-19. I was asked to address the following questions:

What level of data transparency and international collaboration is expected and necessary for a complete investigation into the origins of an infectious disease?

When a threat to the public's health occurs, epidemiologists and/or other relevant experts (e.g. hospital infection prevention specialists) investigate the problem so they can identify sources and risk factors, implement prevention and control measures, and communicate with stakeholders. Whether the event occurs in the hospital setting or in a community, there is a well-described, systematic process for performing a complete investigation into the origins of an infectious disease outbreak [1-5]. The process requires significant collaboration among public health officials, laboratory personnel, clinicians, and other stakeholders in areas affected by the outbreak. The detailed review begins with the initial cases (known or suspected) but should also include a time period preceding the current problem to determine whether earlier cases existed, and if so, the occurrence rates between



the period of the current problem and a comparator period. A high level of data transparency is required, including full and complete access to patient medical records and other pertinent clinical information (e.g., radiology, laboratory, and other studies); to infected patients and their contacts (for interviews); and to laboratory specimens and/or microbiologic isolates (for molecular epidemiology (e.g. genomic sequencing) to support the clinical investigation. As an example, in May 2013, I was invited by the Saudi Ministry of Health to join an international team to investigate a cluster of novel coronavirus infections in the eastern region of the Kingdom. This infection would later become known as Middle Eastern Respiratory Syndrome (MERS). The outcome of our findings was published in the New England Journal of Medicine within weeks of the conclusion of our investigation [6]. As described in the methods section of the manuscript, our team was allowed access to medical records so that we could review clinical and demographic information and determine potential contacts and exposures. In collaboration with Saudi epidemiologists, case patients and contacts were interviewed. Clinical specimens from a sampling of patients were accessed from storage and sequenced to provide additional information on transmission, evolution, and origin, which was later further described in a more extensive subsequent genomic analysis [7]. Although our investigation focused on transmission in the healthcare settings, our investigation yielded important information that would inform subsequent investigations into community origins.

What data collection and preservation standards are in place at hospitals to ensure they can track emerging infectious diseases?

In the United States, the CMS requires that a healthcare facility maintains an infection prevention and control program in order to prevent, recognize, and control, to the extent possible, the onset and spread of infection within the facility, including surveillance and investigation to prevent the spread of infection. It also requires that the hospital complies with



the reportable diseases requirements of the local health authority. To comply with this regulation, accrediting agencies, such as the Joint Commission, survey hospital infection prevention infrastructure and written plans for detecting and controlling infections, including new emerging infections. Public health authorities may also monitor data received from hospitals to look for concerning trends.

As part of standard work, once a trend of concern is identified by the hospital infection control program, case ascertainment and maintaining an epidemiologic description of the outbreak will inform initial hypotheses for explaining the potential cause, source, and mode of spread of the outbreak's causative agent(s). A related step to confirming diagnoses is the need to proactively save specimens (e.g., microbiologic strains already isolated) for longer than conventional holding periods and not discard them so that they are available for further analysis if new questions arise later in an investigation.

What is the relationship between hospitals and national and international health authorities when it comes to investigating the emergence and spread of infectious diseases?

In the United States, the CDC provides some surveillance data and clinical guidance on management of emerging infectious diseases. They also routinely provide consultation and laboratory assistance to healthcare facilities and health departments that are working to solve outbreaks or investigate infection control breaches and other adverse events, but only upon request. Hospitals will reach out to their local/state public health, or vice-versa, if a concerning reportable or a trend is identified. The health department, in turn, may extend a formal invitation to CDC for help leading an on-site team. Public health will help gather additional information from interviews, case/chart reviews, observations and possibly environmental sampling. The team analyzes this information and helps develop control measures. In the case of a multistate outbreak, the CDC coordinates the investigation, working closely public health



partners, who in turn work with their labs and healthcare facilities. The World Health Organization (WHO) will investigate outbreaks if invited by the government, but is more often looked to as an important and exclusive source for global disease surveillance information.

What data must be shared by hospitals, labs, and governments in order to complete a comprehensive investigation of a zoonotic spillover episode?

These are well described by our CDC [8].

The initial steps in an investigation require that one confirming or verifying the diagnosis through:

- Reviewing full medical records of suspected cases and selected non-cases during the time period of initial emergence and for some time period prior to initial emergence. Record coded patient identifiers, age, sex, race/ethnicity, date of illness onset or diagnosis, symptoms, signs, laboratory findings, or other relevant data.
- Verifying the results of laboratory testing
- Saving specimens (e.g., microbiologic strains already isolated) so that they are available for further analysis
- Access to affected persons for clinical examination by health-care personnel when possible
- Interviewing the affected persons and their contacts

Any existing routine surveillance that exists should be reviewed, such as public health agency surveillance data; medical system billing/coding data from other hospitals, laboratories, or ambulatory care settings; institutional setting records (e.g., school and workplace attendance records); and other special surveys.



The outbreak should be characterized in terms of time, place, and person:

- Time of onset of illness (symptoms, signs, or laboratory test positivity) among affected persons; period of likely exposure to the causal agent(s) or risk factor(s); time when treatments were administered or control measures were implemented; and time of potentially related events or unusual exposures.
- Place of residence and occupation; venues for recreational activity; activity sites (e.g., rooms or units in which persons were hospitalized; rooms visited during a convention or meeting; or seating or activity locations on transportation conveyances, such as planes or cruise ships).
- Person /demographic characteristics (e.g., age, sex, and race/ethnicity), occupation, and diagnoses; and features shared by affected persons.

To support the comprehensive clinical investigation, laboratory samples should be accessible for sequencing or other molecular epidemiologic investigations.

These data should inform next steps, i.e., environmental sampling, obtaining specimens from suspect animals (e.g. bats, suspected intermediary host).

Thank you for the opportunity to testify before you today on data and access that investigators need in order to trace an outbreak to a discrete origin. Upholding the principles for transparency, scientific integrity, and objectivity is critical to understanding the origins of any outbreak, especially COVID-19.

References



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Chairman FOSTER. Thank you. And our final witness, Dr. Murray, will now be recognized for five minutes.

**TESTIMONY OF DR. SUZAN MURRAY, PROGRAM DIRECTOR,
SMITHSONIAN GLOBAL HEALTH PROGRAM,
SMITHSONIAN NATIONAL ZOO
& CONSERVATION BIOLOGY INSTITUTE**

Dr. MURRAY. Thank you so much, Chairman Foster, Ranking Members Lucas and Obernolte, and Members of the Subcommittee, as well as the distinguished panelists. Thank you for calling the hearing, and inviting me to participate. My name's Dr. Suzan Murray, and I'm the Director of Smithsonian's Global Health Program based out of the National Zoo and the Conservation Biology Institute. Recognizing that 75 percent of these diseases come from the wildlife population first, and then jump to the human population, our program was built and utilizes experts in wildlife medicine, human medicine, public health, conservation biology, and epidemiology to study and respond to health issues at the human/animal interface. Recognizing that human, wildlife, and environmental health are all inextricably linked, we utilize this multidisciplinary approach to investigate emerging infectious diseases, we build in-country capacity, and train the next generation of health specialists.

Determining the origins of a virus is difficult, and ongoing investigation and identification of new viruses can improve and expedite this process. With a robust sample collection, we can precisely—we can more precisely determine how a virus in question is genetically related to variants. This can tell us much about geographic location, host species, and time period over which the virus may have evolved. We learn a great deal of information from data collection from free-ranging species, including, but not limited to, bats, primates, and rodents, and expanding this data set exponentially is the most critically—the most critical opportunity we have to better understand the origins of this particular virus.

Over the past decade, as part of a larger consortium, we have worked in more than 30 countries and discovered over 1,000 new mammalian viruses. Analysis of these viruses has determined that they represent less than 1 percent of existing viruses. To more fully understand the origins and evolution of these viruses, continued sampling at an increasing rate is needed, and the best way to accomplish this is by forming and utilize a broad and inclusive approach. The wide variety of factors that contribute to disease emergence underscores the need to develop a multi-disciplinary, transparent, and cooperative community that works together to develop and analyze approaches to data collection and protocols for sharing this information. Some of this work is already underway now between multiple agencies and organizations, such as NSTC's (National Science and Technology Council's) Health Threats Subcommittee. Additional coordination could further improve our ability to react to the next pandemic.

Orchestrating this kind of coordination is not easy. Managing across governments, researchers, and impacted communities is a huge undertaking, especially when no one organization is charged with ensuring that coordination. Even when there is a commitment

to work together, differences in how places operate can add difficulty. Principles for transparency and data access range from—and vary from community to community based upon several factors, including, but not limited to, shared trust on how data will be interpreted, utilized, and credited.

We learned a great deal from the current pandemic and other epidemics about what's working well, as well as areas in which we must improve. We developed invaluable partnerships with many countries, forming a strong and growing One Health workforce to build upon the foundational works in the areas of bio-surveillance, viral discovery, laboratory protocol development and implementation, as well as rapid response units and data sharing. Over the past decade our work has shifted from primary disease surveillance to investigating and better understanding the drivers of disease emergence. In this process we have increasingly incorporated such essential practices such as modeling, data sharing, and understanding the role of human behaviors, cultural norms, as well as outreach.

Further, we are recognizing the science is strongest and most impactful when it draws upon these multidisciplinary assets, while being inclusive and contextualized for affected communities, cultures, arcs, and broader societies. For instance, some of the most critical and inter-related drivers of disease emergence include land use change, human behaviors, and education that can affect human behaviors. Recently, with internal partners from the National Air and Space Museum, the National Museum of Natural History, Smithsonian's Occupational Health Program, and our Office of Communication and External Affairs, along with outside partners such as UBS's Global Visionaries Program, we are developing a more comprehensive approach to pandemic prevention by incorporating previously underutilized techniques to assess high risk interfaces more—while—more quickly and truth-find this data. This will ensure that our approach is iterative, always learning, and—while incorporating infected communities from the beginning, and then throughout the process. This will require that we conduct both human and animal surveillance concurrently, utilizing the most advanced data collection methodology, such as smartphone technology and field-based PCR (polymerase chain reaction) machines. Not only will this more quickly inform scientists and pharma companies, but it will ensure that the public is engaged and informed along the way.

Knowledge of pandemic prevention, public health, and vaccinology are helpful to all of us, from communities living at the high-risk interfaces, to scientists who study viruses, to economists interpreting the impacts on global economy, to people visiting exhibits such as National—the Natural History's Outbreak Museum outbreak exhibit, to the general public reading and watching the news. Knowledge is power, and broadly sharing knowledge is absolutely essential to our global health. Thank you again for inviting me to participate in today's hearings, and I look forward to answering any questions you might have.

[The prepared statement of Dr. Murray follows:]

Smithsonian Institution

Written Testimony of

Dr. Suzan Murray, D.V.M., D.A.C.Z.M., Director, Smithsonian Global Health Program
Smithsonian National Zoological Park and Conservation Biology Institute

PRINCIPLES FOR OUTBREAK INVESTIGATION: COVID-19 AND FUTURE INFECTIOUS DISEASES

House Committee on Science, Space, and Technology

Subcommittee on Investigations and Oversight

U.S. House of Representatives

Wednesday July 14, 2021

Chairman Foster, Ranking Member Obernolte, and Members of the Subcommittee, thank you for calling this hearing, and inviting me to participate. My name is Suzan Murray, and I am the Director of Smithsonian's Global Health Program, based out of the National Zoological Park and Conservation Biology Institute. This program utilizes experts in wildlife medicine, human medicine, public health, conservation biology, epidemiology, virology and molecular biology to study and respond to issues emerging at the human/animal interface. Recognizing that human health, wildlife health, and environmental health are inextricably linked, we utilize a multidisciplinary approach to investigate emerging infection diseases that threaten both human and animal life, and we build in country capacity and train the next generation of health specialists.

Determining the origins of a virus is difficult, but ongoing identification of novel viruses can improve and expedite the process. With a robust sample collection, we can more precisely determine how the virus in question is genetically related to known variants. This can tell us much about the geographic location, host species, and time period over which the virus may have evolved. We learn a great deal of information from the data collected from free-ranging species – particularly bats, primates and rodents – and expanding this data set exponentially is the most critical opportunity we have to better understand the origins of a particular virus. Over the past decade, as part of a larger consortium, we have worked in more than 30 countries and discovered over 100 novel mammalian viruses. Analysis of these viruses has determined that they represent less than 1 % of existing viruses. While this is a critical data set, to more fully understand the origins and evolution of these viruses, continued sampling at an increasing rate is needed to round out this data set.

If genetic quality of virus extracted from samples is sufficient, and the virus maps genetically to other well-sampled viruses, it is sometimes possible to conduct a 'time-scaled' phylogenetic analysis, allowing an estimation of the period of time over which the virus is likely to have evolved.

Which species is the most likely to be the reservoir host is a complicated question. From previous research we know that bats, primates, and rodents are the most likely to harbor emerging viruses of zoonotic potential, but they are not the only species able to do so. In fact, domestic livestock are responsible for most cases of zoonotic viral transfer to humans.

The genetic analysis described above, along with geographic information collected from the outbreak, can guide targeted bio surveillance in animals. In addition, the advent of new machine learning

techniques may be able to help elucidate the proximal host species in which the virus may have evolved. Additionally, virus culture and experimental infection studies under laboratory settings can be used to determine the host range outside of humans.

Determining where a spillover occurred and from what species is only part of the story. Did the spillover occur directly from the wildlife reservoir species to humans? or was another intermediate species (e.g., livestock) involved? Often, another host that is not necessarily a 'reservoir' for the pathogen will be responsible for direct transmission to humans. Since the pathogen may not have evolved within this 'bridge' host, genomic methods are not always sufficient to identify the species responsible for direct transfer to humans.

Wild animals don't seek out human interaction, so we must also determine the human-drivers that brought people into contact with a new virus. Possible examples include wildlife trade, habitat destruction and encroachment, and other practices.

The variety of factors underscores the need to develop a multidisciplinary, systematized, transparent and cooperative community that includes not only wildlife veterinarians, physicians, virologists, but ecologists, human behavioralists and public health leaders among others, who must work hand in hand to develop and analyze approaches to data collection and protocols for sharing critical information. Some of this work is already underway now between multiple agencies and organizations. Additional coordination could further improve our ability to react to the next pandemic.

Orchestrating this kind of coordination is not easy. Managing across governments, researchers, and impacted communities is a huge undertaking with no one organization charged with ensuring that coordination. Even where there is commitment to work together, differences in how different places operate can add difficulty. Principles for transparency and data access range and vary from community to community based upon several factors including but not limited to shared trust on how data will be interpreted, utilized, and credited. In addition, there are costs to consider to the groups that spent the time and resources to collect the data up front.

These are complex systems to navigate and even more difficult when such efforts cross work groups, communities, or national borders. There are obvious reasons why data is most useful when shared openly and rapidly and there should be a strong focus on developing protocols in which all parties are encouraged and incentivized to contribute to shared data sets prior to the initiation of data collection, perhaps by engaging a neutral third party to coordinate and promote transparent and rapid sharing of laboratory-based and epidemiological data within and across national boundaries.

Even in a scenario where all the necessary global treaties are in place to enable timely and accurate data sharing, that will not matter if we cannot collect good data on the ground. This is only achieved through the committed and ongoing work to build trust with the communities in spillover hotspots. In addition to trust, creating and supporting the in-country communities with which we work presents a real opportunity to not only address the current pandemic but avoid future outbreaks. Our international exchange training and capacity building programs bring together experts from different countries to facilitate shared knowledge exchange and provide an avenue to have a more robust and sustainable impact.

We have learned a great deal - from the current pandemic and other epidemics - about what is working well and areas in which we must improve. We have developed invaluable partnerships with many countries, forming a strong and growing “One Health Workforce” to build upon the foundational work in areas of wildlife bio-surveillance, concurrent human syndromic surveillance, viral discovery, laboratory protocol development and implementation, rapid response units as well as sharing critical data with scientific counterparts. Over the past decade, our work has shifted from primary disease surveillance to investigating and better understanding the drivers of disease emergence. In this process, we have increasingly incorporated such essential practices as modeling, data sharing, and the role of human behaviors, cultural norms and outreach. Further, we are recognizing that science is strongest and most impactful when it draws upon these multidisciplinary assets while being inclusive and contextualized for affected communities, cultures and broader societies.

We’ve also learned much more about how to fully leverage current technologies including artificial intelligence and computation, which allow us to make more data available to more key players, much more quickly. We’ve also learned to look for weaknesses and gaps more transparently so that they can be addressed more broadly to achieve better solutions. Finally, we’ve learned how critical it is to gather critical data; analyze it thoroughly and quickly; share across many platforms; and then develop interventions to not only neutralize current pandemics, but prevent future ones.

For instance, some of the most critical and inter-related drivers of disease emergence include land use shifts, human behaviors and education that can affect human behaviors. Recently, with internal partners from the National Air and Space Museum, the National Museum of Natural History, the Smithsonian’s Occupational Health Program, and our Office of Communications and External Affairs along with outside partners such as the UBS Global Visionaries program, we are developing a more comprehensive approach to pandemic prevention by more fully utilizing Smithsonian historical collections and incorporating previously under-utilized techniques to assess high-risk interfaces more quickly and truthfully find the data. This will ensure that our approach is iterative and always learning, while incorporating affected communities from the beginning and then throughout the process. This will also require that we conduct both human and animal syndromic surveillance concurrently, utilizing the most advanced data collection methodologies such as smart phone technology and field-based PCR machines. Not only will this better and more quickly inform scientists and Pharma companies, but it will ensure that the public is engaged and informed along the way.

The term “public” is used here in the broadest sense as we seek to increase and diffuse knowledge as widely as possible and appropriate. Knowledge of pandemic prevention, public health and vaccinology are helpful to all of us, from communities living at high risk of human-animal interfaces, to scientists who study viruses, to economists interpreting impacts on the global economy, to people visiting exhibits such as Natural History’s Outbreak exhibit, to the general public reading and watching the news. Knowledge is power, and broadly shared knowledge is absolutely essential to our global health.

Thank you again inviting me to participate in today’s hearing. I look forward to answering any questions you have.



**SMITHSONIAN'S NATIONAL ZOO
& CONSERVATION BIOLOGY INSTITUTE**

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Dr. Suzan Murray is a board-certified zoo veterinarian at the Smithsonian Conservation Biology Institute and serves as both the program director of the Global Health Program and as the SCBI's chief wildlife veterinary medical officer. She leads an interdisciplinary team engaged in worldwide efforts to address health issues in endangered wildlife and combat emerging infectious diseases of global significance, including zoonotic diseases. Dr. Murray also acts as the Smithsonian liaison to the Foreign Animal Disease Threat and Pandemic Preparedness subcommittees of the White House's Office of Science and Technology. Dr. Murray's work focuses on providing clinical care to free-ranging wildlife, pathogen detection, advanced diagnostics, training of international veterinarians and other health professionals, capacity building, and collaboration in infectious disease research at the human-wildlife-domestic animal interface. She previously served as chief veterinarian for the Smithsonian's National Zoo and has a wealth of clinical knowledge and experience with wildlife and zoo animals both free-ranging and in human care.

Dr. Murray earned a bachelor's degree from Amherst College in 1984 and completed her veterinary degree in 1991 from Tufts University. After a surgical internship, she completed a residency in zoological medicine at the Smithsonian's National Zoo in 1995 and became a Diplomate of the American College of Zoological Medicine (DACZM) in 2000. Dr. Murray has been either the principle investigator or co-principle investigator on several research grants including Morris Animal Foundation, Smithsonian Endowment, Smithsonian Women's Committee, and James Bond Funds.

Chairman FOSTER. Thank you. And at this point we will begin our first round of questions, and the Chair will now recognize himself for five minutes. Before I get started, I have a statement here from the American Society for Microbiology to be entered into the hearing record, and, without objection, it will be so ordered. Thank you.

Now, Dr. Price, it strikes me that two of the most significant words in your testimony seem to be “upon invitation.” And, you know, it seems like the very extensive set of data that you’d like to have before, and during, and even after the outbreak of a pandemic are things where you’re going to need exceptional local cooperation. One of the lessons that I take away from this, and probably from other pandemics, is that there’s a big incentive for institutions to cover up the extent. You know, this, happens, you know, at the family level, at the business level, at the local government, State, nation, you know, even at the level of systems of government.

And so how do we—is there an alternative here to doing our best to try to get treaty level commitments in advance that spell out exactly what is necessary here? Because, you know, my fear is that all—the best-intended voluntary data sharing agreements will not survive the first contact with the enemy. And what—could you say a little bit about what the thinking of the scientific community worldwide is on that?

Dr. PRICE. To establish ground rules before this happens is important, so treaty level, whatever it may be, but at the end of the day the host country needs to be collaborative, needs to cooperate, so forcing it upon them, we have to do something where it is an agreed upon standard, and make sure that we are addressing concerns that they may have about negative impact, and minimizing that for the sake of getting the data, and for the sake of understanding the science.

Chairman FOSTER. Um-hum. Are there international efforts to get data repositories, for example, that will be immediately accessible ahead of time so you don’t have to negotiate that, at least? It’s one way around part of this.

Dr. PRICE. Well, I think there are some collaborations internationally, but more ad hoc. One well-established venue we have for sharing information is a very low-tech e-mail platform called ProMED. ProMED’s been around since 1994, and has—was established by the International Society for Infectious Diseases, and basically it is a bunch of frontline doctors, epidemiologists, sometimes veterinarians, just e-mail about things they’re seeing that seem odd. Sometimes it turns into nothing, but sometimes it ends up being something. So, for instance, the first notification the world had of Middle East Respiratory Syndrome was through ProMED. On December 31, four days before the World Health Organization announced to the world the SARS-CoV-2, or what later became known as SARS-CoV-2, that had been already released through ProMED.

So there are platforms like that that allow early communication and direct scientist to scientist communication. The problem is I think sometimes governments, you know, do get involved in maybe stifling communication through those venues.

Chairman FOSTER. Yeah. And, Dr. Murray, you held out some optimism that sort of flooding the zone with technology, cheap handheld PCR machines, potentially, that automatically report what it is that they've measured in the field, so that, when, you know, someone is in the—in Barro Colorado Island and finds a capybara with a weird viral infection—

Dr. MURRAY. Uh-huh.

Chairman FOSTER [continuing]. You can immediately—you know, just to pick a Smithsonian relevant example.

Dr. MURRAY. I caught that. Thank you.

Chairman FOSTER. Yeah.

Dr. MURRAY. Yeah.

Chairman FOSTER. And so—you know, so that this automatically gets reported to the relevant international database. And is there—is that something where potentially the U.S. Congress could put some money into to get that deployed nationally and internationally?

Dr. MURRAY. Yes. Thank you very much. I wanted to also just echo what Dr. Price said, because I think that, in addition to what you're saying, if we—with a lot of the partners that we work with, again, in 30 different countries, we have to establish the ground rules ahead of time, and also incentivize our partners that if you participate and follow these rules, you get something from it, and if you don't, you don't. So I think that's part of it, and I also think that there are some other projects funded by USAID (United States Agency for International Development), as well as the Global Viral Project, to build platforms upon which a lot of the data of all these viruses, the sequences, will sit and be available to everyone. So I think there are some movements afoot.

In combination with that, yes, I think that's one of Smithsonian's strengths, using our field sites to—as—especially those that are within the hot spots, the areas in which the next disease is most likely to occur, and using the—and using the field technologies to identify in the field, and then having the relationships where that can be uploaded and shared very quickly. We have a lot of that existing in Kenya already, some really great relationships with the Kenyan government, the Mpala Research Center, Kenya Wildlife Services, and National Museums of Kenya, whereby that data sharing can happen very rapidly. So I think another thing we might want to look at is if something is going well, how do we replicate that? How do we replicate it broadly and quickly?

Chairman FOSTER. Thank you, and I am now over my time allotment, so I will recognize Ranking Member Obernolte for five minutes.

Mr. OBERNOLTE. Thank you, Mr. Chairman, and thank you very much to our witnesses. This has been a fascinating hearing. I have a question for Dr. Relman. In the early days of the pandemic there was a letter written by a group of scientists that was published in the *Lancet* and *Nature Medicine*, two scientific medical journals, in which the theory that anything other than a natural origin theory for the coronavirus was—should be considered implausible, and I'll read an excerpt of it, because I find it a little disturbing. The letter said, "The rapid, open, and transparent sharing of data on this outbreak is now being threatened by rumors and misinformation

around its origins. We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin. Conspiracy theories do nothing but create fear, rumors, and prejudice that jeopardize or global collaboration in the fight against this virus.”

So after the publication of those—of that letter, and that, you know, was just about a month after the appearance of the first case in the United States, so very early in the pandemic, after the appearance of that letter, media and social media companies would refer back to that letter in having their fact checkers label as untrue anyone that tried to express the theory about anything other than a natural origin, so it became this self-reinforcing cycle. So my question for you, at the time that letter was published, would you consider that a statement of scientific fact, would you consider it a statement of scientific consensus, or would you consider it a statement of opinion?

Dr. RELMAN. Well, I think I share with you your concern about statements that don’t properly reflect what we know and don’t know. Many people have opinions, and I think it’s important that people make clear that when they express a point of view it either is an opinion or based upon something more concrete. And I agree that that letter did have a chilling effect on certain elements of public discussion. I understand where many of those letter writers were coming from. They were—I think to a large degree, operating from a perspective of a priori understanding. Past outbreaks have largely occurred through natural spillover mechanisms, and I think it was a reasonable assumption to say this one may well be as well.

But every time we, as scientists, make these statements, I think to your point, we have to be very clear about the basis on which we make them, and where there is certain—there is certainly—remaining uncertainty, and then what it is we need to do to either disprove the counterargument, or prove our own. And I worry, and found it disheartening early last year, that there was much too little of that.

Mr. OBERNOLTE. So, a related question, then, how do we prevent this from occurring in the future? Because obviously we are in a period of intense polarization in our society. You know, science has been politicized in a lot of different areas. How do we prevent this from happening next time? Because, obviously, I think everyone agrees it was not helpful.

Dr. RELMAN. Yeah. It’s a complicated question. It’s outside my pay grade, but I’ll simply point out that there was a larger sort of cultural problem, societal problem, with the tenor of discussion at that time. And as you well recall, there were others outside the scientific community who were making very forceful, and almost defiant statements that made it difficult for any of us with more measured arguments to insert our positions into this public discourse. So I think the answers come from multiple directions. We need to remember what the scientific process is about, who—and how it’s undertaken, and simply better inform our public as well about how to judge statements that are being made, and the questions they should be asking of all of us.

Mr. OBERNOLTE. Right. Well, thank you. And then, final question for you, you didn’t touch on it a lot in your testimony today, but

in your written testimony I was interested in—when you were talking about the Wuhan Institute of Virology, and the possibility of a lab leak from that institution. You discussed some of the practices for studying viruses there that you consider risky. Can you talk a little bit more about that?

Dr. RELMAN. Sure. Well, I want to start by saying that I think the rest of us, the rest of the panelists here, and I share a lot of similar sort of thinking about this. If there is an origin, or an argument to be made about the possibility of a laboratory origin here, I think we have to consider first the possibility that there was unrecognized presence of this virus either in the laboratory, through samples that were collected, or an encounter by a laboratorian out in the field, and that the infection perhaps was asymptomatic, or simply not recognized.

But I am concerned about the—sort of the—sort of unregulated—lack of proper discussion about how we go about examining diversity in nature. And I fully support the statements that have been made by Doctors Murray, and Price, and Perlman, but when we collect lots of sequences we have to ask what's their relevance? And the way in which some of this relevance is determined is by scientific experimental means, either by trying to grow the entity that we think resides within a sample, or by trying to recreate it, or study a piece of it. And what I think concerns me is that we haven't yet had a proper discussion about where there may be risk in some of these experiments, not the majority, but some of it, that hasn't been properly addressed, and that we need to sort of confront and discuss more openly.

And I'll just call out a certain set of experiments that have been published by the Wuhan Institute that I view as particularly risky. In this work, they started with a natural isolate of bat coronavirus called WIV-1, which a good friend and colleague of mine, Ralph Baric, had already studied and declared to be a virus "poised for human emergence." This is WIV-1, came out of a bat in the Wuhan Institute of Virology. And their approach for studying novel sequences that they found in other samples was to take a piece of those genomes, a piece of that sequence, and swap it into this WIV-1 virus. They then resurrected a few of those viruses and grew them in the laboratory, so now we're talking about a chimeric virus with properties that we don't know yet, we don't—we can't predict well. That was the purpose of the experiment. Those are experiments that concern me. And I'm not saying that they led to this outbreak or pandemic by any means, but it's simply the kind of work that we, as a scientific society, need to think much more clearly and more deliberately about before we undertake it, again, in the search of important—answers to important questions about the diversity in nature.

Mr. OBERNOLTE. Thank you. Well, thank you, Dr. Relman.

Chairman FOSTER. Thank you.

Mr. OBERNOLTE. I see my time's expired. Thank you to all of our panelists for this fascinating discussion. I yield back.

Chairman FOSTER. Yeah. And I gave you a little extra time because of the extraordinary interest in that question. It's one of the threads that this Subcommittee's going to have to be looking at, just trying to understand what the definition and limits of dan-

gerous research are, and how we deal with that internationally. I now recognize Representative Perlmutter for five minutes.

Mr. PERLMUTTER. Thank you, Mr. Chair, and to the panelists, thank you very much for your testimony today. I feel compelled to defend something my—one of my best friends in life, the Chairman, had to say early in his introduction, and that is concerning the scientific method, and the ability of—whether it's a lawyer or a politician, our responsibility is to make decisions based on the best information we have available to us at the time, and it may not be perfect in the scientific method. And, Dr. Relman, you go through the six factors that you consider as part of the scientific method, but, you know, it takes time, obviously, to understand the origin of things, to understand this virus, and decisions had to be made.

And, just for the record, I'll read something President Trump said at the time in January—late January of 2020. "China has been working very hard to contain the coronavirus. The United States greatly appreciates their efforts and transparency. It will all work out well. In particular, on behalf of the American people, I want to thank President Xi." And he goes on for the next two months thanking China for their tremendous efforts with respect to the coronavirus. And when I looked, Dr. Relman, at the points that you make as to the scientific method, you say verifiable and reproduceable, transparent, representative, expert, independent, and without conflicts of interest. And so there are a number of things within that list of components as to how to prove a point where you introduce people. And when you introduce people, then you start losing some of the facts that my Chairman and my physicist friend, Dr. Foster, find so concrete. And so, just to defend lawyers and people who have to make decisions, I want to say it's not all that cut and dry.

Now I want to turn my attention to a Coloradan, and a Buff, and I want to thank her for being part of our panel today. I want to ask Dr. Price, in Colorado we've now seen the Delta variant overtake all the other types of viruses, and variations in viruses, and it makes up three-quarters of the cases that we now see in our State. From your point of view, Dr. Price, what steps can the international public health authorities take to prevent new strains like the Delta variant from emerging?

Dr. PRICE. Well, I think we're starting that now with vaccination, and I think the trick is to get vaccinated before these new strains emerge, and allow to take hold, and circulate enough where they can start infecting others, and potentially even vaccinated people. So that's the first step. And then the second step, and my virology colleagues are already doing this, is keeping track of the evolution of this virus, and when we need to tweak the vaccine or create a booster that will cover different variants, that we are on top of it, and we're constantly surveilling very proactively to be able to design a booster or a new vaccine to overcome it.

Mr. PERLMUTTER. So in your testimony you talked about sort of—there has to be a little level of trust among the scientific community, among the epidemiologists, among the vaccinologists, the virologists. So how do you—how does one build that trust so that you can work with Dr. Perlman, or Dr. Murray, and know that you're getting straight information?

Dr. PRICE. Yeah. Well, you have to respect that when a host country, or when another lab, invites you in to investigate and help them solve a problem that it is their data that you are working with, and the way to get access to it is to be invited, and agree on the objective. And you need to have that collaboration, I mean, both philosophically and practically. So philosophically it's important for the host country, in my experience, to be viewed as the lead for solving the problem, that they want to solve a problem that they might be viewed as creating, so you are there in the capacity of a consultant, not to parachute in, and take data, and then publish on behalf of yourself and the United States. So you have to remember just basic, you know, human consideration.

And then practically it's very important because you're going in and reviewing charts in another country. They may be in a different language, they may have different medical norms, they may—you know, you need their people there alongside you to work together and do this collaboratively. So stay focused on the scientific question you seek to answer, do not make conclusions with incomplete data, resist the urge to start making conclusions, and communicate often with their government authorities. And make sure you're not exploiting the host, whether it's an underdeveloped country that often this happens in, or in a developed country, that you're not going in to try to get something on behalf of, you know, your own country, or your own institution or academic career.

Mr. PERLMUTTER. My time has expired. I thank you, and I'll yield back to the Chair, and I just felt compelled to defend lawyers and politicians. Thank you.

Chairman FOSTER. Thank you. And we'll now recognize the Ranking Member, who is, I believe, neither a lawyer nor a scientist, and yet a tremendous contributor to this Committee.

Mr. LUCAS. Thank you, Mr. Chairman. Being a farmer by trade, and my university training is in agricultural economics, so how to allocate those finite resources, and on the unlimited demands to achieve the greatest possible rate of return. And if I wanted to put you to sleep, we would discuss the inelasticity of the demand for food, but that's a whole different entire subject.

Turning to Dr. Relman, since the early days of the COVID-19 pandemic, China's not been transparent with the rest of the world about how the virus began to spread in Wuhan, China, or shared reliable data that could inform on the possible origins. So thinking about the last exchange between my colleague and one of your wonderful fellow witnesses, recognizing this, how do we move forward in investigating the origins of COVID-19 without cooperation from China, and how must Congress, Federal agencies, and scientific community more broadly work together to do this? I give you the big question, Doc.

Dr. RELMAN. Thank you for that, Congressman. Well, I have to turn back to my co-panelist, Dr. Price, and suggest that we are in dramatic agreement on a number of the points that she made. But I will call—I will make a distinction between the importance of relationships amongst scientists and the kinds of relationships that exist between governments. And here I will again reflect much of the sentiment by Dr. Price. There have been, in fact, very good working relationships between scientists in the United States and

those in China at these very institutes. I've been on trips to those places, and have met many of those scientists, and greatly respect them. And I think, in fact, they have much to share if we could create the right circumstances under which they feel allowed, and empowered to do so.

That's a very complicated task, and I don't mean to suggest that any of this is easy, Congressman Perlmutter. It is not. But it doesn't mean that we shouldn't try. And I'll point out that our National Academies of Sciences and the Chinese Academy of Sciences held a series of meetings last summer, of which I participated, where we had some very fruitful discussions. They did not involve the governments of our two countries, and I think there was some useful progress made. So I'm maybe an idealist or an optimist, but I think that if we can build these kinds of relationships amongst scientists, we can start there, and then find ways in which to take into account the interests of our governments, and other larger institutions. I'll—

Mr. LUCAS. Continuing with you, Doctor, in your written testimony you disagree with the argument that further clarity about the origins of COVID-19 is not needed. So could you explain to us—and remember we are a conduit to the great American people, the folks who ultimately pay and suffer or benefit from our actions. Could you explain to us, and them, why it is so important that we investigate the origins of COVID-19, and what an investigation into the current pandemic would—how it would enable future pandemic preparedness and response?

Dr. RELMAN. Be happy to. I would say there are at least four benefits to an effort to understand the origins. The first is a point that's been made already, that anything we learn will help us devote resources and design strategies to both anticipate and prevent the next pandemic. That's first and foremost. Second, we will learn how better to investigate outbreaks. That's a point that's already been made by my fellow panelists. Third, we may deflect further recriminations, and perhaps some finger pointing, by the gaining of greater clarity. And then fourth, if there is further evidence that perhaps accidents occurred in the conduct of science, then I think we will be forced to have conversations and further discussions beyond that which we have already had in this country that look more carefully about where there is risk that perhaps deserves special attention.

Mr. LUCAS. In your written testimony, again, you discuss the two predominant COVID-19 origin theories. What I would now ask you is, moving forward, how do we better communicate scientific and factual evidence so we can continue to investigate the origins of COVID-19? How do we regain that trust? How do we convince the American public of the merits of what we need to do?

Dr. RELMAN. Yeah. I know we're almost out of time, but let me just suggest that the first place would be something that Dr. Price has already mentioned, the great importance of transparency in all of the information upon which we base our statements. And that come—that has to come from all corners of the globe. Not just China, not just the United States, but every other place where there's useful information about the virus, the early clinical events, and everything else that we have to understand about this eco-

system. So data transparency is where we start, and that should be made available to the world's public as well.

Mr. LUCAS. Thank you, Doctor. And, with that, Mr. Chairman, I would yield back, noting that the general public doesn't necessarily always understand what goes on behind those lab doors where you worked all those years, and we need to make sure they understand it is to their benefit, and the future of everyone, that those things happen. Yield back.

Chairman FOSTER. I agree. And we'll now recognize one of our most valued Members of the Investigation Subcommittee, Dr. Ami Bera, M.D., for five minutes.

Mr. BERA. Great. Thanks, Mr. Chairman. And while my good friend from Colorado's a lawyer and a politician, I happen to be a doctor and a politician, and in a prior life was Chief Medical Officer for Sacramento County, so—did a lot of public health work.

Again, thank you for holding this hearing. I think, just from a pure medical, and scientific, and epidemiologic perspective, it is really important for us to take a look backwards, and get a sense of what's happened. And, you know, as the Member of Congress who held the first hearing on that kind of novel coronavirus, didn't have a name, and had—chaired multiple hearings on this, one of the first recommendations we made in early January 2019 or 2020 was that we had to use every effort and resource to get the best scientists into the hot zone, into Wuhan, to not just understand what was going on, understand how this virus was transmitted, but also to help the Chinese, in terms of containment. Obviously that didn't happen. I think, you know, China's response was different, which really was setting up barriers, and I think we'll look back and certainly see a missed opportunity that slowed down our response.

That said, the scientists in China, you know, there was a hero in China who, again, probably not at the request of the government, released the gene sequence fairly early, and probably did save untold—tens of thousands of life. You know, certainly allowed us to develop the ability to do testing fairly early on, and, you know, it ultimately allowed, you know, Moderna, Pfizer to start working on vaccines as well. So, you know, I'm not disparaging any of the Chinese scientists, per se, because I actually think the scientific community would've wanted to work in a cooperative, collaborative way. It's when the politicians get involved, in this case, you know, the Chinese government, that I think things went awry.

I appreciate all the questions of my colleagues. I do think, just from a purely scientific perspective, it is important for us to understand the origins so we can better prepare ourselves both to address and minimize the next pandemic, and be prepared for that. You know, as one of the witnesses talked about, I think we do have to go through and, you know, get a better understanding of the zoonotic risks that are out there so we can be better prepared for that. But one area that, you know, I think is incredibly important for us to think about as Congress, and—is our own biosecurity. Because there are bad actors in the world, and, you know, for folks watching a virus bring the world to its knees, they've just seen what's possible, and we spend a lot of time—you know, I chair the Subcommittee on Nonproliferation, and we think about nuclear

threats, but what we've just seen is a biological threat bring us to our knees. And as we prepare for the next naturally occurring pathogen, I think we also have to build in systems to address and think about, you know, potential man-made pathogens, whether, intentionally or unintentionally, that could impact us.

I know we were able to get \$1.7 billion in the American Rescue Plan to build out our infrastructure for gene sequencing of these viruses. It's helping us right now as we deal with the pandemic, but, you know, maybe—I'll ask Dr. Relman, as we build out these bio-surveillance tools at the global level, as we try to put in these institutional norms for lab standards, as, you know, Chairman Foster and I have talked about, you know, what is appropriate gene—genetic research, and, you know this CRISPR technology, you know, is readily available to the world. We're teaching our high school students how to do gene editing. There's real risks here. So [inaudible] Dr. Relman some things we should be thinking about, as Congress, to better prepare for the next naturally occurring pathogen, but also to prevent a man-made pathogen from bad actors?

Dr. RELMAN. Those are all really, really important, good questions, and there obviously are no simple answers. I think one response would be say that there are multiple approaches that will be needed, no one of which would be sufficient. Part of this, I think, is going to be a question about whether we think there are discrete, probably very small, defined parts of this science that deserve special attention. In other words, might there be a red line? Might there be a place past which we simply ought not to deliberately go? I personally think that there are. I also think that those are vanishingly few, and that we have to be very careful in perhaps drawing a line, if we do so, so that we don't impair or impede the rest of the critical science upon which we depend.

So these are really tough problems. I would like to see, for example, our government sponsoring further international discussion about whether there are norms of this sort, how we go about defining them, who needs to be at the table, and perhaps not just scientists, and not certainly just life scientists, but social scientists, I think, to some of your points previously made. But that's a start, and, you know, this is as much about people as it is about rules. And we're going to have to have a better understanding of where we all stand on this, and a mechanism for ongoing discussion as unforeseen problems arise.

Mr. BERA. Great. Thank you. And maybe that's a topic for a future hearing, Chairman Foster.

Chairman FOSTER. I agree, and the analogy between nuclear nonproliferation and the dangers of, you know, of bioweapons, and everything from homebrew bioweapons to nation-states. And, without objection, I'd actually like to enter into the record a report and—from the Nuclear Threat Initiative (NTI) that they issued last fall, entitled "Preventing The Next Global Biological Catastrophe". And—recommended, among other things, stronger mechanisms at the United Nations for determining the source of a biological threat regardless of origin. And so, without objection, I'd like to enter that into the record. And next is Representative Gonzalez for five minutes.

Mr. GONZALEZ. Thank you, Chairman Foster, and Ranking Member Obernolte, for holding this timely hearing today, and to our distinguished witnesses for joining us. Dr. Relman, in your testimony you labeled the work being performed at the Wuhan lab as “unusually risky”. I want to focus on that specifically because in 2016 the lab reportedly experimented on a live bat coronavirus that could infect human cells in a BSL-2 lab, a biosafety level compared to a U.S. dentist’s office. Furthermore, in 2018, the lab’s director, Dr. Xi [phonetic] gave a presentation with slides that exposed some of her unmasked colleagues working with bats in their bare hands. And then finally, in 2019, Dr. Peter Daszak, one of the lab’s top contractors, who has coincidentally been charged with leading *The Lancet* and WHO investigations, made comments that researchers in the Wuhan lab have found over 100 coronaviruses, including some that can get into human cells in the lab, and are “untreatable”. I recognize you’ll not offer a personal opinion on this hypothesis, but would you agree that this is a troubling fact pattern that warrants further investigation?

Dr. RELMAN. I think my response would be to say we, as an international community of scientists, have some important issues to address, some of which you’ve just alluded to. I do think that some of the work that they have described, like work that has been done by others elsewhere in the world, deserves special attention. I mentioned already some of the experiments that I think were unusually risky. And it’s not just my opinion, it’s the opinion of other well-known coronavirus experts, in fact. But this is work that they had permission to do, at least by their own government, so I think there are many people here that need to be—you know, that need to be in these discussions to decide how do we go about viewing and assessing risk, and how are we going to manage it going forward? It’s a—

Mr. GONZALEZ. Yeah, I agree.

Dr. RELMAN [continuing]. Not a problem of one place.

Mr. GONZALEZ. Yeah, absolutely. I think this screams for more global coordination and pressure, frankly. Given Dr. Daszak’s professional and financial ties to the Wuhan lab, do you have any—does it give you pause that an investigation completed by the WHO and *Lancet* can be considered credible? Because I struggle with that mightily, if I’m being honest.

Dr. RELMAN. No, I too—and I’ve stated this in various places, I was troubled by the—first of all, the terms of reference with which they began their investigation, their exclusion of certain hypotheses just because, and then second the skewed treatment of just one, and not another. Yes, I think that was a problem.

Mr. GONZALEZ. Thank you. Dr. Perlman, in a 2020 op-ed you applauded Chinese efforts to share relevant COVID-19 information rapidly and responsibly. We now know that the—that following the WHO’s 2020 mission to China, the CCP implemented a gag order to stop the sharing of any information related to the pandemic so as to coordinate the release of the information with the Chinese government. They’ve also silenced their own scientists, deleted data bases, and, of course, blamed the United States military for the outbreak, which is ridiculous. Given how the last year and a half has unfolded, do you still stand by those comments?

Dr. PERLMAN. Yeah, so I think that—I think the points you make are very well taken. I think a lot of those comments were made at a time when the world was a little different. We talked about the 2020 *Lancet* paper, and that did have a big impact, but it was at a time when the world was really divided into those who thought this was all man-made versus a natural origin, and now everything's become more nuanced, and I think it's very appropriate to be more nuanced. I certainly would—if I had to do everything over again, and I was really smart, I would've thought about those nuances back then. But at the time, that's what we were thinking.

And also the other thing I was thinking about was the SARS epidemic, it took—the Chinese government was even more opaque then initially, and it took several months for that to be admitted to. So this felt like it—in those early days felt like it was more rapidly done, information was being spread, but there was no question, even in the very beginning, there was some coverup, because I have friends in China who were telling me that they thought things should've been released more quickly, and this was in the first days of January. So this—and the problems only got worse, because the things you're describing are what—going to make it so difficult to figure out where this virus came from. Destroying farmed animals, not having specimens available, not having information available. This is the only way I think we're going to figure out this particular pandemic.

Mr. GONZALEZ. Yeah. I mean, just following up, I mean, if the Chinese won't permit researchers to conduct field work, and only offer limited access to granular lab records and data, how can we carry out an investigation that is credible and immune to CCP influence when we've already seen such horrible behavior, whether it was SARS or COVID-19?

Dr. PERLMAN. Yeah, I think it's something that has to change, because—if we're going to figure this out.

Mr. GONZALEZ. Thank you. And——

Dr. MURRAY. And if it's possible to add to that, could I make a comment?

Mr. GONZALEZ. Sure.

Dr. MURRAY. Yeah, that—one of the things I would just like to underline as we're talking about this is that, if we're trying to create the entire puzzle, if we're trying to understand the entire picture, and if we think about what we can control, what we do have the access to, if we have a jigsaw puzzle with 100 pieces, and we only have one or two of those pieces currently available, it's hard to understand everything. But I think the more that we can really develop that robust data set that's independent, perhaps, of—that's—you know, from the wildlife surveillance that's perhaps independent of government, it might give us the chance to have a better understanding, and that might be something we can—that we can more easily control.

Mr. GONZALEZ. Thank you. And thank you, Mr. Chairman, for giving me a few extra seconds there. I yield back.

Chairman FOSTER. No, I'm happy to—also, if our witnesses and Members are interested, I think we will continue this sort of informal procedure we have of, after we've gaveled the hearing closed, we'll turn off the live feed, and just have the sort of private discus-

sions that we have typically after in-person hearings as well, which are often very, very productive and useful. And so now I will recognize Representative Beyer for five minutes.

Mr. BEYER. Mr. Chairman, thank you very much, and—to you and Mr. Obernolte for hiring the—holding this really fascinating—I will begin that I am concerned about the low vaccination rates in Republican-led States, continuing to place our economic and societal recovery at risk, and Tennessee’s willful abandonment of any attempt at pandemic response is only the latest example of failed leadership. So while we’re focused today broadly on the origins, which will be very helpful in trying to understand how to prevent both a future lab leak or any other future natural origin for a virus, we have to remember there have been more COVID deaths in 2021 than in all of 19—in all of 2020.

So we need to also be focused on the variants, and if we don’t get serious about addressing COVID, the next variants of concerns are likely to be homegrown. They’re not in China, not in the market, not in a lab, and they’re preventable in light of our access to vaccines. So one of the concerns I have is that—for much of the pandemic is we’re not going to know much about these variants if we don’t have good surveillance. For Dr. Murray, can you offer your perspective on the current status of U.S. COVID surveillance? What’s working well, what should we be thinking about for the future?

Dr. MURRAY. There’s—we—to be honest, I think that our COVID surveillance was much more robust just a few years ago. We knew that—even 3 or 4 years ago that an epidemic—not necessarily a pandemic, but we knew that an epidemic was likely on its way, and we knew that it was likely to be an influenza virus, or a coronavirus, that it was likely to come from bats or primates, and Asia or Africa. So we knew a lot, and I think that we were pretty actively involved in a number of different countries doing that surveillance, and creating the data base from which all of our scientists and pharma companies need to access information. For a variety of reasons, I think in part due to changes in funding structures that—and changing in priorities, that surveillance isn’t as robust right now as it was, and, from my perspective, I think it’s time that we lean in, because that continuous sampling I think was what’s most critical as we look at trying to understand viral evolution.

One other thing I’ll just note is that if we’re trying to understand how the—what—how the viruses might mutate in the future, it’s very helpful to look at past collections as well, to even, like, historical collections from the museums, the Museum of Natural History, to look at the coronaviruses 100 years ago, 50 years ago, where they are now. It gives us some indication of where to go. So we are still, obviously, doing—there’s a number of different groups doing surveillance, but it’s a strange time to be slowing down.

Mr. BEYER. Thank you. Dr. Relman, I was fascinated by your discussion about how, in the Wuhan lab, they had taken previously characterized bat-associated coronavirus poised for human emergence, and then inserting the all-important spike protein from the novel viruses. This sounds awfully dangerous. What was the possible gain from introducing the spike protein into these viruses?

Dr. RELMAN. Well, the purpose was to understand the very diversity that you've been hearing about, and trying to understand what parts of this diversity may prove to be a true threat to humans. One of the approaches, and I would argue an approach that we ought to, you know, step back and perhaps discuss more, is to take the part of the virus that we think is most likely responsible for interaction with human cells, the spike protein, and study it in some way, shape, or fashion. The way they chose to do it was to place it—swap it into another virus whose properties they had just characterized—and a virus that they were able to resurrect and propagate.

So these were the chimeric viruses that they created, and they literally did not know what was going to happen when they did that. Their purpose was to see if this starting virus, WIV-1, had now become even more well adapted to human cells, and more able to grow. They didn't know what the answer would be, and I would say that that was a pretty risky throw of the dice that they were undertaking there.

Mr. BEYER. Yeah. Doctor, one last quick question. You've talked about the COVID Commission Planning Group, Nuclear Threat Initiative, World Economic Forum. Do you think we can actually build this new global entity to track these things ahead of time?

Dr. RELMAN. I do, I do, and I've admitted my conflicts as an advisor to a number of those organizations, but the NTI, for example, the Nuclear Threat Initiative, has a very interesting proposal for a novel international entity that would routinely investigate small outbreaks which are happening all the time as a way of practicing how to do this right for the time that comes when something large happens, and—so I do like some of these proposals.

Mr. BEYER. Great. Thank you very much. Mr. Chairman, I yield back.

Chairman FOSTER. Thank you. And we'll now recognize Representative Waltz for five minutes.

Mr. WALTZ. Thank you, Mr. Chairman. And, just as an opening statement, I certainly want to state my opinion, and I think the vast majority of the American people would agree, that it's a little insane—I mean, it's kind of nuts that 18 months after this outbreak, trillions of dollars spent, millions of lives lost, millions more disrupted, businesses ruined, kids falling further behind in school than they already were, that this is the first hearing that the majority has hosted on the origins of the coronavirus. I mean, I just think the total lack of urgency to get to the bottom of this, again, a year and a half after the outbreak is, just to be candid, a bit outrageous, and I do hope this is the first of many. This is a very complicated issue, and I do hope this is the first of many to get to the bottom of it. But I certainly applaud that—you know, let's get started in having the hearing today.

You know, Dr. Relman, you walk through in your testimony what an investigation should look like. I think we all agree it hasn't looked like that, with all of those elements. Key factors are objectivity and conflicts of interest. And you also stated, I just want to make sure I understand you correctly, neither hypothesis should be ruled out, natural origin or a lab leak conducting very dangerous

research that you just described, is that correct? They're both viable?

Dr. RELMAN. That's correct.

Mr. WALTZ. Thank you. And I would certainly agree. I just—trying to wrap my mind around what I think may be the world's biggest coincidence that the epicenter of this virus happened in a city—the only city in all of China that is conducting research—dangerous research on this virus, 1,000 miles away from natural origin, a city larger than Manhattan, and in a lab that the State Department, in 2018, shared real concerns for its safety in a cable. So that brings me over to Dr. Perlman. And, you know, it was already mentioned, but I think it's worth mentioning again, that in March 2020 you signed a letter that “strongly condemned conspiracy theories that suggested COVID-19 does not have a natural origin.”

And I have to say, the entire world was looking to the scientific community at that time, just months after the outbreak, and that really did set the tone, and it's no surprise, then, we had the media and Big Tech banning any discussion of anything but natural origin. Dr. Perlman, do you still stand by that, that any discussion is a conspiracy theory, that Dr. Relman, who says it's a viable hypothesis that it's not natural origin, is a conspiracy theorist? Do you stand by that statement?

Dr. PERLMAN. So, remember the statement that I said just a few minutes ago, the statement was based on the notion that this was constructed from scratch, so that was really what the letter was about. I don't think it was defined well enough in that letter. But that's what the discussion was. It wasn't about—

Mr. WALTZ. So—

Dr. PERLMAN [continuing]. All these other possibilities.

Mr. WALTZ. —[inaudible] that it is constructed from scratch, you would call it conspiracy theory?

Dr. PERLMAN. Well, it's—I would—I don't know—you know, conspiracy theory obviously has a lot of pejorative terms with it, so it's not a term that one would want to use lightly. I think the idea at the time was, though, that this was all being convoluted so that a whole bunch of possibilities were put into the idea that this virus was made from the beginning.

Mr. WALTZ. Dr. Perlman, what that letter had the effect of doing was shutting off very viable lines of investigation into whether this came from that lab. We—which we are funding with U.S. taxpayer dollars, indirectly or directly. And we can't stop the next pandemic, the last three of which have come from China, without understanding where it came from. And now here we are a year and a half later just trying to start figuring this out, and then you sign the Lancet Commission letter, which Peter Daszak has since resigned and recused himself from because he's receiving funding, and his livelihood is dependent on the very type of research that we're asking him to investigate.

So I think, yes, the Chinese coverup has been abhorrent, but I think, Mr. Chairman, just as inexcusable is a scientific community who has not been objective, who has effectively shut off lines of research, whose livelihoods depend on funding pertaining to that research, and could be compromised. And I think we, as a Com-

mittee, need to take a hard look at those backgrounds, and if there are conflicts of interest, as Dr. Relman pointed out, before we move forward in an objective way. I thank you for holding this, and I yield back.

Chairman FOSTER. Thank you. And we will now recognize Representative Stansbury. If she is willing to activate her video? Yes.

Ms. STANSBURY. Thank you, Mr. Chairman. It's nice to be here this morning. I really appreciate the opportunity ask a couple of questions during this hearing. Mr. Chairman, my questions are for Dr. Relman. The first question I have is about laboratory biosafety. As you pointed out in your testimony, the lab that—lab accidents involving dangerous pathogens occur more frequently when—than we would like to admit, even here in the United States. But, you know, one of the things I'd like to kind of just put on the record is a little bit about the biosafety standards in American laboratories, and your thoughts about how we can ensure that the highest standards are upheld not only in the United States, but proliferated internationally.

Dr. RELMAN. Thank you for the question. I think, from my perspective, safety and security are about both guidelines, rules, or regulations, as well as people, and the people part is as important as any of the other. The finest rules and regulators, if not properly followed or believed in, simply don't serve us to the degree that we need. So I agree that this is a collective responsibility. We here in the United States, I think, have further soul searching to do, but building upon good efforts that have started already. And so I would like to see more attention to this, and more attention to the idea of how we can provide incentives, positive incentives, for people to believe that this is important, and worthy of some attention.

Ms. STANSBURY. Thank you.

Dr. PRICE [continuing]. I can add to that, Representative Stansbury, is that OK?

Ms. STANSBURY. Yes. Thank you.

Dr. PRICE. So in the U.S., you know, the BSL-4 is the level of laboratory that would require work with this type of virus, and would have oversight in clinical samples by the Joint Commission, as—the Clinical Laboratory Standards Institute. And I suppose if research only, Doctors Relman and Perlman can correct me, but the CDC would probably oversee that. And the Joint Commission accredits 22,000 healthcare institutions in the United States, but there's also an international arm that accredits hospitals around the globe, and currently there are 54 such hospitals in China that are accredited by the JCI (Joint Commission International).

The Centers for Medicaid and Medicare Services also regulates some laboratory testing via CLIA (Clinical Laboratory Improvement Amendments), and there is an ability for international laboratories seeking CLIA certification to do so. So I do wonder, in addition to what Dr. Relman said, is if there is a will to have some regulatory oversight more so at a global level using some existing structures that we have.

Ms. STANSBURY. Thank you both, and thank you, Mr. Chairman. My second question is about the kinds of research that we're talking about, including gain of function research. Dr. Relman, you argued in your testimony that we should consider whether or not

there are certain kinds of life sciences research that is so risky that perhaps it shouldn't be undertaken, and I wanted to understand a bit more about gain of function research, and how this research is used in microbiology, how common it is in vaccine development, and what we can understand as to how it might apply to what we're discussing here today.

Dr. RELMAN. Thank you. I'm not a big fan of this term, gain of function, because it's confusing, and used in confusing ways. I would prefer to talk about risky research, risk—research that is unusually risky, and for several reasons. To me, the two properties that matter most that we need to be concerned about the property of something being able to do harm, so that's pathogenicity, and the property of transmission. Anything that has both of those properties, something that can do harm and can transmit easily, is something that we need to pay attention to. And, to make it very simple, I would argue that we should not go about deliberately, intentionally, creating something that has both of those properties that doesn't exist in nature without a very, very thorough and well-informed discussion. That's the kind of thing that gets out of control very quickly, and, if it hasn't yet happened, it will. I'm just very concerned about that kind of work.

Ms. STANSBURY. Thank you, Dr. Relman. And, with that, Mr. Chairman, I yield back. Thank you.

Chairman FOSTER. Thank you. And we will now recognize Representative Feenstra for five minutes.

Mr. FEENSTRA. Thank you, Chairman Foster, and Ranking Member Obernolte, for the opportunity participate in this first official hearing on COVID-19 origins in the U.S. House. And thank you to all the witnesses for your testimony, and sharing your extensive research and experience with us on such a crucial, crucial topic today.

Dr. Relman, in your testimony you mentioned that some say further clarity about the origin of the COVID pandemic is no longer needed. I personally think that's absurd, and I was happy to see that you disagree with them also. Following up on past questions, can you elaborate on the potential consequences of not investigating the origin of the virus?

Dr. RELMAN. Sure. And to clarify, I think the viewpoint that I was seeking to portray was one where a number of people have said this seems so difficult, it seems so unlikely that we are going to gain greater clarity, for some of the reasons that have been discussed here this morning, that perhaps the effort is not worth it, that the effort, which will come with a lot of political difficulty and complication perhaps ought not to be undertaken. I disagree with that. I do think, for a whole number of reasons, there are likelihoods of greater insight into the origins of the pandemic, in part because science is such an international undertaking, and that there are lots of useful kinds of information there that could come from all corners of the globe about how this pandemic began.

But I do think it's critical that we pursue this, and the reason is that, in part, if we do nothing further, one of these two hypotheses, in my view, continues to be so difficult for some of us scientists to discuss, the idea that one of us could have caused an accident, that's—that one of us made an error, that it will not be

pursued with sufficient rigor and attention. And that's my concern, should we simply drop this now.

Mr. FEENSTRA. Yeah. Dr. Relman, I fully agree with you. I mean, I think everyone—we owe it to the world to understand, and that we never let something like this happen again if we can come up with the way it happened, and then to protect ourselves that it never happens again.

Dr. MURRAY, you worked as part of a governmental funded USAID consortium to collect dormant viruses and study infection—and the infectiousness, and sometimes implemented gain of function research to predict disease outbreaks, risk factors, and create predictive models. I've got two questions. How does the COVID-19 emergence and spread compare to your predictive models, and then what are your predictive models and risk factors your team considers for laboratory accidents?

Dr. MURRAY. Just to—thank you for the question. Just to clarify, we were not involved in any gain of function research. We were doing surveillance, both human and wildlife surveillance, in several different countries, and—yeah. So—and then in terms of—we do have modelers on the team who do look at how diseases can spread. The places we were most active in is looking at some of the gaps, and recognizing that if we want to understand how viruses can best spread, there is a missing gap of social scientists, and looking at what humans do to put them in—at times in harm's way, and what the riskiest behaviors are. So we do—we have spent a fair amount of time developing some strategies, and making sure we're incorporating social scientists and behavioralists to investigate cultural norms, and to better understand to build those partnership with our—with folks in different countries so we can address—so we can all be part of the solution as part of the problem, per se.

Mr. FEENSTRA. Well, thank you. And I'm an academic myself. I'm actually doing research and funding. I'm just going through an IRB (institutional review board) hearing as we speak, and it concerns me that, you know, I take into consideration all the risk factors when I'm doing research, and, you know, here we have a situation that we're not sure exactly how it happened, but we have to be very cognizant of the risk factors that happen. And it—you know, it—I'm sure it didn't happen on purpose, but how did this—could it have been a laboratory accident, and how do we make sure that never happens again? I'm not saying it necessarily was, but it could have been. And I just hope, Dr. Murray, that your studies can look into this more, and I would just like to hear a little more of your thoughts on that.

Dr. MURRAY. I think that one of the best things that we can all do is look at what our research so far has identified, and then also identify the gaps. And part of the gaps I think is looking at a risk analysis, a risk analysis across the board. I think my esteemed colleagues on the panel have said the same thing, and there's many places along the way, from the human behaviors in the field, and what—and how people come in contact with wildlife—you know, living, and then also dead wildlife, the—whether it's the—the risk is in the—a restaurant, whether it's eating wildlife, or whether it's preparing wildlife ahead of time, and then what happens when you

bring it back home. So everything, all the way through, looking at risks—risk factors in laboratories as well. And the more that we can all feel comfortable, and develop that—trusting relationships to say, hey, this is an area we should really look at more closely, the better.

Mr. FEENSTRA. Thank you, and I yield back. I fully agree this is very serious, and in all research we take human subjects very, very, very seriously, and I just hope that we look at that further. Thank you, and I yield back.

Chairman FOSTER. Thank you. And now, despite the fact that he only has a Master's Degree in Biochemical Engineering, we will recognize Representative Casten for five minutes.

Mr. CASTEN. Thank you, Doctor. Really appreciate you guys all being here. I'm—want to be careful picking my words here, because I want to ask something that's going to sound controversial, and I hope that my colleagues across the aisle will take it in the spirit it's intended. I've had this fear from the start of COVID that we're repeating some of the mistakes we made in the HIV crisis, in the sense that there are political conversations we're uncomfortable having, and because of our discomfort having those political conversations we've allowed this to spread in ways that it shouldn't. And this is going to sound political, because we're all politicians, but it makes me so sad that we're seeing, you know, certain political populations less likely to get vaccinated, more likely to die. And if we can't talk about that epidemiologically, then we're leaving something out of the conversation.

Layered on top of that is the fact that we have created some real risks to health and welfare because of that politics. You know, I had an Asian-American constituent who was attacked with a stick a year ago and told to go back to China, which was not where he or his family tree was from, but that was the way he looked. And—so I guess my question for you all as scientists who, you know, if you do your job well, you are looking at all the data, and asking questions without any presumed answer, how would you like us as political figures, hopefully political leaders, to speak about those issues in a way that maximizes your ability to do your job effectively? And it—happy for any of you who have answers to that, but I think we're all—in our line of work, we could use your guidance and wisdom to make sure that we don't unnecessarily inflame things, while still making sure that we don't ignore hard questions as we did during the early stages of the HIV epidemic. Any comments would be welcome.

Dr. MURRAY. Personally, I really like the idea of us, it's been mentioned a few times along, making big efforts to bring the public along. As scientists, we do a lot of work, and we collect data, and we share it with other scientists, but we don't always make the effort, or sometimes we don't—we're not effective in translating that information to the general public along the way. I do feel like we have known a lot about the, you know, epidemics that were on their way, and had we more effectively communicated with the general public, I think we would've had been very able to respond more quickly at—that's my thought.

Dr. PRICE. I would follow up and say I've appreciated this Subcommittee's willingness to consider all theories of the origins of

COVID-19, even though some have somehow generated a political view or polarization behind each of those theories. And I think it's important to remember that we don't have the data. We have to use the science, and back off from anything that seems to be going to the left or the right, and just look at the data, and make conclusions based on that alone, and be open until then.

Dr. RELMAN. I would simply add that, from an international point of view, in my humble opinion, it's humility that we, as a nation, need a little bit more of. I think we've been a little quick to point fingers and suggest that we're not equally, you know, culpable in some cases for either promoting factors that encourage natural outbreaks or for promoting certain kinds of science that carry unusual risks. And I think, you know, it's that glass house analogy. We have to be careful about where we throw stones without first saying, look, we're fallible, just like everyone else. Here are some examples of problems that we have discovered amongst our own. Now let's have a more open conversation about where we each see this being the case.

Mr. CASTEN. Well, thank you.

Dr. PERLMAN. Yeah, could I just add something to that?

Mr. CASTEN. Yeah. Yeah, sorry. Please go ahead.

Dr. PERLMAN. Yeah. I'm on the FDA (Food and Drug Administration) and the ACIP (Advisory Committee on Immunization Practices) Vaccine Committee, so we actually think about the first part of your question, about how do we get vaccines into the whole population? And I think it's not only politicians being all in agreement about whether vaccines are necessary or not, but I think we think pretty strongly that bringing in community leaders, and making sure that everybody's on the same page about the importance of vaccination. Because both in the U.S. and the rest of the world, this vaccine hesitancy is a big deal in terms of having problems in ending the pandemic. So there's a lot of places in the world there's not enough vaccine, but even where there is there's still so much hesitancy. So I think that it's just the—it's—making it very clear that everyone thinks vaccination is the way for—to move forward is really an important intervention.

Mr. CASTEN. Thank you. I yield back.

Chairman FOSTER. Thank you. And now, with some relief, we will return to the other Ph.D. on—in the Science Committee, Representative Baird.

Mr. BAIRD. Thank you, Ranking Member Obernolte, and—so now I'm—is that coming through better?

Chairman FOSTER. Yes.

Mr. BAIRD. I really appreciate, Mr. Chairman, holding this kind of discussion, and it seems to me that the purpose of this hearing, and I commend you for taking the opportunity to do that, is to look into how this COVID virus was released. There are two theories at the present time, one about being an animal to human situation, or a zoonotic situation, and the other being the laboratory leak. So my question comes down to—is how we identify, and how we can be helpful, in trying to re-establish or re-affirm, as some of you have suggested, the credibility for our scientific community, because they really haven't had a chance to weigh in.

So I guess I'm going to start one question with Mr.—or Dr. Relman. You—in your testimony you had discussion about international governance mechanism leading to the investigation of the COVID-19 origins. And so, from your perspective, how do we re-establish a protocol that is appropriate and effective for convincing our society that our scientists can make the appropriate conclusions? And so I would appreciate your comments on how we can identify the bipartisan, transparent program for investigating the origins of this COVID-19.

Dr. RELMAN. Yeah, it's a tough question. There are issues of governance and of authorities, and then there are a set of other issues around which I feel I have a little bit familiarity, which is how do we articulate the questions at hand carefully, how do we decide upon the kinds of data that will best address those questions, then how do we decide upon the right expertise to make use of those data in addressing those questions? And, finally, the process that will be pursued. All of those four things, I think, still need some attention. This is where your Committee has, I think, important standing. You're all about how does a proper investigation become well-articulated, and empowered, and defined? And that's what we need here. And I think, once we have those four elements better articulated, we'll have much greater—a better ability to decide who is it that can best do this, and how are we going to proceed?

Mr. BAIRD. Well, I—if I'm understanding you right, I think now is a good time to review how we think that protocol ought to be, and maybe this Committee is a good place to start with reviewing that. And then that adds credibilities if we approve that and move forward. Am I understanding that correctly?

Dr. RELMAN. Yes, I think so. And there may well be good partners for you who have already begun this process. The COVID Planning Commission that I referred to in my testimony, the U.S. National Academies that's begun to have conversations, and other National Academies around the world. So I think partnerships, perhaps informal, between countries with various kinds of standing and roles to play could be a part of this discussion with you as well.

Mr. BAIRD. So thank you very much, and I yield back, Mr. Chairman.

Chairman FOSTER. Well, thank you. I think, at this point, we—unless I—there's someone lurking on Zoom, I think we are now out of Members with questions. As I mentioned earlier, we will—after we gavel this hearing closed, we'll be able to have an opportunity for sort of informal conversation, sort of the electronic equivalent of hanging around in the anteroom after this. And—so I'm—any Members or panelists who are interested in that are very welcome to. So, with that, I will thank our witnesses again, and gavel this hearing closed.

[Whereupon, at 2:02 p.m., the Subcommittee was adjourned.]

Appendix I

ANSWERS TO POST-HEARING QUESTIONS

ANSWERS TO POST-HEARING QUESTIONS

Responses by Dr. David Relman

U.S. HOUSE OF REPRESENTATIVES
 SUBCOMMITTEE ON INVESTIGATION AND OVERSIGHT
 COMMITTEE ON SCIENCE, SPACE, AND TECHNOLOGY

Questions for the Record to:

Dr. David Relman
 Professor of Medicine and of Microbiology and Immunology
 Stanford University School of Medicine

Submitted by Representative Bill Posey

1. The Majority Charter for this hearing notes that it is rare for animal viruses to have the ability to successfully infect an individual human much less have the ability for human-to-human transmission. The Charter also cites a Government Accountability Office (GAO) report that finds that laboratory accidents are not particularly rare.

a. How should the rarity of these different events shape our approach to biosafety and outbreak surveillance?

To start, pandemics are rare, in and of themselves—regardless of cause. This means that we are talking about a series of events for which we have little prior experience and very few examples. Having said that, we do know that the precursor conditions for both natural spillovers and laboratory accidents are increasingly prevalent. Furthermore, spillovers and accidents that fizzle out and fail to start a pandemic happen more often than we know or wish to believe.

Typically, microbes that are indigenous to an animal species do not cause disease in that host, but when they enter a different host species, one to which they are less well-adapted, either the microbe fails to replicate in a sustained manner, or if they do, they cause damage to their new host but typically do not then transmit easily to another new host. There are rare cases where an animal-adapted microbe happens to have a broad host range, but again, usually for it to display sustained transmission in a new host it must undergo new adaptation. Thus, it is surprising how well-adapted SARS-CoV-2 appeared to be for humans from the time that cases exploded in Wuhan. The one caveat is the possibility that there was early adaptation and viral evolution among human cases earlier in the Fall of 2019 that have not been revealed and from which virus has been preserved and characterized.

Meanwhile, yes, lab accidents are not uncommon. Allison Young published in USA Today the results of an investigation she undertook several years ago that revealed approximately 1500 accidents in US labs from 2006 through 2013 involving particularly dangerous infectious agents, called “Select Agents”—800 of which prompted medical treatment (<https://www.usatoday.com/story/news/2015/05/28/biolabs-pathogens-location-incidents/26587505/>).

2. It has been revealed that researchers at the Wuhan Institute of Virology conducted research, including suspected gain-of-function research under BioSafety Level-2 (BSL-2) conditions.

a. How did this revelation affect your personal and your professional opinions about the likelihood of a lab-leak?

I was very concerned when I heard about this, and more so once I read the paper in which WIV scientists openly admitted to this work at BL-2 conditions. This paper, published in 2016 in the Journal of

Virology, a journal of the American Society for Microbiology, Zeng et al (including Peter Daszak) described growth at WIV of a bat coronavirus called WIV1 and a mutant form of this virus, in cells bearing the human ACE2 receptor, under BSL2 conditions (J Virol 90:6573-82, 2016). WIV1 was known to be a potentially dangerous virus because of its ability to grow in primary human cells; in fact, Ralph Baric had called it a virus “poised for human emergence”. Ralph Baric had made clear that he handled this virus in his laboratory in the United States under BSL3 conditions, using additional safeguards. At the same time, I understand that Chinese authorities did not require BSL3 conditions for work on these viruses at that time. To me, the practices of Chinese investigators suggested a reactionary, rather than an anticipatory approach. In China, it seemed, a virus was presumed to be benign until it proved itself not to be. To be clear, because most microbes and viruses in the natural world have not been characterized, and because most microbes have a tough time jumping across the host species barrier, one might become lulled into thinking that most work can be safely undertaken without unusual precautions. But to me, this particular case demonstrates a degree of recklessness. The WIV investigators had deliberately set out to find the next SARS virus, couldn’t have known that they didn’t in fact have it in their hands, and more to the point, already knew about the worrisome properties of WIV1 when they undertook this work. This paper, and the reasonable presumption that much of their other work with poorly characterized bat coronaviruses was also carried out under BSL2 conditions, caused me to think that a lab accident at WIV was even more possible than it might otherwise have been.

Responses by Dr. Stanley Perlman*Department of Microbiology and Immunology*

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August 9, 2021

Chairman Bill Foster
 Subcommittee on Investigations and Oversight
 Committee on Science, Space, and Technology

Dear Dr. Foster,

Please find below my responses to the questions asked by Representative Posey.

Question 1. *The Majority Charter for this hearing notes that it is rare for animal viruses to have the ability to successfully infect an individual human much less have the ability for human-to-human transmission. The Charter also cites a Government Accountability Office (GAO) report that finds that laboratory accidents are not particularly rare. a. How should the rarity of these different events shape our approach to biosafety and outbreak surveillance?*

Response. Thank you for this important question about benefit-risk ratio. I think that there are several issues that need to be considered. While the detection of potential human pathogens is fortunately a rare event, surveillance is critical for identification of potential pathogens, as best illustrated in tracking studies of avian and swine influenza virus. An important part of surveillance is that it needs to be combined with assessment of whether the pathogen in question is likely to cause disease and be transmissible in humans or economically important animals, so that we are not just cataloguing viruses or other pathogens. The results of the surveillance should be made public as soon as possible so that scientists, public health authorities and governmental agencies can be pro-active in dealing with threats. This surveillance must be done carefully and with the correct precautions to make pathogen escape from the laboratory virtually zero.

Second, laboratory accidents have been reported over the past 10-20 years and some of them have been publicized. Most laboratory accidents are minor and did not constitute a risk to the person involved in the accident or anyone else. To address the risk part of the benefit-risk equation, information about lab accidents should be carefully analyzed for their actual risk of causing substantial human disease. This information is critical to designing approaches that balance biosafety and the need to identify pathogens before they cause a threat to humans. This information will help authorities determine appropriate biosafety levels at which putative pathogens from specific animal species should be studied.

2. It has been revealed that researchers at the Wuhan Institute of Virology conducted research, including suspected gain-of-function research under BioSafety Level-2 (BSL-2) conditions. a. How did this revelation affect your personal and your professional opinions about the likelihood of a lab-leak?

Response. I believe that you are referring to the construction of chimeric viruses, that enabled infection of human cells by bat coronaviruses that could not previously infect these cells. I am not well qualified to address this question but my understanding is that these experiments per se would not constitute gain-of-function research as defined by the NIH (increased virulence or interhuman transmissibility). Chimeric viruses were engineered at WIV and have been engineered in the USA. Investigators who use these approaches would be better suited than me to answer these questions.

However, I agree, as implied in your question, that the biosafety level (BSL2) that was used for some of these studies may have been inappropriately low. As I noted in my response to the first question, this issue is worthy of continued investigation and deliberation.

This concern, however, does not change my opinion about the possibility of a lab-leak. I do not think that these chimeric viruses served as precursors to SARS-CoV-2. They are quite distant genetically from SARS-CoV-2.

Please let me know if you have additional questions or comments or wish to continue this discussion.

Sincerely,

A handwritten signature in black ink that reads "Stanley Perlman". The signature is written in a cursive, flowing style.

Stanley Perlman, M.D., Ph.D., Professor
University of Iowa Distinguished Chair
Department of Microbiology and Immunology
Department of Pediatrics

Responses by Dr. Connie Price

U.S. HOUSE OF REPRESENTATIVES

SUBCOMMITTEE ON INVESTIGATION AND OVERSIGHT

COMMITTEE ON SCIENCE, SPACE, AND TECHNOLOGY

Questions for the Record to:

Connie Price, MD

Chief Medical Officer

Denver Health

Submitted by Representative Bill Posey

1. You were on the ground in Saudi Arabia investigating Middle East Respiratory Syndrome (MERS).

a. Does there appear to be a substantial difference in the response of the Government of Saudi Arabia to Middle East Respiratory Syndrome (MERS) Coronavirus and the response of the Chinese Communist Party (CCP) to COVID-19?

I will speak to my direct experience investigating MERS. As I was not on the ground in Wuhan, I can't directly compare the differences in the response.

I was initially invited by the Saudi Deputy Health Minister to investigate a hospital outbreak in Eastern Saudi Arabia in May 2013, almost eight months after what later became known as MERS was initially reported through proMED. I was accompanying another team member from Baltimore and another from Toronto. We shared expertise in healthcare epidemiology and infection control and we also worked together on SARS control in Toronto during the pandemic in 2003. The Deputy Health Minister was a professional colleague through our US-based scientific society as an expert in mass gathering medicine.

The Deputy Health Minister gave our team a very specific charge to investigate a hospital outbreak in Eastern Saudi Arabia. Unlike COVID-19, the case counts from MERS were largely driven by outbreaks in healthcare settings, so there was a clear need for focused intervention in healthcare facilities. Understanding the source and the transmission of MERS in hospitals was crucial to getting the case counts controlled.

It is important to note that we were invited as subject matter experts to advise their public health experts on how to conduct the investigation, interpret data, and make recommendations. We were not invited in to take charge of the investigation and give direct orders. When the investigation concluded and a scientific publication was written, it was important to the Saudis that they be recognized as lead and senior authors on all publications, even if the writing was led by non-Saudis. Our team agreed to not let that be a barrier to publication, as it was important to share our findings, which we did within just a few weeks of the investigation.

We always had Saudis with us when reviewing charts and interviewing personnel. I can't say for sure whether that altered answers, but I don't have any reason to believe it did. We had multiple daily communications with the Deputy Health Minister. Although they were protective of the data, and there were times when I felt we were only given one or two pieces of the puzzle,

and it took work to get the full picture, generally they were cordial and accommodating of our data requests. They seemed to respect our expertise and share our desire to understand the epidemiology of MERS in hospitals. No findings were suppressed in our publication.

I can not say for sure if that would have been the case had our findings been viewed as a threat to national interests.

In fact, in the initial emergence of MERS, the Deputy Health Minister who invited me had been highly criticized by international scientists for a reluctance to collaborate with some specialist laboratories around the world offering to help investigate the possible source of MERS and explore how it spreads. Some experts believed that infections and deaths could have been stopped well within the two years MERS first emerged - and would have been if Saudi authorities had been more open to outside help offered by specialist teams around the world with the technology, know-how and will to conduct scientific studies.

This concern was highlighted in a November 2018 report from Georgetown University's Center for Global Health Science and Security, commissioned by the Wellcome Trust. They published a MERS-CoV Data Sharing Case Study Report,. The report highlighted many perceived inadequacies of Saudi Arabia's data sharing practices associated with the MERS-CoV and reported that the research response was plagued by many of the same problems encountered during other public health emergencies. There were problems associated with ill-defined norms around data sharing, uncertainty about which parties were responsible for sharing certain data and who should bear the associated costs of data curation and maintenance, intellectual property considerations of commercial parties, pressure to publish in scientific journals before data was released to the public, technical barriers associated with appropriately disseminating and securing the data, as well as concerns about data reliability and suitability. There were disputes between parties trying to exercise competing legal rights over the MERS virus and controlling access to virus samples, and there were cultural factors that impacted how data were collected and disseminated. There were even concerns by some about the possible destruction of virus samples and data, and perhaps suppression of earlier knowledge of the virus so as not to disrupt the upcoming Hajj which attracts more than one million pilgrims annually. The lack of a free press in Saudi Arabia has been cited as a major barrier to data sharing during the MERS-CoV outbreak and research response.

The response to both MERS and COVID-19 are a stark contrast to the successful collaboration witnessed during the SARS response in 2003, of which I also took part. When SARS emerged, the WHO established a "closed network" of collaborators, within which data and information were shared. The network comprised 11 research teams from 9 countries. The collaborating teams had to accept a set of confidentiality rules that meant information and data could not be shared outside of the group without the permission of the specific team that generated the data. Within two and a half months, scientists had identified the animal host and the mode of transmission to humans. MERS took much longer, and today the scientific community is still not sure of the natural reservoir or intermediate host of SARS-CoV-2

b. What are some specific differences in openness, sharing of genetic data, laboratory results, theories of origins and overall collaboration between Saudi government and health officials with U.S. and international investigators or public health authorities?

Part of this question is addressed in response to 1a.

The novel coronavirus that led to the outbreak of Middle East respiratory syndrome (MERS) was first identified in a patient in Jeddah, Saudi Arabia. In 2012, a specimen containing the virus was sent to a well known virus research facility, the Erasmus Medical Center in the Netherlands, by pathologist working in Saudi Arabia who initially identified the virus. The Erasmus Medical Center sequenced its genome and distributed samples to other laboratories, subject to those laboratories signing a materials transfer agreement (MTA). Some criticized this MTA for slowing the potential progress of other labs by at least several weeks and for being too restrictive. Erasmus MC representatives argued, and I agree, that MTAs are common practice when shipping viruses and that the company gave the virus to any lab that asked for it.

However, a debate about the commercial rights associated with the virus was triggered after Erasmus University Medical Center filed a patent application. It was alleged in early 2013 that patenting the MERS virus hampered the rapid development of diagnostics, specifically in Saudi Arabia, and that the novelty requirement for patentability may have tempted scientists to delay the publication of data urgently needed to contain the virus and to develop diagnostics. Others refute this claim.

By contrast, the virus causing COVID-19, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a global pandemic, with the public discussion focused on the day-to-day management of the disease. Unlike with MERS, which was geographically limited, most major research institutions are involved in combatting SARS-CoV-2. Nevertheless, the considerable market value of diagnostically and therapeutically useful products creates a legitimate interest in commercial rights. It is difficult to know if that has resulted in suppression of useful data on SARS-CoV-2.

With regard to the origins of emerging viruses, it has been clear throughout history that the interface between humans and animals is of paramount importance. The majority of all human infectious diseases and pandemics have originated through transmission of viruses from animals to humans. However, because most animal pathogens are not readily transmitted to humans, it follows that for an animal pathogen to become a specialized pathogen in humans, it must evolve into a pathogen capable of not only infecting humans, but maintaining long-term human-to-human transmission without the need for reintroduction from the original animal host. This often, but not always, involves an intermediary host. For example, the 2003 SARS outbreak originated from bats and its human emergence is believed to have been facilitated through intermediate hosts (eg civet cats) in the wet markets of southern China. The 1918 pandemic influenza was thought to have emerged directly from avian origin, without an intermediary host. The H1N1 influenza epidemic appears to have arisen in through the reassortment of viruses of swine, possibly avian, origin. The MERS coronavirus has been attributed to a bat source with an intermediary host in camels. Given the precedent, the original theories for the emergence of SARS-CoV-2 is that the virus came from nature, jumping from its natural reservoir in some type

of bat, through a still-unknown intermediary animal (eg pangolin) and into humans (possibly at or near a "wet market" in Wuhan, China), among whom it then spread rapidly.

2. The Majority Charter for this hearing notes that it is rare for animal viruses to have the ability to successfully infect an individual human much less have the ability for human-to-human transmission. The Charter also cites a Government Accountability Office (GAO) report that finds that laboratory accidents are not particularly rare.

a. How should the rarity of these different events shape our approach to biosafety and outbreak surveillance?

There has not yet been a pandemic that has a documented origin from a laboratory accident, but existing knowledge of previous pandemics suggest emergence through cross-transmission of viruses from animals to humans. Still, the potential for a laboratory leak exists, and it has yet to be ruled out as the origin of the COVID-19 pandemic. Regardless of what is determined of its origins, the concern has drawn our attention to the global policy patchwork around laboratory biosafety. There is no international authority with oversight on Biosafety Level 4 labs and safe practices for research on deadly agents. The United States and other leading nations can help proliferate best practices out to the rest of the globe through training and educating international scientists on minimum safety protocols.

3. It has been revealed that researchers at the Wuhan Institute of Virology conducted research, including suspected gain-of-function research under BioSafety Level-2 (BSL-2) conditions.

a. How did this revelation affect your personal and your professional opinions about the likelihood of a lab-leak?

While history and patterns of previous pandemics usually point to a zoonotic source, the revelation underscores the need to a complete a full and thorough investigation into the origins of the pandemic before making conclusions about its origins. While it still seems less likely to me, a laboratory source is plausible.

Responses by Dr. Suzan Murray

Smithsonian Institution

Questions for the Record

Dr. Suzan Murray, D.V.M., D.A.C.Z.M.,

Director, Smithsonian Global Health Program Smithsonian National Zoological Park and Conservation
Biology Institute

PRINCIPLES FOR OUTBREAK INVESTIGATION: COVID-19 AND FUTURE INFECTIOUS DISEASES

House Committee on Science, Space, and Technology Subcommittee on Investigations and Oversight
U.S. House of Representatives

Wednesday July 14, 2021

1. The Majority Charter for this hearing notes that it is rare for animal viruses to have the ability to successfully infect an individual human much less have the ability for human-to-human transmission. The Charter also cites a Government Accountability Office (GAO) report that finds that laboratory accidents are not particularly rare. a. How should the rarity of these different events shape our approach to biosafety and outbreak surveillance?

The degree of spillover of viruses from the animal to human population is currently not known, but we believe this might be much more common than initially understood. Since this is often the first opportunity to identify an illness and develop functional and appropriate interventions, increasing research and syndromic surveillance efforts would undoubtedly help teams to identify this initial mechanism of disease spread. While spillover is likely not to be rare, so far the ability for viruses to develop the capability to not only colonize the human population but begin to spread from person to person does currently appear to be thankfully more rare. Our approach to biosafety and outbreak surveillance can indeed be guided and prioritized by studying the more common mechanisms of spread over the less common. Given that we know that approximately 1 % of all known viruses have been discovered, and therefore a great many more exist in nature, this certainly underscores the need for upscaling biological surveillance.

2. It has been revealed that researchers at the Wuhan Institute of Virology conducted research, including suspected gain-of-function research under BioSafety Level-2 (BSL-2) conditions. a. How did this revelation affect your personal and your professional opinions about the likelihood of a lab-leak.

Our team's expertise is in field surveillance, pathogen discovery, and capacity building and as such have not been involved in this type of research so we are therefore unable to comment in a meaningful way.

Appendix II

ADDITIONAL MATERIAL FOR THE RECORD

DOCUMENTS SUBMITTED BY REPRESENTATIVE BILL FOSTER



**Statement from the American Society for Microbiology
in response to the
House Science, Space & Technology Investigations and Oversight Subcommittee Hearing:
"Principles for Outbreak Investigation: COVID-19 and Future Infectious Diseases"**

July 14, 2021

On behalf of our 30,000 members in the United States and around the world, the American Society for Microbiology (ASM) thanks Subcommittee Chairman Bill Foster, Ranking Member Jay Obernolte, and members of the House Science, Space & Technology Investigations and Oversight Subcommittee for holding this hearing to discuss principles for outbreak investigation, particularly in light of the discourse around the origins of the SARS-CoV-2 virus and the source of the COVID-19 pandemic.

As Congress exercises its oversight authority over the response to the COVID-19 pandemic, we urge you to lead with science. Scientists, who have the appropriate subject-matter expertise, provide the best opportunity to answer key questions regarding the origin and transmission of the pandemic and any future infectious disease outbreak. Congress should follow an evidence-based process to ensure answers are grounded in sound science, are as complete as possible, and based in fact.

Below are guiding principles from ASM on the process of investigating COVID-19 or any pandemic outbreak.

1. In order to achieve the most complete and accurate understanding of how the COVID-19 pandemic originated, **it is essential to lead with the scientific method to conduct a free and open inquiry.**
2. Sound scientific principles led to the rapid development of effective vaccines to protect against the worst outcomes of COVID-19 infection. **These same principles of free and open inquiry, global collaboration, objectivity and data-sharing must guide investigations into the origin of the pandemic.** We support continued curiosity-driven, peer-reviewed fundamental research on viral pathogens.
3. Regardless of how the pandemic emerged, **continued support for research and countermeasure development is essential to combatting emerging infectious diseases and preventing the next pandemic.**
4. **Assumptions about the origins of the COVID-19 pandemic should not result in decisions about the future direction of pandemic-related research and policies that could have unintended consequences for potentially lifesaving research.** Such decisions should be rooted in sound scientific principles, with appropriate precautions to protect public health.
5. Questions about key clinical and virus sequence data from the earliest cases of COVID-19 remain unanswered. **It is essential for scientists with appropriate expertise to be free to explore these questions objectively regardless of where the scientific discoveries may lead, without fear of political retribution.**

6. **Governments, international agencies and scientists should work together and share their findings publicly in an honest, transparent effort to provide greater certainty about the earliest relevant events that preceded the pandemic.** Uncovering the answers to critical questions surrounding the pandemic origins will allow stakeholders to craft appropriate and effective strategies to prevent future pandemics.

Whether through the pathway of natural emergence or laboratory escape, the question of how the COVID-19 pandemic started, leading to infection of 180 million people and the deaths of nearly 4 million, is a complex one that includes a spectrum of possibilities. Unraveling the origins of the pandemic is necessary to prepare for future pandemics.

###

The American Society for Microbiology is one of the largest professional societies dedicated to the life sciences and is composed of 30,000 scientists and health practitioners. ASM's mission is to promote and advance the microbial sciences.

ASM advances the microbial sciences through conferences, publications, certifications and educational opportunities. It enhances laboratory capacity around the globe through training and resources. It provides a network for scientists in academia, industry and clinical settings. Additionally, ASM promotes a deeper understanding of the microbial sciences to diverse audiences.



NTI:bio

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BUILDING A SAFER WORLD

Agenda for the Next Administration: Biosecurity

Preventing the Next Global Biological Catastrophe

The United States should support and fund new global platforms and capabilities to improve pandemic preparedness, reduce biotechnology risks, and decrease the likelihood of a globally catastrophic biological event.

There is little doubt that on Inauguration Day in January 2021, the United States and the world still will be struggling to contain the catastrophic human and economic effects of the COVID-19 pandemic. Even as work continues to develop and distribute a safe and effective vaccine and to rebuild economies, U.S. and global leaders also must focus on strengthening capabilities to prevent, prepare for, and respond to the next high-consequence biological event. It likely will not be anywhere near 100 years in coming. Our interconnected world means events on the scale of—or even worse than—COVID-19 could develop any time, and they could come from a naturally emerging pathogen, laboratory accident, or deliberate misuse by state or non-state actors. Indeed, in the latter cases, COVID-19 may have shown the way.

Strengthening International Preparedness for Biological Events

The COVID-19 pandemic has illustrated just how poorly prepared the world was to handle a pandemic at a time when biological threats are increasing. Now and for the future, the security of the United States depends substantially on other countries being prepared and on strong global institutions. The World Health Organization (WHO) must be strengthened to combat the ongoing global COVID-19 pandemic and to get ahead of the next pandemic—or worse, a global catastrophic biological event of even greater proportions.



Terminating the U.S. relationship with the WHO will significantly impair the international response to the COVID-19 pandemic, cause needless deaths globally, threaten American and global health, and undermine international security.

To complement WHO leadership, the world urgently needs a robust, dedicated global health security funding mechanism to catalyze and accelerate investments needed to help prevent the next pandemic. Despite slow, incremental progress made during crises—such as the 2001 anthrax attacks, 2009 H1N1 flu pandemic, and 2014 West African Ebola epidemic—most countries are still unprepared for a major biological event, and no mechanism has been created to finance identified gaps. Leading by example, the United States must work to secure sustained support from heads of state so that countries prioritize biological threat preparedness as an international security imperative and work together to build a United Nations system that is in a stronger position to help coordinate a global response.

Recommendations

- **Rescind U.S. withdrawal from the WHO:** Terminating the U.S. relationship with the WHO will significantly impair the international response to the COVID-19 pandemic, cause needless deaths globally, threaten American and global health, and undermine international security. The WHO provides needed testing and protective gear to safeguard citizens and health workers around the world, promotes research and development toward vaccines for the world's deadliest diseases, and enables external assessment of country capacity for biosecurity, disease detection, and emergency response. Instead of walking away from the WHO, it
- is in the United States' interest to strengthen the WHO's ability to reduce biological risks, detect threats early, and respond rapidly and effectively.
- **Call for and support a 2021 heads-of-state summit on biological threats:** Given the enormous global gaps in preparedness for high-consequence biological threats, a heads-of-state-level summit, chaired by the United Nations (UN) Secretary-General in coordination with the WHO and other relevant UN entities, would create political will for sustainable health security financing and international emergency response capabilities. U.S. support for and participation in this summit is critical to advance global health security priorities worldwide, force high-level attention on this issue, and work to rebuild the international system for future threats.
- **Promote the establishment of a Global Health Security (GHS) Challenge Fund and work with Congress to provide a significant U.S. investment:** A new dedicated, catalytic financing mechanism would prioritize and help close the massive global gaps in pandemic preparedness, disburse loans and grants that are managed within a country's national budget, and be administered over an agreed period of time to increase country accountability and create a sustainable way to shift accounting lines away from donor balance sheets to national budgets. This fund would complement the WHO and other development banks and institutions, leveraging and building on

efforts to evaluate country preparedness by providing a financial and political incentive to governments to address their preparedness gaps. U.S. leadership and significant financing will catalyze multi-sectoral global investment and prioritization.

Reducing Biotechnology Risks

Scientific advances in the life sciences, like DNA synthesis and gene editing, are outpacing the ability of governments to provide effective oversight. As a result, there is an uneven patchwork of security and safety practices across researchers, facilities, countries, and regions. Laboratories around the world already are capable of creating and modifying dangerous viruses, and widespread use of bench-top DNA synthesis technology is just around the corner. A key barrier to developing globally scalable solutions that reduce these urgent biological risks is a lack of incentives and coordination among funders, companies, research institutions, and publishers to promote adequate biosecurity measures.

Recommendation

- **Support the launch of a Dedicated Global Entity Focused on Reducing the Risk of Biotechnology Catastrophe:** Although biotechnology promises to transform American health and society, no global governance approaches exist to identify and reduce biotechnology risks arising from quickly

expanding international biotechnology capabilities. A new, dedicated organization would create a platform to extend American biosecurity practices to a global audience while facilitating the development of novel multi-sectoral approaches and incentives that catalyze companies, researchers, funders, investors, publishers, insurers, and other stakeholders to urgently reduce biotechnology risks.

Mitigating Global Catastrophic Biological Threats

COVID-19 has demonstrated the need for strong international institutions to rapidly detect, identify, and respond to disease outbreaks wherever they arise. In the absence of such mechanisms, fear, mistrust, disinformation campaigns, and confusion inevitably follow, with major potentially negative impacts on human health and global security. The United States and international community lack a robust system for maintaining transparency and building confidence around bioscience research and development. Ongoing transparency efforts are critical for reducing the risk of dangerous misperceptions and suspicions about country capabilities and government intentions, which could inadvertently foster bioweapon arms race dynamics among nations.

Measures to reduce these risks should include the development of an internationally credible, transparent, and science-based mechanism to rapidly investigate the source of an outbreak

COVID-19 has demonstrated the need for strong international institutions to rapidly detect, identify, and respond to disease outbreaks wherever they arise.



of unknown origin during a crisis. Partners in academia, industry, and the international community will be critical in developing and piloting enhanced transparency measures to build trust and increase clarity about national bioscience and biotechnology research and development enterprises in the United States and around the world.

Recommendations

- **Advocate for the establishment of a permanent UN facilitator and unit within the Office of the UN Secretary-General dedicated to responding to high-consequence biological events:** The Office of the UN Secretary-General, working in concert with the WHO, the UN Office for the Coordination of Humanitarian Affairs, and the UN Office for Disarmament Affairs, should designate

a permanent facilitator and unit to address high-consequence biological events with the potential to overwhelm the capacities of the current international epidemic response architecture and result in mass casualties.

- **Strengthen international capabilities to rapidly investigate biological events of unknown origin:** The United States must work with the UN Secretary-General to strengthen UN mechanisms for determining the source of a biological event regardless of origin. These capabilities will need to rely on a robust international laboratory network and effective integration into public health or law enforcement responses upon outbreak attribution.



This is one of two papers prepared by the Nuclear Threat Initiative for the next U.S. administration. Find the papers on nuclear policy and biosecurity at www.nti.org/priorities



The Nuclear Threat Initiative is a nonprofit global security organization focused on reducing nuclear and biological threats imperiling humanity.

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DOCUMENTS SUBMITTED BY REPRESENTATIVE BILL POSEY

February 17, 2021

Chairwoman Eddie Bernice Johnson
Committee on Science, Space & Technology
2321 Rayburn HOB
Washington, DC. 20515

Ranking Member Frank Lucas
Committee on Science, Space & Technology
2321 Rayburn HOB
Washington, DC 20515

Dear Chairwoman Johnson and Ranking Member Lucas,

The SARS-CoV-2 virus (COVID-19) has [cost the lives](#) of more than 465,000 Americans and killed nearly 2.4 million worldwide.¹ It has disrupted the life of every American, causing unprecedented economic harm and costing Americans up to \$16 trillion by an [estimate in the Journal of the American Medical Association](#).² As Members of Congress, we have a duty not only to address the effects of this pandemic but also to fully understand its origins. Where did this virus, which first broke out in the shadow the Wuhan Institute of Virology (WIV), come from?

Since the beginning of the pandemic, various media outlets and scientific journals have considered this question. Last month, [New York magazine asked](#) the pressing question: "For decades, scientists have been hot-wiring viruses in hopes of preventing a pandemic, not causing one. But what if ...?"³ Their reporter concludes that "what happened was fairly simple, I've come to believe. It was an accident. A virus spent some time in a laboratory, and eventually it got out."

Even more recently, the State Department issued a [statement](#) and [fact sheet](#) relating to the coronavirus research that was performed at the Wuhan Institute of Virology.⁴ These call for a full investigation into the origins of the virus, including into the possibility that the pandemic was a result of a laboratory accident.

In November, *The Washington Post* editorial board [called for](#) "a credible, impartial investigation to better prepare for future pandemics."⁵ Leading biosafety expert and microbiologist, Richard Ebright [noted that](#) "The question whether the outbreak virus entered humans through an accidental infection of a lab worker is a question of historical fact, not a question of scientific fact. The question can be answered only through a forensic investigation, not through a scientific investigation."⁶

The reporting mentioned here raise many questions that are relevant to our committee's March 5, 2020, hearing on coronaviruses and their origins. Over time, these issues have gained further attention but there has yet to be a full inquiry into the circumstances surrounding the origins of COVID-19. I would encourage you to take the time to read these materials, and consider the issues raised.

In addition to the issues raised below, the State Department's "[Fact Sheet: Activity at the Wuhan Institute of Virology](#)" focuses on three points of concern.⁷ The first is that there are reasons to be concerned about the account that researchers at the WIV have provided. The State Department "has reason to believe that several researchers inside the WIV became sick in autumn 2019, before the first identified case of the outbreak, with symptoms consistent with both COVID-19 and common seasonal illnesses." Despite this, WIV researchers have

claimed that there was “zero infection” among the WIV staff. The fact sheet also focuses on the activity of secret military research conducted at WIV that may be implicated in the pandemic’s eruption. The last concern raised by the State Department is a chief concern for our committee—the research being conducted, using taxpayer dollars, at the WIV could be closely related to the emergence of SARS-CoV-2 but key data and evidence has been covered up and taken down by Chinese authorities.

We do not know with certainty the origins of the COVID-19 virus. Several of the first explanations – that it evolved in a pangolin or came from a wet market – have been subsequently [dismissed](#).⁸ The suggestion that it may have escaped from the Wuhan lab has been disparaged by some as a “conspiracy theory” yet such a possibility was [acknowledged in 2012](#) by Dr. Anthony Fauci as a possible risk associated with gain-of-function (GOF) research, the same type of research being conducted on coronaviruses at the WIV.⁹ GOF research is when researchers take a virus and manipulate it to be more dangerous among humans. This is done for further study and the preparation of vaccines and therapeutics before a disease emerges.

A 2017 study [published in PLOS Pathogens](#) credits the National Institutes of Allergy and Infectious Disease (NIAID) for providing funding for research at the Wuhan lab where coronaviruses were engineered to be easily transmitted in human cells.¹⁰ This would indicate that these viruses could be highly infectious in humans which would also indicate some risk that researchers could be exposed to and infected by these viruses. These risks are very well-documented, many reporters and researchers have pointed out the widespread and frequent escapes, releases, and infections of pathogens from biosafety labs across the world.

[Washington Post reporting](#) highlights 2018 State Department memos which describe specific safety concerns at the Wuhan Institute of Virology.¹¹ Moreover, [a recent publication](#) in a peer reviewed journal, *Frontiers of Public Health*, raises possible connections between SARS-CoV-2 and a SARS-like coronavirus which killed three miners in China in 2012.¹² These miners’ infections closely resembled COVID-19; this mystery virus and other viruses from the vicinity were stored and studied by the WIV.

Examining all possible origins of this virus, including a lab leak, is a necessary step in determining the exact way that this virus emerged and ravaged the globe. If it emerged from a lab, we would have the opportunity to examine this and develop safer protocols for such research. If it didn’t emerge from a lab, we can put the issue to rest and devote our full attention to determining how it arose in order to prevent future pandemics.

Some efforts have been made to conduct such an investigation, but these are rife with their own issues. The *Lancet* COVID-19 Commission has a team investigating aspects of the virus including its origins. Unfortunately, this team is led by a researcher that funded and coauthored GOF research, manipulating highly infectious coronaviruses with the very Chinese researchers at the Wuhan lab that may have been the source if this was the result of a lab leak. This very fact could compromise the study’s findings.

The World Health Organization also has a team of researchers investigating the origins of COVID-19 but the composition of this team was decided by China. Even with this power, China [initially barred](#) the team from entry into the country.¹³ China has recently [decided to permit](#) the team’s entry but the baseless delay ought to raise further inquiry into China’s conduct on the origins of the virus.¹⁴ In [publicly available emails](#), WHO leadership and officials made praising China a priority which further undermines the grounds for full acceptance of their collaboration with the Chinese on an issue in which China will be reticent to faithfully represent events which may implicate the work of their scientists in the outbreak of the pandemic.¹⁵

Finally, China has very recently provided further evidence of a possible cover-up by [deleting numerous critical records on coronaviruses including an overwhelming number from the Wuhan Institute of Virology](#).¹⁶ Additionally, journalists and researchers already in China have had their [investigation stymied by the Chinese government](#). Their evidence and samples were confiscated and they have been tracked by police and prevented from visiting sites that are implicated in the emergence of this virus, including the caves in the region where the most closely related viruses to SARS-CoV-2 originated.¹⁷ These actions do not instill confidence that there is a commitment to a transparent and thorough investigation but rather a priority on serving self-interest, reputation, and international good-standing.

The issues raised in this letter and the various resources that it points to were not known to the committee or its members prior to the March 5th hearing but they are very relevant. Had we known these things in early March, we could have conducted a more thorough examination of all possible theories for the origins of COVID-19 and developed a more effective defense against emerging infectious diseases.

This Committee has a duty to the American people who have been ravaged by this disease to fully investigate the origins of SARS-COV-2 and ensure that the full force of the federal government is deployed to confidently affirm the origins.

I ask the Committee to hold a series of hearings on this subject as soon as possible. There are few subjects more pressing given the magnitude of the disruption and loss of life stemming from this pandemic.

I look forward to working with you and other Members of the Committee as we seek answers.

Sincerely,



Bill Posey
Member of Congress

¹ <https://coronavirus.jhu.edu/map.html>

² <https://jamanetwork.com/journals/jama/fullarticle/2771764>

³ <https://nv.mag.com/intelligencer/article/coronavirus-lab-escape-theory.html>

⁴ Statement: <https://2017-2021.state.gov/ensuring-a-transparent-thorough-investigation-of-covid-19s-origin//index.html>
Fact Sheet: <https://2017-2021.state.gov/fact-sheet-activity-at-the-wuhan-institute-of-virology//index.html>

⁵ https://www.washingtonpost.com/opinions/global-opinions/the-coronavirus-origins-are-still-a-mystery-we-need-a-full-investigation/2020/11/13/cb4390e-2450-11eb-8672-c281c7a2c96e_story.html

⁶ <https://www.washingtonpost.com/politics/2020/05/01/was-new-coronavirus-accidentally-released-wuhan-lab-its-doubtful/>

⁷ <https://www.state.gov/fact-sheet-activity-at-the-wuhan-institute-of-virology/>

⁸ <https://www.dailymail.co.uk/sciencetech/article-8369085/Wuhan-wet-market-NOT-origin-coronavirus-pandemic.html>

⁹ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3484390/>

¹⁰ <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006698>

¹¹ <https://www.washingtonpost.com/opinions/2020/04/14/state-department-cables-warned-safety-issues-wuhan-lab-studying-bat-coronaviruses/>

¹² <https://doi.org/10.3389/fpubh.2020.581569>

¹³ <https://www.bloomberg.com/news/features/2020-12-30/china-is-making-it-harder-to-solve-the-mystery-of-how-covid-began>

¹⁴ <https://www.globaltimes.cn/page/202101/1212426.shtml>

¹⁵ <https://www.judicialwatch.org/documents/dcnf-v-hhs-production-oct-2020-01149/>

¹⁶ <https://www.dailymail.co.uk/news/article-9129681/New-cover-fears-Chinese-officials-delete-critical-data-Wuhan-lab.html>

¹⁷ <https://hosted.ap.org/TPGIMAGES/article/24fbadc58ccc3a40bca2ddf7a14d2955/china-clamps-down-hidden-hunt-coronavirus-origins>

February 22, 2021

Chairwoman Carolyn B. Maloney
Committee on Oversight and Reform
2308 Rayburn HOB
Washington, DC. 20515

Ranking Member James Comer
Committee on Oversight and Reform
2410 Rayburn HOB
Washington, DC 20515

Dear Chairwoman Maloney and Ranking Member Comer,

The SARS-CoV-2 virus (COVID-19) has [cost the lives](#) of more than 465,000 Americans and killed nearly 2.4 million worldwide.¹ It has disrupted the life of every American, causing unprecedented economic harm and costing Americans up to \$16 trillion by an [estimate in the Journal of the American Medical Association](#).² As Members of Congress, we have a duty not only to address the effects of this pandemic but also to fully understand its origins. Where did this virus, which first broke out in the shadow the Wuhan Institute of Virology (WIV), come from?

Since the beginning of the pandemic, various media outlets and scientific journals have considered this question. Recently, [New York magazine asked](#) the pressing question: “For decades, scientists have been hot-wiring viruses in hopes of preventing a pandemic, not causing one. But what if ...?”³ Their reporter concludes that “what happened was fairly simple, I’ve come to believe. It was an accident. A virus spent some time in a laboratory, and eventually it got out.”

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The reporting mentioned here raises many questions that are relevant to your committee’s March 11, 2020, hearing on coronavirus preparedness and response. Over time, these issues have gained further attention but there has yet to be a full inquiry into the circumstances surrounding the origins of COVID-19. I would encourage you to take the time to read the materials and consider the issues raised.

In addition to the issues raised below, the State Department’s “[Fact Sheet: Activity at the Wuhan Institute of Virology](#)” focuses on three points of concern.⁷ The first is that there are reasons to be concerned about the account that researchers at the WIV have provided. The State Department “has reason to believe that several researchers inside the WIV became sick in autumn 2019, before the first identified case of the outbreak, with symptoms consistent with both COVID-19 and common seasonal illnesses.” Despite this, WIV researchers have

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The World Health Organization also has a team of researchers investigating the origins of COVID-19 but the composition of this team was decided by China. Even with this power, China [initially barred](#) the team from entry into the country.¹³ China has recently [decided to permit](#) the team’s entry but the baseless delay ought to raise further inquiry into China’s conduct on the origins of the virus.¹⁴ In [publicly available emails](#), WHO leadership and officials made praising China a priority which further undermines the grounds for full acceptance of their collaboration with the Chinese on an issue in which China will be reticent to faithfully represent events which may implicate the work of their scientists in the outbreak of the pandemic.¹⁵

China has also very recently provided further evidence of a possible cover-up by [deleting numerous critical records](#) on coronaviruses including an overwhelming number from the Wuhan Institute of Virology.¹⁶ Additionally, journalists and researchers already in China have had their [investigation stymied by the Chinese government](#). Their evidence and samples were confiscated and they have been tracked by police and prevented from visiting sites that are implicated in the emergence of this virus, including the caves in the region where the most closely related viruses to SARS-CoV-2 originated.¹⁷ These actions do not instill confidence that there is a commitment to a transparent and thorough investigation but rather a priority on serving self-interest, reputation, and international good-standing.

Finally, these issues are particularly important for the House Committee on Oversight and Reform to address because there have been several U.S. Departments and agencies as well as universities that have been directly involved in research related to coronaviruses and other pathogens at the Wuhan Institute of Virology. There are numerous examples [of funding](#) from the National Institutes of Health and the Department of Defense being used to finance the [researchers and studies](#) on coronaviruses.¹⁸ There should be a full accounting of how the researchers at the WIV used United States taxpayer funds. This is particularly important in light of the continued efforts by the Chinese government to block full and transparent inquiry into the origins of the pandemic.

The issues raised in this letter and the various resources that it points to were not known to the Committee or its members prior to the March 11, 2020, hearing but they are very relevant. Had the Committee been aware of these issues in early March, a more thorough examination of all possible theories for the origins of COVID-19 might have been conducted.

I urge the Committee to hold a series of hearings on this subject as soon as possible. There are few subjects more pressing given the magnitude of the disruption and loss of life stemming from this pandemic. as we need to have a better understanding of COVID-19's origins in order to adequately prepare for the possibility of other emerging diseases. We have a sworn duty to the American people to ensure a full examination of this matter and demand concrete answers from federal officials.

I look forward to working with you and other Members of the Committee as we seek answers.

Sincerely,



Bill Posey
Member of Congress

Enclosure

¹ <https://coronavirus.jhu.edu/map.html>

² <https://jamanetwork.com/journals/jama/fullarticle/2771764>

³ <https://ny mag.com/intelligencer/article/coronavirus-lab-escape-theory.html>

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- ⁴ Statement: <https://2017-2021.state.gov/ensuring-a-transparent-thorough-investigation-of-covid-19s-origin/index.html> Fact Sheet: https://2017-2021.state.gov/fact-sheet-activity-at-the-wuhan-institute-of-virology/index.html#nav_primary-nav
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- ⁷ <https://www.state.gov/fact-sheet-activity-at-the-wuhan-institute-of-virology/>
- ⁸ <https://www.dailymail.co.uk/sciencetech/article-8369085/Wuhan-wet-market-NOT-origin-coronavirus-pandemic.html>
- ⁹ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3484390/>
- ¹⁰ <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006698>
- ¹¹ <https://www.washingtonpost.com/opinions/2020/04/14/state-department-cables-warned-safety-issues-wuhan-lab-studying-bat-coronaviruses/>
- ¹² <https://doi.org/10.3389/fpubh.2020.581569>
- ¹³ <https://www.bloomberg.com/news/features/2020-12-30/china-is-making-it-harder-to-solve-the-mystery-of-how-covid-began>
- ¹⁴ <https://www.globaltimes.cn/page/202101/1212426.shtml>
- ¹⁵ <https://www.judicialwatch.org/documents/dcnf-v-hhs-production-oct-2020-01149/>
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- ¹⁷ <https://hosted.ap.org/TPGIMAGES/article/24fbadc58ccc3a40bca2dd7a14d2955/china-clamps-down-hidden-hunt-coronavirus-origins>
- ¹⁸ Funding being awarded to EcoHealth Alliance was being used to support dangerous coronavirus research at the WIV https://www.usaspending.gov/keykeyword_search/%22ECOHEALTH%20ALLIANCE%20INC.%22 including GoF studies: <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006698>

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November 9, 2020

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Dr. Jeffrey Sachs
The Lancet COVID-19 Commission
475 Riverside Drive, Suite 1040
New York NY 10115

Dear Dr. Sachs,

Thank you for the ongoing work that you and your colleagues at *The Lancet* COVID-19 Commission have done to fight the worldwide pandemic. The work you are doing is important in our battle against COVID-19 and will inform future pandemic battles.

None of us know where or how this virus arose, but the world is owed an answer to this question with full transparency, accountability. These are the purposes of my letter and I appreciate that *Lancet* is looking into this matter. I do have some questions regarding one of the eleven sections of the Commission, "the Origins of COVID-19 and Future Threats." This section is essential to the proactive work of preventing the emergence of infectious diseases which could cause future epidemics or even pandemics. It is the importance of this work which prompts me to write. I have heard from interested parties who are very concerned about the role of Dr. Peter Daszak on this section of the Commission. They have raised questions about his close financial, professional, and academic ties to the Wuhan Institute of Virology personally and through his organization, EcoHealth Alliance. Though Dr. Daszak is a well-respected virologist and emerging infectious disease expert, some have suggested that his role on the Commission raises sufficient conflicts of interest to create doubts about the reliability of the Commission's findings.

Dr. Daszak has received more than \$50 million in funding from the US government for various projects. Some of these funds, including NIAID grant R01AI110964, and supported bat coronavirus research at the Wuhan Institute of Virology. Moreover, inquiries into the emergence of bat coronaviruses, including the original SARS, form a bulk of Dr. Daszak's work. He has a clear interest in continuing his work with the Wuhan Institute of Virology and his own investigations of bat coronaviruses. This could draw into question the veracity of his findings for *The Lancet*.

Additionally, Dr. Daszak appears to have already publicly stated his conclusions about the Wuhan Institute of Virology. He wrote a letter to *The Lancet*, with other coauthors, which declared that "we stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin." Additionally, his tweets on the origins of COVID-19 include the following, though this is not an exhaustive list.

On June 13th Dr. Daszak [tweeted](#):

“Either @PeterNavarro45 believes these conspiracy theories & is therefore unfit to serve in WH, or he's prepared to go to any lengths by pushing a conspiracy theory to detract from the awful reality of spiking COVID cases in the USA. Disastrous situation... Roll on November!”

A June 23rd [tweet](#) stated:

“But this type of research has nothing to do with the origin of COVID unless you believe the conspiracy theories. Why mix the two together if the virus came from bats, which is what all the evidence suggests?”

On July 13th, Dr. Daszak [tweeted](#):

“BSL4 labs are audited regularly. WIV lab was built w/ French Govt scientists incl. Inst. Pasteur & leading US BSL4 lab. Regular visits by foreign scientists & continual collab. w/ peers from other countries. The lab hypothesis is hype based on conspiracy w/ no evidence at all.”

A July 16th [tweet](#) stated:

“These & other papers are strong evidence SARS-CoV-2 evolved naturally in bats, spilled over directly/via intermediate host (unlikely pangolins) in S. China, trickled through to mkts via wildlife traders/animals. @WHO team will follow science, not conspiracy theorists, I hope.”

And on July 29th, he [tweeted](#):

“With more evidence coming out every week to undermine these conspiracy theories, it's hard not to see the continuing drumbeat as anything other than politically motivated efforts to divert opinion from failed control of COVID.”

Again, on August 10th, in another [tweet](#), he stated that the:

“Wuhan Inst. Virol. is just like all BSL-4 labs - high security, well-managed, efficiently run. Conspiracy theories, politics are the issue.”

Dr. Daszak has also [written in The Guardian](#) conflating lab-origins theories with genetic engineering and conspiracy theories: “suggestions that Covid-19 is a manmade virus are the latest chapter in a tale of blame, misinformation and finger-pointing. Cue the conspiracy theorists, marching out their narrative about the high-security BSL-4 lab in Wuhan, where mysterious experiments to design “frankenviruses” led to the tragic global pandemic.”

The complete rejection of the possibility of a lab-origin of SARS-CoV-2 flies in the face of the [history of lab losses and releases of various pathogens](#), including [SARS-like coronaviruses](#) from biosafety labs like those in Wuhan. Moreover, officials from the U.S. Department of State [issued memos](#) in 2018 which warned of biosecurity concerns at the very lab in question, the Wuhan Institute of Virology. Dr. Daszak, in [one tweet on July 13](#), dismisses the notion of safety concerns at the Wuhan lab. Even if the preponderance of evidence suggests a zoonotic origin, there must be a full investigation into every possible origin of this pandemic virus, and it seems that it ought to include the possible escape from a lab. It is clear that Dr. Daszak has foregone

conclusions about the impossibility of a lab escape, even from a lab with known [safety concerns](#), which may cloud his judgement.

To suggest the possible escape of a virus from a lab is not to endorse conspiracy theory. The escape of dangerous pathogens has long been a topic of discussion, debate, and concern related to pathogenic and gain-of-function research. These fears have been motivated by actualized risks of lab-escaped viruses. Over the past decades, there have been escapes from labs across the world. In 2004, lab workers and several of their contacts were infected by [SARS from a lab in Beijing](#). There were also lab-acquired SARS infections in [Singapore](#) and [Taiwan](#). More recently, in 2018, there were [201 lab releases or losses](#) of dangerous pathogens within the United States alone from some of the highest security biosafety labs in the world.

Further, the notion that a virus could escape from a lab, and even indirectly from a well-regulated lab, was [suggested in 2012 by Dr. Fauci](#) in a letter defending gain-of-function research:

“Consider this hypothetical scenario: an important gain-of-function experiment involving a virus with serious pandemic potential is performed in a well-regulated, world-class laboratory by experienced investigators, but the information from the experiment is then used by another scientist who does not have the same training and facilities and is not subject to the same regulations. In an unlikely but conceivable turn of events, what if that scientist becomes infected with the virus, which leads to an outbreak and ultimately triggers a pandemic?”

It is a misrepresentation of reality to claim that investigating the possibility of such an occurrence is simply promoting further “conspiracy theory” and does a disservice to an open investigation of the origins of SARS-CoV-2.

Those familiar with Dr. Daszak’s statements and his funding and research interests at the Wuhan Institute of Virology may give little credence to the Commission’s findings, and his leadership of this section would only fan the flames of those who speculate there may have been a lab origin to COVID-19.

Moreover, in the Commission’s [“Statement on the occasion of the 75th session of the UN General Assembly,”](#) the declaration of interests includes two sentences dedicated to Daszak which include mention of some of these concerns which could impact the credibility of the Commission and of *The Lancet*:

“PD reports grants from Johnson & Johnson, II-XI, Swiss Re, and Reckitt-Benckiser, outside the submitted work; and reports collaboration with a range of governmental and academic organisations in China, including those targeted by conspiracy theorists as being the origin of COVID-19.”

In an age when misinformation regularly makes headlines and vital public health messaging is highly scrutinized, it is essential, for the sake of the public trust, that any possible cause for doubt be clearly and quickly resolved. While Dr. Daszak is a leading virologist and emerging disease scholar, other experts without such conflicts of interest may be better suited to oversee this important section of the Commission’s work. This would bolster public trust in the Commission’s findings against accusations involving conflicting interests.

As can be seen from the recent increases in vaccine hesitancy related to the COVID-19 vaccine programs, there is a pressing need for clear, honest and transparent investigations into every issue surrounding the virus. Each investigation should be free from preconceptions and allowed to follow every line of evidence to its final conclusion.

Should the *Lancet* Commission's inquiry into the origins of COVID-19 proceed under Dr. Daszak's leadership, I fear that it may only add more questions to answers that it is seeking. Another chairperson for this section should be chosen to safeguard the public trust in the COVID-19 Commission and *The Lancet* generally. Dr. Daszak's continued involvement in and leadership of this section will only serve to undermine the credibility of *The Lancet's* COVID-19 Commission.

I appreciate your attention to this matter and look forward to your timely response.

Sincerely,



Bill Posey
Member of Congress



Congressman Bill Posey
Washington DC
December 10, 2020

Dear Congressman Posey,

Thank you for your letter. The Lancet Covid-19 Commission is interested in an objective analysis of the origins of the Covid-19 epidemic, both in the emergence of the virus, SARS-Cov-2, and in the early handling of the outbreak in Wuhan, China. These issues are important not only to understand the history of this calamity but to better prevent a similar disaster in the future. All of the various theories that have been raised, from a natural zoonotic event to a laboratory release of the virus, will be explored objectively according to the available evidence. They all deserve and require a careful analysis.

The Commission has established a Task Force for this purpose that will report to the entire Commission at the end of 2021. The Task Force does not have any official investigative powers nor a large budget. It will operate mainly by reviewing thoroughly and objectively all of the publicly available evidence, especially the peer-reviewed literature, and by conducting interviews with as many knowledgeable parties and scientists as possible. This will be an open, transparent, and carefully documented process. The Commission will encourage experts and parties to these issues to submit evidence to the Task Force. The members of the Task Force are included at the bottom of this letter. This is a very highly regarded and experienced global group of scientists who will be volunteering their time for this effort.

The Chairman of the Task Force, Dr. Peter Daszak, is one of the world's experts on zoonotic diseases, including those of coronaviruses, and is a member of the National Academy of Medicine. He has been involved in research at the Wuhan Institute of Virology (WIV) on bat coronaviruses, and so is in a position to query senior China's officials at WIV and elsewhere regarding Covid-19 and its antecedents. He will be acting collegially with the rest of the Task Force, most of whom have not had any affiliation with WIV, and he will be acting under my direction and on behalf of the entire Lancet Covid-19 Commission. Dr. Daszak has emphasized to the Commission that all theories that have been raised about the emergence of SARS-CoV-2 will be considered carefully. The Commission and the Task Force have no preconceived conclusions in the outcome of this work. We are interested in ascertaining the truth.

I would very much welcome your suggestions on data and literature to consider, as well as scientists and other knowledgeable parties for the Task Force to interview during the course of 2021. I thank you for your interest in the work of the Commission and will be happy to stay in contact during the coming year.

Sincerely yours, and with best wishes for the New Year,

Jeffrey D. Sachs
Chair of the Lancet Covid-19 Commission

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John Amuasi MD Ph.D., Director of Africa Ctr for Neglected Tropical Diseases & Sr. Lecturer, Kwame Nkrumah University of Science and Technology (KNUST), Accra, Ghana. MD, One Health, Global Health Policy

Danielle Anderson Ph.D., Director BSL-3 lab, Duke-NUS, Singapore. First non-Chinese citizen to work in the Wuhan Institute of Virology BSL-4 lab. Lab Biosafety, virology.

Isabella Eckerle MD, Head of Centre for Emerging Diseases, Univ. Geneva. MD, Virologist.

Hume E. Field DVM Ph.D., School of Veterinary Science, Univ Queensland – Led the original WHO veterinary investigation into the origin of SARS-CoV in wet markets in Guangdong. Veterinarian, One Health

Gerald Keusch MD, Boston University, Head of BSL-4 lab (NEIDL), Former Director of NIH Fogarty Intl. Center, Member National Academy of Medicine. Lab Biosafety.

Dato' Sai Kit (Ken) Lam Ph.D., Professor Emeritus Univ Malaya. Discovered Nipah virus, Member Malaysian Academy of Science. Medical emerging disease virologist.

Carlos das Neves DVM Ph.D., Director for Research & Internationalization, Norwegian Veterinary Institute, President of International Wildlife Disease Association, Former Hon. Consul of Portuguese Republic in Norway. One Health.

Malik Peiris Ph.D. FRS Legion d'honneur, Hong Kong University. Key researcher with deep knowledge of coronaviruses, influenza viruses and Chinese research. Medical virology

Stanley Perlman MD Ph.D., Univ Iowa, Coll Medicine, Rapid Falls. Long term Coronavirus virologist.

Linda Saif Ph.D. – Ohio State Univ, Columbus. Has worked on coronaviruses pre-SARS and was one of the team that inspected the Wuhan lab a few years ago from the NAS. Member of US National Academy of Sciences. Long term Coronavirus research animal models.

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January 22, 2021

Dr. Jeffrey Sachs
The *Lancet* COVID-19 Commission
475 Riverside Drive, Suite 1040
New York NY 10115

Dear Dr. Sachs,

Thank you for your response to my letter. I am grateful to have made you and the *Lancet* Commission aware of the concerns regarding Dr. Daszak's presence on the Commission as chair of the Task Force.

I have no doubts about either Dr. Daszak's credentials or his experience. I believe that it is important to take the concerns that I enumerated in my letter seriously as the credibility of the report could be seriously compromised. Even the most highly regarded and experienced researchers and epidemiologists are subject to the unfortunate reality that we are less likely to notice those things which we would prefer not to notice. This principle extends even to the likes of the Supreme Court where, at times, Justices must recuse themselves from cases for various reasons.

To reiterate, Dr. Daszak's funding and long-standing personal relationship with the Wuhan Institute of Virology (WIV) and his publicly stated presuppositions regarding the outcomes of the Commission's investigation jeopardize the acceptance of the Commission's report as clear, honest, and transparent. His leadership of the Commission is of significant issue.

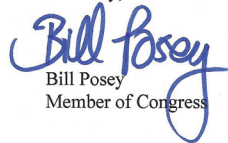
The public is likely to view as suspect any report issued under the leadership of someone who has been outspoken in dismissing, from the earliest days, the suggestion that SARS-COV-2 may have escaped from the WIV as "conspiracy theories".

I am extremely disappointed that my letter did not result in further examination of Dr. Daszak's role on your Commission. While I appreciate your assurances that the Commission and the Task Force do not have preconceived conclusions regarding the outcome of their work, I fear that Dr.

Daszak's public statements regarding the verdict he has already reached will damage the credibility of the Task Force's report.

I appreciate your attention to this matter and look forward to your timely response.

Sincerely,


Bill Posey
Member of Congress

FULLY EMBARGOED AND NOT FOR RELEASE
BEFORE 10 AM USA EDT ON 4 MARCH 2021

OPEN LETTER

**Call for a Full and Unrestricted
International Forensic Investigation into the
Origins of COVID-19**

March 4, 2021

1. Introduction

Finding the origins of SARS-CoV-2 is critically important to both better addressing the current pandemic and reducing the risks of future ones. Unfortunately, well over a year after the initial outbreak the origins of the pandemic remain unknown.

As scientists, social scientists, and science communicators who have been independently and collectively looking into the origins of the COVID-19 pandemic, we believe it essential that all hypotheses about the origins of the pandemic be thoroughly examined and full access to all necessary resources be provided without regard to political or other sensitivities.

Based on our analysis, and as confirmed [by the global study](#) convened by the World Health Organization (WHO) and Chinese authorities, there is as yet no evidence demonstrating a fully natural origin of this virus. The zoonosis hypothesis, largely based on patterns of previous zoonosis events, is only one of a number of possible SARS-CoV-2 origins, alongside the research-related accident hypothesis.

Although the “collaborative” process of discovery mandated by the World Health Assembly in May 2020 was meant to enable a full examination of the origins of the pandemic, we believe that [structural limitations](#) built into this endeavor make it all but impossible for the WHO-convened mission to realize this aspiration.

In particular, we wish to raise public awareness of the fact that half of the joint team convened under that process is made of Chinese citizens whose scientific independence may be limited, that international members of the joint team had to rely on information the Chinese authorities chose to share with them, and that any joint team report must be approved by both the Chinese and international members of the joint team.

We have therefore reached the conclusion that the joint team did not have the mandate, the independence, or the necessary accesses to carry out a full and unrestricted investigation into all the relevant SARS-CoV-2 origin hypotheses - whether natural spillover or laboratory/research-related incident.

We are also concerned that the joint team's work has been inaccurately reported by the media as an independent investigation whose conclusions reflect those of the WHO. The February 9, 2021 [Wuhan joint press conference](#) was a good example of this misunderstanding. Although the findings were those of the joint team, they were [widely reported as representing the WHO itself](#).

As strong supporters of the WHO and its mission, we believe it must be made clear that any findings of the joint committee, while potentially useful to a limited extent, represent [neither the official position of the WHO](#) nor the result of an unrestricted, independent investigation.

For this reason, we believe it is essential that the contours of a full and unrestricted investigation be outlined to set a standard against which current and future efforts can be evaluated.

2. Limitations of the WHO-convened global study

The following core problems undermine the existing WHO-convened joint study:

- The [Terms of Reference](#), which govern the exact objectives, duties and responsibilities of the joint-investigation, were negotiated without sufficient transparency (July 2020), and then made public after considerable delay (November 2020).
- These Terms of Reference were significantly limited, for instance by stipulating that most of the field work had to be conducted by the Chinese side with the results simply communicated to the international members of the joint team for review and discussion, [in a format chosen by the Chinese side](#).
- The intermediate and final reports of the joint study rely on a consensus among the 17 Chinese members and 17 international members of the joint team. It is likely, therefore, that reports resulting from this process will ultimately represent a necessary [compromise](#) based partly on political and other limitations.
- The selection process of international experts on the team did not adequately screen for conflicts of interest.
- The skills represented in the team are purely focused on public health and zoonosis at the detriment of [forensic skills suitable for investigating a laboratory or research-related accident](#).
- The team did not have the mandate or [access to granular lab records](#), data, and personnel to an extent that would allow them to confidently evaluate the various hypotheses.
- The team operated in the context of a [broad Chinese State Council gag order](#) which prevented any spontaneous sharing of any information about the pandemic, and which coordinated the careful release of any such information with the Chinese government. That gag order was put in place a week after the departure of the Feb 2020 WHO joint mission from China.
- The international members of the joint team, [by their own admission](#), have often relied on verbal assurances given to them by their Chinese counterparts rather than independent investigation, particularly regarding the possibility of a lab or research-related accident.
- At least one international team member had expressed a strong [conviction](#) towards the pure zoonosis hypothesis before joining the investigation - when hardly any data about the SARS-CoV-2 virus were available - and was [dismissive of the lab-related origins](#). These public statements cast serious doubts as to his scientific objectivity.

3. What a full investigation should look like

Because the joint team investigation falls short of the mark, we believe it essential for the international community to outline how a full and unrestricted investigation could be organized. Such an investigation would need to:

- Be carried out by a truly independent team with no unresolved conflicts of interest and no full or partial control by any specific agenda or country.
- Be multidisciplinary by including epidemiologists, virologists, wildlife experts, public health specialists, forensic investigators, biosafety and biosecurity experts, etc.
- Include several individuals with Chinese-language skills and with an understanding of Chinese culture, who can help to interpret behaviors during the original events and also help decode the dynamic during the investigation itself.
- Start its study by considering all possible scenarios for each pathway. These should include:
 - A pure zoonosis event with/without intermediate host;
 - Infection at a sampling site of a lab employee or of some accompanying non-lab personnel;
 - Infection during transport of collected animals and/or samples;
 - Lab Acquired Infection (LAI) in one of the laboratories in Wuhan;
 - Lab-escape without LAI, for instance via waste handling or animals that escaped or were disposed of inappropriately.
- Follow a common forensic investigation approach, based on:
 - Traditional rigorous on-the-ground investigation;
 - Desk-based analyst work to make sense of the elements collected;
 - Open-source intelligence to help gather additional information;
 - A review of previous zoonosis events and lab-related accidents, from which technical and institutional insights may be gained.
- Have full or significant access to all sites, records, samples, and personnel of interest, including:
 - Key Wuhan markets;
 - All laboratories and institutions, Chinese or international, known to have worked on coronaviruses or shared facilities or equipment with groups that worked on coronaviruses;
 - Hospital records from fall 2019 of early or suspect patients, including interviews with patients or contacts;
 - Important pathogen sampling sites, such as the [Mojiang mine](#);
 - Current and past personnel, such as employees of the labs in 2019 and people present on specific sampling sites.
- Have full access to all relevant records of the labs and institutions involved in coronavirus research, including:
 - Environmental reports;
 - Inspection reports;
 - Maintenance logs;
 - Lab experiment logs;
 - Raw sequence reads;
 - Records of shipments of samples;
 - Specimen destruction records;
 - Personnel logs;
 - Incident reports;
 - Animal breeding records;
 - Sampling trip records, including the [2013 Mojiang sampling trip](#);
 - Key databases of pathogens, samples, and isolates, including those taken offline.

- Have full access to granular data, preferably directly from the source and in its raw form, not summarized data. This data can be anonymized if necessary; there should be no legal reason to limit access.
- Have full access to market samples, environmental samples, hospital samples and any potential samples such as waste waters and blood banks with full permission to perform independent sequencing or other testing.
- Have full access to the Chinese CDC case records and related primary hospital and/or clinic records.
- Have full access to other Chinese case databases describing pneumonia cases.
- Be able to conduct confidential interviews, including of [early cases](#) and their relatives and past and present personnel associated with the sites or institutions of interest such as markets, hospitals, sampling sites, and laboratories.
- Deploy a secure reporting channel for people to confidentially contribute information, wherever they are based, without fear of punishment or retribution.

4. Conclusion and Next Steps

We recognize that as an international agency that must rely on the collaboration of its member states, the World Health Organization is limited in what it can achieve in this type of investigation. It is not our intention to undermine the WHO, which is working under challenging circumstances at a time of tremendous global need.

Although the joint team investigation was a significant opportunity for the international community to gain some limited and highly curated information, it has unfortunately proven opaque and restrictive, greatly compromising the scientific validity of the investigation.

With more than two million deaths, more than a hundred million infected by COVID-19 worldwide, and a massive global disruption impacting some of the world's most vulnerable populations, we cannot afford an investigation into the origins of the pandemic that is anything less than absolutely thorough and credible. If we fail to fully and courageously examine the origins of this pandemic, we risk being unprepared for a potentially worse pandemic in the future.

Because we believe the joint team process and efforts to date do not constitute a thorough, credible, and transparent investigation, we call on the international community to put in place a structure and process that does.

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OPEN LETTER

April 7, 2021

CALL FOR A FULL INVESTIGATION INTO THE ORIGINS OF COVID-19

Understanding how the SARS-CoV-2 virus emerged and spread to humans is an essential foundation for prioritizing future pandemic prevention and response strategies. Yet well over a year after the initial outbreak, no robust process for examining the origins of the pandemic has been established and critical records and biological samples that could provide essential insights into pandemic origins remain inaccessible. These shortcomings pose a significant threat to everyone and future generations.

As scientists, social scientists, and science communicators, including signatories of the March 4, 2021 [open letter](#) on COVID-19 origins, we believe there is a better way forward.

- Reaction to the China-WHO joint study team report -

In our previous [open letter](#), we outlined our fears that the joint international committee/Chinese government team *“did not have the mandate, the independence, or the necessary accesses to carry out a full and unrestricted investigation into all the relevant SARS-CoV-2 origin hypotheses.”* Having read the report entitled ‘WHO-convened Global Study of Origins of SARS-CoV-2: China part’ and reviewed the statements made in the March 30, 2021 WHO-organized [press event](#) announcing the report’s release, we have regrettably concluded that our concerns were fully justified.

In addition to the issues regarding the joint mission structure and process outlined in our previous open letter, we wish to express the following concerns regarding the joint study process and report conclusions:

- The joint study team saw its priority [as seeking a zoonotic origin](#), not as fully [examining all possible sources](#) of the pandemic. Its [Terms of Reference](#) did not mention any possible lab-pathway and on the contrary explicitly stated a strict zoonosis origin from the very start (*“identify the zoonotic source of the virus”*).

- The published data supporting the mission report mostly present reviews of Chinese studies that have not been published, shared with, or reviewed by the international scientific community.
- Well over a year after the initial outbreak, critical records and biological samples that could provide essential insights into pandemic origins remain inaccessible. This withholding of key resources that could and should have been made available undermined the credibility of the joint study team work.
- The joint study team used different evidentiary standards for the four origin theories it considered. No solid justification is provided for why a 'lab-related accident' (whether a lab-leak or sampling accident) should be considered 'extremely unlikely', or why a natural spillover via an unknown animal host should be considered 'likely to very likely'. At this stage there is still no direct evidence for either pathway nor any verified data or evidence sufficient to rule any one out, while historical evidence amply supports both.
- In particular, a primary conclusion of the report, that SARS-CoV-2 was most probably introduced into the human population through an intermediate host, is not supported by the negative results of all the 80,000 tested samples of wildlife, livestock (35 species) and poultry. That pathway remains entirely theoretical, which at the very least shows the necessity to remain open to other pathways.
- The joint study report spends a mere 440 words examining the lab-accident pathway - less than 1% of the whole report - and does so in a dismissive and superficial way without considering all the possible versions of that pathway, including a possible infection of [a sampling team member](#) by a virus that may never have been isolated or sequenced. The joint study report also makes no mention of the Gain of Function research on bat coronaviruses that was being carried out in Wuhan in the second half of 2019.
- The 440-word assessment of the lab-accident pathway is supplemented by Annex D7 of the report, which labels the lab-pathway as a 'conspiracy theory' five times while containing disputed, incorrect, imprecise, and contradictory assertions (as detailed in the [supplement](#) below).
- The final process utilized by the joint study team for assessing the likelihood of the lab pathway – essentially a show of hands by the joint study team members based on an extremely superficial review – failed to reach some most basic standards of credible analysis and assessment. Further, it is at best unclear whether the Chinese joint study team members had the leeway to express their fair evaluation of all hypotheses in the presence of Chinese government minders.

Against these significant limitations and procedural failures that call the conclusions of the China-WHO joint study report into question, we fully support the March 30, 2021 statement by [WHO Director-General](#) Dr. Tedros Adhanom Ghebreyesus that all origin hypotheses must still be examined, including the possibility of a lab-related incident, that China must be more forthright in sharing essential data and biological samples, and that WHO is prepared to send additional missions and experts to China in order to thoroughly examine all origin hypotheses. We welcome this courageous defense of the WHO's integrity and recognize the organization's potential to lead a comprehensive investigation into pandemic origins, if given the mandate and necessary support.

We further welcome the March 30, 2021 [Joint Statement on the WHO-convened COVID-19 Origins Study](#) by 14 countries underscoring the need for “a transparent and independent analysis and evaluation, free from interference and undue influence” and voicing their shared concern that the joint study “lacked access to complete, original data and samples.” We also recognize the [European Union Statement on the WHO-led COVID-19 Origins Study](#) underlining that the identification of the source of the SARS-CoV-2 virus will “require full and transparent cooperation by all WHO Member States and a collaborative effort by scientists from various disciplines.”

- Next steps -

The principles articulated by the WHO Director General and the two joint statements mentioned above will require a renewed commitment by WHO and all Member States to a full and unrestricted forensic and scientific investigation based on access, transparency and timeliness. This essential goal must be realized in at least one of three possible concrete steps forward.

1. *Revise existing Terms of Reference*

Given the extreme limitations of the present joint study, and in line with Dr. Tedros Adhanom Ghebreyesus's [statement](#), we do not believe a comprehensive study of the *origins of SARS-CoV-2* has yet been undertaken. Accordingly, the most logical next step would be for the existing [Terms of Reference](#) between the WHO and China for such search to be revised to make such an examination possible. These revised terms should:

- Replace the veto power by any government over the composition of the international experts team with a provision requiring final decisions regarding the make-up of the international expert group to be made by the WHO Executive Board;

- Ensure the incorporation of a wider skill-set in the international experts team, including biosafety and biosecurity experts, biodata analysts and experienced forensic investigators;
- Guarantee a transparent selection of the experts team, backed by a more robust process for dealing with possible conflicts of interest. Such a process should prevent any conflict of interest involving prior professional association with laboratories and institutions relevant to the investigation;
- Provide an official mandate allowing international experts to request full or significant access to all sites, records, and samples of interest, and to interview relevant people without the presence of government authorities and with the assistance of translators provided by the WHO; and
- Make the revised Terms of Reference public immediately after negotiation so as to be available for review by the international community and scientists.

2. *Pass new World Health Assembly resolution*

If these revisions cannot be agreed upon and implemented in the very near term, a second option would be for interested governments to propose a new resolution at the May 2021 World Health Assembly. In addition to incorporating the points above, this new resolution should:

- Call for an unrestricted international forensic and scientific investigation into the origins of the pandemic, fully examining all COVID-19 origin hypotheses, with full access to all records, samples, and personnel relevant to the investigation irrespective of location;
- Call for the timely and comprehensive sharing of raw data relevant to the emergence of COVID-19 within the framework of [Open Science](#) and the [FAIR principles](#);
- Call for improved national and international regulatory oversight of laboratories doing high risk virology research, with specific emphasis on “gain of function research of concern” ([GOFROC](#)); and
- Empower the World Health Organization to negotiate new Terms of Reference for this full investigation based on the principles articulated above.

3. *Establish parallel international investigation*

If it should prove impossible for the Terms of Reference to be quickly revised or for a new and sufficient World Health Assembly resolution to be passed in the coming session, the best

remaining alternative would be for governments seeking a full and credible examination into the origins of the pandemic to come together to develop a new and independent process, with China's cooperation if possible but without it if not. Such a process would:

- Outline what a full and unrestricted investigation of all origins hypotheses would entail;
- Draw on the combined knowledge of government agencies and global experts to examine all origin hypotheses to the full extent possible;
- Pool knowledge and intelligence of cooperating governments in a collaborative effort to probe essential questions;
- Establish a secure whistleblower system allowing scientists and others in China and other countries to share relevant information without fear of retribution; and
- Hold transparent public hearings designed to examine all hypotheses in the most data-driven and responsible manner possible.

Calling for a full investigation into the origins of the pandemic by the best available means is not intended to point fingers at any one country. Its purpose is to leave no stone unturned in seeking to understand how this catastrophe began so we can prioritize efforts to address our greatest shortcomings for the benefit of all people and all nations.

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Supplement:

Some issues regarding Annex D7 (entitled ‘Wuhan Institute of Virology’) of the ‘WHO-convened Global Study of Origins of SARS-CoV-2: China part’

- **Incorrect Statement:** In Annex D7 Prof. Shi Zhengli states that the *‘miners had been to the cave 2 - 3 times’* in relation to the Mojiang mine deaths in 2012. Actually the miners developed symptoms after working [8 to 14 days in the mine](#) for around [7 hours a day](#).
- **Disputed Statement:** In Annex D7 the ‘WIV experts’ are cited as saying that the Mojiang miners’ deaths were *‘more likely explained by fungal infections’*. The fungal infection diagnosis is however in contradiction with [the diagnostic of Prof. Zhong Nanshan](#), the foremost Chinese SARS expert at the time, who diagnosed a most likely primary infection from a SARS-like coronavirus, with a possible secondary fungal infection in some cases (pulmonary aspergillosis). Further, the diagnosis of the ‘WIV experts’ also contradicts the positive bat SARS coronavirus antibody tests (IgM and/or IgG) obtained for 4 of the 6 miners (these four tests were carried out at the WIV itself and described in this [PhD thesis](#)).
- **Incorrect Statement:** Annex D7 wrongly describes the deleted database as an *‘Excel spreadsheet that had been on the website for 10 years’* when actually it is a [61.5MB MySQL database](#) that had been released only a few years ago. This database represents only [one of multiple WIV](#) databases that have been taken offline.
- **Contradictory Statement:** Annex D7 states that the database was taken offline after being attacked by hackers. On [another occasion](#) Prof. Shi Zhengli explicitly stated that the database was attacked by hackers during the pandemic and then taken offline. This is in contradiction with the fact that the database was taken offline on [September 12, 2019](#), before the official start of the pandemic.
- **Incorrect Statement:** In Annex D7 Prof. Shi Zhengli is quoted as saying that *‘all fieldwork is done with full PPE’*. This is in contradiction with statements, photographic and video records, showing limited PPE use by WIV personnel during field work. This was additionally confirmed by Shi Zhengli herself [in a presentation](#) (starting from 16:30) she gave in June 2018 (see [transcript and translation](#)) *‘Although bats carry many viruses, the chance of directly infecting humans is very small. Higher-level protection measures will be taken when we know that bats in a certain location carry viruses that may be transmitted to humans, and in most cases only ordinary protection will be taken.’*

- Imprecise/Incorrect Statement:** Annex D7 does not specify the number of people tested for antibodies (IgG) within the Wuhan Institute of Virology and other labs in Wuhan working on BatCoV. From the results (all negative) we can only infer that a small fraction of the 590 WIV staff and students were actually tested. Indeed with the reported prevailing background antibodies (IgG) positive rate of [around 4%](#) in urban Wuhan around April 2020, the probability of no positive antibodies test (IgG+) amongst any reasonable number of potentially directly exposed staff and students becomes quickly extremely small, and is effectively null for the full population of 590. A proper review of tests should instead encompass all staff (including maintenance staff), all students and all construction and temporary workers on site at the WIV, WIBP and at the other Wuhan P3 labs of interest, plus all sample collection staff (some of which may not work at labs).
- Imprecise/Incorrect Statement:** Annex D7 states that *'all samples are stored, but not all have been examined yet'*, so there is not certitude that the WIV never collected or handled SARS-CoV-2 samples since it may still be in a non-examined sample.
- Incorrect Statement:** Annex D7 states that *"The only SARS-CoV-2-like virus found by this group is RaTG13"*, but 8 other SARS-CoV-2 most related viruses (the 7896 clade) can be seen in [slides shown by Dr. Shi Zheng Li in webinars](#).
- Imprecise/Contradictory Statement:** Annex D7 states that *"none of [the viruses from the mine] has higher similarity to SARS-CoV-2 than the RaTG13 has"*, but it is actually not possible to make such an assertion when the [7896 clade](#) viruses are also very similar to SARS-CoV-2 based on their RdRp, but have still not been fully published more than a year after the start of the pandemic (only the short RdRp sections have been published). Moreover on other occasions the existence [of a 100 or so related viruses \(28:20\)](#) has been mentioned.
- Imprecise Statement:** Annex D7 states that *"None [virus from the mine] could be isolated"*. It is not clear whether this refers generally to viruses found in the Mojiang mine, or strictly to viruses similar to SARS-CoV-2. In any case, Dr. Shi herself has previously reported that some bat [adenoviruses had been isolated](#) from samples *'collected in Mojiang'*, and the WIV has also previously [successfully isolated SARS-like viruses](#) from Yunnan sites. Hence the statement may not preclude the possibility that some similar coronaviruses may also have been successfully isolated from Mojiang or elsewhere. We further note that the WIV uses a very specific [naming order for live viruses isolated in their laboratory](#) according to which there should be a WIV6 (not WIV06) isolate and a WIV15 one, which however are not mentioned anywhere in the literature.

- **Imprecise/Contradictory Statement:** In Annex D7 Yuan Zhiming (the director of the WIV P4 lab) refutes categorically any possibility of a lab-leak. Dr. Yuan Zhiming had nevertheless repeatedly denounced structural issues with many labs in China before the pandemic. [As recently as October 2019](#), he wrote that:
 - *“Due to different investment sources, affiliations, and management systems, the implementation of these laboratories faces difficulties converging objectives and cooperation workflows. This scenario puts laboratory biosafety at risk since the implementation efficiency and timely operations are relatively compromised.”*
 - *“several high-level BSLs have insufficient operational funds for routine yet vital processes. Due to the limited resources, some BSL-3 laboratories run on extremely minimal operational costs or in some cases none at all.”*
 - *“Currently, most laboratories lack specialized biosafety managers and engineers. In such facilities, some of the skilled staff is composed by part-time researchers. This makes it difficult to identify and mitigate potential safety hazards in facility and equipment operation early enough.”*
- **Imprecise Statement:** The few visits detailed in the Annexes (mostly D7) only cover some the institutions in Wuhan with P2 and P3 labs that were known to be involved in BatCoV research. A proper investigation should first clearly identify and then cover all the [labs in Wuhan](#) that were actively working on BatCoVs in 2019 without exception, and also the WIBP (next door to the WIV) which may have been involved in a [BatCoV vaccine development program \(30:20\)](#).
- **Imprecise Statement:** Annex D7 provides neither the nature nor the details of research involving bat coronaviruses in Wuhan laboratories in 2019 - including but not limited to possible BatCoV vaccine development programs, pathogenicity studies involving animal experiments, usage of passaging, Gain-Of-Function and [genetic manipulation techniques \(29:50\)](#) - which should be one of the very first steps towards rationally identifying some of the main risk factors in a lab-related accident scenario.

EMBARGOED UNTIL 4 AM EDT ON APRIL 30, 2021

Open Letter to the World Health Organization and the Members of its Executive Board

April 30, 2021

Understanding the origins of the pandemic is essential to addressing our vulnerabilities and preventing future crises. Unfortunately, as outlined in previous open letters released on [March 4](#) and [April 7](#), structural, procedural, and analytical shortcomings of the WHO-convened joint study into COVID-19 origins have created unnecessary barriers to this understanding.

On March 30, 2021, World Health Organization Director General Tedros Adhanom made a number of [important assertions](#) following the release of the WHO-convened [joint study report](#). These include:

- “I do not believe that [the joint’s team] assessment [of a possible lab incident] was extensive enough. Further data and studies will be needed to reach more robust conclusions... potentially with additional missions involving specialist experts, which I am ready to deploy.”
- “As far as WHO is concerned all hypotheses remain on the table... We have not yet found the source of the virus, and we must continue to follow the science and leave no stone unturned as we do... It is clear that we need more research across a range of areas, which will entail further field visits.”

As scientists and science communicators, we welcome this courageous defense of the scientific method and of the WHO’s integrity. We also hope that Dr. Tedros’s clear articulation of critical next steps will be fully supported by all concerned countries and parties. This open letter lays out specific recommendations for what a full investigation into pandemic origins should entail.

Objective of further Studies:

The stated objective of the further studies recommended by Dr. Tedros must be “to *investigate all possible origins of the COVID-19 pandemic*, including an exclusively “natural” zoonosis in the wild, human contamination in an animal farm, and a research-related accident.

This would contrast with the reductive language of [World Health Assembly resolution 73.1](#), which tasked the WHO “to work closely with the World Organisation for Animal Health and

the Food and Agriculture Organization of the United Nations, and countries, as part of the One Health approach, to identify the *zoonotic* source of the virus.”

Additionally the WHO has [previously stated](#) that the mission’s objectives were only to recommend, help design, and review scientific studies. As later confirmed by a [joint-team member](#), this meant that performing an investigation, let alone a forensic audit of laboratories, was beyond the remit of the joint mission.

To address this shortcoming, we recommend that the stated objective be reformulated as: *to conduct a full scientific and forensic investigation into all possible origins of COVID-19, be it zoonotic or not*. Such a reformulation of the objective would ensure compliance with the scientific method of deriving the conclusion from data and facts, not the reverse.

Methods and Protocols:

Given the stakes, the “COVID-19 origins investigation” must follow the highest standards of data-driven, peer-reviewed science. To make this possible in the next phase of the COVID-19 origins study, we recommend:

- ☐ Clearly listing the possible paths for virus evolution and human infection (Annexes A and B below provide a succinct review of such possible paths).
- ☐ Allocating proper time and efforts to examining all hypotheses without any *a priori* assumptions.
- ☐ Ensuring all assumptions and key steps in the analysis are supported by factual data.
- ☐ Guaranteeing access to required raw data (relevant records, samples, project applications, project reports, personnel information, field trip information, relevant emails, laboratory notebooks, etc.) and not solely semi-aggregated data or summaries.
- ☐ Sharing of all relevant medical records, anonymized according to local laws for confidentiality protection, with the joint study team.
- ☐ Ensuring that the team of scientists and specialists is able to undertake their studies at key meetings and visits with no [unnecessary presence](#) of host government non-scientific personnel.
- ☐ Ensuring mission members can conduct interviews, as needed, confidentially and/or anonymously, and with assistance of translators appointed by the WHO if necessary.

Team Selection:

The Terms of Reference for the initial joint study gave Chinese authorities an effective veto over participation in the international team, thereby compromising its independence. In order to support the revised objectives of the further studies, we recommend that:

- ☐ Any veto power be removed and the selection process of the members be made transparent to the WHO Executive Board.

- ❑ International experts with conflicts of interest, including those who may have served in the first phase of the study, should not be included in the WHO-organized independent committee going forward.
- ❑ The selection process should ensure that the team has the skill sets required to assess all pandemic origin hypotheses and to conduct any necessary scientific and forensic audit. This requires the inclusion of biosafety and biosecurity experts, biodata analysts, and forensic investigators, as the WHO previously very successfully did following the SARS lab-leaks in Singapore and Taiwan in 2003/04.

Essential Resources:

The joint-study work has suffered from limited access to granular data, relevant records and samples. We recommend that this type and level of access should be mandated for the next phase of the joint-study work, with specific mention of the following key resources:

- ❑ Anonymized data and biological samples from early COVID-19 patients, close contacts and possibly infected persons.
- ❑ Records of laboratories and institutions involved in coronavirus research in Wuhan.
- ❑ Key databases of pathogens, samples and isolates. These databases are essential as they contain data about viruses not yet published, and some of these viruses may be closely related to SARS-CoV-2. We recommend access to the histories of changes and the previous versions since 2018 for:
 - The 62MB MySQL [database batvirus.whiov.ac.cn](http://batvirus.whiov.ac.cn), including access to the password-protected private section.
 - The www.virus.org.cn portal and its underlying 15 databases.
- ❑ The full sequence of the 8 coronaviruses sampled in the Mojiang mine several years ago, which are some of the closest relatives of SARS-CoV-2 and were mentioned in an [Addendum](#) to a Nature paper published in November 2020.
- ❑ Documents previously prepared (grant applications, detailed progress reports, final reports, scientific publications) by the researchers involved in the following research projects:
 - USA National Institute of Health research projects entitled "Understanding the Risk of Bat Coronavirus Emergence": [1R01AI110964-01](#) and [2R01AI110964-06](#).
 - USA research projects whose goal is to strengthen global capacity for detection of viruses with pandemic potential: [PREDICT](#) and [PREDICT 2](#).
 - Research projects in China investigating various coronaviruses: [31770175](#), [31800142](#), [2013FY113500](#), [81290341](#), [XDPB0301](#), [XDB20101010](#).
 - The July 2019 [tender](#) of China's Ministry of Science and Technology, which aimed to isolate new viral pathogens, standardize their preservation and establish a shared database.

- Research [project](#) initiated by the Chinese Academy of Sciences in 2018 to study several viruses and develop vaccines.
- [China Virome Project](#), also named [China National Global Virome Initiative](#) (CNGVI), which aims to identify unknown viruses from wildlife and is part of the [Global Virome Project](#).

Essential Questions

Because the first phase of the joint study process focused primarily on examining the zoonosis hypothesis, commensurate efforts should now be expended in the next phase of the study examining the possibility of a lab-related incident, including by addressing the following questions:

1. In April 2012, after clearing bat guano in an abandoned mine in Mojiang (Yunnan), six men contracted [severe pneumonia with COVID-19-like symptoms](#). All were sent to Kunming hospital where three eventually died. An on-duty doctor at the hospital at the time [reported](#) a 'potential epidemic outbreak' of unknown pneumonia to the local Center for Disease Control, on arrival of the 5th patient. Unspecified samples from these patients were sent to the Wuhan Institute of Virology and other labs in 2012. Dr. Shi Zhengli recently announced that the [WIV tested the serum samples again](#).
 - > Why were these six miners sent to clean guano in the mine in April 2012? Who hired them and sent them all to the same distant Kunming hospital?
 - > Why are these pneumonia cases absent from the Chinese CDC statistics for 2012 and why were they not reported to the WHO, despite a 'potential epidemic outbreak' alert having been reported to the local CDC?
 - > Why were these lethal pneumonia cases not mentioned in any scientific research article after 2014, despite the [PREDICT](#) program showing a high [interest](#) for potential coronavirus disease outbreaks via bat guano shortly after these events?
 - > Were any SARS-like coronaviruses isolated from the patient samples?
 - > What samples were taken from these six patients and sent to the WIV and other labs? Are any of these samples available for independent analysis?
 - > Is it possible to interview the three surviving miners, their relatives and some Mojiang villagers - and also to take serum samples from them - in order to better understand in which condition these miners fell sick and what their exact pathology was?
2. To this day all the coronaviruses most closely related to SARS-CoV-2 come from that Mojiang mine. Some scientists who went sampling at the mine had their [samples confiscated](#) while investigative journalists have been systematically turned away.
 - > Can Chinese authorities offer unfettered access to the mine to international scientists for the required continued sampling effort?

3. Animal sampling and testing is the only way to establish a zoonotic origin, be it in the wild or in a farm. This data should be shared with the international scientific community.
 - Which animals were, are and will be tested in China, in which wild ecosystems and in which breeding farms?
 - Can the full data for these tests be shared with international experts and the scientific community?
4. Chinese authorities have asserted difficulties sharing human health data with the international members of the WHO-China joint team because of strict domestic privacy laws. However informed consent can normally be waived when de-identified data is used, as was done for instance in this recent Chinese [publication](#) involving 35,040 Wuhan citizens tested for COVID-19.
 - Why was such a waiver not available when requests for very similar data were made by the joint-mission team members?
 - Can this type of waiver be made now?
5. Dr. Shi Zhengli has stated that Wuhan Institute of Virology virus databases were taken offline [during the pandemic](#). However the key [bat virus database](#) was taken offline in September 2019, three months before the official start of the outbreak.
 - Can Chinese officials explain this contradiction?
 - Can Chinese officials explain why the [scientific paper](#) describing the [key database](#) (digital object identifier: [10.11922/csddata.2019.0018.zh](#)) was taken [offline](#) from the corresponding Chinese journal website “China Science Data” in mid-2020?
 - Can Chinese officials also explain why the [full website](#) of “China Science Data”, where the database was described, became [inaccessible](#) in March-April 2021?
 - Can these databases, in their form as of September 2019, be shared with the WHO study group?
6. A bat coronavirus sampled in the Mojiang mine in 2013 ('RaTG13') is still the virus most closely-related to SARS-CoV-2. Dr. Shi Zhengli and Yanyi Wang, director of the WIV, said in interviews that there is “[no more sample](#)” of RaTG13, so that no further sequencing is possible, and that the virus was no longer “[in our lab](#).” Based on the raw data provided, it has unfortunately not been possible to [assemble](#) the RaTG13 genome sequence.
 - When was the RaTG13 sample fully depleted?
 - How was the RaTG13 genome sequence assembled and how was the [5' end](#) sequence determined?
 - Did the WIV or any other laboratory ever attempt to recreate RaTG13 or any other coronaviruses by assembling them from synthetic gene sequences?

- Why are a few of the [RaTG13 amplicons dated as June 2017](#) and named “7896”, which is the [name](#) of another closely related virus collected in the same mine?
 - Given that RaTG13 shows [weak binding](#) to bat receptor ACE-2 and binds only to one of the ACE2 orthologs of *Rhinolophus affinis*, is RaTG13 the true whole genome sequence of the sample BtCoV/4991 collected in 2013 by the WIV?
7. A striking feature of the SARS-CoV-2 genome which increases its pathogenicity, is the presence of a so-called “furin cleavage site”. This site was noted as a “cleavage site” in a January 2020 [publication](#) by Dr. Shi Zheng-Li and colleagues.
- Why was this so-called “furin cleavage site,” clearly an important and novel feature of the SARS-CoV-2 virus, not mentioned in the February 2020 Nature [publication](#)?
8. WIV acknowledged [isolating three strains of live SARS-related coronaviruses](#), but based on the WIV naming [convention](#) for their live viruses isolates it appears that the WIV did not disclose two potential isolates, WIV6 (not WIV06) and [WIV15](#), as these names are not mentioned anywhere in the literature.
- Do these isolates exist? If no, what is the explanation for why these isolate names were skipped in the series?
 - In any case, can the sequences, additional relevant data and the live isolates themselves for all viruses?, plus their clones and mutants (if any) be provided to the WHO Study Group?
9. WIV laboratories were involved in specific government-sponsored research projects in 2019, in collaboration with [EcoHealth Alliance](#). The key objective for one of these [projects](#) (‘bat coronavirus surveillance’) was to identify potentially dangerous viruses based on their spike proteins and involved so-called “gain of functions” experiments, in which viruses were specifically manipulated to acquire new pathogenic features.
- Can Chinese authorities provide the laboratory notebooks and electronic records of the WIV and of any other laboratory that was involved in virus “gain of function” research, as well as any results, including related sequences and isolates?
 - Can [Peter Daszak](#), President of EcoHealth Alliance and also a member of the [joint study team](#), clarify the context of the [experiments](#) he was referring to in late 2019 and make all relevant records available to the study team?
10. The influenza diagnosis and treatment [plan](#) issued by China's National Health Commission on 13 November 2019 advised against isolating virus specimens from patients who did not test positive for influenza, while the [2018 plan](#) had previously encouraged it. This change in policy may have had the unfortunate consequence of facilitating an unreported circulation of SARS-CoV-2 in the last few weeks of 2019.
- What was the reason for this change in policy?

11. Chinese authorities have stated [that staff at four Wuhan labs](#) all tested negative for SARS-CoV-2 antibodies.
 - How many people were tested, in which Wuhan labs, on which days, and as part of which teams or services within these labs?
 - Were any of these serum samples retained?
 - Are independent international investigators able to retest the samples of the lab staff to confirm the results?
12. [Dr. Shi Zhengli](#) and Dr. [Yuan Zhiming](#) have both stated that ‘all staff tested negative for SARS-CoV-2 antibodies’ at the WIV in March 2020. Yet, this is statistically unlikely (roughly less than one chance in a billion) given that there are more than 590 staff and students at the WIV and about 4.4% of the Wuhan urban population [tested positive](#) at around that time. Even if only 85 people were tested, the chance of no positive test would still be less than 4%.
 - How can this contradiction be explained?
 - Can Chinese authorities make available the anonymized raw data of these tests and the test samples for further examination?

Next Steps:

As terrible as COVID-19 has been, this is almost certainly not the last pandemic we will face – and possibly not even the worst. Taking all necessary measures to understand the origins of this pandemic as an essential foundation for addressing our dangerous vulnerabilities is therefore a matter of great urgency. Doing so will also establish an important precedent for fully and transparently investigating any such outbreaks in the future wherever and however they might originate.

We call on the World Health Organization and its Executive Board to fully address the recommendations and questions raised in this letter as a critical step toward protecting everyone on earth and future generations.

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Annex A: Lab-related Accident scenarios

Lab-Related Accident Scenario:	Field sampling accident	Lab acquired infection (LAI) of Wuhan lab personnel	Lab escape without LAI
	LS1	LS2	LS3
Description	Infection during field sampling by or on behalf of a Wuhan laboratory	Infection inside a Wuhan institution with laboratories	Infection outside a Wuhan institution with laboratories
Index case	Personnel present at field sampling site, went back to Wuhan or infected someone who went back to Wuhan	Can be lab personnel, staff, student, or anybody present in the institution (including temporary worker or visitor).	Someone out of the lab, in proximity typically, or in relation to lab activities (such as waste processing)
Actual Biosafety Level	Very limited to equivalent of Biosafety Level 2 (BSL-2) laboratory, full PPE commonly not worn	Bat SARS-related coronaviruses research officially performed at BSL-2 & BSL-3 levels	Bat SARS-related coronaviruses research officially performed at BSL-2 & BSL-3 levels
Incident	Either via contact with animal hosts or animal waste on site	Either: - infection in lab suite handling virus - infection in common facilities shared with lab suite handling virus - infection in institution precinct via aerosols, wastes or stray lab animal	Either - infection via aerosol outside lab precinct - infection via incompletely neutralized liquid or solid lab wastes outside lab - infection via stray lab animal
Virus [See Annex B on virus genesis]	Present in nature	Either present in nature or a lab product	Either present in nature or a lab product
First person infected in a lab/institution?	Possibly - could also be employee, contractor, contract worker, collaborator, associate, or visitor, accompanying university student	Probably - could be employee, contractor, contract worker, collaborator, associate, or visitor	First person is infected outside of lab/institution
Virus in field samples?	Not necessarily. Could be an infection from a bat that may not even have been sampled	Not if virus is a lab product, or from live bat collected in nature and bred in lab	Not if virus is a lab product, or from live bat collected in nature and bred in lab
Virus isolated? (cell culture)	Not necessarily. May not be in a field sample, and, even if the virus is in a field sample, the field sample may not have been processed yet	Not necessarily. Could be from non-processed field sample or a non isolated lab construct	Not necessarily. Could be from non-processed field sample or a non isolated lab construct

Virus sequenced?	Not necessarily. May not be in a field sample, and, even if the virus is in a field sample, the field sample may not have been processed yet	Not necessarily. Could be from non-processed field sample or a non isolated or non sequenced lab construct	Not necessarily. Could be from non-processed field sample or a non isolated or non sequenced lab construct
Argument #1: SARS-CoV-2 not present in lab	Irrelevant	Relevant only in case of a sequenced virus	Relevant only in case of a sequenced virus
Argument #2: No lab-employee tested positive for SARS-CoV-2 antibodies (IgG)	Irrelevant (unless all field sampling personnel, contractors, contract workers, associates, and visitors were tested)	Irrelevant (unless all lab personnel, contractors, contract workers, associates, and visitors were tested)	Irrelevant

Annex B: Virus genesis scenarios

Virus Scenario:	Found in nature	Routine processing in lab	Serial passage in lab, Gain of function selected	Genetic manipulation in lab, Gain of function created and selected
	VS1	VS2	VS3	VS4
Lab Product?	No	Yes	Yes	Yes
Description	Virus arose in nature	Virus arose naturally in lab via mutations or recombinations (without artificial selection)	Virus arose in lab through serial passage (directed evolution), artificially selected mutations or recombinations	Virus arose in lab through genetic manipulation via mutations and recombinations created and selected
Host	Natural host (bat or other animal)	Lab cultures and/or lab animals	Lab cultures and/or lab animals	Lab cultures and/or lab animals

Note that all combinations of VS2, VS3 and VS4 are also possible, for instance VS3 followed by VS2.

Open Letter

Call for a Comprehensive Investigation of the Origin of SARS-CoV-2, if Possible with Chinese Government Participation

Embargoed for Release at 7 AM USA EDT on June 28, 2021

On June 12, 2021, World Health Organization Director General Dr. Tedros Adhanom Ghebreyesus [stated](#) that the families of the many millions who have died from COVID-19 “deserve knowing what the origin of this virus is, so we can prevent it from happening again.”

In line with this critical aspiration, G7 leaders released a [joint statement](#) on the following day calling for “a timely, transparent, expert-led, and science-based WHO-convened Phase 2 COVID-19 Origins study including, as recommended by the experts’ report, in China.”

As scientists and science communicators who have called for a comprehensive investigation into pandemic origins in three open letters earlier this year ([#1](#), [#2](#) and [#3](#)), we welcome these statements and again call for a comprehensive scientific and forensic investigation into all plausible origin hypotheses with unrestricted access to all relevant records, samples, and personnel in China and, as appropriate, beyond.

All people and every nation, including China, have a direct interest in the origin of the pandemic being identified and our greatest vulnerabilities being addressed. It is therefore particularly unfortunate that no comprehensive investigation of all plausible origins has yet been carried out and that none is currently planned.

For reasons articulated in earlier letters, we believe that the WHO-convened joint-study process currently in place does not in its present form clear the bar of credibility due to grave structural shortcomings ([Annex A](#)).

Although the WHO-convened joint-study has been called by many a “WHO investigation,” it was neither led by the WHO nor intended to be an investigation. As Dr Tedros [recently clarified](#), “there is a misunderstanding. The [study] group came from different institutions and different countries, and they’re independent. There were only two WHO staff who joined them. They came up with their study.” The leader of this independent expert committee, Peter Ben Embarek, has also [repeatedly explained](#) that the joint study process was neither an investigation nor designed to be one.

Further, the Chinese government’s well documented measures to hide records and prevent Chinese experts from sharing critical information and granular data make it very clear that the current process has no possibility, without significant changes, of fully and credibly investigating all plausible origin hypotheses.

Because failing to comprehensively investigate pandemic origins puts everyone and future generations at unnecessary risk, we call on world leaders to adopt a **two-track** approach for ensuring the fullest possible investigation of pandemic origins.

The **first track** is to invite China to fully cooperate with an improved WHO-convened investigation that is data-driven, independent, and meets essential conditions for a credible process. Such an investigation should:

- Be based on an explicit mandate to fully investigate all plausible origin hypotheses, including all plausible scenarios for both a zoonotic spillover (in the wild or at a farm or market) and a research-related incident. It is important to note that a research-related incident need not necessarily involve a non-natural virus, as multiple research-related accidental infection scenarios are compatible with a virus that was collected in the wild and not voluntarily genetically altered in a laboratory. These include researchers getting infected on a bat sampling field-trip, researchers becoming infected while working in one of the various Wuhan laboratories, and people outside laboratories becoming infected due to an accidental pathogen leak from faulty wastewater treatment, an air filter failure, or some other source of environmental contamination ([Annex B](#));
- Be led by a multidisciplinary team of international experts with the expertise domains necessary for an examination of both the zoonotic spillover and the research-related incident pathways, such as virology, epidemiology, zoology but also traditional forensics skills, laboratory audit experience, engineering skills, data acquisition and data analysis skills;
- Avoid any conflict of interest and undue bias within the experts team, which may be suggested by unsubstantiated statements and repeated dismissal of plausible non-zoonotic origins or by previous strong research partnership with parties of interest;
- Empower the experts team to carry out a full scientific and forensic investigation, which must include being given full access to all relevant records, samples, and personnel, upon request (anonymized if necessary) and being able to hold confidential interviews with Chinese experts without Chinese government oversight or control with the assistance of independent translators; and
- Share raw data with international experts for further studies, whenever possible, for constructive feedback and transparency.

Because these represent the most basic conditions under which any credible process can be carried out, the full acceptance of all of these terms by the Chinese government should be a necessary precondition for any next phase of the WHO-convened joint-study.

To prevent a situation where the negotiation and planning process is drawn out over many months or even years, a firm deadline of two months following the articulation of these terms should be set for their acceptance by Chinese authorities and for the commencement of a full investigation on the ground in China. It is our sincere hope that, for the good of humanity, the Chinese government will join such a comprehensive and science-based investigation process as a full partner.

Should Chinese authorities not allow this type of comprehensive investigation within this time frame, however, it would then be clear that a **second track** would need to be established, under which groups of nations, coordinating around some other organization or mechanism, should set up an alternative science-based and data-driven investigation.

Although the decision of how to best mandate and coordinate such an investigation should be left to interested governments and partners, possible entities might include the OECD, G7, the Quad, or other structures and institutions.

While this type of alternate investigation, by unfortunate necessity carried out without the full participation of the Chinese government, would suffer from a lack of access to many important records, samples, and personnel inside China, a great deal of highly relevant information could still be gathered without the participation of Chinese authorities.

In fact, many governmental and individual investigators around the world have already gathered and begun analysing significant amounts of relevant data. A well organized and concerted effort, free of interference and drawing on all available information sources and involving a large pool of experts, could well end-up providing unambiguous evidence supporting one particular origin hypothesis or another.

An investigation carried out without the assistance of Chinese authorities should include:

- Careful testing and analysis of hospital samples and environmental samples from various countries to better understand the initial emergence and the early spread of SARS-CoV-2 around the world;
- A thorough evaluation of the farm-animal and wild-life trades from South-East Asia to China, and within China, and their potential roles in the pandemic;
- A detailed analysis of all the known coronaviruses related to SARS-CoV-2 to reconstruct the evolutionary history of SARS-CoV-2 (its geography and its phylogenetic relationships);
- A systematic quantitative evaluation of the key features of SARS-CoV-2 in order to clarify statements about its origin;
- A broad semi-quantitative assessment of the current weights of evidence in favor of each possible scenario in order to foster a rational debate and help drive further research efforts on key points;
- A systematic search for documents and missing information about key virus sequences, including:
 - The main WIV database of pathogens, samples and isolates that was taken offline in Sep. 2019 and never reinstated, and the other databases managed by the WIV which were taken offline;
 - Materials from NCBI GenBank sequence database, including the emails exchanged regarding the submission of the first genome sequence of SARS-CoV-2 (MN908947.1) by Zhang et al and made accessible on Genbank on [12th Jan 2020](#);
 - Materials from the GISAID database, including the virus sequence and metadata associated with accession number EPI_ISL_402122, which is no longer available at GISAID and which likely corresponds to an early COVID-19 patient;
- The acquisition and review, in accordance with the joint statements in favor of a full and transparent investigation by the [United States](#) and the [European Commission](#), of relevant communications, documents and data from the following parties:
 - North-American partners of the WIV:
 - EcoHealth Alliance, in particular studies in Asia of [pangolins](#) trade and of [bats](#).

- United States Agency for International Development (USAID), particularly with regard to their work in [China](#) under the projects “[PREDICT](#)” and “[PREDICT-2](#)”, and [sampling collection](#) in Asia;
- National Institutes of Health, especially regarding projects [1R01AI110964-01](#) and [2R01AI110964-06](#) entitled “Understanding the Risk of Bat Coronavirus Emergence”;
- o European partners of the WIV:
 - European Commission, in particular the documents related to funds allocated to WIV within the framework of [Horizon 2020](#) and [EVA-G](#), including those reserved until the period of 2023, the correspondence with the WIV, plus the WIV interim report which was [mentioned](#) by the European Commission;
 - French research institutions (Inserm, Institut Pasteur, Fondation Mérieux), regarding the documentation related to their [cooperation](#) established in 2014 with the WIV, and also the funds that were allocated to the WIV since;
- The inspection of documents (submitted manuscripts, submitted sequences, cover letters, reviewers’ comments, authors’ replies) associated with the peer review process of the key scientific articles listed in [Annex C](#), which may recover additional data and relevant information;
- The creation of a secure whistleblower program allowing for the safe sharing of information from within China and abroad;
- The examination of the forensic evidence collected by intelligence agencies and Open Source Intelligence specialists, including a detailed mapping out of key research teams, their stated objectives, their work at the time of the outbreak, their means, and an evaluation of the safety conditions within their laboratories, and any events of interest within or around these laboratories.

While the Chinese government must be offered every opportunity to join a comprehensive investigation into pandemic origins, it should not be afforded a veto over whether or not the rest of the world carries out the fullest possible investigation.

The two-track process proposed here encourages China to participate in a comprehensive, science-based, and data-driven investigation should it wish to do so, as other countries have done before both with zoonotic outbreaks and laboratory-related accidents.

In the unfortunate case the Chinese government chooses not to join this process, however, a thorough investigation into the origins of SARS-CoV-2 is still possible, has a realistic chance of success, and must be pursued for the common good.

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Annex A: Shortcomings of the WHO-China joint-study process

The main shortcomings of the joint-study process and its resulting Phase 1 report include:

- The World Health Assembly mandate called for a joint study of “the zoonotic origin of the virus” rather than a comprehensive investigation into all plausible origins, setting the stage for a process predominantly focused around examining the natural origins hypotheses with extremely little effort expended on a research-related accident;
- The Terms of Reference (ToRs) for that essential process were negotiated behind closed doors between China and the WHO, without known input from member states other than China, and were only rendered public months after they were finalized;
- The ToRs granted the Chinese government an effective veto over who could participate in the international expert committee;
- The selection process for the international expert committee [was not transparent](#);
- Several members of the international committee had significant conflicts of interest;
- No provisions were made ensuring international experts had access to all relevant records, data, and personnel;
- The raw data was not provided to the international expert committee, including upon request;
- No provisions were made allowing Chinese experts the opportunity to provide confidential input to international experts without fear of retribution by Chinese government authorities;
- A mere 2 paragraphs in the 120 pages of the main body of the Phase-1 report were dedicated to a possible research-related origin;
- The likelihood of each scenario was decided by a show of hands in front of Chinese government officials;
- Multiple inconsistencies have been noticed in Annex D7 of the report and were listed in [the Supplement of our letter #2](#).

Annex B: The three accidental infection scenarios

The laboratory-related accident hypothesis includes three **scenarios of accidental infection** which were detailed in the [Annex A of our letter #3](#):

1. A researcher or assistant getting infected on a bat sampling site and bringing the infection back to Wuhan.
2. A researcher, student or employee getting infected in one of the various Wuhan laboratories known to be working on Bat Coronaviruses, typically at level P2 or P3 (a Laboratory Acquired Infection - LAI). Examples of previous LAIs include:
 - Hantavirus outbreak at a College in Yunnan (China) in 2003, associated with laboratory rats and lax safety practices ([paper by Shi Zhengli et al](#))
 - [SARS LAIs](#) in a Singapore P3 and a Taiwan P4 in 2003, fully investigated by the WHO.
 - Several [SARS LAIs](#) at the top P3 in China in 2004, with a limited investigation from which the WHO eventually dropped out.
 - [Brucellosis LAI](#) in Harbin (China) in 2011 due to lax safety practices.
3. Someone outside of a laboratory getting infected due to an escaped pathogen, without anybody in the laboratory itself being infected - for instance due to bad wastewater treatment or to an air filter failure (a Laboratory Escape without LAI).
Examples:
 - Respiratory anthrax accident in Sverdlovsk (ex USSR) [in 1979](#), covered up for many years.
 - Foot and Mouth outbreak in Britain [in 2007](#)
 - Brucellosis outbreak in Lanzhou in China in [Nov 2019](#)

For the various **scenarios about the virus** (natural vs. product of research), see [Annex B of our letter #3](#).

Annex C: Scientific papers of interest

Here is a list of articles for which documents associated with the peer review process may help retrieve additional data and information, for instance in relation to early sequences of SARS-CoV-2 and to related viruses:

- [Andersen et al. 2020](#),
- [Calisher et al. 2020](#),
- [Hong Zhou et al. 2020](#),
- [Lam et al. 2020](#),
- [Latinne et al. 2020](#),
- [Li et al. 2020](#),
- [Liu et al. 2019](#),
- [Xiao et al. 2020](#)
- [Xiao et al. 2021](#),
- [Zhang et al. 2020](#),
- [Zhang & Holmes 2020](#),
- [Zhou et al. 2020](#)



Did the SARS-CoV-2 virus arise from a bat coronavirus research program in a Chinese laboratory? Very possibly.

By Milton Leitenberg | June 4, 2020

This scanning electron microscope image shows SARS-CoV-2 in yellow. (Photo credit: Credit: NIAID-RML)

On May 15, the *Bulletin of the Atomic Scientists* published a short commentary titled, “Let evidence, not talk radio, determine whether the outbreak started in a lab,” by Ali Nouri, a biologist and president of the Federation of American Scientists. “The outbreak” referred to the pandemic of SARS-CoV-2 now circling the globe. It is a thin commentary, and it is puzzling why the *Bulletin* thought it desirable to publish it at all. Only two weeks earlier the journal had published **a reasoned and competent appraisal** by Kings College London biosecurity expert Filippa Lentzos titled, “Natural spillover or research lab leak? Why a credible investigation is needed to determine the origin of the coronavirus pandemic.”

The Nouri article very correctly pilloried the statements by President Donald Trump, Secretary of State Mike Pompeo, presidential legal advisor Rudy Giuliani, and radio

personality Rush Limbaugh. These are as notorious a gang of four fabricators as will ever likely be recorded in American history. They were ably assisted by Fox News, which the Nouri critique also mentions. Nouri ended his commentary with these lines: "Our leaders ought to ... take steps to prevent the next pandemic, instead of diverting our attention to unsupported sensationalist theories spread by cable TV and talk radio."

Perhaps the most damaging blows to efforts to obtain a certain answer as to the origin of the SARS-CoV-2 "outbreak" have been the pronouncements by Trump, Pompeo, and their echo chambers. But they and their remarks are not the measure by which the question of the possibility that a laboratory escape began the pandemic should be examined. Trump's diversionary ranting comes from a president who did nothing for two months in the face of an oncoming lethal pandemic, actively denied and denigrated intelligence warnings of the imminent danger, and said that SARS-CoV-2 would "just go away ... like a miracle" and that "within a couple of days is going to be down close to zero." All this has been widely and thoroughly chronicled.¹

But long before Trump, Pompeo and Co. sought a Chinese scapegoat for the president's gross and willful incompetence, researchers understood that the possibility of laboratory escape of the pathogen was a plausible, if unproven, possibility. It is most definitely not "a conspiracy theory."

The circumstantial evidence for a lab escape. By way of introduction, there are two virology institutes in Wuhan to consider, not one: The Wuhan Center for Disease Control and Prevention (WHCDC) and the Wuhan Institute of Virology (WIV). Both have conducted large projects on novel bat viruses and maintained large research collections of novel bat viruses, and at least the WIV possessed the virus that is the most closely related known virus in the world to the outbreak virus, bat virus RaTG13. This virus was isolated in 2013 and had its genome published on January 23, 2020. Seven more years of bat coronavirus collection followed the 2013 RaTG13 isolation.

One component of the novel-bat-virus project at the Wuhan Institute of Virology involved infection of laboratory animals with bat viruses. Therefore, the possibility of a lab accident includes scenarios with direct transmission of a bat virus to a lab worker, scenarios with transmission of a bat virus to a laboratory animal and then to a lab worker, and scenarios involving improper disposal of laboratory animals or laboratory waste.

A May 20, 2020, photo of the Wuhan Institute of Virology in Wuhan, where research on bat coronaviruses was conducted. (Photo by Kyodo News via Getty Images)

Documentary evidence indicates that the novel-bat-virus projects at Wuhan CDC and the Wuhan Institute of Virology used personal protective equipment and biosafety standards that would pose high risk of accidental infection of a lab worker upon contact with a virus having the transmission properties of the outbreak virus.

In assessing the possibility of a lab accident, one must take into consideration each of the following eight elements of circumstantial evidence:

1. *Official Chinese government recognition early in the SARS-CoV-2 outbreak of biosafety inadequacies in China's high containment facilities.* In February 2020, several weeks after the outbreak of the disease in Wuhan, China's President Xi Jinping stressed the need to ensure "biosafety and biosecurity of the country."² This was followed immediately by a China Ministry of Science & Technology announcement of new guidelines for laboratories, especially in handling viruses.³ Almost at the same time, the Chinese newspaper Global Times published an article on "chronic inadequate management issues at laboratories, including problems of biological wastes."⁴

A [PBS NewHour presentation](#) on May 22, 2020 provided the following information:

On January 1, Wuhan Institute of Virology's director general, Yanyi Wang,

messed her colleagues, saying the National Health Commission told her the lab's COVID-19 data shall not be published on social media and shall not be disclosed to the media. And on January 3, the commission sent this document, never posted online, but saved by researchers, telling labs to destroy COVID-19 samples or send them to the depository institutions designated by the state. Late Friday [May 16, 2020] the Chinese government admitted to the destruction ... but said it was for public safety.

The Chinese government explanation for the destruction of SARS-CoV-2 samples has no scientific credibility. For purposes of "public safety" any samples would surely be stored and studied, exactly as with the ones that were isolated from patients, and their RNA genomes decoded and published.

2. *Recognition by Zhengli Shi, a renowned scientist who leads a research team at the Wuhan Institute of Virology, that a laboratory escape was a possibility.* Shi took the possibility of a laboratory escape perfectly seriously. Jonna Mazat of the University of California-Davis, a collaborator with Dr Shi, told Josh Rogin of the Washington Post, "Absolutely, accidents can happen." In an interview with Scientific American, Shi admitted that her very first thought was "If coronaviruses were the culprit, she remembers thinking 'Could they have come from our lab?'"

Meanwhile she frantically went through her own lab's records from the past few years to check for any mishandling of experimental materials, especially during disposal. She breathed a sigh of relief when the results came back: none of the sequences matched those of the viruses her team had sampled from bat caves. 'That really took a load off my mind,' she says. 'I had not slept a wink for days.'

3. *Questions surrounding Chinese government attribution of the Wuhan's Huanan South China Seafood Market as the source of the SARS-CoV-2 virus.* Many China scholars noted that it was quite unusual for Chinese government authorities to identify Wuhan's Huanan South China Seafood Market so quickly as the source of the outbreak. They thought this behavior so uncharacteristic that it raised suspicions in their minds. The authors of a newly published paper wrote that

...we were surprised to find that SARS-CoV-2 resembles SARS-CoV in the late phase of the 2003 epidemic after SARS-CoV had developed several advantageous adaptations for human transmission. Our observations suggest that by the time SARS-CoV-2 was first detected in late 2019, it was already pre-adapted to human

transmission to an extent similar to late epidemic SARS-CoV. However, no precursors or branches of evolution stemming from a less human-adapted SARS-CoV-2-like virus have been detected.... It would be curious if no precursor or branches of SARS-CoV-2 evolution are discovered in humans or animals....Even the possibility that a non-genetically-engineered precursor could have adapted to humans while being studied in a laboratory should be considered, regardless of how likely or unlikely.⁵

It is important to note that no intermediary host has yet been identified for the SARS-CoV-2 virus. The authors also noted that “[n]o animal sampling prior to the shutdown and sanitization [of the Wuhan fish market] was done.”

The question of whether the index case appeared in the Wuhan fish market appears to be moot in any case. Chinese researchers have published data showing that there were 41 cases of SARS-CoV-2 between December 1, 2019 and January 2, 2020. Fourteen of these had no contact with the Huanan seafood market, including the very first recorded case on December 1, 2019.⁶ And that supposes that the true index case was December 1, which is doubtful.

On May 26, the Chinese government scrapped the previous official story about the Wuhan fish market:

China's top epidemiologist said Tuesday that testing of samples from a Wuhan food market, initially suspected as a path for the virus's spread to humans, failed to show links between animals being sold there and the pathogen. Gao Fu, director of the Chinese Center for Disease Control and Prevention, said in comments carried in China state media.⁷

No SARS-CoV-2 isolates were detected in any of the animals or fish sold at the market, only in environmental samples, including sewage. Gao Fu added, "At first, we assumed the seafood market might have the virus, but now the market is more like a victim. The novel coronavirus had existed long before."⁸

4. *Suppression of information and individuals by Chinese authorities.* A publication by two Chinese university academics discussed both the WHCDC and the WIV and concluded that "the killer coronavirus probably originated from a laboratory in Wuhan"; the publication was removed from the internet by Chinese government officials. The paper had been posted on Research Gate but was blocked after 24 hours. After being placed on an archive file by internet users, it was again blocked after a week, and the two Chinese authors were pressured to retract the paper. However, it is still available on Web archives.⁹

The Chinese government closed the laboratory in Shanghai that first published the genome of COVID-19 on January 10, explaining that it had been shuttered for "rectification"; the closure happened on January 11. The government then permitted the same genome to be published by Shi on January 12.¹⁰ Chinese citizens who reported on the coronavirus were censured and, in some cases, "disappeared."¹¹ These have included businessman Fang Bin, lawyer Chen Qiushi, former state TV reporter Li Zehua and, most recently, Zhang Zhan, a lawyer. They are reportedly being held in extrajudicial detention centers for speaking out about China's response to the pandemic. They are usually accused of "picking quarrels and provoking trouble."¹²

Another aspect of Chinese government secrecy involved in the SARS-CoV-2 pandemic relates to official reporting by Chinese government officials on the severity of the outbreak in China and on levels of mortality. The number of cases and deaths are suspected of being undercounted by at least an order of magnitude, and possibly two, meaning that the reported figures could be as little as one percent of the actual totals.

In the last week of April 2020, Caixin, one of the most reliable publications in China, reported that a serological study had been carried out in Wuhan on 11,000 inhabitants. Extrapolating from its results, which showed that five to six percent of the sample of 11,000 persons carried antibodies for SARS-CoV-2, Caixin estimated that 500,000 people in the city had been infected, or 10 times the level of official Chinese government reporting. The publication was quickly deleted by Chinese government censors.¹³

The Chinese government has also attempted to obscure the origins of the pandemic with disinformation. On March 13, Chinese Foreign Ministry spokesperson Zhao Lijian suggested that the United States might have introduced the coronavirus to Wuhan.¹⁴ A month later, Zhao Lijian again posted Russian coronavirus and biowarfare-related disinformation, this time followed by online posts from Chinese ambassadors in 13 countries spread across the world.¹⁵ This was unprecedented diplomatic behavior for China, but not an accident. It was a concerted, deliberate, and preposterous disinformation campaign, repeated in May by CGTN, the China Global Television Network, which reposted the disinformation to the social media sites Weibo, Facebook, and Twitter.¹⁶ The history of Soviet and then Russian government biowarfare disinformation suggests that a country spreading such disinformation has or may have something to hide.¹⁷

5. *Laboratory accidents and the escape of highly dangerous pathogens from laboratories are frequent occurrences worldwide.* The accidental infection of researchers in the highest containment biosafety facilities—labelled BSL-2, BSL-3 and BSL-4—occurs worldwide, as do accidental releases by other means. In [an excellent review published in February 2019](#), Lynn Klotz of the Center for Arms Control and Non-Proliferation noted that three releases of Ebola and Marburg viruses from BSL-4 and lower-containment facilities in the United States had occurred due to incomplete inactivation of cultures. Releases via infection of researchers took place in the highest containment facilities in the United States—at the Centers for Disease Control and Prevention (CDC) in Atlanta and at the US Army Medical Research Institute of Infectious Diseases (USAMRIID)—but in all cases only the researcher became ill, and there was no further transmission of the pathogen.

“In an analysis circulated at the 2017 meeting for the Biological Weapons Convention, a conservative estimate shows that the probability is about 20 percent for a release of a mammalian-airborne-transmissible, highly pathogenic avian influenza virus into the

community from at least one of 10 labs over a 10-year period of developing and researching this type of pathogen,” Klotz wrote. “This percentage was calculated from FSAP [US Federal Select Agent Program] data for the years 2004 through 2010. Analysis of the FOIA NIH (National Institutes of Health) data gives a much higher release probability—that is, a factor five to 10 times higher, based on a smaller number of incident reports.”¹⁸

Between 2009 and 2015, the FSAP recorded 749 incidents in seven categories—not solely releases or researcher infections—from 276 facilities. In addition, Klotz recorded 11 confirmed releases of select agents that resulted in a laboratory-acquired infection in roughly 280 specifically approved laboratories in the United States between 2003 and 2017, a rate of just under one per year.¹⁹ A second publication in the *Bulletin* that covered closely-related subject matter and a personal communication from its author suggested that federally reported cases involving select agents were likely to be substantially undercounted:²⁰

There is a fundamental problem of using the defined select agents as a surrogate for potential pandemic agent releases from research labs. The vast majority of ‘classical BW agents’ that initially defined select agents in the US were selected specifically to be NOT capable of sustained transmission so as to better define the military tactical limits of a military employment and because the establishment of progressive transmission was considered unpredictable and possibly counterproductive in military operations, at least on the US side of offensive development in the 1940s-1960s.

As my historical review of lab escapes that resulted in pandemics or wide area epidemics published in the BAS found, most pandemic, continental or large scale community outbreaks originating from lab escapes came from civilian labs working with public or veterinary pathogens of non-military interest.

It takes only one superspreading graduate student or maintenance worker to start a pandemic.

It is known that a very large percentage of the individuals infected with the SARS-CoV-2 virus show no symptoms and do not become clinically ill, which would facilitate an unrecognized infection of one or more laboratory researchers.

6. *There have been laboratory accidents and escapes of highly dangerous pathogens*

in China in general and biosafety issues at the Wuhan Institute of Virology in particular. After the SARS epidemic in 2002-2003, which originated naturally in China and which China initially kept secret, work on the coronavirus pathogen that was responsible for the outbreak was undertaken in laboratories around the world. This research led to six cases of infection in laboratory workers: four in the National Institute of Virology in Beijing and one each in laboratories in Singapore and Taiwan.

The A1 Verse: The limit of the marginal

The laboratory-acquired infections of lab workers in Beijing led to short-lived outbreaks of SARS in the Beijing region in 2004.²¹

A second case of infected researchers in China resulted in brief outbreaks of disease in early December 2019. An outbreak of brucellosis began in an agricultural laboratory in Lanzhou (Gansu Province, central China) and spread to China's premier bird flu laboratory in Harbin (Heilongjiang Province, northeast China). It was linked to index cases involving graduate students who were exposed while conducting research and included at least 96 people.²²

7. Under what biosafety conditions was bat coronavirus research carried out at the Wuhan Institute of Virology? Most work—including all published work using live bat coronaviruses that were not SARS-CoV and MERS-CoV—was conducted under BSL-2 conditions.²³ This was consistent with both WHO and CDC recommendations.²⁴ BSL-2 provides only minimal protection against infection of laboratory researchers, and these regulations were almost certainly too lenient for working with bat coronaviruses. All such work should have been carried out under BSL-3 conditions. However, extremely high-risk gain of function (GoF) studies with bat SARS-related coronaviruses were carried out at BSL-3 or BSL-4. Statements made by various commentators claiming that the WIV worked only with RNA isolates and not with live viruses are untrue (as discussed in further detail in a following section).

In regard to the Wuhan Institute of Virology in particular, relevant information is again available from both Chinese and Western sources. Information from official Chinese government sources appeared in a Voice of America report which noted:

[T]here is Chinese evidence that the lab had safety problems. VOA has located

state media reports showing that there were security incidents flagged by national inspections as well as reported accidents that occurred when workers were trying to catch bats for study.

About a year before the corona virus outbreak, a security review conducted by a Chinese national team found the lab did not meet national standards in five categories.

The document on the lab's official website said after a rigorous and meticulous review, the team gave a high evaluation of the lab's overall safety management. "At the same time, the review team also put forward further rectification opinions on the five non-conformities and two observations found during the review."

In addition to problems in the lab, state media also reported that national reviewers found scientists were sloppy when they were handling bats.

One of the researchers working at the Wuhan Center for Disease Control & Prevention described to China's state media that he was once attacked by bats, and he ended up getting bat blood on his skin.

In another incident, the same researcher forgot to take protective measures, and the urine of a bat dripped "like rain onto the top of his head," reported China's Xinhua state news agent.²⁵

Also, information was leaked from the US Department of State and published in the *Washington Post* on April 14:

Two years before the novel coronavirus pandemic upended the world, U.S. Embassy officials visited a Chinese research facility in the city of Wuhan several times and sent two official warnings back to Washington about inadequate safety at the lab, which was conducting risky studies on corona viruses from bats. The cables have fueled discussions inside the U.S. government about whether this or another Wuhan lab was the source of the virus – even though conclusive proof has yet to emerge.

In January 2018, the U.S. Embassy in Beijing took the unusual step of repeatedly sending U.S. science diplomats to the Wuhan Institute of Virology, which had in 2015 become China's first laboratory to achieve the highest level of international bioresearch safety (known as BSL-4). WIV issued a news release in English about

the last of these visits, which occurred on March 27, 2018. The U.S. delegation was led by Jamison Fouss, the consul general in Wuhan, and Rick Switzer, the embassy's counselor of environment, science, technology and health. Last week, WIV erased that statement from its website, though it remains archived on the Internet.

What the U.S. officials learned during their visits concerned them so much that they dispatched two diplomatic cables categorized as Sensitive But Unclassified back to Washington. The cables warned about safety and management weaknesses at the WIV lab and proposed more attention and help. The first cable ... also warns that the lab's work on bat coronaviruses and their potential human transmission represented a risk of a new SARS-like pandemic.

"During interactions with scientists at the WIV laboratory, they noted the new lab has a serious shortage of appropriately trained technicians and investigators needed to safely operate this high-containment laboratory," states the Jan. 19, 2018, cable, which was drafted by two officials from the embassy's environment, science and health sections who met with the WIV scientists.

"Most importantly," the cable states, "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 diseases. 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."²⁶

The US government had supplied a portion of the funds to build the Wuhan Institute of Virology and these cables were an appeal for funds to support additional training in biosafety and biosecurity. There were similar concerns about the nearby Wuhan Center for Disease Control and Prevention lab, which operates entirely at BSL-2. Chinese government authorities did not provide the US government with samples of the virus obtained from either the earliest cases or from the Wuhan fish market. A US intelligence official commented: "The idea that it was just a totally natural occurrence is circumstantial. The evidence it leaked from the lab is circumstantial. Right now, the ledger on the side of it leaking from the lab is packed with bullet points, and there's almost nothing on the other side."²⁷

8. *What is the nature of the research being carried out in Zhengli Shi's laboratory at the Wuhan Institute of Virology?* Details of the most recent National Institute of Allergy and Infectious Diseases (NIAID) grant for WIV bat coronavirus surveillance and WIV bat coronavirus gain of function research are publicly available. The key activity for bat coronavirus surveillance is "Aim 1 ... We will sequence receptor binding domains (spike proteins) to identify viruses with the highest potential for spillover which we will include in our experimental investigations (Aim 3)." The key activity for bat coronavirus gain of function investigation is "Aim 3.... We will use S protein sequence data, infectious clone technology, in vitro and in vivo infection experiments, and analysis of receptor binding to test the hypothesis that % divergence thresholds in S protein sequences predict spillover potential."²⁸

Translated into something approaching lay language, Aim 3 states that de novo synthesis is to be used to construct a series of novel chimeric viruses, comprising recombinant hybrids using different spike proteins from each of a series of unpublished natural coronaviruses in an otherwise-constant genome of a bat coronavirus. The ability of the resulting novel viruses to infect human cells in culture and to infect laboratory animals would be tested. The underlying hypothesis is that a direct correlation would be found between the receptor-binding affinity of the spike protein and the ability to infect human cells in culture and to infect laboratory animals. This hypothesis would be tested by asking whether novel viruses encoding spike proteins with the highest receptor-binding affinity have the highest ability to infect human cells in culture and laboratory animals.

The WIV began its gain of function research program for bat coronaviruses in 2015. Using a natural virus, institute researchers made "substitutions in its RNA coding to make it more transmissible. They took a piece of the original SARS virus and inserted a snippet from a SARS-like bat coronavirus, resulting in a virus that is capable of infecting human cells."²⁹ This meant it could be transmitted from experimental animal to experimental animal by aerosol transmission, which means that it could do the same for humans. In other words, gain of function techniques were used to turn bat coronaviruses into human pathogens capable of causing a global pandemic.

There have been three publications, in 2015,³⁰ 2016 and 2017, describing the WIV gain of function research. The WIV, having learned both basic and traceless infectious-clone technology from joint research with a laboratory at the University of North Carolina (UNC) in 2015, initiated construction of novel chimeric coronaviruses without

UNC immediately thereafter. WIV's first publication on the use of basic infectious-clone technology to construct novel chimeric coronaviruses at WIV appeared in 2016.³¹ WIV's first publication on the use of traceless, signature-free infectious-clone technology also appeared in 2016.³²

As this article was being edited, two excellent publications appeared that provide greater technical detail on WIV's gain of function research, and readers should certainly examine these with care.³³ The two papers strongly support the argument that the SARS-CoV-2 outbreak was the results of an escape from one of the two Chinese virology laboratories in Wuhan.

The Chinese government has proudly stated that the WIV "preserves more than 1,500 strains of virus," the largest collection in Asia of bat and other coronaviruses.³⁴ (The government statement probably should have said 1,500 isolates rather than "strains.") The 2019 interview with Shi in *Scientific American* reports that the WIV had at least hundreds of individual strains.³⁵ These numbers have been reported by Chinese government authorities, and they are being taken at face value here.

From 2004 on, the WIV published many dozens of partial or full genome sequences of coronaviruses in their collection. On June 1, Daszak and Shi published partial genetic sequences of 781 Chinese bat coronaviruses, more than one-third of which had never been published previously.³⁶ There are also multiple published records of animal infection research with bat coronaviruses at the WIV. In order to carry out the research program described above, the WIV laboratory needs to use live viruses, and not just RNA fragments. This contradicts two of the assertions, made by some commentators, that Shi worked only with RNA fragments and that her laboratory did not maintain live viruses. On May 24, 2020, the director of the WIV acknowledged that the laboratory did have "three live strains of bat corona viruses on site," but implied only three.³⁷ Knowledgeable virologists assume that the number must be much higher, probably hundreds of live viral isolates.³⁸

It is precisely in the course of the kind of gain of function research that the WIV conducted that there would be the greatest likelihood of infection of a laboratory researcher. Many commentators have noted that millions of people in several western Chinese provinces, as well as in other South Asian countries, live their lives in daily proximity to bat caves and that serological testing has shown a fraction of these villagers to have antibodies to bat coronaviruses, showing that natural infection had

occurred. The commentators argue therefore that “the odds” are in favor of SARS-CoV-2 having arisen in the field, and that a laboratory escape is so implausible that it is out of consideration. The logic of “the odds” is specious: It would take only a single laboratory infection to overcome “the odds,” if such could in fact be reckoned. That is essentially what happened in the four SARS laboratory infections that occurred in the Beijing laboratory in 2004; “the odds” for exposure of villagers in Yunnan province were irrelevant.

Since the SARS-CoV-2 genome was decoded and published, there have been numerous statements from virologists that the genome shows no indication of genetic manipulation, and that this too supports the argument that it arose in the field and did not escape from a laboratory. Although this argument implicitly recognizes that the WIV laboratory was using genetic engineering technology, there is no reason to arbitrarily assume that only a bat coronavirus that was genetically modified might have escaped from the laboratory. Nevertheless, the second portion of the NIAID research grant design made absolutely clear that the WIV would be applying genetic engineering techniques to bat coronaviruses. Using the current standard genetic engineering technology, many alterations of several bases in the RNA genome would be undetectable, including construction of a chimeric coronavirus encoding an unpublished spike protein in an unpublished genome. This would be the equivalent of a natural mutation in several bases that coded for the spike proteins.

An article in Independent Science News by Jonathan Latham and Allison Wilson discusses another mechanism, described by Nikolai Petrovsky of Flinders University in Australia, that could have resulted in the SARS-CoV-2 virus that produced the pandemic:

Take a bat coronavirus that is not infectious to humans, and force its selection by culturing it with cells that express human ACE2 receptor, such cells having been created many years ago to culture SARS coronaviruses and you can force the bat virus to adapt to infect human cells via mutations in its spike protein, which would have the effect of increasing the strength of its binding to human ACE2, and inevitably reducing the strength of its binding to bat ACE2.

Viruses in prolonged culture will also develop other random mutations that do not affect its function. The result of these experiments is a virus that is highly virulent in humans but is sufficiently different that it no longer resembles the original bat

virus. Because the mutations are acquired randomly by selection there is no signature of a human gene jockey, but this is clearly a virus still created by human intervention.³⁹

Final comments. On April 30, Newsweek described a report produced by the US Defense Intelligence Agency which stated that “in early February, China’s Academy for Military Medical Sciences ‘concluded that it was impossible for them to scientifically determine whether the Covid-19 outbreak was caused naturally or accidentally from a laboratory incident.’” The author of a newly published paper analyzing the genome of SARS-COV-2 reported that “the COVID-19 virus is exquisitely adapted to infect humans... The virus’s ability to bind protein on human cells was far greater than its ability to bind the same protein in bats, which argues against bats being a direct source of the human virus.”⁴⁰

Overall, the data indicates that SARS-CoV-2 is uniquely adapted to infect humans, raising important questions as to whether it arose in nature by a rare chance event or whether its origins might lie elsewhere.

In climate change era, was Surfside collapse really an unpredictable “Black Swan” event?

Geng Shuang, a spokesman for the Chinese Ministry of Foreign Affairs, said. “China has mentioned many times that the origin of the virus is a scientific question, which should be evaluated by scientists and medical experts, and should not be politicized.”⁴¹ Essentially the same position on the possibility that a lab leak originated the pandemic was expressed by Xiao Qiang, a research scientist at the School of Information at the University of California, Berkeley. “I don’t think it is a conspiracy theory. I think it’s a legitimate question that needs to be investigated and answered. To understand exactly how this originated is critical knowledge for preventing this from happening in the future.”⁴²

But Chinese officials reacted angrily in April when Australian officials suggested that the World Health Organization should be able to quickly investigate a disease outbreak that could lead to a pandemic, retaliating by instituting trade restrictions on several Australian agricultural exports to China. In early May, the World Health Organization’s representative in China, Gauden Galea, publicly complained that China had refused

repeated requests to permit the WHO to participate in whatever investigations the Chinese government was undertaking itself. He said that the WHO had not been given access to laboratory logs at the WIV or the Wuhan Chinese Center for Disease Control and Prevention.⁴³

On May 18, prior to a meeting of the WHO governing board, the European Union submitted a draft resolution supported by 100 nations that asked the WHO “to work with other United Nations agencies to ‘identify the zoonotic source of the virus and the route of introduction to the human population, including the possible role of intermediate hosts. ... The document does not propose a review to identify missteps in how countries handled the outbreak and is instead forward-looking. It calls on the WHO to potentially arrange ‘scientific and collaborative field missions’ to help prevent similar future outbreaks. It also appears to rule out the possibility that the virus was man-made or experimented upon.”⁴⁴

The draft resolution did not mention Wuhan or China.⁴⁵

On May 18, China’s President Xi addressed the meeting of the governing body of the WHO via video. Ironically, Xi asked that countries “step up information sharing” but declared that China would support a review of the pandemic led by the WHO as long as it was “objective and impartial” and held after the pandemic was under control or over. The operative WHO resolution focuses on identifying “the zoonotic source of the virus” and says nothing about any forensic investigation.⁴⁶ The Chinese Foreign Ministry spokesman Zhao Lijian declared that “China supported an international inquiry all along,” and China’s *Global Times* asked: “Will China oppose scientific research into the virus’ origin? No, because it is a necessary move to fight COVID-19 in a scientific way and conducive to prevention measures and development of vaccines and medicine,” but added “Not only China-related factors but also those related to the US and other countries need to be included. Earlier confirmed cases than the previously known first infected case have continuously been found in the US. Among those diagnosed as having flu last winter, how many were coronavirus infections? All these clues shouldn’t be missed.”⁴⁷

What does this all mean at the present time? We have in China:

- a record of laboratory escape of the SARS virus in 2004 from a premier Chinese research institute.

- a record of poor biosafety in some of its high-containment facilities, including in the Wuhan institutes.
- a record of suppression of information in general, and in the case of SARS-CoV-2 in particular.
- the initiation of a disinformation campaign in regard to the origin of SARS-CoV-2, targeting US biological laboratories.
- a record of gain of function research at the Wuhan Institute of Virology, including passage of a bat coronavirus construct through experimental animals.

Writing in the *Bulletin of the Atomic Scientists* before the WHO governing board was convened, Filippa Lentzos advocated for a forensic investigation and described what it would entail:

Investigating the range of possible spillover sites—from the wet market, to an accidental lab or fieldwork infection, or an unnoticed lab leak—requires a forensic investigation. Obtaining case histories, epidemiological data, and viral samples from different times and places, including the earliest possible samples from infected individuals and samples from wildlife, is paramount... A forensic investigation would additionally involve auditing and sampling viral collections at relevant labs that had been studying coronaviruses, examining the types of experiments carried out and the viruses used, and reviewing the safety and security practices in place. Key data would also come from documents, including standard operating procedures at the labs and during fieldwork, risk assessments of individual experiments, experiment logs and fieldwork notebooks, training records, waste management logs, accident and infection records, facility maintenance and automated systems records, access logs, security camera footage and communications logs. ...A COVID-19 origins investigation will need to be negotiated and begun rapidly before relevant data diminishes or disappears entirely as time passes.⁴⁸

There is no semblance of any of this in the WHO resolution, and one can scarcely imagine that any of it would be permitted by the current administration in China. An investigation held after the pandemic “is under control” cannot possibly be carried out “rapidly,” and is in fact postponed into the indefinite future. Unfortunately, if there were any documentation in either of the two Wuhan virology institutes that recorded the infection of a laboratory researcher or an escape, or that either had a virus sample that

was extremely similar to SARS-CoV-2, one has to assume that such information has already been removed or destroyed.

Others have suggested that an international “commission, independent of the WHO, needs to be set up with the broad objective of how to ameliorate the next pandemic. Its mandate should go well beyond that of the WHO and part of its work could be to look at how COVID-19 started.”⁴⁹ Unfortunately it is equally difficult to envision such a commission coming to pass.

At present, the origin of SARS-CoV-2 remains unknown. The pros and cons regarding the two alternative possibilities—first, that it arose in the field as a natural evolution, as many virologists maintain, or second, that it may have been the consequence of bat coronavirus research in one of the two virology research institutes located in Wuhan that led to the infection of a laboratory researcher and subsequent escape—are equally based on inference and conjecture. The points gathered in this paper can be no more than suggestive. There is no hard scientific evidence to support either position. Both are inferences from circumstantial evidence. The US administration’s political hectoring only assures that it will be very difficult if not impossible to ever find out which is true.

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Notes

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The origin of COVID: Did people or nature open Pandora's box at Wuhan?

By Nicholas Wade | May 5, 2021



Members of the World Health Organization (WHO) team investigating the origins of the COVID-19 coronavirus arrive by car at the Wuhan Institute of Virology on February 3. (Photo by HECTOR RETAMAL/AFP via Getty Images)

The COVID-19 pandemic has disrupted lives the world over for more than a year. Its death toll will soon reach three million people. Yet the origin of pandemic remains uncertain: The political agendas of governments and scientists have generated thick

clouds of obfuscation, which the mainstream press seems helpless to dispel.

In what follows I will sort through the available scientific facts, which hold many clues as to what happened, and provide readers with the evidence to make their own judgments. I will then try to assess the complex issue of blame, which starts with, but extends far beyond, the government of China.

By the end of this article, you may have learned a lot about the molecular biology of viruses. I will try to keep this process as painless as possible. But the science cannot be avoided because for now, and probably for a long time hence, it offers the only sure thread through the maze.

The virus that caused the pandemic is known officially as SARS-CoV-2, but can be called SARS2 for short. As many people know, there are two main theories about its origin. One is that it jumped naturally from wildlife to people. The other is that the virus was under study in a lab, from which it escaped. It matters a great deal which is the case if we hope to prevent a second such occurrence.

I'll describe the two theories, explain why each is plausible, and then ask which provides the better explanation of the available facts. It's important to note that so far there is *no direct evidence* for either theory. Each depends on a set of reasonable conjectures but so far lacks proof. So I have only clues, not conclusions, to offer. But those clues point in a specific direction. And having inferred that direction, I'm going to delineate some of the strands in this tangled skein of disaster.

A tale of two theories. After the pandemic first broke out in December 2019, Chinese authorities reported that many cases had occurred in the wet market—a place selling wild animals for meat—in Wuhan. This reminded experts of the SARS1 epidemic of 2002, in which a bat virus had spread first to civets, an animal sold in wet markets, and from civets to people. A similar bat virus caused a second epidemic, known as MERS, in 2012. This time the intermediary host animal was camels.

The decoding of the virus's genome showed it belonged a viral family known as beta-coronaviruses, to which the SARS1 and MERS viruses also belong. The relationship supported the idea that, like them, it was a natural virus that had managed to jump from bats, via another animal host, to people. The wet market connection, the major point of similarity with the SARS1 and MERS epidemics, was soon broken: Chinese researchers found earlier cases in Wuhan with no link to the wet market. But that

seemed not to matter when so much further evidence in support of natural emergence was expected shortly.

Wuhan, however, is home of the Wuhan Institute of Virology, a leading world center for research on coronaviruses. So the possibility that the SARS2 virus had escaped from the lab could not be ruled out. Two reasonable scenarios of origin were on the table.

From early on, public and media perceptions were shaped in favor of the natural emergence scenario by strong statements from two scientific groups. These statements were not at first examined as critically as they should have been.

“We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin,” a group of virologists and others wrote in the [Lancet](#) on February 19, 2020, when it was really far too soon for anyone to be sure what had happened. Scientists “overwhelmingly conclude that this coronavirus originated in wildlife,” they said, with a stirring rallying call for readers to stand with Chinese colleagues on the frontline of fighting the disease.

Contrary to the letter writers’ assertion, the idea that the virus might have escaped from a lab invoked accident, not conspiracy. It surely needed to be explored, not rejected out of hand. A defining mark of good scientists is that they go to great pains to distinguish between what they know and what they don’t know. By this criterion, the signatories of the Lancet letter were behaving as poor scientists: They were assuring the public of facts they could not know for sure were true.

It later turned out that the Lancet letter had been [organized and drafted](#) by Peter Daszak, president of the EcoHealth Alliance of New York. Daszak’s organization funded coronavirus research at the Wuhan Institute of Virology. If the SARS2 virus had indeed escaped from research he funded, Daszak would be potentially culpable. This acute conflict of interest was not declared to the Lancet’s readers. To the contrary, the letter concluded, “We declare no competing interests.”

Virologists like Daszak had much at stake in the assigning of blame for the pandemic. For 20 years, mostly beneath the public’s attention, they had been playing a dangerous game. In their laboratories they routinely created viruses more dangerous than those that exist in nature. They argued that they could do so safely, and that by getting ahead of nature they could predict and prevent natural “spillovers,” the cross-over of viruses from an animal host to people. If SARS2 had indeed escaped from such a laboratory

Peter Daszak, a member of the World Health Organization (WHO) team investigating the origins of the COVID-19 coronavirus, talks on his cellphone at the Hilton Wuhan Optics Valley in Wuhan. (Photo by HECTOR RETAMAL/AFP via Getty Images)

experiment, a savage blowback could be expected, and the storm of public indignation would affect virologists everywhere, not just in China. "It would shatter the scientific edifice top to bottom," an *MIT Technology Review* editor, Antonio Regalado, said in March 2020.

A second statement that had enormous influence in shaping public attitudes was a letter (in other words an opinion piece, not a scientific article) published on 17 March 2020 in the journal *Nature Medicine*. Its authors were a group of virologists led by Kristian G. Andersen of the Scripps Research Institute. "Our analyses clearly show that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus," the five virologists declared in the second paragraph of their letter.

Unfortunately, this was another case of poor science, in the sense defined above. True, some older methods of cutting and pasting viral genomes retain tell-tale signs of manipulation. But newer methods, called "no-see-um" or "seamless" approaches, leave no defining marks. Nor do other methods for manipulating viruses such as serial passage, the repeated transfer of viruses from one culture of cells to another. If a virus has been manipulated, whether with a seamless method or by serial passage, there is no way of knowing that this is the case. Andersen and his colleagues were assuring their readers of something they could not know.

The discussion part of their letter begins, "It is improbable that SARS-CoV-2 emerged through laboratory manipulation of a related SARS-CoV-like coronavirus." But wait, didn't the lead say the virus had *clearly* not been manipulated? The authors' degree of certainty seemed to slip several notches when it came to laying out their reasoning.

The reason for the slippage is clear once the technical language has been penetrated. The two reasons the authors give for supposing manipulation to be improbable are decidedly inconclusive.

First, they say that the spike protein of SARS2 binds very well to its target, the human ACE2 receptor, but does so in a different way from that which physical calculations suggest would be the best fit. Therefore the virus must have arisen by natural selection, not manipulation.

If this argument seems hard to grasp, it's because it's so strained. The authors' basic assumption, not spelt out, is that anyone trying to make a bat virus bind to human cells could do so in only one way. First they would calculate the strongest possible fit between the human ACE2 receptor and the spike protein with which the virus latches onto it. They would then design the spike protein accordingly (by selecting the right string of amino acid units that compose it). Since the SARS2 spike protein is not of this calculated best design, the Andersen paper says, therefore it can't have been manipulated.

But this ignores the way that virologists do in fact get spike proteins to bind to chosen targets, which is not by calculation but by splicing in spike protein genes from other viruses or by serial passage. With serial passage, each time the virus's progeny are transferred to new cell cultures or animals, the more successful are selected until one emerges that makes a really tight bind to human cells. Natural selection has done all the heavy lifting. The Andersen paper's speculation about designing a viral spike protein through calculation has no bearing on whether or not the virus was manipulated by one of the other two methods.

The authors' second argument against manipulation is even more contrived. Although most living things use DNA as their hereditary material, a number of viruses use RNA, DNA's close chemical cousin. But RNA is difficult to manipulate, so researchers working on coronaviruses, which are RNA-based, will first convert the RNA genome to DNA. They manipulate the DNA version, whether by adding or altering genes, and then arrange for the manipulated DNA genome to be converted back into infectious RNA.

Only a certain number of these DNA backbones have been described in the scientific literature. Anyone manipulating the SARS2 virus "would probably" have used one of these known backbones, the Andersen group writes, and since SARS2 is not derived from any of them, therefore it was not manipulated. But the argument is conspicuously inconclusive. DNA backbones are quite easy to make, so it's obviously possible that SARS2 was manipulated using an unpublished DNA backbone.

And that's it. These are the two arguments made by the Andersen group in support of their declaration that the SARS2 virus was clearly not manipulated. And this conclusion, grounded in nothing but two inconclusive speculations, convinced the world's press that SARS2 could not have escaped from a lab. A technical critique of the Andersen letter takes it down in [harsher words](#).

Science is supposedly a self-correcting community of experts who constantly check each other's work. So why didn't other virologists point out that the Andersen group's argument was full of absurdly large holes? Perhaps because in today's universities speech can be very costly. Careers can be destroyed for stepping out of line. Any virologist who challenges the community's declared view risks having his next grant application turned down by the panel of fellow virologists that advises the government grant distribution agency.

The Daszak and Andersen letters were really political, not scientific, statements, yet were amazingly effective. Articles in the mainstream press repeatedly stated that a consensus of experts had ruled lab escape out of the question or extremely unlikely. Their authors relied for the most part on the Daszak and Andersen letters, failing to understand the yawning gaps in their arguments. Mainstream newspapers all have science journalists on their staff, as do the major networks, and these specialist reporters are supposed to be able to question scientists and check their assertions. But the Daszak and Andersen assertions went largely unchallenged.

Doubts about natural emergence. Natural emergence was the media's preferred theory until around February 2021 and the visit by a World Health Organization (WHO) commission to China. The commission's composition and access were heavily controlled by the Chinese authorities. Its members, who included the ubiquitous Daszak, kept asserting before, during, and after their visit that lab escape was extremely unlikely. But this was not quite the propaganda victory the Chinese authorities may have been hoping for. What became clear was that the Chinese had no evidence to offer the commission in support of the natural emergence theory.

This was surprising because both the SARS1 and MERS viruses had left copious traces in the environment. The intermediary host species of SARS1 was identified **within four months** of the epidemic's outbreak, and the host of MERS within nine months. Yet some 15 months after the SARS2 pandemic began, and after a presumably intensive search, Chinese researchers had failed to find either the original bat population, or the intermediate species to which SARS2 might have jumped, or any serological evidence that any Chinese population, including that of Wuhan, had ever been exposed to the virus prior to December 2019. Natural emergence remained a conjecture which, however plausible to begin with, had gained not a shred of supporting evidence in over a year.

And as long as that remains the case, it's logical to pay serious attention to the alternative conjecture, that SARS2 escaped from a lab.

Why would anyone want to create a novel virus capable of causing a pandemic? Ever since virologists gained the tools for manipulating a virus's genes, they have argued they could get ahead of a potential pandemic by exploring how close a given animal virus might be to making the jump to humans. And that justified lab experiments in enhancing the ability of dangerous animal viruses to infect people, virologists asserted.

With this rationale, they have recreated the 1918 flu virus, shown how the almost extinct polio virus can be synthesized from its published DNA sequence, and introduced a smallpox gene into a related virus.

These enhancements of viral capabilities are known blandly as gain-of-function experiments. With coronaviruses, there was particular interest in the spike proteins, which jut out all around the spherical surface of the virus and pretty much determine which species of animal it will target. In 2000 Dutch researchers, for instance, earned the gratitude of rodents everywhere by **genetically engineering** the spike protein of a mouse coronavirus so that it would attack only cats.

The spike proteins on the coronavirus's surface determine which animal it can infect. Image credit: CDC.gov

Virologists started studying bat coronaviruses in earnest after these turned out to be the source of both the SARS1 and MERS epidemics. In particular, researchers wanted to understand what changes needed to occur in a bat virus's spike proteins before it could infect people.

Researchers at the Wuhan Institute of Virology, led by China's leading expert on bat viruses, Shi Zheng-li or "Bat Lady," mounted frequent expeditions to the bat-infested caves of Yunnan in southern China and collected around a hundred different bat

coronaviruses.

Shi then teamed up with Ralph S. Baric, an eminent coronavirus researcher at the University of North Carolina. **Their work** focused on enhancing the ability of bat viruses to attack humans so as to “examine the emergence potential (that is, the potential to infect humans) of circulating bat CoVs [coronaviruses].” In pursuit of this aim, in November 2015 they created a novel virus by taking the backbone of the SARS1 virus and replacing its spike protein with one from a bat virus (known as SHC014-CoV). This manufactured virus was able to infect the cells of the human airway, at least when tested against a lab culture of such cells.

The SHC014-CoV/SARS1 virus is known as a chimera because its genome contains genetic material from two strains of virus. If the SARS2 virus were to have been cooked up in Shi’s lab, then its direct prototype would have been the SHC014-CoV/SARS1 chimera, the potential danger of which concerned many observers and prompted intense discussion.

“If the virus escaped, nobody could predict the trajectory,” **said** Simon Wain-Hobson, a virologist at the Pasteur Institute in Paris.

Baric and Shi referred to the obvious risks in their paper but argued they should be weighed against the benefit of foreshadowing future spillovers. Scientific review panels, they wrote, “may deem similar studies building chimeric viruses based on circulating strains too risky to pursue.” Given various restrictions being placed on gain-of function (GOF) research, matters had arrived in their view at “a crossroads of GOF research concerns; the potential to prepare for and mitigate future outbreaks must be weighed against the risk of creating more dangerous pathogens. In developing policies moving forward, it is important to consider the value of the data generated by these studies and whether these types of chimeric virus studies warrant further investigation versus the inherent risks involved.”

That statement was made in 2015. From the hindsight of 2021, one can say that the value of gain-of-function studies in preventing the SARS2 epidemic was zero. The risk was catastrophic, if indeed the SARS2 virus was generated in a gain-of-function experiment.

Inside the Wuhan Institute of Virology. Baric had developed, and taught Shi, a general method for engineering bat coronaviruses to attack other species. The specific targets

were human cells grown in cultures and humanized mice. These laboratory mice, a cheap and ethical stand-in for human subjects, are genetically engineered to carry the human version of a protein called ACE2 that studs the surface of cells that line the airways.

Shi returned to her lab at the Wuhan Institute of Virology and resumed the work she had started on genetically engineering coronaviruses to attack human cells. How can we be so sure?

A May 20, 2020, photo of the Wuhan Institute of Virology in Wuhan, where research on bat coronaviruses was conducted. (Photo by Kyodo News via Getty Images)

Because, by a strange twist in the story, her work was funded by the National Institute of Allergy and Infectious Diseases (NIAID), a part of the US National Institutes of Health (NIH). And grant proposals that funded her work, which are a matter of public record, specify exactly what she planned to do with the money.

The grants were assigned to the prime contractor, Daszak of the EcoHealth Alliance, who subcontracted them to Shi. Here are extracts from the grants for fiscal years 2018

and 2019. (“CoV” stands for coronavirus and “S protein” refers to the virus’s spike protein.)

“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 assays, and virus infection experiments across a range of cell cultures from different species and **humanized mice**.”

“We will use S protein sequence data, **infectious clone technology**, in vitro and in vivo infection experiments and analysis of receptor binding to test the hypothesis that % divergence thresholds in S protein sequences predict spillover potential.”

What this means, in non-technical language, is that Shi set out to create novel coronaviruses with the highest possible infectivity for human cells. Her plan was to take genes that coded for spike proteins possessing a variety of measured affinities for human cells, ranging from high to low. She would insert these spike genes one by one into the backbone of a number of viral genomes (“reverse genetics” and “infectious clone technology”), creating a series of chimeric viruses. These chimeric viruses would then be tested for their ability to attack human cell cultures (“in vitro”) and humanized mice (“in vivo”). And this information would help predict the likelihood of “spillover,” the jump of a coronavirus from bats to people.

The methodical approach was designed to find the best combination of coronavirus backbone and spike protein for infecting human cells. The approach could have generated SARS2-like viruses, and indeed may have created the SARS2 virus itself with the right combination of virus backbone and spike protein.

It cannot yet be stated that Shi did or did not generate SARS2 in her lab because her records have been sealed, but it seems she was certainly on the right track to have done so. “It is clear that the Wuhan Institute of Virology was systematically constructing novel chimeric coronaviruses and was assessing their ability to infect human cells and human-ACE2-expressing mice,” says Richard H. Ebright, a molecular biologist at Rutgers University and leading expert on biosafety.

“It is also clear,” Ebright said, “that, depending on the constant genomic contexts chosen for analysis, this work could have produced SARS-CoV-2 or a proximal progenitor of SARS-CoV-2.” “Genomic context” refers to the particular viral backbone used as the testbed for the spike protein.

The lab escape scenario for the origin of the SARS2 virus, as should by now be evident, is not mere hand-waving in the direction of the Wuhan Institute of Virology. It is a detailed proposal, based on the specific project being funded there by the NIAID.

Even if the grant required the work plan described above, how can we be sure that the plan was in fact carried out? For that we can rely on the word of Daszak, who has been much protesting for the last 15 months that lab escape was a ludicrous **conspiracy theory** invented by China-bashers.

On December 9, 2019, before the outbreak of the pandemic became generally known, Daszak gave an **interview** in which he talked in glowing terms of how researchers at the Wuhan Institute of Virology had been reprogramming the spike protein and generating chimeric coronaviruses capable of infecting humanized mice.

“And we have now found, you know, after 6 or 7 years of doing this, over 100 new SARS-related coronaviruses, very close to SARS,” Daszak says around minute 28 of the interview. “Some of them get into human cells in the lab, some of them can cause SARS disease in humanized mice models and are untreatable with therapeutic monoclonals and you can’t vaccinate against them with a vaccine. So, these are a clear and present danger...”

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“Interviewer: You say these are diverse coronaviruses and you can’t vaccinate against them, and no anti-virals—so what do we do?”

“Daszak: Well I think...coronaviruses—you can manipulate them in the lab pretty easily. Spike protein drives a lot of what happen with coronavirus, in zoonotic risk. So you can get the sequence, you can build the protein, and we work a lot with Ralph Baric at UNC to do this. Insert into the backbone of another virus and do some work in the lab. So you can get more predictive when you find a sequence. You’ve got this diversity. Now the logical progression for vaccines is, if you are going to develop a vaccine for SARS, people are going to use pandemic SARS, but let’s insert some of these other things and get a better vaccine.” The insertions he referred to perhaps included an element called the furin cleavage site, discussed below, which greatly increases viral infectivity for human cells.

In disjointed style, Daszak is referring to the fact that once you have generated a novel coronavirus that can attack human cells, you can take the spike protein and make it the basis for a vaccine.

One can only imagine Daszak's reaction when he heard of the outbreak of the epidemic in Wuhan a few days later. He would have known better than anyone the Wuhan Institute's goal of making bat coronaviruses infectious to humans, as well as the weaknesses in the institute's defense against their own researchers becoming infected.

But instead of providing public health authorities with the plentiful information at his disposal, he immediately launched a public relations campaign to persuade the world that the epidemic couldn't possibly have been caused by one of the institute's souped-up viruses. "The idea that this virus escaped from a lab is just pure baloney. It's simply not true," he declared in an April 2020 [interview](#).

The safety arrangements at the Wuhan Institute of Virology. Daszak was possibly unaware of, or perhaps he knew all too well, the [long history](#) of viruses escaping from even the best run laboratories. The smallpox virus escaped three times from labs in England in the 1960's and 1970's, causing 80 cases and 3 deaths. Dangerous viruses have leaked out of labs almost every year since. Coming to more recent times, the SARS1 virus has proved a true escape artist, leaking from laboratories in Singapore, Taiwan, and no less than four times from the Chinese National Institute of Virology in Beijing.

One reason for SARS1 being so hard to handle is that there were no vaccines available to protect laboratory workers. As Daszak mentioned in the December 19 interview quoted above, the Wuhan researchers too had been unable to develop vaccines against the coronaviruses they had designed to infect human cells. They would have been as defenseless against the SARS2 virus, if it were generated in their lab, as their Beijing colleagues were against SARS1.

A second reason for the severe danger of novel coronaviruses has to do with the required levels of lab safety. There are four degrees of safety, designated BSL1 to BSL4, with BSL4 being the most restrictive and designed for deadly pathogens like the Ebola virus.

The Wuhan Institute of Virology had a new BSL4 lab, but its state of readiness

considerably alarmed the State Department inspectors who visited it from the Beijing embassy in 2018. “The new lab has a serious shortage of appropriately trained technicians and investigators needed to safely operate this high-containment laboratory,” the inspectors wrote in a [cable](#) of January 19, 2018.

The real problem, however, was not the unsafe state of the Wuhan BSL4 lab but the fact that virologists worldwide don’t like working in BSL4 conditions. You have to wear a space suit, do operations in closed cabinets, and accept that everything will take twice as long. So the rules assigning each kind of virus to a given safety level were laxer than some might think was prudent.

Before 2020, the rules followed by virologists in China and elsewhere required that experiments with the SARS1 and MERS viruses be conducted in BSL3 conditions. But all other bat coronaviruses could be studied in BSL2, the next level down. BSL2 requires taking fairly minimal safety precautions, such as wearing lab coats and gloves, not sucking up liquids in a pipette, and putting up biohazard warning signs. Yet a gain-of-function experiment conducted in BSL2 might produce an agent more infectious than either SARS1 or MERS. And if it did, then lab workers would stand a high chance of infection, especially if unvaccinated.

Much of Shi’s work on gain-of-function in coronaviruses was performed at the BSL2 safety level, as is stated in her publications and other documents. She has said in an [interview](#) with *Science* magazine that “[t]he coronavirus research in our laboratory is conducted in BSL-2 or BSL-3 laboratories.”

“It is clear that some or all of this work was being performed using a biosafety standard—biosafety level 2, the biosafety level of a standard US dentist’s office—that would pose an unacceptably high risk of infection of laboratory staff upon contact with a virus having the transmission properties of SARS-CoV-2,” Ebright says.

“It also is clear,” he adds, “that this work never should have been funded and never should have been performed.”

This is a view he holds regardless of whether or not the SARS2 virus ever saw the inside of a lab.

Concern about safety conditions at the Wuhan lab was not, it seems, misplaced. According to a [fact sheet](#) issued by the State Department on January 15, 2021, “The

U.S. government has reason to believe that several researchers inside the WIV became sick in autumn 2019, before the first identified case of the outbreak, with symptoms consistent with both COVID-19 and common seasonal illnesses.”

David Asher, a fellow of the Hudson Institute and former consultant to the State Department, provided more detail about the incident at a [seminar](#). Knowledge of the incident came from a mix of public information and “some high end information collected by our intelligence community,” he said. Three people working at a BSL3 lab at the institute fell sick within a week of each other with severe symptoms that required hospitalization. This was “the first known cluster that we’re aware of, of victims of what we believe to be COVID-19.” Influenza could not completely be ruled out but seemed unlikely in the circumstances, he said.

Comparing the rival scenarios of SARS2 origin. The evidence above adds up to a serious case that the SARS2 virus could have been created in a lab, from which it then escaped. But the case, however substantial, falls short of proof. Proof would consist of evidence from the Wuhan Institute of Virology, or related labs in Wuhan, that SARS2 or a predecessor virus was under development there. For lack of access to such records, another approach is to take certain salient facts about the SARS2 virus and ask how well each is explained by the two rival scenarios of origin, those of natural emergence and lab escape. Here are four tests of the two hypotheses. A couple have some technical detail, but these are among the most persuasive for those who may care to follow the argument.

1) The place of origin. Start with geography. The two closest known relatives of the SARS2 virus were collected from bats living in caves in Yunnan, a province of southern China. If the SARS2 virus had first infected people living around the Yunnan caves, that would strongly support the idea that the virus had spilled over to people naturally. But this isn’t what happened. The pandemic broke out 1,500 kilometers away, in Wuhan.

Beta-coronaviruses, the family of bat viruses to which SARS2 belongs, infect the horseshoe bat *Rhinolophus affinis*, which ranges across southern China. The bats’ range is 50 kilometers, so it’s unlikely that any made it to Wuhan. In any case, the first cases of the COVID-19 pandemic probably occurred in September, when [temperatures in Hubei province](#) are already cold enough to send bats into hibernation.

What if the bat viruses infected some intermediate host first? You would need a longstanding population of bats in frequent proximity with an intermediate host, which in turn must often cross paths with people. All these exchanges of virus must take place somewhere outside Wuhan, a busy metropolis which so far as is known is not a natural habitat of *Rhinolophus* bat colonies. The infected person (or animal) carrying this highly transmissible virus must have traveled to Wuhan without infecting anyone else. No one in his or her family got sick. If the person jumped on a train to Wuhan, no fellow passengers fell ill.

It's a stretch, in other words, to get the pandemic to break out naturally outside Wuhan and then, without leaving any trace, to make its first appearance there.

For the lab escape scenario, a Wuhan origin for the virus is a no-brainer. Wuhan is home to China's leading center of coronavirus research where, as noted above, researchers were genetically engineering bat coronaviruses to attack human cells. They were doing so under the minimal safety conditions of a BSL2 lab. If a virus with the unexpected infectiousness of SARS2 had been generated there, its escape would be no surprise.

2) Natural history and evolution. The initial location of the pandemic is a small part of a larger problem, that of its natural history. Viruses don't just make one time jumps from one species to another. The coronavirus spike protein, adapted to attack bat cells, needs repeated jumps to another species, most of which fail, before it gains a lucky mutation. Mutation—a change in one of its RNA units—causes a different amino acid unit to be incorporated into its spike protein and makes the spike protein better able to attack the cells of some other species.

Through several more such mutation-driven adjustments, the virus adapts to its new host, say some animal with which bats are in frequent contact. The whole process then resumes as the virus moves from this intermediate host to people.

In the case of SARS1, researchers have documented the successive changes in its spike protein as the virus evolved step by step into a dangerous pathogen. After it had gotten from bats into civets, there were six further changes in its spike protein before it became a mild pathogen in people. After a further 14 changes, the virus was much better adapted to humans, and with a further four, the epidemic took off.

But when you look for the fingerprints of a similar transition in SARS2, a strange surprise awaits. The virus has changed hardly at all, at least until recently. From its very first appearance, it was well adapted to human cells. Researchers led by Alina Chan of the Broad Institute compared SARS2 with late stage SARS1, which by then was well adapted to human cells, and found that the two viruses were similarly well adapted. “By the time SARS-CoV-2 was first detected in late 2019, it was already pre-adapted to human transmission to an extent similar to late epidemic SARS-CoV,” they wrote.

Even those who think lab origin unlikely agree that SARS2 genomes are remarkably uniform. Baric writes that “early strains identified in Wuhan, China, showed limited genetic diversity, which suggests that the virus may have been introduced from a single source.”

A single source would of course be compatible with lab escape, less so with the massive variation and selection which is evolution’s hallmark way of doing business.

The uniform structure of SARS2 genomes gives no hint of any passage through an intermediate animal host, and no such host has been identified in nature.

Proponents of natural emergence suggest that SARS2 incubated in a yet-to-be found human population before gaining its special properties. Or that it jumped to a host animal outside China.

All these conjectures are possible, but strained. Proponents of a lab leak have a simpler explanation. SARS2 was adapted to human cells from the start because it was grown in humanized mice or in lab cultures of human cells, just as described in Daszak’s grant proposal. Its genome shows little diversity because the hallmark of lab cultures is uniformity.

Proponents of laboratory escape joke that of course the SARS2 virus infected an intermediary host species before spreading to people, and that they have identified it—a humanized mouse from the Wuhan Institute of Virology.

3) *The furin cleavage site.* The furin cleavage site is a minute part of the virus’s anatomy but one that exerts great influence on its infectivity. It sits in the middle of the SARS2 spike protein. It also lies at the heart of the puzzle of where the

virus came from.

The spike protein has two sub-units with different roles. The first, called S1, recognizes the virus's target, a protein called angiotensin converting enzyme-2 (or ACE2) which studs the surface of cells lining the human airways. The second, S2, helps the virus, once anchored to the cell, to fuse with the cell's membrane. After the virus's outer membrane has coalesced with that of the stricken cell, the viral genome is injected into the cell, hijacks its protein-making machinery and forces it to generate new viruses.

But this invasion cannot begin until the S1 and S2 subunits have been cut apart. And there, right at the S1/S2 junction, is the furin cleavage site that ensures the spike protein will be cleaved in exactly the right place.

The virus, a model of economic design, does not carry its own cleaver. It relies on the cell to do the cleaving for it. Human cells have a protein cutting tool on their surface known as furin. Furin will cut any protein chain that carries its signature target cutting site. This is the sequence of amino acid units proline-arginine-arginine-alanine, or PRRA in the code that refers to each amino acid by a letter of the alphabet. PRRA is the amino acid sequence at the core of SARS2's furin cleavage site.

Viruses have all kinds of clever tricks, so why does the furin cleavage site stand out? Because of all known SARS-related beta-coronaviruses, only SARS2 possesses a furin cleavage site. All the other viruses have their S2 unit cleaved at a different site and by a different mechanism.

How then did SARS2 acquire its furin cleavage site? Either the site evolved naturally, or it was inserted by researchers at the S1/S2 junction in a gain-of-function experiment.

Consider natural origin first. Two ways viruses evolve are by mutation and by recombination. Mutation is the process of random change in DNA (or RNA for coronaviruses) that usually results in one amino acid in a protein chain being switched for another. Many of these changes harm the virus but natural selection retains the few that do something useful. Mutation is the process by which the SARS1 spike protein gradually switched its preferred target cells from those of bats to civets, and then to humans.

Mutation seems a less likely way for SARS2's furin cleavage site to be generated, even though it can't completely be ruled out. The site's four amino acid units are all together, and all at just the right place in the S1/S2 junction. Mutation is a random process triggered by copying errors (when new viral genomes are being generated) or by chemical decay of genomic units. So it typically affects single amino acids at different spots in a protein chain. A string of amino acids like that of the furin cleavage site is much more likely to be acquired all together through a quite different process known as recombination.

Recombination is an inadvertent swapping of genomic material that occurs when two viruses happen to invade the same cell, and their progeny are assembled with bits and pieces of RNA belonging to the other. Beta-coronaviruses will only combine with other beta-coronaviruses but can acquire, by recombination, almost any genetic element present in the collective genomic pool. What they cannot acquire is an element the pool does not possess. And no known SARS-related beta-coronavirus, the class to which SARS2 belongs, possesses a furin cleavage site.

Proponents of natural emergence say SARS2 could have picked up the site from some as yet unknown beta-coronavirus. But bat SARS-related beta-coronaviruses evidently don't need a furin cleavage site to infect bat cells, so there's no great likelihood that any in fact possesses one, and indeed none has been found so far.

The proponents' next argument is that SARS2 acquired its furin cleavage site from people. A predecessor of SARS2 could have been circulating in the human population for months or years until at some point it acquired a furin cleavage site from human cells. It would then have been ready to break out as a pandemic.

If this is what happened, there should be traces in hospital surveillance records of the people infected by the slowly evolving virus. But none has so far come to light. According to the WHO [report on the origins of the virus](#), the sentinel hospitals in Hubei province, home of Wuhan, routinely monitor influenza-like illnesses and "no evidence to suggest substantial SARSCoV-2 transmission in the months preceding the outbreak in December was observed."

So it's hard to explain how the SARS2 virus picked up its furin cleavage site naturally, whether by mutation or recombination.

That leaves a gain-of-function experiment. For those who think SARS2 may have escaped from a lab, explaining the furin cleavage site is no problem at all. “Since 1992 the virology community has known that the one sure way to make a virus deadlier is to give it a furin cleavage site at the S1/S2 junction in the laboratory,” **writes** Steven Quay, a biotech entrepreneur interested in the origins of SARS2. “At least 11 gain-of-function experiments, adding a furin site to make a virus more infective, are published in the open literature, including [by] Dr. Zhengli Shi, head of coronavirus research at the Wuhan Institute of Virology.”

4) *A question of codons.* There’s another aspect of the furin cleavage site that narrows the path for a natural emergence origin even further.

As everyone knows (or may at least recall from high school), the genetic code uses three units of DNA to specify each amino acid unit of a protein chain. When read in groups of 3, the 4 different kinds of DNA unit can specify $4 \times 4 \times 4$ or 64 different triplets, or codons as they are called. Since there are only 20 kinds of amino acid, there are more than enough codons to go around, allowing some amino acids to be specified by more than one codon. The amino acid arginine, for instance, can be designated by any of the six codons CGU, CGC, CGA, CGG, AGA or AGG, where A, U, G and C stand for the four different kinds of unit in RNA.

Here’s where it gets interesting. Different organisms have different codon preferences. Human cells like to designate arginine with the codons CGT, CGC or CGG. But CGG is coronavirus’s least popular codon for arginine. Keep that in mind when looking at how the amino acids in the furin cleavage site are encoded in the SARS2 genome.

Now the functional reason why SARS2 has a furin cleavage site, and its cousin viruses don’t, can be seen by lining up (in a computer) the string of nearly 30,000 nucleotides in its genome with those of its cousin coronaviruses, of which the closest so far known is one called RaTG13. Compared with RaTG13, SARS2 has a 12-nucleotide insert right at the S1/S2 junction. The insert is the sequence T-CCT-CGG-CGG-GC. The CCT codes for proline, the two CGG’s for two arginines, and the GC is the beginning of a GCA codon that codes for alanine.

There are several curious features about this insert but the oddest is that of the two side-by-side CGG codons. Only 5 percent of SARS2’s arginine codons are CGG, and the double codon CGG-CGG has not been found in any other beta-

coronavirus. So how did SARS2 acquire a pair of arginine codons that are favored by human cells but not by coronaviruses?

Proponents of natural emergence have an up-hill task to explain all the features of SARS2's furin cleavage site. They have to postulate a recombination event at a site on the virus's genome where recombinations are rare, and the insertion of a 12-nucleotide sequence with a double arginine codon unknown in the beta-coronavirus repertoire, at the only site in the genome that would significantly expand the virus's infectivity.

"Yes, but your wording makes this sound unlikely—viruses are specialists at unusual events," is the riposte of David L. Robertson, a virologist at the University of Glasgow who regards lab escape as a conspiracy theory. "Recombination is naturally very, very frequent in these viruses, there are recombination breakpoints in the spike protein and these codons appear unusual exactly because we've not sampled enough."

Robertson is correct that evolution is always producing results that may seem unlikely but in fact are not. Viruses can generate untold numbers of variants but we see only the one-in-a-billion that natural selection picks for survival. But this argument could be pushed too far. For instance, any result of a gain-of-function experiment could be explained as one that evolution would have arrived at in time. And the numbers game can be played the other way. For the furin cleavage site to arise naturally in SARS2, a chain of events has to happen, each of which is quite unlikely for the reasons given above. A long chain with several improbable steps is unlikely to ever be completed.

RELATED: Caltech's David Baltimore discusses the debate over origins of SARS-CoV-2

For the lab escape scenario, the double CGG codon is no surprise. The human-preferred codon is routinely used in labs. So anyone who wanted to insert a furin cleavage site into the virus's genome would synthesize the PRRA-making sequence in the lab and would be likely to use CGG codons to do so.

"When I first saw the furin cleavage site in the viral sequence, with its arginine codons, I said to my wife it was the smoking gun for the origin of the virus," said

David Baltimore, an eminent virologist and former president of CalTech. “These features make a powerful challenge to the idea of a natural origin for SARS2,” he said. [1]

A third scenario of origin. There’s a variation on the natural emergence scenario that’s worth considering. This is the idea that SARS2 jumped directly from bats to humans, without going through an intermediate host as SARS1 and MERS did. A leading advocate is the virologist David Robertson who notes that SARS2 can attack several other species besides humans. He believes the virus **evolved a generalist capability while still in bats**. Because the bats it infects are widely distributed in southern and central China, the virus had ample opportunity to jump to people, even though it seems to have done so on only one known occasion. Robertson’s thesis explains why no one has so far found a trace of SARS2 in any intermediate host or in human populations surveilled before December 2019. It would also explain the puzzling fact that SARS2 has not changed since it first appeared in humans—it didn’t need to because it could already attack human cells efficiently. One problem with this idea, though, is that if SARS2 jumped from bats to people in a single leap and hasn’t changed much since, it should still be good at infecting bats. And it seems it isn’t.

“Tested bat species are poorly infected by SARS-CoV-2 and they are therefore unlikely to be the direct source for human infection,” **write a scientific group** skeptical of natural emergence.

Still, Robertson may be onto something. The bat coronaviruses of the Yunnan caves can infect people directly. In April 2012 six miners clearing bat guano from the Mojiang mine contracted severe pneumonia with COVID-19-like symptoms and three eventually died. A virus isolated from the Mojiang mine, called RaTG13, is still the closest known relative of SARS2. Much mystery surrounds the origin, reporting and strangely low affinity of RaTG13 for bat cells, as well as the nature of 8 similar viruses that Shi **reports** she collected at the same time but has not yet published despite their great relevance to the ancestry of SARS2. But all that is a story for another time. The point here is that bat viruses can infect people directly, though only in special conditions.

So who else, besides miners excavating bat guano, comes into particularly close contact with bat coronaviruses? Well, coronavirus researchers do. Shi says she and her group collected more than 1,300 bat samples during some eight visits to the Mojiang cave between 2012 and 2015, and there were doubtless many expeditions to other Yunnan caves.

Imagine the researchers making frequent trips from Wuhan to Yunnan and back, stirring up bat guano in dark caves and mines, and now you begin to see a possible missing link between the two places. Researchers could have gotten infected during their collecting trips, or while working with the new viruses at the Wuhan Institute of Virology. The virus that escaped from the lab would have been a natural virus, not one cooked up by gain of function.

The direct-from-bats thesis is a chimera between the natural emergence and lab escape scenarios. It's a possibility that can't be dismissed. But against it are the facts that 1) both SARS2 and RaTG13 seem to have only feeble affinity for bat cells, so one can't be fully confident that either ever saw the inside of a bat; and 2) the theory is no better than the natural emergence scenario at explaining how SARS2 gained its furin cleavage site, or why the furin cleavage site is determined by human-preferred arginine codons instead of by the bat-preferred codons.

Where we are so far. Neither the natural emergence nor the lab escape hypothesis can yet be ruled out. There is still no direct evidence for either. So no definitive conclusion can be reached.

That said, the available evidence leans more strongly in one direction than the other. Readers will form their own opinion. But it seems to me that proponents of lab escape can explain all the available facts about SARS2 considerably more easily than can those who favor natural emergence.

It's documented that researchers at the Wuhan Institute of Virology were doing gain-of-function experiments designed to make coronaviruses infect human cells and humanized mice. This is exactly the kind of experiment from which a SARS2-like virus could have emerged. The researchers were not vaccinated against the viruses under study, and they were working in the minimal safety conditions of a BSL2 laboratory. So escape of a virus would not be at all surprising. In all of China, the pandemic broke out on the doorstep of the Wuhan institute. The virus was already well adapted to humans, as expected for a virus grown in humanized mice. It possessed an unusual enhancement, a furin cleavage site, which is not possessed by any other known SARS-related beta-coronavirus, and this site included a double arginine codon also unknown among beta-coronaviruses. What more evidence could you want, aside from the presently unobtainable lab records documenting SARS2's creation?

Proponents of natural emergence have a rather harder story to tell. The plausibility of

their case rests on a single surmise, the expected parallel between the emergence of SARS2 and that of SARS1 and MERS. But none of the evidence expected in support of such a parallel history has yet emerged. No one has found the bat population that was the source of SARS2, if indeed it ever infected bats. No intermediate host has presented itself, despite an intensive search by Chinese authorities that included the testing of 80,000 animals. There is no evidence of the virus making multiple independent jumps from its intermediate host to people, as both the SARS1 and MERS viruses did. There is no evidence from hospital surveillance records of the epidemic gathering strength in the population as the virus evolved. There is no explanation of why a natural epidemic should break out in Wuhan and nowhere else. There is no good explanation of how the virus acquired its furin cleavage site, which no other SARS-related beta-coronavirus possesses, nor why the site is composed of human-preferred codons. The natural emergence theory battles a bristling array of implausibilities.

The records of the Wuhan Institute of Virology certainly hold much relevant information. But Chinese authorities seem unlikely to release them given the substantial chance that they incriminate the regime in the creation of the pandemic. Absent the efforts of some courageous Chinese whistle-blower, we may already have at hand just about all of the relevant information we are likely to get for a while.

So it's worth trying to assess responsibility for the pandemic, at least in a provisional way, because the paramount goal remains to prevent another one. Even those who aren't persuaded that lab escape is the more likely origin of the SARS2 virus may see reason for concern about the present state of regulation governing gain-of-function research. There are two obvious levels of responsibility: the first, for allowing virologists to perform gain-of-function experiments, offering minimal gain and vast risk; the second, if indeed SARS2 was generated in a lab, for allowing the virus to escape and unleash a world-wide pandemic. Here are the players who seem most likely to deserve blame.

1. *Chinese virologists.* First and foremost, Chinese virologists are to blame for performing gain-of-function experiments in mostly BSL2-level safety conditions which were far too lax to contain a virus of unexpected infectiousness like SARS2. If the virus did indeed escape from their lab, they deserve the world's censure for a foreseeable accident that has already caused the deaths of three million people. True, Shi was trained by French virologists, worked closely with American virologists and was following international rules for the containment of

coronaviruses. But she could and should have made her own assessment of the risks she was running. She and her colleagues bear the responsibility for their actions.

I have been using the Wuhan Institute of Virology as a shorthand for all virological activities in Wuhan. It's possible that SARS2 was generated in some other Wuhan lab, perhaps in an attempt to make a vaccine that worked against all coronaviruses. But until the role of other Chinese virologists is clarified, Shi is the public face of Chinese work on coronaviruses, and provisionally she and her colleagues will stand first in line for opprobrium.

2. Chinese authorities. China's central authorities did not generate SARS2, but they sure did their utmost to conceal the nature of the tragedy and China's responsibility for it. They suppressed all records at the Wuhan Institute of Virology and closed down its virus databases. They released a trickle of information, much of which may have been outright false or designed to misdirect and mislead. They did their best to manipulate the WHO's inquiry into the virus's origins, and led the commission's members on a fruitless run-around. So far they have proved far more interested in deflecting blame than in taking the steps necessary to prevent a second pandemic.

3. The worldwide community of virologists. Virologists around the world are a loose-knit professional community. They write articles in the same journals. They attend the same conferences. They have common interests in seeking funds from governments and in not being overburdened with safety regulations.

Virologists knew better than anyone the dangers of gain-of-function research. But the power to create new viruses, and the research funding obtainable by doing so, was too tempting. They pushed ahead with gain-of-function experiments. They lobbied against the moratorium imposed on Federal funding for gain-of-function research in 2014, and it was raised in 2017.

The benefits of the research in preventing future epidemics have so far been nil, the risks vast. If research on the SARS1 and MERS viruses could only be done at the BSL3 safety level, it was surely illogical to allow any work with novel coronaviruses at the lesser level of BSL2. Whether or not SARS2 escaped from a lab, virologists around the world have been playing with fire.

Their behavior has long alarmed other biologists. In 2014 scientists calling themselves the Cambridge Working Group urged caution on creating new viruses. In prescient words, they specified the risk of creating a SARS2-like virus. “Accident risks with newly created ‘potential pandemic pathogens’ raise grave new concerns,” they wrote. “Laboratory creation of highly transmissible, novel strains of dangerous viruses, especially but not limited to influenza, poses substantially increased risks. An accidental infection in such a setting could trigger outbreaks that would be difficult or impossible to control.”

When molecular biologists discovered a technique for moving genes from one organism to another, they held a public conference at Asilomar in 1975 to discuss the possible risks. Despite much internal opposition, they drew up a list of stringent safety measures that could be relaxed in future—and duly were—when the possible hazards had been better assessed.

When the CRISPR technique for editing genes was invented, biologists convened a joint report by the US, UK and Chinese national academies of science to urge restraint on making heritable changes to the human genome. Biologists who invented gene drives have also been open about the dangers of their work and have sought to involve the public.

You might think the SARS2 pandemic would spur virologists to re-evaluate the benefits of gain-of-function research, even to engage the public in their deliberations. But no. Many virologists deride lab escape as a conspiracy theory, and others say nothing. They have barricaded themselves behind a Chinese wall of silence which so far is working well to allay, or at least postpone, journalists’ curiosity and the public’s wrath. Professions that cannot regulate themselves deserve to get regulated by others, and this would seem to be the future that virologists are choosing for themselves.

4. *The US role in funding the Wuhan Institute of Virology.*[2] From June 2014 to May 2019, Daszak’s EcoHealth Alliance had a grant from the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, to do gain-of-function research with coronaviruses at the Wuhan Institute of Virology. Whether or not SARS2 is the product of that research, it seems a questionable policy to farm out high-risk research to foreign labs using minimal safety precautions. And if the SARS2 virus did indeed escape from the Wuhan

institute, then the NIH will find itself in the terrible position of having funded a disastrous experiment that led to the death of more than 3 million worldwide, including more than half a million of its own citizens.

The responsibility of the NIAID and NIH is even more acute because for the first three years of the grant to EcoHealth Alliance there was a moratorium on funding gain-of-function research. When the moratorium expired in 2017, it didn't just vanish but was replaced by a reporting system, the Potential Pandemic Pathogens Control and Oversight (P3CO) Framework, which required agencies to report for review any dangerous gain-of-function work they wished to fund.

The moratorium, referred to officially as a "pause," specifically barred funding any gain-of-function research that increased the pathogenicity of the flu, MERS or SARS viruses. It **defined gain-of-function** very simply and broadly as "research that improves the ability of a pathogen to cause disease."

But then a **footnote** on p.2 of the moratorium document states that "[a]n exception from the research pause may be obtained if the head of the USG funding agency determines that the research is urgently necessary to protect the public health or national security."

This seemed to mean that either the director of the NIAID, Anthony Fauci, or the director of the NIH, Francis Collins, or maybe both, would have invoked the exemption in order to keep the money flowing to Shi's gain-of-function research, and later to avoid notifying the federal reporting system of her research.

"Unfortunately, the NIAID Director and the NIH Director exploited this loophole to issue exemptions to projects subject to the Pause –preposterously asserting the exempted research was 'urgently necessary to protect public health or national security'—thereby nullifying the Pause," Dr. Richard Ebright said in an **interview** with Independent Science News.

But it's not so clear that the NIH thought it necessary to invoke any loopholes. Fauci told a Senate hearing on May 11 that "the NIH and NIAID categorically has not funded gain-of-function research to be conducted in the Wuhan Institute of Virology."

This was a surprising statement in view of all the evidence about Shi's

experiments with enhancing coronaviruses and the language of the moratorium statute defining gain-of-function as “any research that improves the ability of a pathogen to cause disease.”

The explanation may be one of definition. Daszak’s EcoHealth Alliance, for one, believes that the term gain-of-function applies only to enhancements of viruses that infect humans, not to animal viruses. “So gain-of-function research refers specifically to the manipulation of human viruses so as to be either more easily transmissible or to cause worse infection or be easier to spread,” an Alliance official told The Dispatch Fact Check.

If the NIH shares the EcoHealth Alliance view that “gain of function” applies only to human viruses, that would explain why Fauci could assure the Senate it had never funded such research at the Wuhan Institute of Virology. But the legal basis of such a definition is unclear, and it differs from that of the moratorium language which was presumably applicable.

Definitions aside, the bottom line is that the National Institutes of Health was supporting research of a kind that could have generated the SARS2 virus, in an unsupervised foreign lab that was doing work in BSL2 biosafety conditions.

In conclusion. If the case that SARS2 originated in a lab is so substantial, why isn’t this more widely known? As may now be obvious, there are many people who have reason not to talk about it. The list is led, of course, by the Chinese authorities. But virologists in the United States and Europe have no great interest in igniting a public debate about the gain-of-function experiments that their community has been pursuing for years.

Nor have other scientists stepped forward to raise the issue. Government research funds are distributed on the advice of committees of scientific experts drawn from universities. Anyone who rocks the boat by raising awkward political issues runs the risk that their grant will not be renewed and their research career will be ended. Maybe good behavior is rewarded with the many perks that slosh around the distribution system. And if you thought that Andersen and Daszak might have blotted their reputation for scientific objectivity after their partisan attacks on the lab escape scenario, look at the second and third names on this list of recipients of an \$82 million grant announced by the National Institute of Allergy and Infectious Diseases in August 2020.

The US government shares a strange common interest with the Chinese authorities: Neither is keen on drawing attention to the fact that Shi's coronavirus work was funded by the US National Institutes of Health. One can imagine the behind-the-scenes conversation in which the Chinese government says, "If this research was so dangerous, why did you fund it, and on our territory too?" To which the US side might reply, "Looks like it was you who let it escape. But do we really need to have this discussion in public?"

Fauci is a longtime public servant who served with integrity under President Trump and has resumed leadership in the Biden Administration in handling the COVID-19 epidemic. Congress, no doubt understandably, may have little appetite for hauling him over the coals for the apparent lapse of judgment in funding gain-of-function research in Wuhan.

To these serried walls of silence must be added that of the mainstream media. To my knowledge, no major newspaper or television network has yet provided readers with an in-depth news story of the lab escape scenario, such as the one you have just read, although some have run brief editorials or opinion pieces. One might think that any plausible origin of a virus that has killed three million people would merit a serious investigation. Or that the wisdom of continuing gain-of-function research, regardless of the virus's origin, would be worth some probing. Or that the funding of gain-of-function research by the NIH and NIAID during a moratorium on such funding would bear investigation. What accounts for the media's apparent lack of curiosity?

The virologists' *omertà* is one reason. Science reporters, unlike political reporters, have little innate skepticism of their sources' motives; most see their role largely as purveying the wisdom of scientists to the unwashed masses. So when their sources won't help, these journalists are at a loss.

Another reason, perhaps, is the migration of much of the media toward the left of the political spectrum. Because President Trump said the virus had escaped from a Wuhan lab, editors gave the idea little credence. They joined the virologists in regarding lab escape as a dismissible conspiracy theory. During the Trump administration, they had no trouble in rejecting the position of the intelligence services that lab escape could not be ruled out. But when Avril Haines, President Biden's director of national intelligence, said the same thing, she too was largely ignored. This is not to argue that editors should have endorsed the lab escape scenario, merely that

they should have explored the possibility fully and fairly.

People round the world who have been pretty much confined to their homes for the last year might like a better answer than their media are giving them. Perhaps one will emerge in time. After all, the more months pass without the natural emergence theory gaining a shred of supporting evidence, the less plausible it may seem. Perhaps the international community of virologists will come to be seen as a false and self-interested guide. The common sense perception that a pandemic breaking out in Wuhan might have something to do with a Wuhan lab cooking up novel viruses of maximal danger in unsafe conditions could eventually displace the ideological insistence that whatever Trump said can't be true.

And then let the reckoning begin.

Notes

[1] This quotation was added to the article after initial publication.

[2] Section revised May 18, 2021

Acknowledgements

The first person to take a serious look at the origins of the SARS2 virus was Yuri Deigin, a biotech entrepreneur in Russia and Canada. In a long and brilliant [essay](#), he dissected the molecular biology of the SARS2 virus and raised, without endorsing, the possibility that it had been manipulated. The essay, published on April 22, 2020, provided a roadmap for anyone seeking to understand the virus's origins. Deigin packed so much information and analysis into his essay that some have doubted it could be the work of a single individual and suggested some intelligence agency must have authored it. But the essay is written with greater lightness and humor than I suspect are ever found in CIA or KGB reports, and I see no reason to doubt that Deigin is its very capable sole author.

In Deigin's wake have followed several other skeptics of the virologists' orthodoxy. Nikolai Petrovsky calculated how tightly the SARS2 virus binds to the ACE2 receptors of various species and found to his surprise that it seemed [optimized for the human receptor](#), leading him to infer the virus might have been generated in a laboratory. Alina Chan published a [paper](#) showing that SARS2 from its first appearance was very well adapted to human cells.

One of the very few establishment scientists to have questioned the virologists' absolute rejection of lab escape is Richard Ebright, who has long warned against the dangers of gain-of-function research. Another is David A. Relman of Stanford University. "Even though strong opinions abound, none of these scenarios can be confidently ruled in or ruled out with currently available facts," he wrote. Kudos too to Robert Redfield, former director of the Centers for Disease Control and Prevention, who told CNN on March 26, 2021 that the "most likely" cause of the epidemic was "from a laboratory," because he doubted that a bat virus could become an extreme human pathogen overnight, without taking time to evolve, as seemed to be the case with SARS2.

Steven Quay, a physician-researcher, has applied statistical and bioinformatic tools to ingenious explorations of the virus's origin, showing for instance how the hospitals receiving the early patients are clustered along the Wuhan N°2 subway line which connects the Institute of Virology at one end with the international airport at the other, the perfect conveyor belt for distributing the virus from lab to globe.

In June 2020 Milton Leitenberg published an early survey of the evidence favoring lab escape from gain-of-function research at the Wuhan Institute of Virology.

Many others have contributed significant pieces of the puzzle. "Truth is the daughter," said Francis Bacon, "not of authority but time." The efforts of people such as those named above are what makes it so.

As the coronavirus crisis shows, we need science now more than ever.

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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 prevalently 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	<i>R. sinicus</i> (1)
October, 2011	anal swab	8	3	3	<i>R. sinicus</i> (3)
May, 2012	anal swab & feces	54	9	4	<i>R. sinicus</i> (4)
September, 2012	feces	39	20	19	<i>R. sinicus</i> (16)
					<i>R. ferrumequinum</i> (3)
April, 2013	feces	52	21	16	<i>R. sinicus</i> (16)
July, 2013	anal swab & feces	115	9	8	<i>R. sinicus</i> (8)
May, 2014	feces	131	8	4	<i>A. stoliczkanus</i> (3)
					<i>R. affinis</i> (1)
October, 2014	anal swab	19	4	4	<i>R. sinicus</i> (4)
May, 2015	feces	145	3	0	
October, 2015	anal swab	25	6	5	<i>R. sinicus</i> (5)
Total		602	84	64	R (61) A (3)

<https://doi.org/10.1371/journal.ppat.1006698.t001>

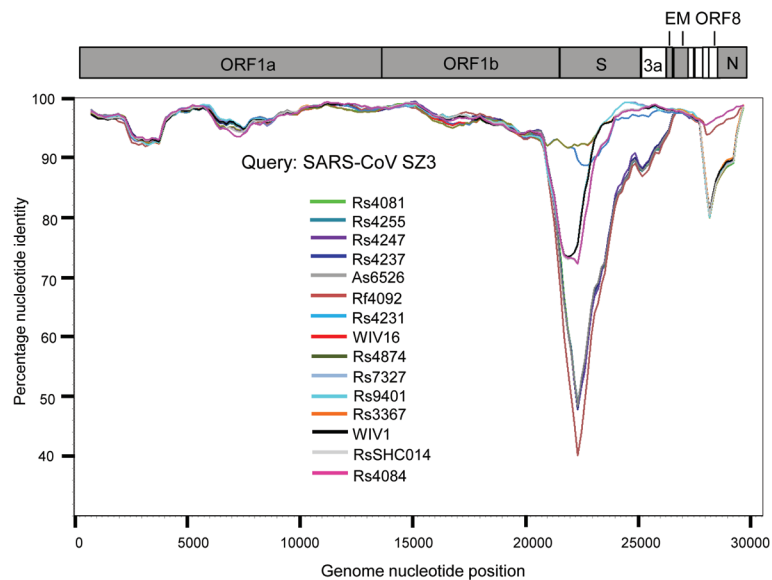


Fig 1. Similarity plot based on the full-length genome sequence of civet SARS-CoV SZ3. Full-length genome sequences of all SARS-CoV detected in bats from the cave investigated in this study were used as reference sequences. The analysis was performed with the Kimura model, a window size of 1500 base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

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,

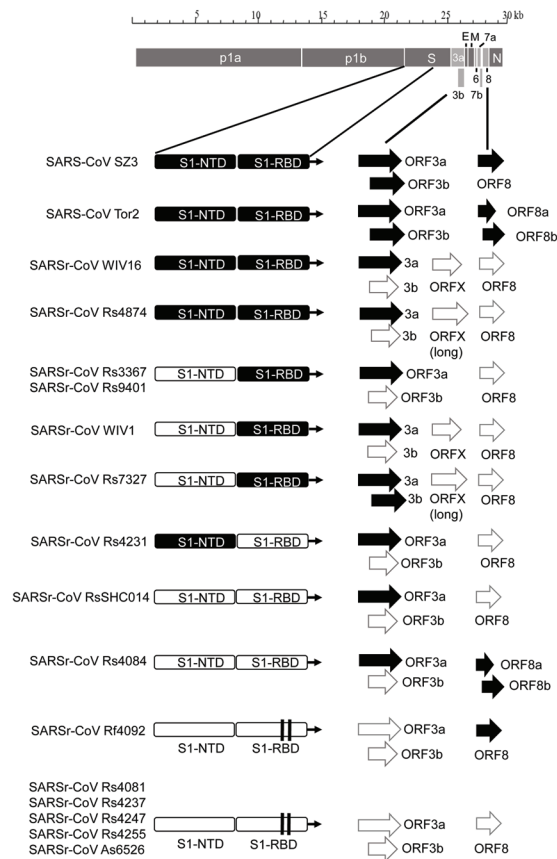


Fig 2. Schematic diagram illustrating the genomic regions or ORFs with most variation between different SARS-CoV and SARSr-CoV isolates. Coding regions of the N-terminal domain (NTD) and receptor-binding domain (RBD) of the spike protein, ORF3a/b and ORF8 (8a/b) in bat SARSr-CoV genomes highly similar 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.

<https://doi.org/10.1371/journal.ppat.1006698.g002>

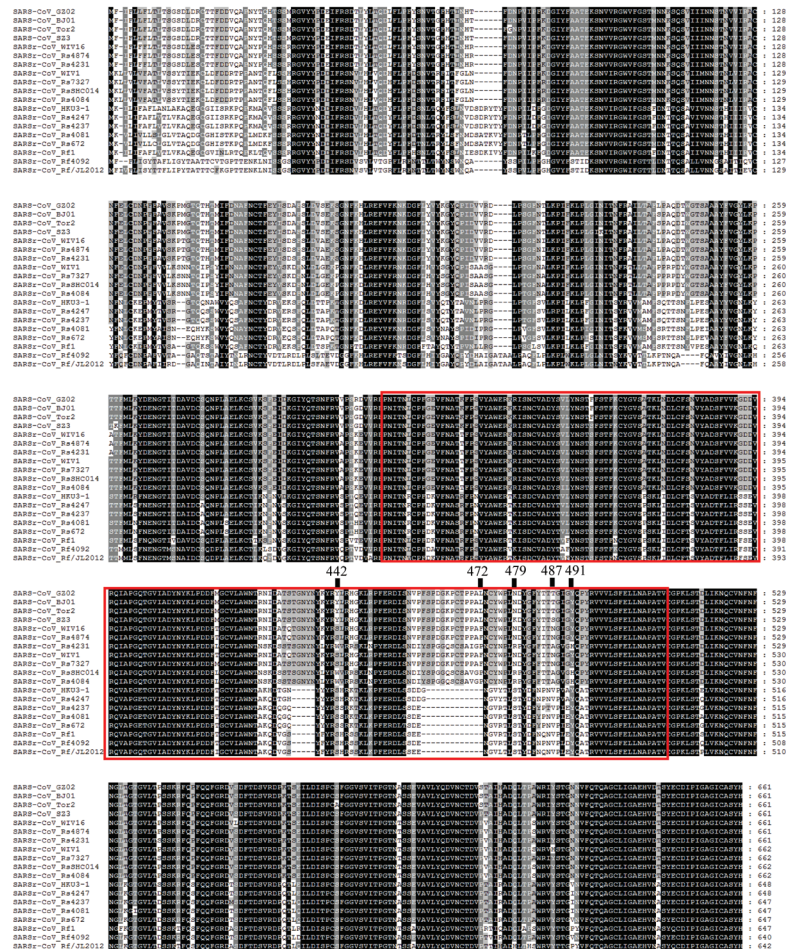


Fig 3. Amino acid sequence comparison of the S1 subunit (corresponding to aa 1–660 of the spike protein of SARS-CoV). The receptor-binding domain (aa 318–510) of SARS-CoV and the homologous region of bat SARSr-CoVs are indicated by the red box. The key aa

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.

<https://doi.org/10.1371/journal.ppat.1006698.g003>

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

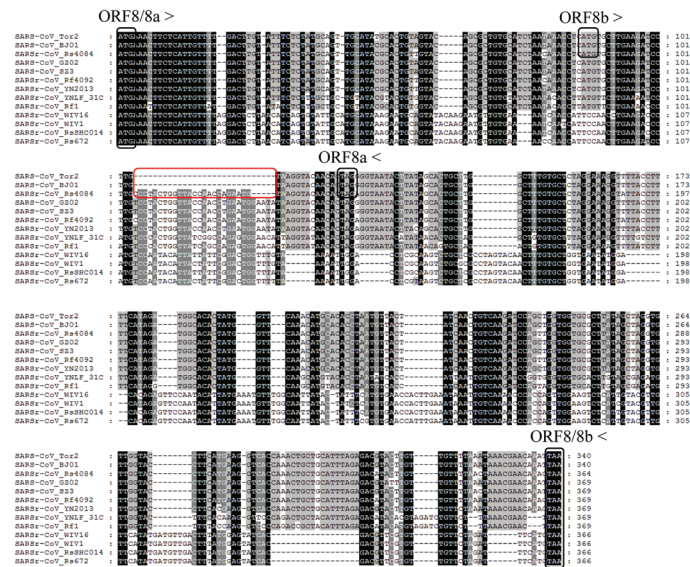


Fig 4. Alignment of nucleotide sequences of ORF8 or ORF8a/8b. The start codons and stop codons of ORF8, 8a and 8b are marked with black boxes and the forward and reverse arrows, respectively. The deletion responsible for the split ORF8a and 8b in human SARS-CoV BJ01, Tor2 and bat SARSr-CoV Rs4084 is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

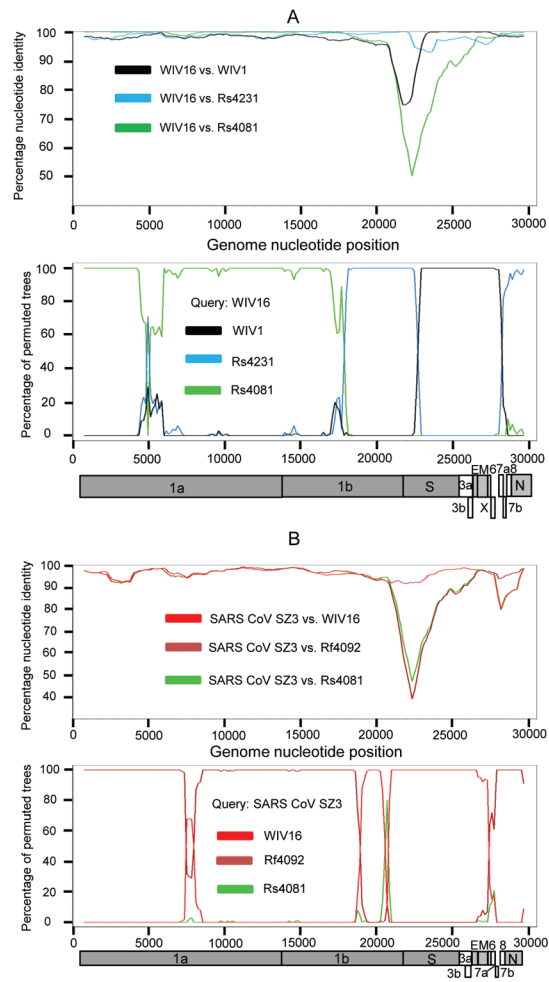


Fig 5. Detection of potential recombination events by similarity plot and boot scan analysis. (A) Full-length genome sequence of SARSr-CoV WIV16 was used as query sequence and WIV1, Rs4231 and Rs4081 as reference sequences. (B) Full-length genome sequence of SARS-CoV SZ3 was used as query sequence and 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

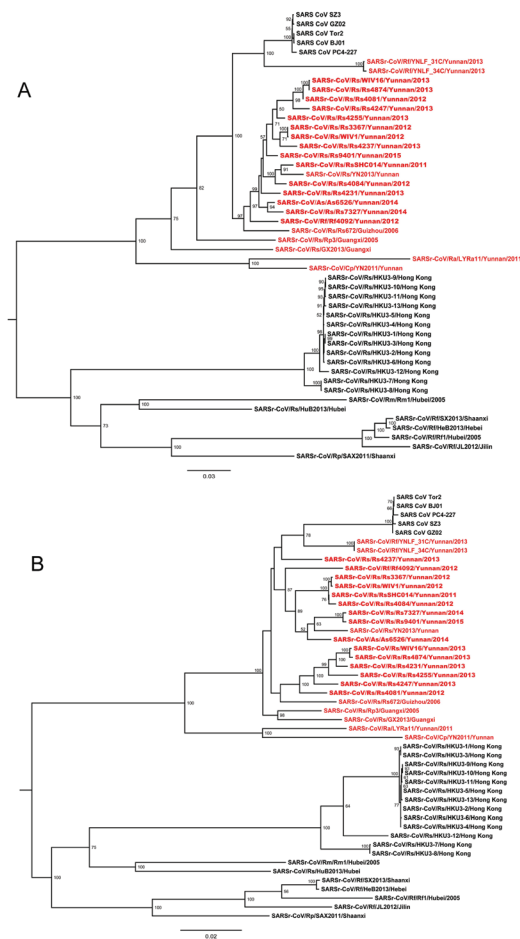


Fig 6. Phylogenetic trees based on nucleotide sequences of ORF1a (A) and ORF1b (B). The trees were constructed by the maximum likelihood method using the LG model with bootstrap values determined by 1000 replicates. Only bootstraps > 50% are shown. The scale bars represent 0.03 (A) and 0.02 (B) substitutions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

nucleotide position. Rs, *Rhinolophus sinicus*; Rf, *Rhinolophus ferreus*; 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.

<https://doi.org/10.1371/journal.ppat.1006698.g006>

increased by 4.42 fold by the SARS-CoV GZ02 ORF8. As a control, the treatment with tunicamycin (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

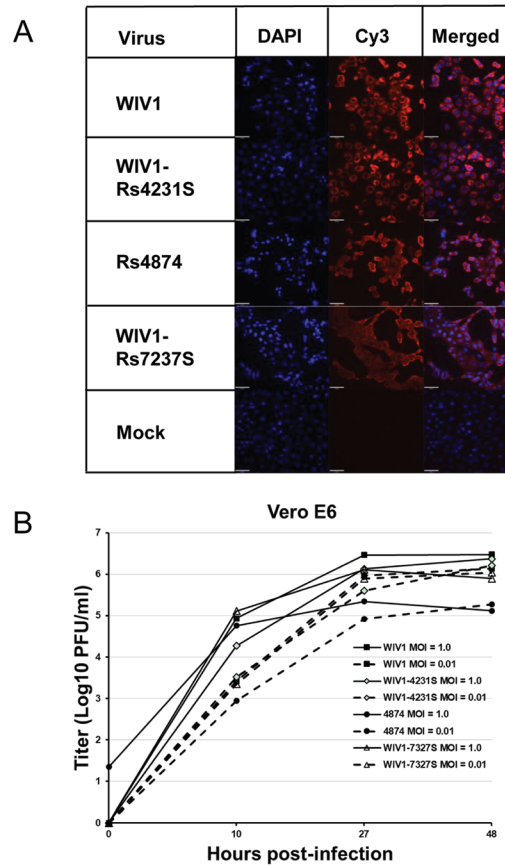


Fig 7. Infection of Vero E6 cells by bat SARS-CoV WIV1, Rs4874, WIV1-Rs4231S and WIV1-Rs7327S. (A) The successful infection was confirmed by immunofluorescent antibody staining using rabbit antibody against the SARS-CoV Rn3 nucleocapsid protein. The columns (from left to right) show staining of nuclei (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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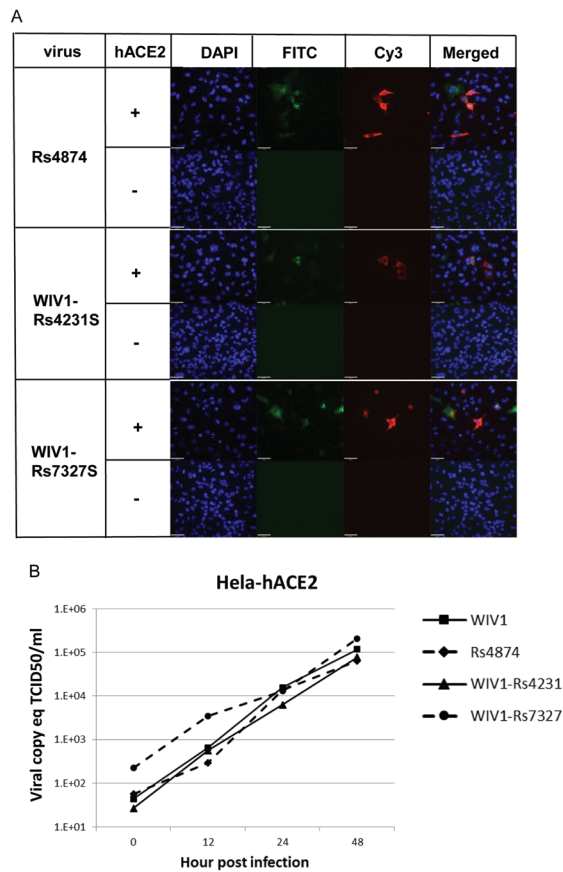


Fig 8. Analysis of receptor usage by immunofluorescence assay (A) and real-time PCR (B). Virus infectivity of Rs4874, WIV1-Rs4231S and WIV1-Rs7327S was determined in HeLa cells with and without the expression of human ACE2. ACE2 expression was detected with goat anti-human ACE2 antibody followed by fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat IgG. Virus replication was detected with rabbit antibody against the SARS-CoV Rn3 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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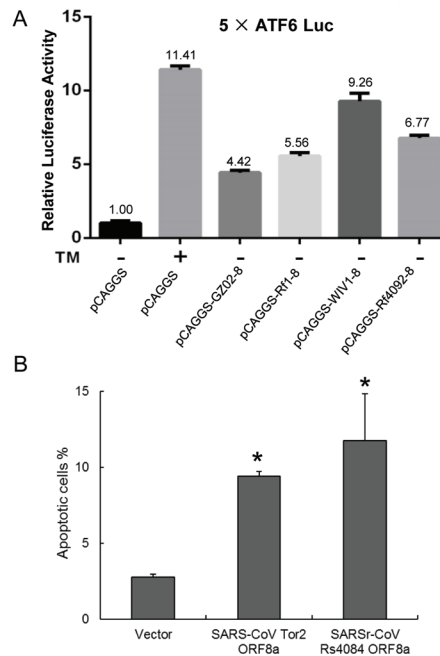


Fig 9. Functional characterization of diverse ORF8 and ORF8a proteins of bat SARS-CoVs. (A) The ORF8 proteins of SARS-CoV and bat SARS-CoVs induces the ATF6-dependent transcriptional activity. HeLa cells were transiently transfected with the pcAGGS expression plasmids of the ORF8 of SARS-CoV GZ02, bat SARS-CoV RI1, WIV1 and RI4092 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 SARS-CoV triggered apoptosis. 293T cells were transfected with the expression plasmids of the ORF8a of SARS-CoV Tor2 and bat SARS-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 SARS-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 SARSr-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.

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 ORF3b 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.

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 1 ml 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 1 min. 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 MgSO₄, 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 MgCl₂, 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.

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 μ l 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 μ g/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'-AGGGCCACCTGGCACTGGTAAGAGTCATTTTGC-3', R-EsBsaI, 5'-ACTGGTCTCTTCGTTTAGTTATTAATAAAATATCACTAGACACC-3') and (F-FsBsaI, 5'-TGAGGTCTCCGAACCTATGGATTGTGTTATGAG-3', RF, 5'-AGGTAGGCCTCTAGGGGCA GCTAAC-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'-AGTCGTCTCAACGAACATGTTATTTTCTTATCTTTCTCACTCTCAC-3' and R-Rs4231-BsmBI, 5'-TCACGTCTCAGTTCGTTTATGTGAATGTAATTTGACAC CTTG-3'). The S gene sequence of Rs7327 was amplified with primer pair (F-Rs7327-BsaI, 5'-AGTGGTCTCAACGAACATGAAATGTTAGTTTGTGCTAC-3' and R-

Rs7327-BsaI, 5'- TCAGGTCTCAGTTCGTTTATGTGTAATGTAATTTAACACCCCTTG-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-AAAGAGCTCAGCCCAGATG-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.

Plasmids

The ORF8 genes of bat SARS-CoV WIV1 and Rf4092 and the ORF8a gene of bat SARS-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 SARS-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 SARS-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 SARS-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 (Yeasten 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 SARS-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 ferreus*; 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)

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

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A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence

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The emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS)-CoV underscores the threat of cross-species transmission events leading to outbreaks in humans. Here we examine the disease potential of a SARS-like virus, SHC014-CoV, which is currently circulating in Chinese horseshoe bat populations¹. Using the SARS-CoV reverse genetics system², we generated and characterized a chimeric virus expressing the spike of bat coronavirus SHC014 in a mouse-adapted SARS-CoV backbone. The results indicate that group 2b viruses encoding the SHC014 spike in a wild-type backbone can efficiently use multiple orthologs of the SARS receptor human angiotensin converting enzyme II (ACE2), replicate efficiently in primary human airway cells and achieve *in vitro* titers equivalent to epidemic strains of SARS-CoV. Additionally, *in vivo* experiments demonstrate replication of the chimeric virus in mouse lung with notable pathogenesis. Evaluation of available SARS-based immune-therapeutic and prophylactic modalities revealed poor efficacy; both monoclonal antibody and vaccine approaches failed to neutralize and protect from infection with CoVs using the novel spike protein. On the basis of these findings, we synthetically re-derived an infectious full-length SHC014 recombinant virus and demonstrate robust viral replication both *in vitro* and *in vivo*. Our work suggests a potential risk of SARS-CoV re-emergence from viruses currently circulating in bat populations.

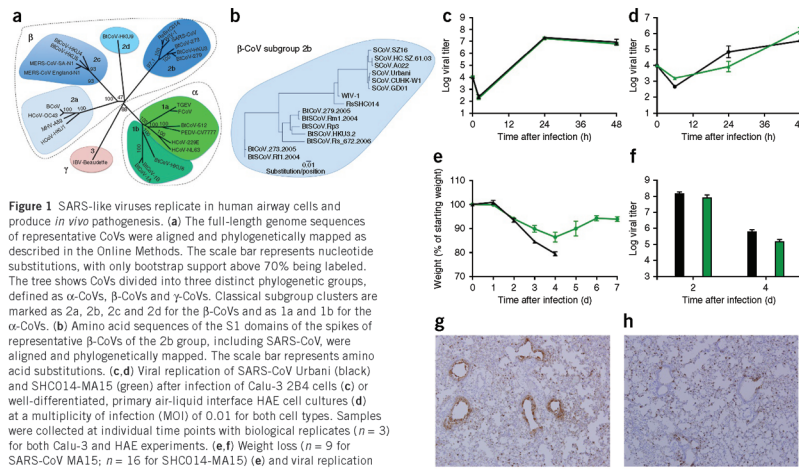
The emergence of SARS-CoV heralded a new era in the cross-species transmission of severe respiratory illness with globalization leading to rapid spread around the world and massive economic impact^{3,4}. Since then, several strains—including influenza A strains H5N1, H1N1 and H7N9 and MERS-CoV—have emerged from animal populations, causing considerable disease, mortality and economic hardship for

the afflicted regions⁵. Although public health measures were able to stop the SARS-CoV outbreak¹, recent metagenomics studies have identified sequences of closely related SARS-like viruses circulating in Chinese bat populations that may pose a future threat^{1,6}. However, sequence data alone provides minimal insights to identify and prepare for future prepandemic viruses. Therefore, to examine the emergence potential (that is, the potential to infect humans) of circulating bat CoVs, we built a chimeric virus encoding a novel, zoonotic CoV spike protein—from the RsSHC014-CoV sequence that was isolated from Chinese horseshoe bats¹—in the context of the SARS-CoV mouse-adapted backbone. The hybrid virus allowed us to evaluate the ability of the novel spike protein to cause disease independently of other necessary adaptive mutations in its natural backbone. Using this approach, we characterized CoV infection mediated by the SHC014 spike protein in primary human airway cells and *in vivo*, and tested the efficacy of available immune therapeutics against SHC014-CoV. Together, the strategy translates metagenomics data to help predict and prepare for future emergent viruses.

The sequences of SHC014 and the related RsWIV1-CoV show that these CoVs are the closest relatives to the epidemic SARS-CoV strains (Fig. 1a,b); however, there are important differences in the 14 residues that bind human ACE2, the receptor for SARS-CoV, including the five that are critical for host range: Y442, L472, N479, T487 and Y491 (ref. 7). In WIV1, three of these residues vary from the epidemic SARS-CoV Urbani strain, but they were not expected to alter binding to ACE2 (Supplementary Fig. 1a,b and Supplementary Table 1). This fact is confirmed by both pseudotyping experiments that measured the ability of lentiviruses encoding WIV1 spike proteins to enter cells expressing human ACE2 (Supplementary Fig. 1) and by *in vitro* replication assays of WIV1-CoV (ref. 1). In contrast, 7 of 14 ACE2-interaction residues in SHC014 are different from those in SARS-CoV, including all five residues critical for host range (Supplementary Fig. 1c and Supplementary Table 1). These changes, coupled with

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the failure of pseudotyped lentiviruses expressing the SHC014 spike to enter cells (Supplementary Fig. 1d), suggested that the SHC014 spike is unable to bind human ACE2. However, similar changes in related SARS-CoV strains had been reported to allow ACE2 binding^{7,8}, suggesting that additional functional testing was required for verification. Therefore, we synthesized the SHC014 spike in the context of the replication-competent, mouse-adapted SARS-CoV backbone (we hereafter refer to the chimeric CoV as SHC014-MA15) to maximize the opportunity for pathogenesis and vaccine studies in mice (Supplementary Fig. 2a). Despite predictions from both structure-based modeling and pseudotyping experiments, SHC014-MA15 was viable and replicated to high titers in Vero cells (Supplementary Fig. 2b). Similarly to SARS, SHC014-MA15 also required a functional ACE2 molecule for entry and could use human, civet and bat ACE2 orthologs (Supplementary Fig. 2c, d). To test the ability of the SHC014 spike to mediate infection of the human airway, we examined the sensitivity of the human epithelial airway cell line Calu-3 2B4 (ref. 9) to infection and found robust SHC014-MA15 replication, comparable to that of SARS-CoV Urbani (Fig. 1c). To extend these findings, primary human airway epithelial (HAE) cultures were infected and showed robust replication of both viruses (Fig. 1d). Together, the data confirm the ability of viruses with the SHC014 spike to infect human airway cells and underscore the potential threat of cross-species transmission of SHC014-CoV.

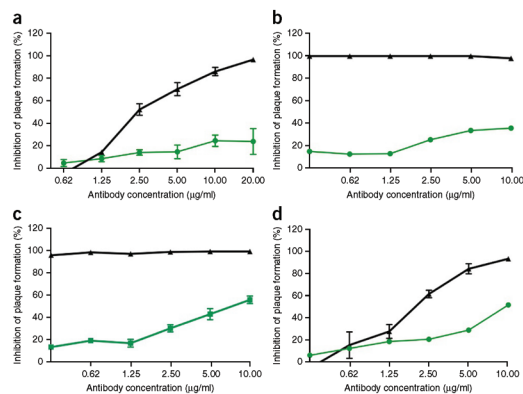
To evaluate the role of the SHC014 spike in mediating infection *in vivo*, we infected 10-week-old BALB/c mice with 10^4 plaque-forming units (p.f.u.) of either SARS-MA15 or SHC014-MA15 (Fig. 1e–h). Animals infected with SARS-MA15 experienced rapid

weight loss and lethality by 4 d post infection (d.p.i.); in contrast, SHC014-MA15 infection produced substantial weight loss (10%) but no lethality in mice (Fig. 1e). Examination of viral replication revealed nearly equivalent viral titers from the lungs of mice infected with SARS-MA15 or SHC014-MA15 (Fig. 1f). Whereas lungs from the SARS-MA15-infected mice showed robust staining in both the terminal bronchioles and the lung parenchyma 2 d.p.i. (Fig. 1g), those of SHC014-MA15-infected mice showed reduced airway antigen staining (Fig. 1h); in contrast, no deficit in antigen staining was observed in the parenchyma or in the overall histology scoring, suggesting differential infection of lung tissue for SHC014-MA15 (Supplementary Table 2). We next analyzed infection in more susceptible, aged (12-month-old) animals. SARS-MA15-infected animals rapidly lost weight and succumbed to infection (Supplementary Fig. 3a, b). SHC014-MA15 infection induced robust and sustained weight loss, but had minimal lethality. Trends in the histology and antigen staining patterns that we observed in young mice were conserved in the older animals (Supplementary Table 3). We excluded the possibility that SHC014-MA15 was mediating infection through an alternative receptor on the basis of experiments using *Ace2*^{−/−} mice, which did not show weight loss or antigen staining after SHC014-MA15 infection (Supplementary Fig. 4a, b and Supplementary Table 2). Together, the data indicate that viruses with the SHC014 spike are capable of inducing weight loss in mice in the context of a virulent CoV backbone.

Given the preclinical efficacy of Ebola monoclonal antibody therapies, such as ZMAPP¹⁰, we next sought to determine the efficacy of SARS-CoV monoclonal antibodies against infection with

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Figure 2 SARS-CoV monoclonal antibodies have marginal efficacy against SARS-like CoVs. **(a–d)** Neutralization assays evaluating efficacy (measured as reduction in the number of plaques) of a panel of monoclonal antibodies, which were all originally generated against epidemic SARS-CoV, against infection of Vero cells with SARS-CoV Urbani (black) or SHC014-MA15 (green). The antibodies tested were fm6 ($n = 3$ for Urbani; $n = 5$ for SHC014-MA15)^{11,12} **(a)**, 230.15 ($n = 3$ for Urbani; $n = 2$ for SHC014-MA15) **(b)**, 227.15 ($n = 3$ for Urbani; $n = 5$ for SHC014-MA15) **(c)** and 109.8 ($n = 3$ for Urbani; $n = 2$ for SHC014-MA15)¹³ **(d)**. Each data point represents the group mean and error bars define the s.e.m. Note that the error bars in SARS-CoV Urbani-infected Vero cells in **b,c** are overlapped by the symbols and are not visible.



SHC014-MA15. Four broadly neutralizing human monoclonal antibodies targeting SARS-CoV spike protein had been previously reported and are probable reagents for immunotherapy^{11–13}. We examined the effect of these antibodies on viral replication (expressed as percentage inhibition of viral replication) and found that whereas wild-type SARS-CoV Urbani was strongly neutralized by all four antibodies at relatively low antibody concentrations (Fig. 2a–d), neutralization varied for SHC014-MA15. Fm6, an antibody generated by phage display and escape mutants^{11,12}, achieved only background levels of inhibition of SHC014-MA15 replication (Fig. 2a). Similarly, antibodies 230.15 and 227.14, which were derived from memory B cells of SARS-CoV-infected patients¹³, also failed to block SHC014-MA15 replication (Fig. 2b,c). For all three antibodies, differences between the SARS and SHC014 spike amino acid sequences corresponded to direct or adjacent residue changes found in SARS-CoV escape mutants (fm6 N479R; 230.15 L443V; 227.14 K390Q/E), which probably explains the absence of the antibodies' neutralizing activity against SHC014. Finally, monoclonal antibody 109.8 was able to achieve 50% neutralization of SHC014-MA15, but only at high concentrations (10 µg/ml) (Fig. 2d). Together, the results demonstrate that broadly neutralizing antibodies against SARS-CoV may only have marginal efficacy against emergent SARS-like CoV strains such as SHC014.

To evaluate the efficacy of existing vaccines against infection with SHC014-MA15, we vaccinated aged mice with double-inactivated whole SARS-CoV (DIV). Previous work showed that DIV could neutralize and protect young mice from challenge with a homologous virus¹⁴; however, the vaccine failed to protect aged animals in which augmented immune pathology was also observed, indicating the possibility of the animals being harmed because of the vaccination¹⁵. Here we found that DIV did not provide protection from challenge with SHC014-MA15 with regards to weight loss or viral titer (Supplementary Fig. 5a,b). Consistent with a previous report with other heterologous group 2b CoVs¹⁵, serum from DIV-vaccinated, aged mice also failed to neutralize SHC014-MA15 (Supplementary Fig. 5c). Notably, DIV vaccination resulted in robust immune pathology (Supplementary Table 4) and eosinophilia (Supplementary Fig. 5d–f). Together, these results confirm that the DIV vaccine would not be protective against infection with SHC014 and could possibly augment disease in the aged vaccinated group.

In contrast to vaccination of mice with DIV, the use of SHC014-MA15 as a live, attenuated vaccine showed potential cross-protection against challenge with SARS-CoV, but the results have important caveats. We infected young mice with 10^4 p.f.u. of SHC014-MA15 and observed them for 28 d. We then challenged the mice with SARS-MA15 at day 29 (Supplementary Fig. 6a). The prior infection of the mice with the high dose of SHC014-MA15 conferred protection against challenge with a lethal dose of SARS-MA15, although there was only a minimal SARS-CoV neutralization response from the antisera elicited 28 d after SHC014-MA15 infection (Supplementary Fig. 6b, 1:200). In the absence of a secondary antigen boost, 28 d.p.i. represents the expected peak of antibody titers and implies that there will be diminished protection against SARS-CoV over time^{16,17}. Similar results showing protection against challenge with a lethal dose of SARS-CoV were observed in aged BALB/c mice with respect to weight loss and viral replication (Supplementary Fig. 6c,d). However, the SHC014-MA15 infection dose of 10^4 p.f.u. induced >10% weight loss and lethality in some aged animals (Fig. 1 and Supplementary Fig. 3). We found that vaccination with a lower dose of SHC014-MA15 (100 p.f.u.), did not induce weight loss, but it also failed to protect aged animals from a SARS-MA15 lethal dose challenge (Supplementary Fig. 6e,f). Together, the data suggest that SHC014-MA15 challenge may confer cross-protection against SARS-CoV through conserved epitopes, but the required dose induces pathogenesis and precludes use as an attenuated vaccine.

Having established that the SHC014 spike has the ability to mediate infection of human cells and cause disease in mice, we next synthesized a full-length SHC014-CoV infectious clone based on the approach used for SARS-CoV (Fig. 3a)². Replication in Vero cells revealed no deficit for SHC014-CoV relative to that for SARS-CoV (Fig. 3b); however, SHC014-CoV was significantly ($P < 0.01$) attenuated in primary HAE cultures at both 24 and 48 h after infection (Fig. 3c). *In vivo* infection of mice demonstrated no significant weight loss but showed reduced viral replication in lungs of full-length SHC014-CoV infection, as compared to SARS-CoV Urbani (Fig. 3d,e). Together, the results establish the viability of full-length

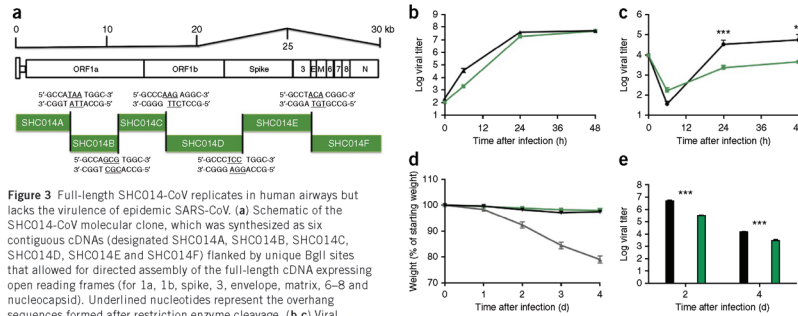


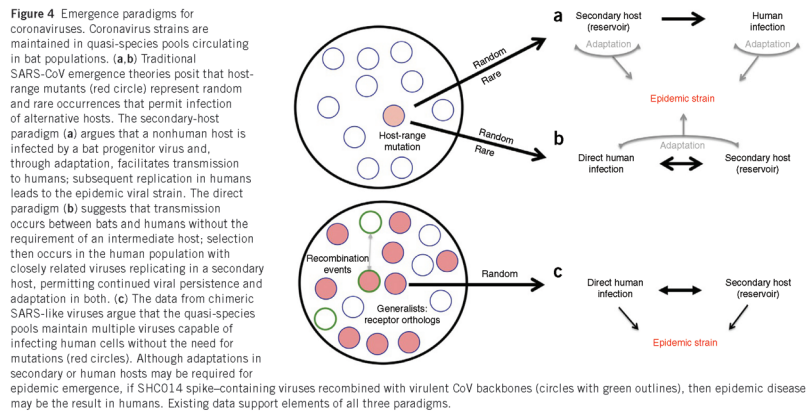
Figure 3 Full-length SHC014-CoV replicates in human airways but lacks the virulence of epidemic SARS-CoV. (a) Schematic of the SHC014-CoV molecular clone, which was synthesized as six contiguous cDNAs (designated SHC014A, SHC014B, SHC014C, SHC014D, SHC014E and SHC014F) flanked by unique BglI sites that allowed for directed assembly of the full-length cDNA expressing open reading frames (for 1a, 1b, spike, 3, envelope, matrix, 6–8 and nucleocapsid). Underlined nucleotides represent the overhang sequences formed after restriction enzyme cleavage. (b, c) Viral replication of SARS-CoV Urbani (black) or SHC014-CoV (green) after infection of Vero cells (b) or well-differentiated, primary air-liquid interface HAE cell cultures (c) at an MOI of 0.01. Samples were collected at individual time points with biological replicates ($n = 3$) for each group. Data represent one experiment for both Vero and HAE cells. (d, e) Weight loss ($n = 3$ for SARS-CoV MA15, $n = 7$ for SHC014-CoV, $n = 6$ for SARS-Urbani) (d) and viral replication in the lungs ($n = 3$ for SARS-Urbani and SHC014-CoV) (e) of 10-week-old BALB/c mice infected with 1×10^5 p.f.u. of SARS-CoV MA15 (gray), SHC014-CoV (green) or SARS-CoV Urbani (black) via the i.n. route. Each data point represents the group mean, and error bars define the s.e.m. $**P < 0.01$ and $***P < 0.001$ using two-tailed Student's *t*-test of individual time points.

SHC014-CoV, but suggest that further adaptation is required for its replication to be equivalent to that of epidemic SARS-CoV in human respiratory cells and in mice.

During the SARS-CoV epidemic, links were quickly established between palm civets and the CoV strains that were detected in humans⁴. Building on this finding, the common emergence paradigm argues that epidemic SARS-CoV originated as a bat virus, jumped to civets and incorporated changes within the receptor-binding domain (RBD) to improve binding to civet Ace2 (ref. 18). Subsequent exposure to people in live-animal markets permitted human infection with

the civet strain, which, in turn, adapted to become the epidemic strain (Fig. 4a). However, phylogenetic analysis suggests that early human SARS strains appear more closely related to bat strains than to civet strains¹⁸. Therefore, a second paradigm argues that direct bat-human transmission initiated SARS-CoV emergence and that palm civets served as a secondary host and reservoir for continued infection (Fig. 4b)¹⁹. For both paradigms, spike adaptation in a secondary host is seen as a necessity, with most mutations expected to occur within the RBD, thereby facilitating improved infection. Both theories imply that pools of bat CoVs are limited and that host-range mutations are

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both random and rare, reducing the likelihood of future emergence events in humans.

Although our study does not invalidate the other emergence routes, it does argue for a third paradigm in which circulating bat CoV pools maintain 'poised' spike proteins that are capable of infecting humans without mutation or adaptation (Fig. 4c). This hypothesis is illustrated by the ability of a chimeric virus containing the SHC014 spike in a SARS-CoV backbone to cause robust infection in both human airway cultures and in mice without RBD adaptation. Coupled with the observation of previously identified pathogenic CoV backbones^{3,20}, our results suggest that the starting materials required for SARS-like emergent strains are currently circulating in animal reservoirs. Notably, although full-length SHC014-CoV probably requires additional backbone adaption to mediate human disease, the documented high-frequency recombination events in CoV families underscores the possibility of future emergence and the need for further preparation.

To date, genomics screens of animal populations have primarily been used to identify novel viruses in outbreak settings²¹. The approach here extends these data sets to examine questions of viral emergence and therapeutic efficacy. We consider viruses with the SHC014 spike a potential threat owing to their ability to replicate in primary human airway cultures, the best available model for human disease. In addition, the observed pathogenesis in mice indicates a capacity for SHC014-containing viruses to cause disease in mammalian models, without RBD adaptation. Notably, differential tropism in the lung as compared to that with SARS-MA15 and attenuation of full-length SHC014-CoV in HAE cultures relative to SARS-CoV Urbani suggest that factors beyond ACE2 binding—including spike processivity, receptor bio-availability or antagonism of the host immune responses—may contribute to emergence. However, further testing in nonhuman primates is required to translate these findings into pathogenic potential in humans. Importantly, the failure of available therapeutics defines a critical need for further study and for the development of treatments. With this knowledge, surveillance programs, diagnostic reagents and effective treatments can be produced that are protective against the emergence of group 2b-specific CoVs, such as SHC014, and these can be applied to other CoV branches that maintain similarly heterogeneous pools.

In addition to offering preparation against future emerging viruses, this approach must be considered in the context of the US government-mandated pause on gain-of-function (GOF) studies²². On the basis of previous models of emergence (Fig. 4a,b), the creation of chimeric viruses such as SHC014-MA15 was not expected to increase pathogenicity. Although SHC014-MA15 is attenuated relative to its parental mouse-adapted SARS-CoV, similar studies examining the pathogenicity of CoVs with the wild-type Urbani spike within the MA15 backbone showed no weight loss in mice and reduced viral replication²³. Thus, relative to the Urbani spike-MA15 CoV, SHC014-MA15 shows a gain in pathogenesis (Fig. 1). On the basis of these findings, scientific review panels may deem similar studies building chimeric viruses based on circulating strains too risky to pursue, as increased pathogenicity in mammalian models cannot be excluded. Coupled with restrictions on mouse-adapted strains and the development of monoclonal antibodies using escape mutants, research into CoV emergence and therapeutic efficacy may be severely limited moving forward. Together, these data and restrictions represent a crossroads of GOF research concerns; the potential to prepare for and mitigate future outbreaks must be weighed against the risk of creating more dangerous pathogens.

In developing policies moving forward, it is important to consider the value of the data generated by these studies and whether these types of chimeric virus studies warrant further investigation versus the inherent risks involved.

Overall, our approach has used metagenomics data to identify a potential threat posed by the circulating bat SARS-like CoV SHC014. Because of the ability of chimeric SHC014 viruses to replicate in human airway cultures, cause pathogenesis *in vivo* and escape current therapeutics, there is a need for both surveillance and improved therapeutics against circulating SARS-like viruses. Our approach also unlocks the use of metagenomics data to predict viral emergence and to apply this knowledge in preparing to treat future emerging virus infections.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

V.D.M. designed, coordinated and performed experiments, completed analysis and wrote the manuscript. B.L.Y. designed the infectious clone and recovered chimeric viruses; S.A. completed neutralization assays; L.E.G. helped perform mouse experiments; T.S. and J.A.B. completed mouse experiments and plaque assays; X.-Y.G. performed pseudotyping experiments; K.D. generated structural figures and predictions; E.F.D. generated phylogenetic analysis; R.L.G. completed RNA analysis; S.H.R. provided primary HAE cultures; A.L. and W.A.M. provided critical monoclonal antibody reagents; and Z.-L.S. provided SHC014 spike sequences and plasmids. R.S.B. designed experiments and wrote manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Viruses, cells, *in vitro* infection and plaque assays. Wild-type SARS-CoV (Urbani), mouse-adapted SARS-CoV (MA15) and chimeric SARS-like CoVs were cultured on Vero E6 cells (obtained from United States Army Medical Research Institute of Infectious Diseases), grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, CA) and 5% fetal clone serum (FCS) (Hyclone, South Logan, UT) along with antibiotic/antimycotic (Gibco, Carlsbad, CA). DBT cells (Baric laboratory, source unknown) expressing *ACE2* orthologs have been previously described for both human and civet; bat *Ace2* sequence was based on that from *Rhinolophus leschenaulti*, and DBT cells expressing bat *Ace2* were established as described previously⁸. Pseudotyping experiments were similar to those using an HIV-based pseudovirus, prepared as previously described¹⁰, and examined on HeLa cells (Wuhan Institute of Virology) that expressed *ACE2* orthologs. HeLa cells were grown in minimal essential medium (MEM) (Gibco, CA) supplemented with 10% FCS (Gibco, CA) as previously described²⁴. Growth curves in Vero E6, DBT, Calu-3 2B4 and primary human airway epithelial cells were performed as previously described^{8,25}. None of the working cell line stocks were authenticated or tested for mycoplasma recently, although the original seed stocks used to create the working stocks are free from contamination. Human lungs for HAE cultures were procured under University of North Carolina at Chapel Hill Institutional Review Board-approved protocols. HAE cultures represent highly differentiated human airway epithelium containing ciliated and non-ciliated epithelial cells as well as goblet cells. The cultures are also grown on an air-liquid interface for several weeks before use, as previously described²⁶. Briefly, cells were washed with PBS and inoculated with virus or mock-diluted in PBS for 40 min at 37 °C. After inoculation, cells were washed three times and fresh medium was added to signify time '0'. Three or more biological replicates were harvested at each described time point. No blinding was used in any sample collections nor were samples randomized. All virus cultivation was performed in a biosafety level (BSL) 3 laboratory with redundant fans in the biosafety cabinets, as described previously by our group². All personnel wore powered air purifying respirators (Breathe Easy, 3M) with Tyvek suits, aprons and booties and were double-gloved.

Sequence clustering and structural modeling. The full-length genomic sequences and the amino acid sequences of the S1 domains of the spike of representative CoVs were downloaded from Genbank or Pathosystems Resource Integration Center (PATRIC), aligned with ClustalX and phylogenetically compared by using maximum likelihood estimation using 100 bootstraps or by using the PhyML (<https://code.google.com/p/phyml/>) package, respectively. The tree was generated using maximum likelihood with the PhyML package. The scale bar represents nucleotide substitutions. Only nodes with bootstrap support above 70% are labeled. The tree shows that CoVs are divided into three distinct phylogenetic groups defined as α -CoVs, β -CoVs and γ -CoVs. Classical subgroup clusters are marked as 2a, 2b, 2c and 2d for β -CoVs, and 1a and 1b for the α -CoVs. Structural models were generated using Modeller (Max Planck Institute Bioinformatics Toolkit) to generate homology models for SHC014 and Rs3367 of the SARS RBD in complex with ACE2 based on crystal structure 2AJF (Protein Data Bank). Homology models were visualized and manipulated in MacPyMol (version 1.3).

Construction of SARS-like chimeric viruses. Both wild-type and chimeric viruses were derived from either SARS-CoV Urbani or the corresponding mouse-adapted (SARS-CoV MA15) infectious clone (ic) as previously described²⁷. Plasmids containing spike sequences for SHC014 were extracted by restriction digest and ligated into the E and F plasmid of the MA15 infectious clone. The clone was designed and purchased from Bio Basic as six contiguous cDNAs using published sequences flanked by unique class II restriction endonuclease sites (BglII). Thereafter, plasmids containing wild-type, chimeric SARS-CoV and SHC014-CoV genome fragments were amplified, excised, ligated and purified. *In vitro* transcription reactions were then performed to synthesize full-length genomic RNA, which was transfected into Vero E6 cells as previously described². The medium from transfected cells was harvested and served as seed stocks for subsequent experiments. Chimeric and full-length viruses were confirmed by sequence analysis before use in these

studies. Synthetic construction of chimeric mutant and full-length SHC014-CoV was approved by the University of North Carolina Institutional Biosafety Committee and the Dual Use Research of Concern committee.

Ethics statement. This study was carried out in accordance with the recommendations for the care and use of animals by the Office of Laboratory Animal Welfare (OLAW), NIH. The Institutional Animal Care and Use Committee (IACUC) of The University of North Carolina at Chapel Hill (UNC, Permit Number A-3410-01) approved the animal study protocol (IACUC #13-033) used in these studies.

Mice and *in vivo* infection. Female, 10-week-old and 12-month-old BALB/cAnNHsd mice were ordered from Harlan Laboratories. Mouse infections were done as previously described²⁰. Briefly, animals were brought into a BSL3 laboratory and allowed to acclimate for 1 week before infection. For infection and live-attenuated virus vaccination, mice were anesthetized with a mixture of ketamine and xylazine and infected intranasally, when challenged, with 50 μ l of phosphate-buffered saline (PBS) or diluted virus with three or four mice per time point, per infection group per dose as described in the figure legends. For individual mice, notations for infection including failure to inhale the entire dose, bubbling of inoculum from the nose, or infection through the mouth may have led to exclusion of mouse data at the discretion of the researcher; post-infection, no other pre-established exclusion or inclusion criteria are defined. No blinding was used in any animal experiments, and animals were not randomized. For vaccination, young and aged mice were vaccinated by footpad injection with a 20- μ l volume of either 0.2 μ g of double-inactivated SARS-CoV vaccine with alum or mock PBS; mice were then boosted with the same regimen 22 d later and challenged 21 d thereafter. For all groups, as per protocol, animals were monitored daily for clinical signs of disease (hunching, ruffled fur and reduced activity) for the duration of the experiment. Weight loss was monitored daily for the first 7 d, after which weight monitoring continued until the animals recovered to their initial starting weight or displayed weight gain continuously for 3 d. All mice that lost greater than 20% of their starting body weight were grounded and further monitored multiple times per day as long as they were under the 20% cutoff. Mice that lost greater than 30% of their starting body weight were immediately sacrificed as per protocol. Any mouse deemed to be moribund or unlikely to recover was also humanely sacrificed at the discretion of the researcher. Euthanasia was performed using an isoflurane overdose and death was confirmed by cervical dislocation. All mouse studies were performed at the University of North Carolina (Animal Welfare Assurance #A3410-01) using protocols approved by the UNC Institutional Animal Care and Use Committee (IACUC).

Histological analysis. The left lung was removed and submerged in 10% buffered formalin (Fisher) without inflation for 1 week. Tissues were embedded in paraffin and 5- μ m sections were prepared by the UNC Lineberger Comprehensive Cancer Center histopathology core facility. To determine the extent of antigen staining, sections were stained for viral antigen using a commercially available polyclonal SARS-CoV anti-nucleocapsid antibody (Imgenex) and scored in a blinded manner by for staining of the airway and parenchyma as previously described²⁰. Images were captured using an Olympus BX41 microscope with an Olympus DP71 camera.

Virus neutralization assays. Plaque reduction neutralization titer assays were performed with previously characterized antibodies against SARS-CoV, as previously described^{11–13}. Briefly, neutralizing antibodies or serum was serially diluted twofold and incubated with 100 p.f.u. of the different infectious clone SARS-CoV strains for 1 h at 37 °C. The virus and antibodies were then added to a 6-well plate with 5×10^5 Vero E6 cells/well with multiple replicates ($n \geq 2$). After a 1-h incubation at 37 °C, cells were overlaid with 3 ml of 0.8% agarose in medium. Plates were incubated for 2 d at 37 °C, stained with neutral red for 3 h and plaques were counted. The percentage of plaque reduction was calculated as $(1 - (\text{no. of plaques with antibody} / \text{no. of plaques without antibody})) \times 100$.



CORRIGENDA

Corrigendum: Multiphoton imaging reveals a new leukocyte recruitment paradigm in the glomerulus

Sapna Devi, Anqi Li, Clare L V Westhorpe, Camden Y Lo, Latasha D Abeynaike, Sarah L Snelgrove, Pam Hall, Joshua D Ooi, Christopher G Sobey, A Richard Kitching & Michael J Hickey

Nat. Med. 19, 107–112 (2013); published online 16 December 2012; corrected after print 12 August 2015

In the published article, in the Online Methods section, it is stated that the dose of DHE used is 20 mg/kg, when in fact DHE was administered at 2 mg/kg. The error has been corrected in the HTML and PDF versions of the article.

Corrigendum: PAR1 signaling regulates the retention and recruitment of EPCR-expressing bone marrow hematopoietic stem cells

Shiri Gur-Cohen, Tomer Itkin, Sagarika Chakrabarty, Claudine Graf, Orit Kollet, Aya Ludin, Karin Golan, Alexander Kalinkovich, Guy Lederger, Eitan Wong, Elisabeth Niemeyer, Ziv Porat, Ayelet Erez, Irit Sagi, Charles T Esmon, Wolfram Ruf & Tsvee Lapidot

Nat. Med. 21, 1307–1317 (2015); published online 12 October 2015; corrected after print 18 November 2015

In the version of this article initially published, the first author's name was incorrect. The error has been corrected in the HTML and PDF versions of the article.

Corrigendum: Myeloid-derived growth factor (C19orf10) mediates cardiac repair following myocardial infarction

Mortimer Korf-Klingebiel, Marc R Rebell, Stefanie Klede, Torben Brod, Andreas Pich, Felix Polten, I Christian Napp, Johann Bauersachs, Arnold Ganser, Eva Brinkmann, Ines Reimann, Tibor Kempf, Hans W Niessen, Jacques Mizrahi, Hans-Joachim Schönfeld, Antonio Iglesias, Maria Bobadilla, Yong Wang & Kai C Wollert

Nat. Med. 21, 140–149 (2015); published online 12 January 2015; corrected after print 19 November 2015

In the version of this article initially published, the article number in reference 13 is incorrectly stated as '100ra190' and should be '100ra90'. The error has been corrected in the HTML and PDF versions of the article.

Corrigendum: A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence

Vineet D Menachery, Boyd L Yount Jr, Kari Debbink, Sudhakar Agnihothram, Lisa E Gralinski, Jessica A Plante, Rachel L Graham, Trevor Scobey, Xing-Yi Ge, Eric F Donaldson, Scott H Randell, Antonio Lanzavecchia, Wayne A Marasco, Zhengli-Li Shi & Ralph S Baric

Nat. Med.; doi:10.1038/nm.3985; corrected 20 November 2015

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Corrigendum: Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice

Arturo J Vegas, Omid Veischi, Mads Gürtler, Jeffrey R Millman, Felicia W Pagliuca, Andrew R Bader, Joshua C Doloff, Jie Li, Michael Chen, Karsten Olejnik, Hok Hei Tam, Siddharth Jhunjhunwala, Erin Langan, Stephanie Aresta-Dasilva, Srujan Gandham, James J McGarrigle, Matthew A Bochenek, Jennifer Hollister-Lock, Jose Oberholzer, Dale I Greiner, Gordon C Weir, Douglas A Melton, Robert Langer & Daniel G Anderson

Nat. Med.; doi:10.1038/nm.4030; corrected online 18 February 2016

In the version of this article initially published online, the authors omitted acknowledgment recognizing the histology core of the Harvard Stem Cell Institute and several individuals for their assistance. The error has been corrected for the print, PDF and HTML versions of this article.

Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response

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ABSTRACT

Bats harbor severe acute respiratory syndrome (SARS)-like coronaviruses (SL-CoVs) from which the causative agent of the 2002–2003 SARS pandemic is thought to have originated. However, despite the fact that a large number of genetically diverse SL-CoV sequences have been detected in bats, only two strains (named WIV1 and WIV16) have been successfully cultured *in vitro*. These two strains differ from SARS-CoV only in containing an extra open reading frame (ORF) (named ORFX), between ORF6 and ORF7, which has no homology to any known protein sequences. In this study, we constructed a full-length cDNA clone of SL-CoV WIV1 (rWIV1), an ORFX deletion mutant (rWIV1-ΔX), and a green fluorescent protein (GFP)-expressing mutant (rWIV1-GFP-ΔX). Northern blotting and fluorescence microscopy indicate that ORFX was expressed during WIV1 infection. A virus infection assay showed that rWIV1-ΔX replicated as efficiently as rWIV1 in Vero E6, Calu-3, and HeLa-hACE2 cells. Further study showed that ORFX could inhibit interferon production and activate NF-κB. Our results demonstrate for the first time that the unique ORFX in the WIV1 strain is a functional gene involving modulation of the host immune response but is not essential for *in vitro* viral replication.

IMPORTANCE

Bats harbor genetically diverse SARS-like coronaviruses (SL-CoVs), and some of them have the potential for interspecies transmission. A unique open reading frame (ORFX) was identified in the genomes of two recently isolated bat SL-CoV strains (WIV1 and -16). It will therefore be critical to clarify whether and how this protein contributes to virulence during viral infection. Here we revealed that the unique ORFX is a functional gene that is involved in the modulation of the host immune response but is not essential for *in vitro* viral replication. Our results provide important information for further exploration of the ORFX function in the future. Moreover, the reverse genetics system we constructed will be helpful for study of the pathogenesis of this group of viruses and to develop therapeutics for future control of emerging SARS-like infections.

Severe acute respiratory syndrome coronavirus (SARS-CoV) is a zoonotic pathogen that caused the 2002–2003 SARS pandemic, which originated in China (1). Since then, genetically diverse SARS-like coronaviruses (SL-CoVs) have been reported in bats in China, Europe, and Africa (2–11), indicating a wide geographic distribution of this group of viruses. However, most bat SL-CoVs have been identified only by sequences and are not fully characterized due to the lack of cultured viruses. Thus, their potential for transmission to and likely pathogenesis in domestic animals and humans remain untested. WIV1 and WIV16 are two recently identified SL-CoV strains with high genomic similarity to human SARS-CoV. These two strains have been successfully cultured *in vitro* and have been shown to use the same molecule (angiotensin-converting enzyme [ACE2]) for cellular entry as SARS-CoV (2, 10). Recently, another bat SL-CoV strain, SHC014, has been demonstrated to use human ACE2 by the construction of an infectious cDNA clone (12). Furthermore, animal infection experiments indicated that SL-CoV WIV1 and SHC014 could replicate efficiently and caused low pathogenesis in ACE2 transgenic mice (12, 13). The fact that the native bat SL-CoVs could use human ACE2 without any mutations indicates a high risk of interspecies transmission for these and similar coronaviruses that may exist in natural reservoirs.

Coronaviruses have the largest genomes among RNA viruses. Their genomes consist of a positive, single-stranded RNA of around 30,000 nucleotides (nt), with two-thirds at the 5' end en-

coding genome replication proteins (ORF1ab) and one-third at the 3' end encoding structural proteins, including a spike glycoprotein (S), a small envelope protein (E), a membrane protein (M), and a nucleocapsid protein (N). Coronaviruses carry a set of open reading frames (ORFs) expressed from full-length mRNAs and subgenomic-length mRNAs (sgRNAs), which have a common 3' end originating at distinct transcription regulatory sequences (TRS) and joined with a common leader sequence encoded at the 5' end of genomic RNA (14). Currently, coronaviruses are divided into the genera *Alphacoronavirus*, *Betacoronavirus*, and *Gammacoronavirus* and the proposed genus *Deltacoronavirus* (15). SARS-CoV and SL-CoVs are grouped into the same coronavirus species, SARS-related coronavirus (SARSr-CoV),

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within the genus *Betacoronavirus*. Besides the family-conserved genes, SARS-CoV possesses several accessory genes, including ORF3, ORF6, ORF7, ORF8, and ORF9, which are specific for this group of coronaviruses but not essential for *in vitro* viral replication (16–18). Accessory genes in coronavirus genomes play important roles in regulating the host immune response (19). The SARS-CoV ORF3a, ORF3b, and ORF6 have been reported to inhibit the host interferon (IFN) response during virus infection and contribute to pathogenesis (20, 21). ORF3a and ORF7a activate NF- κ B and upregulate interleukin-8 (IL-8) and CCL5 production (22, 23). Bat SL-CoVs display great genetic diversity and share overall nucleotide sequence identities of 88 to 97% with human SARS-CoV (2–11). Bat SL-CoVs WIV1 and WIV16 are the closest relatives to human SARS-CoV discovered so far. These two viruses are identical in genomic structures except that WIV1 and -16 have an extra ORF (named ORFX) between ORF6 and ORF7 with no homology to any known protein sequences (2, 10).

In this study, we explored the function of ORFX in modulating the host immune response through the use of eukaryotic overexpression assays and recombinant viruses generated through reverse genetics techniques.

MATERIALS AND METHODS

Virus and cells. The SL-CoV WIV1 strain (GenBank accession number KF367457) and other viruses were propagated as described previously (2). Sendai virus (SeV) strain Cantell (kindly provided by Hanzhong Wang) was propagated in 10-day-old embryonated chicken eggs at 37°C for 48 h (24). All experiments using live virus was conducted under biosafety level 2 (BSL2) conditions. HeLa cells stably expressing human ACE2 (HeLa-hACE2) were described previously (25). 293T, Vero E6, HeLa, and HeLa-hACE2 cells were grown and propagated in Dulbecco's modified Eagle's medium (GIBCO, Invitrogen) supplemented with 10% fetal bovine serum (Life Technologies). Calu-3 cells were grown and propagated in Dulbecco's modified Eagle's medium–nutrient mixture F-12 medium supplemented with 15% fetal bovine serum. Cells were grown at 37°C in a humidified atmosphere with 5% CO₂.

Plasmids. The coding region of ORFX was amplified by reverse transcription-PCR (RT-PCR) from viral RNA using the Superscript one-step RT-PCR kit (Invitrogen). The amplified gene was cloned into plasmid pCAGGS with a C-terminal hemagglutinin (HA) tag (pCAGGS-ORFX) for eukaryotic expression. Reporter plasmids used included pIFN κ -Luc (expressing firefly luciferase under the control of the IFN- β promoter), pNF- κ B-Luc (expressing firefly luciferase under the control of the NF- κ B promoter), and pRL-TK (expressing *Renilla* luciferase under the control of the herpes simplex virus thymidine kinase promoter), as well as an expression plasmid for influenza virus NS1, as described previously (24). Plasmids expressing subcellular organelle markers, including SecY β -green fluorescent protein (GFP) (endoplasmic reticulum [ER] marker), B4Cal-Ti-red fluorescent protein (RFP) (Golgi apparatus marker), and Mito-yellow fluorescent protein (YFP) (mitochondrion marker), were kindly provided by Yanyi Wang of the Wuhan Institute of Virology.

Viral infection assays. Vero E6, Calu-3, and HeLa-hACE2 cells were infected with viruses at a multiplicity of infection (MOI) of 1.0, 0.1, or 0.001 in 25-cm² flasks with a 1-h adsorption period, followed by two washes with D-Hanks solution and culturing by adding 3 ml of medium. The viral supernatants were harvested, at 0, 2, 6, 12, 18, 24, 36, 48, and 72 h postinoculation, with 300 μ l removed and 300 μ l medium added back at each time point. The virus concentration was titrated by plaque assay in Vero E6 cells.

Vero E6 cells were infected by rWIV1-GFP- Δ X or mock infected. After 24 h, fluorescence micrographs were taken to check the expression of green fluorescent protein.

Cloning of WIV1 cDNAs. The virus genome was divided into 8 continuous fragments (A to G) and amplified using specific primers (primer sequences are available upon request). Viral RNA was extracted from the supernatant of WIV1-infected cultures and reverse transcribed with Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega) and random hexamer deoxynucleotide primers. The cDNA was denatured for 5 min at 95°C and amplified by PCR with KOD DNA polymerase (Toyobo) for 20 cycles of 95°C for 30 s, 60°C for 30 s with a 0.5°C decrease per cycle, and 68°C for 5 min, 15 cycles of 95°C for 30 s, 50°C for 30 s, and 68°C for 5 min, and a final extension at 68°C for 10 min. The amplicons were cloned into pGEM-T Easy (Promega). Besides three natural BglII sites, several BglII sites were introduced by synonymous mutations in the PCR process to make all contiguous cDNA fragments capable of unidirectional ligation. SacII and AscI sites were introduced into the 5' terminus of fragment A and the 3' terminus of fragment G, respectively. A poly(A) sequence (25 nt) was added to the 3' terminus of fragment G. At least three colonies of each cDNA clone were sequenced, and the one identical to or with some synonymous mutations to the reported sequence was selected for assembly.

To ablate a natural BglII site at position 1575, primers FA, F-c1575a, R-c1575a, and RA were used for overlap extension PCR (OE-PCR) to introduce the synonymous mutation C1575A (primer sequences are available upon request). Based on previous *in vitro* transcription tests, the synonymous mutation T27527C was also introduced to interrupt a potential T7 termination site via OE-PCR.

Strategy for modifying pBeloBAC11. The cytomegalovirus (CMV) promoter was amplified from pcDNA3.1(+) (Thermo Fisher Scientific) with forward primer 5'-TGAGGATCCCGTTGACATTGATTATTGACTAG-3' and reverse primer 5'-CCTGACTGCAGGTCGACTGCCGCGGAGCTCTGCTTATATAGACC-3'. Hepatitis delta virus (HDV) ribozyme was synthesized as described previously (26), and amplified with forward primer 5'-CAGTCGACCTGCAGTCAGGCGCGCGGTCGGCATGGCATCTCC-3' and reverse primer 5'-CTAGAAGGCACAGCTCCCTTATGCCATCCGAGTGG-3'. The bovine growth hormone (BGH) transcription terminal signal was amplified from pcDNA3.1(+) with forward primer 5'-GGATGGCTAAGGGAGCTGTGCTTCTAGTTGCCACAGC-3' and reverse primer 5'-TGAAAGCTTCCATAGAGCCGACCGCATCC-3'. The three PCR products then were ligated using OE-PCR, with BamHI and HindIII sites flanking the amplicon and SacII and AscI sites between the CMV promoter and HDV ribozyme. The amplicon was then inserted into pBeloBAC11 (New England BioLabs) between BamHI and HindIII sites. The construct was designated pBAC-CMV.

Construction of infectious bacterial artificial chromosome (BAC) clones of WIV1. Subclone A and subclone G were first digested with SacII and AscI (New England BioLabs), respectively, followed by treatment with calf intestinal alkaline phosphatase (CIAP) (TaKaRa), chloroform extraction, and isopropanol precipitation, and then restricted with BglII (TaKaRa). Subclones B to F were digested with BglII. pBAC-CMV was digested with SacII and AscI. All digestion products were then separated using 1% agarose gels, excised, and purified by using a gel extraction kit (Omega). Digested fragments A to G and pBAC-CMV were ligated overnight at 4°C, transformed into DH10B competent cells, and plated on *Chl*^r LB culture. Ten clones were screened by restriction fragment length polymorphism (RFLP) analysis with NcoI, StuI, or HindIII. The correct clone was named pBAC-CMV-rWIV1 (Fig. 1).

Construction of WIV1 mutants. To delete ORFX, the fragment F was PCR amplified with primers FF (5'-ACCTGTGGCCTTTTGGCGAGGT TTTAATGCTACTAC-3') and RFox (5'-GCCTCTAGGGCTCAAGGA TAATCTATCTCCATAGG-3'). Fragment G was PCR amplified with FGox (5'-GCCCTAGAGGCAACGAACATGAAATATTCTCTCTCC-3') and RG (5'-ACTGGGCGGCCCTTTTCTTTTCTTTTCTTTTCTTTT GTCATCTCTCTGAGAAGC-3'). This new fragment was named Gox. These two products were then cloned into pGEM-T Easy. The two fragments were inserted into the BAC along with the other fragments as described above. The rescued mutant was named as rWIV1- Δ X. To place

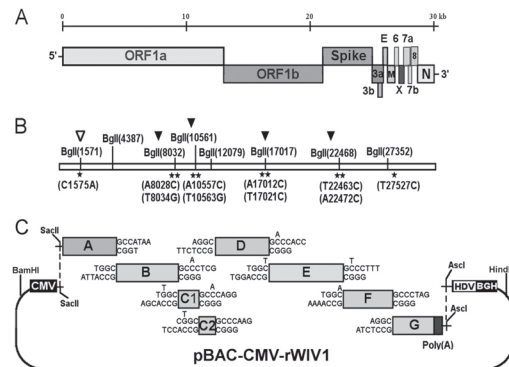


FIG 1 Strategy for construction of an infectious WIV1 BAC clone. (A) Genomic structure of WIV1. (B) The mutations are indicated under the stars. C1575A was used to ablate a natural BglII site at nucleotide 1571 (V), and T27527C was used to disrupt a potential T7 stop site. The others were for introducing BglII sites (▼). (C) The WIV1 genome was split into eight contiguous cDNAs (A to G): A, nt 1 to 4387; B, nt 4388 to 8032; C1, nt 8033 to 10561; C2, nt 10562 to 12079; D, nt 12080 to 17017; E, nt 17018 to 22468; F, nt 22469 to 27352; G, nt 27353 to 30309. Unique BglII sites were introduced into the fragments by synonymous mutations to make these fragments capable of unidirectional ligation along with native BglII sites in the genome. The original nucleotides are shown above the flanking sequences of corresponding fragments. A poly(A) sequence was added to the 3' terminus of fragment G. A CMV promoter, HDV ribozyme, and BglII transcriptional terminal signal were inserted into pBacBAC11 between BamHI and HindIII sites. SacII and AscI sites were introduced between the CMV promoter and ribozyme. Fragments A to G were inserted into the pBAC-CMV plasmid in a single step.

GFP into the open reading frame of ORFX, the F fragment was amplified with primers FF and RFoeGFP (5'-GCTCACCATAGTGGTTCGTTTAT CAAGGATAATCTATCTOC-3'). The GFP gene was amplified with primers 5'-CCTTGATAAAGCAACCACTATGGTGAGCAAGGCGAG GAGC-3' and 5'-TGCCTCTAGGGCTTACTGTACAGCTGTCCAT GCC-3'. The two PCR products were ligated by OE-PCR, and the product was inserted into pGEM-T Easy. The rescued mutant was named rWIV1-GFP-ΔX.

Transfection of infectious WIV1 BAC clones. Vero E6 cells were seeded in a 6-well plate a day in advance, and then one well was transfected with 6 μg infectious BAC plasmids constructed as described above with Lipofectamine LTX and Plus reagent (Life Technologies). Virus progeny was plaque purified once. One clone was passaged once in Vero E6 cells for 72 h and used to generate a stock for future use.

RFLP. RNAs extracted from wild-type and recombinant viruses were reverse transcribed with random hexamer primers. RT-PCR was used to generate five amplicons containing the five mutations designed in the strategy. These amplicons included a 1,124-bp amplicon (nucleotide positions 1312 to 2435) spanning a naturally occurring BglII site at nucleotide 1571 that had been ablated in recombinant viruses, a 1,438-bp amplicon spanning the B/C1 junction (nucleotide positions 7560 to 8997), a 1,437-bp amplicon spanning the C1/C2 junction (nucleotide positions 10196 to 11632), a 1,437-bp amplicon spanning the D/E junction (nucleotide positions 16793 to 18229), and a 1,438-bp amplicon spanning the E/F junction (nucleotide positions 21908 to 23345) (these amplicons correspond to fragments F1 to F5 in Fig. 1). The first amplicon of wild-type WIV1 (wtWIV1) that contains nucleotide 1571 can be cleaved by BglII, but the other four amplicons cannot. In contrast, the five amplicons of recombinant viruses are different from those of wild-type virus in the capability of being cut by BglII.

Northern blot analysis. The N gene was amplified with primers WIV1-NF (5'-ATGTCTGATAATGGACCCCA-3') and WIV1-3R (5'-G TCATTCTCTGAGAAGCTA-3') and used as a template for probe prep-

aration according to the description in the DIG-High Prime DNA labeling and detection starter kit II (Roche). Vero E6 cells were infected with wild-type and recombinant viruses at an MOI of 1.0. At 24 h postinfection, intracellular RNA was isolated using TRIzol reagent (Ambion). RNA (20 μg) was precipitated, treated with 17 μl sample buffer (50% formamide, 2.2 M formaldehyde [37%], 1× morpholinepropanesulfonic acid [MOPS]) at 65°C for 10 min, supplemented with 3 μl 10× dye solution (50% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanole FF), and then separated in a denaturing 0.8% agarose-2.2 M formaldehyde gel at 28 V for ~17 h. The RNA was hydrolyzed with 0.05 M NaOH for 40 min, transferred to a Hybond-N+ membrane (GE Healthcare) for ~18 h, and then cross-linked to the membrane using UV light. The membrane was prehybridized, probed with a digoxigenin (DIG)-labeled probe for the N gene, and washed, and detection was performed according to the instructions for the DIG-High Prime DNA labeling and detection starter kit II (Roche).

RT-PCR of leader-containing transcripts. Intracellular RNA was isolated from wtWIV1. A forward primer (Leader-F) located in the leader sequence, along with various reverse primers located in several ORFs, was used for amplifying leader-containing sequences (primer sequences are available upon request). Leader-containing amplicons were sequenced with the corresponding reverse primers.

ORFX subcellular location. HeLa cells were transfected with an ORFX-expressing plasmid and cotransfected with organelle markers expressing plasmid SecG1B-GFP, B4Gal-Ti-RFP, or Mito-YFP. After 24 h, the cells were fixed and stained with a mouse anti-HA IgG (Promoter). A Cy3-conjugated goat anti-mouse IgG (Promoter) was used for secondary detection in cells expressing ER or mitochondrial markers. A fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Promoter) was used for secondary detection in cells expressing the Golgi marker. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). Staining patterns were examined with an Olympus Fluoview upright confocal microscope (Olympus).

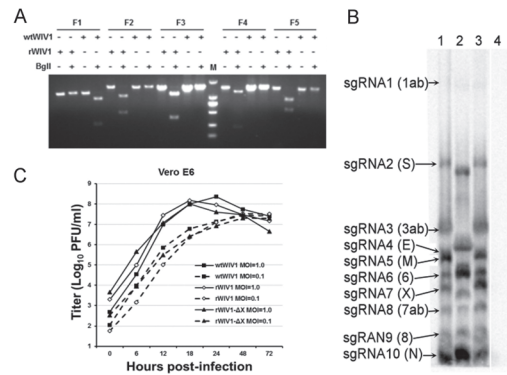


FIG 2 Recovery and characterization of recombinant viruses. (A) Restriction fragment length polymorphism. Amplicons flanking five mutated sites of wild-type and recombinant viruses were digested by BglI. The first amplicon (F1) of wild-type virus can be digested by BglI, and its other four amplicons (F2 to F5) cannot be. In contrast, for amplicons of rWIV1, the first amplicon (F1) cannot be digested by BglI and its other four amplicons (F2 to F5) can be. Lane M, DL2000 DNA ladder (TaKaRa). (B) Detection of viral genomic transcription and replication by Northern blotting. Vero E6 cells were infected with wild-type or recombinant viruses, and intracellular RNA was extracted for Northern blot analysis. Lane 1, wtWIV1; lane 2, rWIV1-ΔX; lane 3, rWIV1; lane 4, uninfected control. (C) Growth kinetics of wild-type and recombinant viruses. Vero E6 cells were infected with wtWIV1 (■), rWIV1 (◇), or rWIV1-ΔX (▲) at an MOI of 1.0 or 0.1 PFU/cell. Cell supernatants were taken at the indicated time points postinfection, and virus titers were determined by plaque assay in Vero E6 cells.

Luciferase assays and quantitative PCR. For the OREX-mediated IFN promoter assay, 293T cells were seeded in 12-well plates and cotransfected with empty vector plasmid pCAGGS, plasmid pCAGGS-NS1, or increasing amounts (100, 200, 400, 600, and 800 ng) of pCAGGS-OREX with the indicated reporter plasmids. At 24 h posttransfection, cells were infected with Sendai virus (SeV) (100 hemagglutinin units [HAU]/ml) for 12 h to induce IFN production or were treated with tumor necrosis factor alpha (TNF-α) for 1 h to activate NF-κB. Cell lysates were prepared, and luciferase activity was measured using dual-luciferase assay kits (Promega) according to the manufacturer's instructions.

293T cells were transfected with empty vector, NS1-expressing plasmid, or increasing amounts (100, 300, and 600 ng) of OREX-expressing plasmid. After 24 h, the cells were infected with SeV (100 HAU/ml). At 12 h postinfection, the cells were lysed. The mRNA was extracted and reverse transcribed with PrimeScript RT master mix (TaKaRa). The expression level of IFN-β mRNA was determined by quantitative PCR using SYBR Premix Ex Taq II (TaKaRa). The GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA was quantified as an internal control. 293T cells were transfected as described above. After 24 h, the cells were treated with TNF-α for 6 h, and the cell RNA was extracted and used for quantification of the expression of IL-8 mRNA. All experiments were performed in triplicate and repeated at least three times. All primer sequences used in the quantitative PCRs are available upon request.

IRF3 translocation assay. 293T cells were transfected with empty vector, NS1, or OREX-expressing plasmid. After 24 h, IFN regulatory factor 3 (IRF3) nuclear translocation was induced by infecting the cells with SeV for 8 h. The cells were fixed and stained with a rabbit anti-IRF3 polyclonal IgG (Proteintech) and a mouse anti-HA IgG (Promoter). An Alexa Fluor 488-conjugated donkey anti-rabbit IgG (Yeast) and an Alexa Fluor 555-conjugated donkey anti-mouse IgG (Beyotime) were used to detect IRF3 and OREX, respectively. The cells transfected with empty vector were stained with a rabbit anti-IRF3 polyclonal IgG and a goat anti-SeV IgG (kindly provided by Lin-Fa Wang, Duke-NUS Graduate Medical School, Singapore) as an indication of infection efficiency. An Alexa Fluor 488-

conjugated donkey anti-rabbit IgG and a Cy3-conjugated donkey anti-goat IgG (Promoter) were used to detect IRF3 and SeV, respectively. Nuclei were stained with DAPI.

Quantification of mRNA expression of cytokines in infected Calu-3 cells. Calu-3 cells grown in 24-well plates were mock infected or infected with rWIV1 or rWIV1-ΔX at an MOI of 5 or with SeV (100 HAU/ml). The cells were lysed at 4, 12, 24, and 30 h postinfection. The mRNA expression levels of IFN-β, IL-6, IL-8, and TNF-α were quantified by quantitative PCRs. The expression of GAPDH mRNA was measured as an internal control. All primer sequences used in the quantitative PCRs are available upon request. The experiment was performed twice.

IFN-β sensitivity assay. Vero E6 cells were seeded a day in advance. The cells were pretreated with 10, 100, or 1,000 U/ml IFN-β (PBL, Piscataway, NJ) for 24 h, infected with wtWIV1, rWIV1, and rWIV1-ΔX at an MOI of 0.1 PFU/cell, and posttreated with the same amount of IFN-β as used previously. At 24 h postinfection, the viral replication was analyzed by plaque assay. The experiment was performed in triplicate.

Statistics. The statistical significance of the obtained data was analyzed using a Student *t* test in GraphPad Prism (GraphPad Software, San Diego, CA). A *P* value of <0.05 was considered statistically significant. Data are presented as the means ± standard errors of the means (SEM).

RESULTS

Strategy for construction of an infectious WIV1 BAC. Originally, the genome was split into seven contiguous cDNAs (A to G) (Fig. 1A and C). Due to plasmid instability, fragment C was separated into two segments (C1 and C2). Besides three naturally occurring BglI sites (GCCNNNN ↓ NGGC), four BglI sites were successfully introduced by synonymous mutations in the genome (Fig. 1B). Different asymmetric 3-nt overhangs at the junctions of each two contiguous fragments were created by these BglI sites. The eight fragments were then linked in one direction. A SacI site was added to the 5' terminus of fragment A. A poly(A) sequence

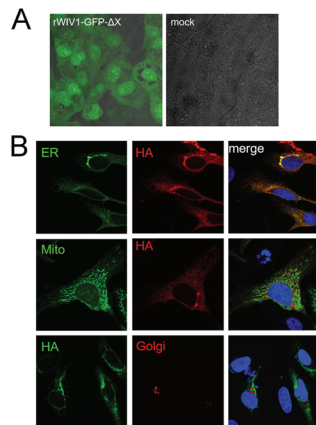


FIG 3 Expression and subcellular location of ORFX protein. (A) The open reading frame of ORFX was replaced by the GFP sequence, and the recombinant virus was rescued. Vero E6 cells were infected with the recombinant virus or mock infected. Green fluorescence was visualized at 24 h postinfection. (B) ORFX protein with an HA tag at the C terminus was expressed in HeLa cells, along with SecG1β-GFP (ER marker), Mito-YFP (mitochondria marker), or B4Gal-T1-RFP (Golgi marker). The cells were fixed after 24 h and stained with a mouse anti-HA IgG. A Cy3-conjugated goat anti-mouse IgG was used for secondary detection in cells expressing an ER or mitochondrial marker. An FITC-conjugated goat anti-mouse IgG was used for secondary detection in cells expressing a Golgi marker. ORFX protein showed a cytoplasmic distribution and colocalized with the ER marker SecG1β.

(25 nt) and an *Ascl* site were added to the 3' terminus of fragment G. A naturally occurring *Bgl*II site at nucleotide 1571 was removed by the synonymous mutation C1575A (Fig. 1B). Other unexpected synonymous mutations also occurred, including T1422C, T12984C, T14213C, T17130C, C17934T, and T26068G.

The plasmid pBAC-CMV was constructed by inserting the cytomegalovirus (CMV) promoter, hepatitis delta virus (HDV) ribozyme, and bovine growth hormone (BGH) transcription terminal signal sequences into pBeloBAC11, along with the introduction

of the *Sac*II and *Ascl* sites between the CMV promoter and HDV ribozyme (Fig. 1C). The eight genomic fragments were inserted into pBAC-CMV in one step. Recombinant viruses could be rescued by direct transfection with the BAC constructs.

Rescue of recombinant viruses. To rescue recombinant WIV1 (rWIV1), fragments A and G were digested with *Sac*II and *Ascl*, respectively. Following calf intestinal alkaline phosphatase (CIAP) dephosphorylation, the two fragments, along with fragments B to F, were digested using *Bgl*II and inserted into pBAC-CMV between *Sac*II and *Ascl* sites in one step. The constructed clone (pBAC-CMV-rWIV1) was transfected into Vero E6 cells. A cytopathic effect was observed at 72 h posttransfection. The one ablated natural *Bgl*II site and four introduced *Bgl*II sites in the rescued viral genome were confirmed by restriction fragment length polymorphism (RFLP) analysis with *Bgl*II digestion (Fig. 2A). Using this method, we also rescued an ORFX deletion mutant virus (rWIV1-ΔX) (Fig. 2B, lane 2) and a mutant with a GFP sequence placed in the coding region of ORFX (rWIV1-GFP-ΔX) (Fig. 3A).

ORFX is a functional gene not essential for virus replication.

The one-step growth curves for the two rescued recombinant viruses (rWIV1-ΔX and rWIV1) and wild-type WIV1 (wtWIV1) determined by plaque assay showed that rWIV1-ΔX and rWIV1 both replicated to titers close to those of wild-type virus (Fig. 2C). The expected set of appropriately sized 10 sgRNAs, including sgRNA7 (ORFX), were observed in Northern blot analysis in cells infected with wtWIV1 and rWIV1 (Fig. 2B, lanes 1 and 3). As expected, sgRNA7 was not observed in rWIV1-ΔX infected cells (Fig. 2B, lane 2). Analysis of leader-containing sequences indicated that all 10 sgRNAs in wtWIV1 share an identical core sequence, ACGAAC (Table 1), which further confirmed that ORFX is expressed as sgRNA7. The fact that GFP was expressed in rWIV1-GFP-ΔX-infected cells further confirmed that the open reading frame of ORFX could be expressed (Fig. 3A). Subcellular location analyses showed that the ORFX protein colocalized with the ER marker but not with the Golgi and mitochondrial markers (Fig. 3B).

ORFX protein inhibits production of IFN-β. To determine whether ORFX inhibits the induction of IFN, 293T cells were transfected with plasmids pIFNβ-Luc and pRL-TK and a plasmid expressing ORFX, influenza virus strain PR8 NS1 (positive control), or empty vector (negative control). As expected, SeV activated IFN production in cells transfected with empty vector. The positive control, influenza virus NS1 protein dramatically inhibited the expression from the IFN promoter. ORFX protein exhibited an inhibition effect, but the effect decreased when more

TABLE 1 Leader-containing sequences of sgRNAs

sgRNA	ORF(s)	Leader-containing sequence ^a	Consensus sequence positions
1	1a/b	GTAGATCTGTTCTCTAAACGAACCTTTAAATCTGT	67–72
2	S	GTAGATCTGTTCTCTAAACGAACATGAAATGTTA	21486–21491
3	3a/b	GTAGATCTGTTCTCTAAACGAACCTTATGGATTGT	25263–25268
4	E	GTAGATCTGTTCTCTAAACGAACCTTATGTAATCAT	26112–26117
5	M	GTAGATCTGTTCTCTAAACGAACCTAATATTATTA	26351–26356
6	6	GTAGATCTGTTCTCTAAACGAACGCTTTCTTATTA	26916–26921
7	X	GTAGATCTGTTCTCTAAACGAACCACTATGTTACT	27272–27277
8	7a/b	GTAGATCTGTTCTCTAAACGAACATGAAAATTATT	27794–27799
9	8	GTAGATCTGTTCTCTAAACGAACATGAAAATTCTC	28300–28305
10	N	GTAGATCTGTTCTCTAAACGAACAACTAAATATGT	28672–28677

^a The consensus sequence is in bold. Underlining indicates the initiation codon.

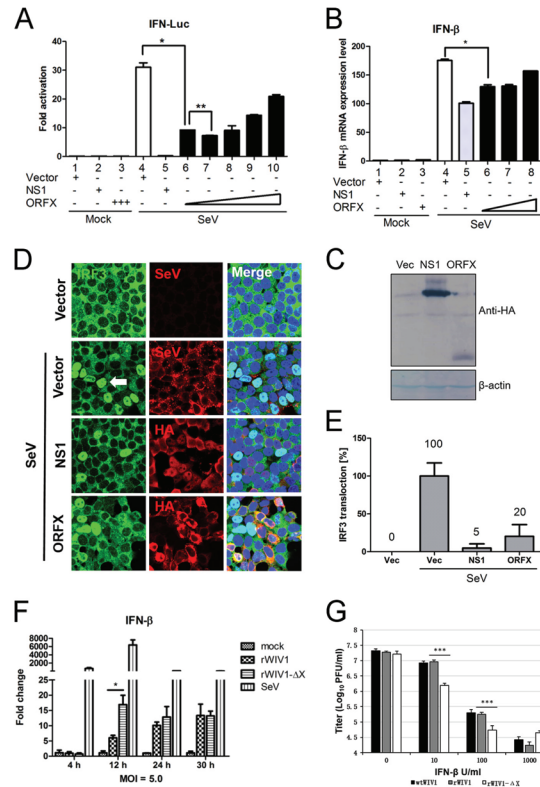


FIG 4 ORFX protein inhibits the production of type I interferon. (A and B) 293T cells seeded in 12-well plates were transfected with 100 ng pIFN-β-Luc, 5 ng pRL-TK, empty vector, an influenza A NS1-expressing plasmid, or increasing doses (100, 200, 400, 600, and 800 ng) of an ORFX-expressing plasmid. Empty vector was added appropriately to ensure that cells in each well were transfected with the same amount of plasmids. The cells were infected with Sendai virus (100 hemagglutinating units/ml) at 24 h posttransfection. Samples were collected at 12 h postinfection, followed by dual-luciferase assay. The results were expressed as the firefly luciferase value normalized to that of *Renilla* luciferase. The relative expression of IFN-β mRNA was determined by quantitative RT-PCR and normalized to the expression level of GAPDH mRNA. (C) The expression of the NS1 and ORFX proteins was analyzed by Western blotting with an antibody against HA tag. The experiments were replicated three times. (D and E) For the IRF3 translocation assay, 293T cells were transfected with empty vector-, NS1-, or ORFX-expressing plasmid. After 24 h, the cells were infected with Sendai virus to induce IRF3 nuclear translocation. The cells were fixed at 8 h postinfection and stained with anti-HA IgG. A goat anti-Sendai virus polyclonal IgG was used to stain the cells transfected with empty vector. A rabbit anti-IRF3 polyclonal IgG was used to label IRF3. The white arrow indicates IRF3 nuclear translocation. The relative IRF3 translocation ratios were calculated for each group by counting the number of IRF3 nuclear translocation cells (randomly selected from at least 4 fields) and dividing by the total number of infected or transfected cells. The IRF3 nuclear translocation efficiency of each group was expressed as the percentage of their relative IRF3 translocation ratios to that of the control (cells transfected with empty vector). (F) Calu-3 cells were mock infected or infected with rWIV1 or rWIV1-ΔX (MOI of 5) or SeV (100 HAU/ml). At 4, 12, 24, and 30 h postinfection, the cell RNA was extracted and used for quantitative RT-PCR of the expression level of IFN-β mRNA. The experiment was performed in triplicate and replicated twice. (G) Vero E6 cells were pretreated with indicated amount of IFN-β, infected with wtWIV1, rWIV1, or rWIV1-ΔX at an MOI of 0.1 PFU/cell, and posttreated with IFN-β. Viral replication was analyzed at 24 h postinfection by plaque assay. The experiment was performed in triplicate and replicated twice. The differences between selected groups were significant, with *P* values of less than 0.05, as follows: 0.0049 (*) in panel A, 0.0008 (**) in panel B, 0.0072 (*) in panel A, 0.0018 (*) in panel B, 0.018 (*) in panel F, and <0.0001 (***) in panel G.

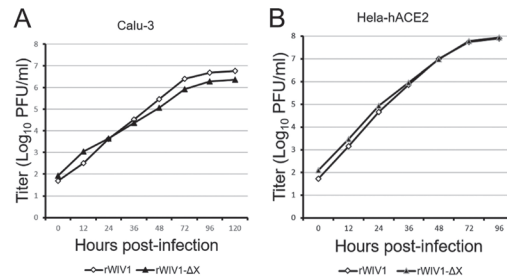


FIG 5 Comparison of viral replication efficiencies of rWIV1-ΔX and rWIV1 in IFN-competent cells. Calu-3 (A) and HeLa-hACE2 (B) cells were infected with rWIV1 or rWIV1-ΔX at an MOI of 0.001. Samples were collected at 0, 12, 24, 36, 48, 72, 96, and 120 h postinfection. The viral titers were measured by plaque assay.

ORFX protein was expressed (Fig. 4A). Similar results were observed for IFN-β mRNA quantification (Fig. 4B and C).

An IRF3 nuclear translocation assay was performed to see whether ORFX protein inhibits IFN production through inhibiting this process. 293T cells were transfected with an empty vector, NS1-, or ORFX-expressing plasmid. After 24 h, IRF3 nuclear translocation was induced by infection with SeV for 8 h. The relative IRF3 translocation ratios were calculated for each group by counting the number of the IRF3 nuclear translocation cells (randomly selected from at least 4 fields) divided by the number of total infected or transfected cells. The IRF3 nuclear translocation efficiency of each group was expressed as the percentage of their relative IRF3 translocation ratios to that of the control (cells transfected with empty vector). As expected, NS1 strongly inhibited translocation of IRF3, while ORFX protein also showed inhibition of IRF3 translocation but less efficiently (Fig. 4D and E).

To further investigate the IFN inhibition activity of ORFX, the deletion mutant and wild-type recombinant virus were used to infect Calu-3 cells at an MOI of 5. Mock-infected cells were used as negative control. Calu-3 cells infected with SeV were used as positive control. Samples were collected at 4, 12, 24, and 30 h postinfection. The relative expression of IFN-β mRNA was determined by quantitative PCR and normalized to the expression of GAPDH mRNA. Compared to SeV, WIV1 recombinants induced low levels of IFN-β mRNA in Calu-3 cells (Fig. 4F). The ORFX deletion mutant induced a significantly higher level of IFN-β mRNA than wild-type recombinant virus in infected cells at 12 h postinfection, but there were no significant differences at 24 and 30 h postinfection (Fig. 4F). These results indicate that ORFX protein may play a role in antagonizing IFN only at early times during WIV1 infection.

An ORFX deletion mutant shows increased sensitivity to IFN-β. To further investigate the effect of ORFX on the viral sensitivity of IFN, we tested the replication efficiencies of wtWIV1, rWIV1, and rWIV1-ΔX in Vero E6 cells which were pretreated and posttreated with IFN-β. The replication of rWIV1-ΔX was inhibited and reduced by ~0.5 log compared to that of wtWIV1 and rWIV1 at concentrations of 10 and 100 U/ml IFN-β (Fig. 4G), whereas at a higher IFN-β concentration (1,000 U/ml), the

rWIV1-ΔX titers did not show an obvious decrease compared to those of wild-type virus. We expected that the ORFX deletion mutant would replicate less efficiently than the wild-type virus in IFN-competent cells. However, we did not find a significant difference when we grew the two viruses in Calu-3 and HeLa-hACE2 cells, even at a very low MOI of 0.001 (Fig. 5).

ORFX protein activates NF-κB. NF-κB plays an important role in regulating the immune response to viral infection and is also a key factor frequently targeted by viruses for taking over the host cell (27). Several proteins (Nsp1, N, and 3a) encoded by SARS-CoV have activities in both IFN antagonism and NF-κB activation (28). In this study, we also tested whether ORFX protein could activate NF-κB. 293T cells were transfected with pNF-κB-Luc, pRL-TK, empty vector, NS1, or increasing amounts (200, 400, and 600 ng) of ORFX expressing plasmid. After 24 h, the cells were mock treated or treated with TNF-α for 6 h, and luciferase activity was determined. ORFX protein obviously activated NF-κB, no matter whether the cells were treated with TNF-α or not (Fig. 6A), whereas IL-8 was upregulated only when the cells were treated with TNF-α (Fig. 6B). However, no significant difference was observed for IL-6 and IL-8 transcription levels between the rWIV1-ΔX- and rWIV1-infected Calu-3 cells (Fig. 6C and D). A significant difference was observed only for the induction of TNF-α mRNA at the late time of virus infection, when the ORFX deletion mutant induced less TNF-α mRNA (Fig. 6E).

DISCUSSION

In this study, we have developed a fast and cost-effective method for reverse genetics of coronaviruses by combining two approaches developed by others (29, 30). Our method allows the genomes of coronaviruses to be split into multiple fragments and inserted into a BAC plasmid with a single step. Recombinant viruses can then be efficiently rescued by direct transfection of the BAC constructs. As the genomes can be divided into multiple short fragments, mutations can be introduced into individual fragments easily (31). Using this method, we successfully rescued three recombinant viruses derived from SL-CoV WIV1 (rWIV1, rWIV1-ΔX, and rWIV1-GFP-ΔX). The recombinant rWIV1 and rWIV1-ΔX replicated to titers close to those of wtWIV1 in Vero

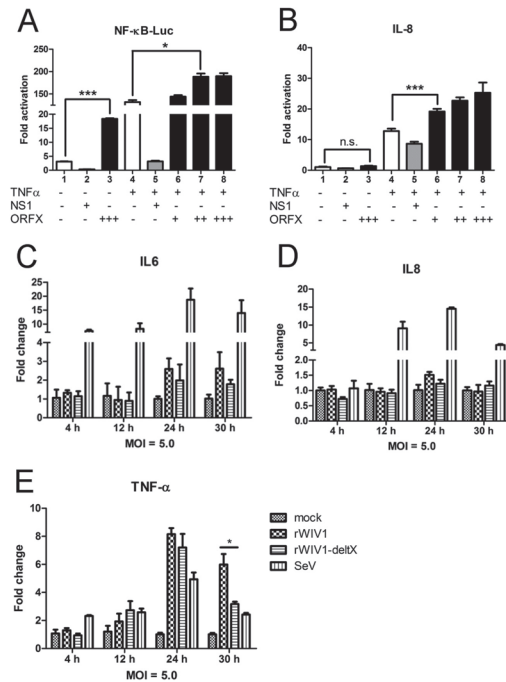


FIG 6 ORFX protein activates NF- κ B. 293T cells were transfected with 100 ng pNF- κ B-Luc, 10 ng pRL-TK, empty vector, an NS1-expressing plasmid, or increasing amounts (200, 400, and 600 ng) of an ORFX-expressing plasmid. After 24 h, the cells were treated with TNF- α . (A) Dual-luciferase activity was determined after 6 h. The results were expressed as the firefly luciferase activity normalized to that of *Renilla* luciferase. (B) The relative expression of IL-8 mRNA was quantified through quantitative RT-PCR and normalized to that of GAPDH mRNA. Differences between selected groups were significant, with *P* value less than 0.05, as follows: <0.0001 (***) (bars 1 and 3 in panel A), 0.0339 (*) (bars 4 and 7 in panel A), and 0.0002 (***) (bars 4 and 6 in panel B). n.s., not significant. The experiments were performed three times. (C to E) The RNA extracted from Calu-3 cells for Fig. 4 was used for quantification of the expression of IL-6 (C), IL-8 (D), and TNF- α (E) mRNAs.

E6 cells (Fig. 2C), suggesting that the deletion of ORFX did not affect WIV1 replication *in vitro*. Northern blotting and fluorescence microscopy further confirmed that ORFX is transcribed as sgRNA7 and translated in virus-infected cells. These results demonstrated that the unique ORFX in SL-CoV WIV1 is a functional gene but is not essential for virus replication. We propose that the ORFX sgRNA is the template for the translation of a novel 11-kDa accessory protein of WIV1, bringing the total number of group-specific accessory proteins to ten.

In previous studies, it has been proved that SARS-CoV group-specific accessory genes ORF3b and ORF6 inhibit host IFN production and/or signaling during virus infection and contribute to viral pathogenesis (20). It is interesting to know whether the

ORFX has a similar function in antagonizing IFN. In this study, ORFX protein showed an inhibitory effect on IFN production, but the effect decreased when more ORFX protein was expressed (Fig. 4A and B). Moreover, the ORFX deletion mutant had a significantly lower inhibitory effect on IFN production than wild-type recombinant virus in infected Calu-3 cells, but only at an early time after infection (Fig. 4F). Furthermore, the IFN sensitivity assay indicated that the ORFX deletion mutant was more sensitive to IFN- β (Fig. 4G), suggesting that ORFX protein may participate in subverting the antiviral state stimulated by IFN- β . All these results suggested that ORFX participates in the modulation of the IFN response. Previous studies showed that SARS-CoV ORF3a and ORF7a activate NF- κ B and upregulate IL-8 and CCL5 pro-

duction (22, 23). In our study, we also found through a dual-luciferase assay that overexpressed ORFX can activate NF- κ B (Fig. 6A). Furthermore, the level of TNF- α mRNA induced by wild-type recombinant virus was significantly higher than that induced by the ORFX deletion mutant, but only at the late stage of infection (Fig. 6E). These results indicated that ORFX also participates in activation of NF- κ B. We noted that the IFN inhibition activity of ORFX was not dose dependent and decreased when there was more ORFX expression. One possible hypothesis is that ORFX inhibits IFN only at the early stage of infection. At the late stage, it activates NF- κ B, which in turn stimulate IFN expression, and this leads to the attenuation of its IFN antagonist activity.

Coronavirus was previously shown to induce the unfolded-protein response (UPR) and ER stress in infected cell culture (32). Normally, ER is an active organelle for protein folding and modification. Loss of protein folding homeostasis would cause ER stress and induce the UPR, leading to the activation of three ER stress transducers. These transducers work in concert to attenuate translation and improve ER folding capacity to restore ER homeostasis (33). In this process, NF- κ B is activated, and apoptosis will be induced if ER stress is prolonged (32, 33). In this study, we observed that the overexpression of ORFX protein led to cell death and the decrease of *Renilla* values (data not shown). This may imply that ORFX has a cytotoxic effect and an influence on overall protein translation. We also found that ORFX colocalizes with an ER marker. We hypothesize that ORFX may induce the UPR and cause ER stress which would activate NF- κ B and induce apoptosis, promoting viral release at the late stage of infection.

It should be noted that the IFN and NF- κ B detection systems used in this study were derived from and used in human cells. Since the innate immune system of bats is special and probably deficient in some aspects compared to the human system (34), it will be interesting to conduct the same studies in bat cells to determine whether ORFX protein has the same profiles as those observed in the human cell system. The development of different cell lines from the *Rhinolophus* bat, which is the reservoir host of SL-CoV, will facilitate this research in the future.

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STATEMENT SUBMITTED BY THE ASSOCIATION OF PUBLIC HEALTH LABORATORIES

Response from the Association of Public Health Laboratories.

The Association of Public Health Laboratories (APHL) is pleased to submit a Statement for the Record in response to the Hearing on *“Principles for Outbreak Investigation: COVID-19 and Future Infectious Diseases”* held on July 14, 2021, at 12:00 p.m.

My name is Scott Becker and I am the Chief Executive Officer of APHL which represents state, local and large city public health laboratories in the United States and its territories. These laboratories were the first to test for the SARS-CoV-2 virus when it was apparent that it was circulating in the United States, and continue to play a crucial role in the laboratory response to the COVID-19 pandemic.

There is currently considerable discussion in the scientific community, academic journals and popular media about the origin of the pandemic, with the two plausible origins being a zoonotic spillover event or a laboratory leak. APHL fully supports the need for a thorough investigation into the origin, with the goal of learning as much as possible so that the event will not be repeated in the future. However, the reality is that the actual origin of the first appearance of the SARS-CoV-2 virus in humans may never be recognized.

Given the unfathomable cost of the pandemic in both morbidity and mortality, as well as catastrophic losses to the national economy, APHL believes that future detection and control of emergent pathogens with pandemic potential is a matter of national security. As such, the major focus, at this point in time, should be on developing and sustaining the national infrastructure necessary to detect the introduction of emerging pathogens and the ability to move quickly to limit the spread of the outbreak. APHL recommends three areas that we believe are essential for the health and security of our nation.

Firstly, the focus of the federal government should be on developing nationwide systems for enhanced genomic surveillance of pathogens using the most advanced technologies available. The surveillance technology should be pathogen agnostic, rather than targeted at detecting specific known pathogens as is now the case. This surveillance technology should reside in a public health laboratory system that is sustainably funded, and staffed by highly qualified and well-trained scientists. The public health surveillance system must be seamlessly, and bi-directionally linked to the Centers for Disease Control and Prevention (CDC) as well as to equivalent public health organizations in other countries, for rapid and transparent alert notification and exchange of data.

Secondly, all aspects of the national laboratory system should be inter-connected. The first contact with the health care system of a patient infected with an emergent pathogen is likely to occur at a hospital Emergency Room or an Urgent Care Center. Specimens from the index patient may be tested on-site with a point-of-care test, or tested, using more complex methods, in a hospital laboratory. For a new pathogen, this preliminary testing will confirm that a common pathogen is not present in the sample, but will not identify the causative agent. The sample will then be forwarded to the state or local public health laboratory, or the CDC, for identification by a range of molecular analyses, including whole genome sequencing. The mission of public health laboratories has always been to perform geographically relevant surveillance and early detection of pathogens and provide a “first to respond” function. Public health laboratories, while equipped and staffed to respond to local and regional outbreaks, are not prepared to perform the high throughput testing required in a pandemic. Here the role of commercial laboratories, and sometimes, accredited academic laboratories, becomes essential in achieving the necessary scale. Given this involvement of many laboratories with distinct, but overlapping

missions and roles at different phases of a large outbreak or pandemic, it is essential, that we recognize and support the concept of an interconnected national laboratory system, rather than the functional silos that now exist.

Thirdly, during the pandemic of 2020 and 2021, it has become clear that silos also exist in our public health data systems. Public health surveillance encompasses the interactive system of public health agencies at various levels (including federal, state, and local) working with health care providers and the public to detect, report, and prevent illness and death. These activities depend on the availability of accurate and timely data that can be analyzed using modern epidemiologic methods. In the United States, public health surveillance and data systems are poorly funded, exist in silos and are based on outdated technologies¹.

To this end, APHL applauds the use of funds appropriated in the American Rescue Plan. These funds will support public health data surveillance and analytics infrastructure modernization initiatives at the Centers for Disease Control and Prevention, and establish, expand, and maintain efforts to modernize the United States disease warning system to forecast and track hotspots for COVID–19, its variants, and emerging biological threats.²

1. Identifying and Tracking SARS-CoV-2 Variants — A Challenge and an Opportunity (2021). Scott J. Becker, M.S., Jill Taylor, Ph.D., and Joshua M. Sharfstein, M.D. *New England Journal of Medicine*. Doi: 10.1056/NEJMp2103859.
2. H.R. 1319. American Rescue Plan Act of 2021. Sec. 2404. Funding for data modernization and forecasting center (<https://www.congress.gov/bills/117th/congress/house-bill/1319/text>).

LETTER SUBMITTED BY DR. STEVEN QUAY, ET AL.

Submitted to House Subcommittee on Investigations and Oversight Hearing
Principles for outbreak investigation: COVID-19 and future infectious diseases

Science Not Speculation Should Inform the Investigation of the Origin of COVID-19

Summary. Two hypotheses of the origin of the COVID-19 pandemic exist: a natural zoonosis or a laboratory-related event. An investigation of the origin of the COVID-19 pandemic needs to be conducted to a science-based resolution of these competing hypotheses. Until new evidence is found, an investigation of the pathway for the origin of COVID-19 should proceed with the assumption that a laboratory-acquired infection or laboratory accident is at least as likely, if not more likely, than a natural zoonotic transmission.

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Two Lancet letters¹ have put forth a plea to use scientific evidence, not speculation, in determining the origin of the COVID-19 outbreak. We agree that only well collected scientific data and rigorous analysis can bring us closer to understanding how the responsible virus, SARS-CoV-2, entered the human population.

We also agree with the four open letters from concerned scientists and science communicators (including some of the signatories of this communication) that have previously carefully laid out the case for a further scientific investigation and provided a road map for such an investigation, including detailed methods to use, the documents to seek, and the skills needed by the investigators to conduct such work.²

At the same time, we note that the assessment by the WHO-convened joint study-team of the likelihood of each possible pathway was as follows: “direct zoonotic spillover is considered to be a possible-to-likely pathway; introduction through an intermediate host is considered to be a likely to very likely pathway; introduction through cold/ food chain products is considered a possible pathway; introduction through a laboratory incident was considered to be an extremely unlikely pathway.”

We also note that the assessment was immediately qualified by the declarations of Dr Tedros himself, who asserted that, “As far as WHO is concerned, all hypotheses remain on the table and further reiterated that the conclusions were not those of the WHO. The [study] group came from different institutions and different countries, and they’re independent. There were only two WHO staff who joined them. They came up with their study.”³ In agreement with Dr. Tedros’ statement, available evidence as well as a preliminary re-analysis⁴ of the primary data in the study group report and additional scientific studies lead show that both a traditional zoonosis and a laboratory-acquired infection or research-related accident remain fully plausible hypotheses at this stage.⁵

Based on this information, we address here some limitations of these two Lancet letters, and, where

¹ Calisher C, Carroll D, Colwell R, et al. Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19. The Lancet, 395:E42-E43, 2020. [Lancet 2020](#) ; Calisher C, Carroll D, Colwell R, et al. Science, not speculation, is essential to determine how SARS-CoV-2 reached humans. The Lancet, 2021. [Lancet 2021](#)

² C.D. Butler, B. Canard, H. Cap, et al. Reaction to the China-WHO joint press conference on 9 Feb 2021, (March 4, 2021). [Open Letter 1](#) ; C.D. Butler, H. Cap, J-M Claverie, et al. Reaction to the China-WHO joint study team report, (April 7, 2021). [Open Letter 2](#) ; C.D. Butler, H. Cap, J-M Claverie, et al. Open Letter to the World Health Organization and the Members of its Executive Board ahead of the World Health Assembly (May 2021), (April 30, 2021). [Open Letter 3](#) ; Adadi, R. Bahulikar, C.D. Butler, J-M Claverie, et al. Call for a Comprehensive Investigation of the Origin of SARS-CoV-2, if Possible with Chinese Government Participation, (June 28, 2021). [Open Letter 4](#).

³ See Science Magazine, 18 June 2021.

⁴ A - Quay S. OPEN LETTER TO THE WHO: Evidence that is Undisputed Favoring Lab Origin of COVID Needs to Be Acknowledged. [Preprint, Zenodo, March 2021](#) ; B - Quay S. A Bayesian analysis concludes beyond a reasonable doubt that SARS-CoV-2 is not a natural zoonosis but instead is laboratory derived. [Preprint, Zenodo, January 2021](#)

⁵ See for instance this recent summary of the situation by Stanford professor David Relman.

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appropriate, a recent preprint entitled, “The Origins of SARS-CoV-2: A Critical Review,” by Holmes et al.^{6,7}

Wrong Focus on Biosafety Level-4 Research

Neither Holmes et al nor the two Lancet letters acknowledge the now well documented fact that bat coronaviruses were worked on at BSL-2 and BSL-3 levels in various labs in Wuhan, including the Wuhan Institute of Virology (WIV), and never at BSL-4.⁸ Respected Western scientists, including a signatory of the first Lancet letter, have since expressed their dismay at this low level of biosafety. Holmes et al. describe the WIV BSL-4 site in Wuhan, but this is irrelevant to a discussion of possible lab origins.

No acknowledgement of a possible field research-related scenario

Neither Holmes et al. nor the two Lancet letters properly consider the various research related scenarios⁹. In particular they fail to consider the possibility of an infection of a Wuhan based personnel on a bat sampling trip, for which there is abundant evidence¹⁰ that limited PPEs were used (despite the declarations of the Annex D7 of the China-WHO report). Contrary to what Holmes et al assert, such a scenario is highly parsimonious; it would explain the location (Wuhan), the absence of infection at the source (possibly some cave in Yunnan), the absence of such virus in a lab to date (as it may well never have been encountered until that fateful sampling trip) and could have involved an asymptomatic personnel making detection much more difficult.

No proper consideration to the location or virus factor

Neither Holmes et al nor the two Lancet letters pay proper attention to the fact that the outbreak started in Wuhan, China. Considerations of a purported link to wildlife markets and transport hubs fail to acknowledge that there are at least 110 cities in China with more than one million residents, transport hubs, and wildlife markets. There is nothing unique to Wuhan in having these three, commonly found urban features.

An analysis of the relationship between the location of the Wuhan hospitals with early admission (December 1, 2019 to January 10, 2020) and the catchment zone of the Wuhan Metro subway system establishes that the pandemic began along Line 2 of the Metro (a likelihood of 1 in 68,500 this occurred by chance). Because Line 2 has stations at the Wuhan Institute of Virology, the Huanan Seafood Market, the high-speed rail system to reach all points of China within hours and the international airport, with non-stop destinations in the US, Europe, and the Middle East, the

⁶ Holmes et al. The Origins of SARS-CoV-2: A Critical Review. [Holmes et al. 2021 Zenodo pre-print](#)

⁷ Another detailed review of Holmes et al., entitled, “A response to “The Origins of SARS-CoV-2: A Critical Review” was also recently published.: [Chan A. Response to Holmes et al. Medium, 2021.](#)

⁸ See for instance Shi Zhengli answer to Science Magazine: ‘The coronavirus research in our laboratory is conducted in BSL-2 or BSL-3 laboratories.’ or the classification of pathogens as per the National Virus Resource Centre affiliated to the WIV.

⁹ See Annex A of the 3rd open letter for a description of the research-related scenarios.

¹⁰ See for instance this transcript of a video presentation by Shi Zhengli, or these images from a WIV promotional video.

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pandemic has been said to have started and been amplified by the “Wuhan Line 2 COVID Conduit.”¹¹

There is also no acknowledgement that, of the 209 possible viral diseases to cause an outbreak in Wuhan, COVID-19 was a SARS-like coronavirus. Before 2019, 60% of all publications in the world on SARS-like coronaviruses came from the Wuhan Institute of Virology.¹²

No pre-pandemic COVID-19 clinical cases

In Andersen et al.¹³ the authors speculated on the timing of the acquisition of the polybasic cleavage site in a progenitor virus that had been pre-adapted to the human ACE2 receptor. In doing so they wrote:

“Hence, this scenario presumes a period of unrecognized transmission in humans between the initial zoonotic event and the acquisition of the polybasic cleavage site. Sufficient opportunity could have arisen if there had been many prior zoonotic events that produced short chains of human-to-human transmission over an extended period. Studies of banked human samples could provide information on whether such cryptic spread has occurred.”

Previous human SARS-like coronavirus exposure in Wuhan would have been surprising. A 2018 study¹⁴ by Daszak, Shi, and colleagues used blood bank specimens from Wuhan as a control for a study in Southern China of coronavirus seroconversion in people living in close proximity to large bat caves. The stated reason was the “much lower likelihood of exposure to bats due to its urban environment.” All Wuhan specimens were negative.

The WHO Report documents three independent studies of human blood-derived specimens, totaling over 9000, drawn before January 2020, that were tested for serological evidence of COVID-19 antibodies, and all were found to be negative.¹⁵ No cryptic spread was found. A seropositivity of 2.2% in specimens from patients collected from January to April 2020 documented the sensitivity of the assay used. A similar analysis for the previous zoonotic coronavirus epidemics, SARS-CoV-1 and MERS, found about 0.6% community resident pre-epidemic seroconversion and up to 19.9% in supply chain workers.¹⁶ The WHO data establishes

¹¹ Quay, S. Where Did the 2019 Coronavirus Pandemic Begin and How Did it Spread? The People's Liberation Army Hospital in Wuhan China and Line 2 of the Wuhan Metro System Are Compelling Answers. October 28, 2020. [Zenodo Preprint](#)

¹² Quay S. Personal communication.

¹³ Andersen, K.G., Rambaut, A., Lipkin, W.I. et al. The proximal origin of SARS-CoV-2. *Nat Med* 26, 450–452 (2020). [Andersen et al Proximal Origin paper](#)

¹⁴ Wang N, Li SY, Yang XL, et al. Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. *Virol Sin.* 2018 Feb;33(1):104-107. doi: 10.1007/s12250-018-0012-7.

¹⁵ Reference 4B, p. 50.

¹⁶ MERS pre-epidemic seroconversion was 2.3% in shepherds and 3.6% in slaughterhouse workers [MERS seroconversion](#); SARS-CoV-1 pre-epidemic seroconversion among wild animal workers was 19.9%. [SARS-CoV-1 seroconversion](#); MERS and SARS-CoV-1 pre-epidemic seroconversion in community residents of epidemic outbreaks averaged 0.6% (Reference 4-B, p. 66).

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that the incidence of COVID-19 in the community before December 2019 must be less than 0.03% (95% CI, Upper Bound).

A corollary dataset came from a multi-disciplinary clinical team that reviewed 76,253 episodes of fever, influenza-like illness, acute respiratory infection, or pneumonia unspecified that had presented to Wuhan health institutions in individuals of all ages in the period from 1 October to 10 December 2019. Following initial review by the health institutions, only 92 of the 76,253 episodes were considered to have an illness clinically compatible with SARS-CoV-2 infection. Following further review by the external multidisciplinary clinical team, all these cases were assessed not to be cases of SARS-CoV-2 infection.¹⁷ These data establish the upper limit of the incidence of COVID-19 in Wuhan residents in the fall of 2019 at 0.004%.

As noted by Holmes et al. “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). Subsequent serological surveys found ~3% positivity rates to SARS-CoV related (SARSr-CoV) viruses in residents of Yunnan province living close to bat caves, demonstrating regular exposure in rural locations.”

No hypothesis has been offered to explain the lack of any evidence of pre-pandemic seroconversion anywhere in the community.

No intermediate animal host has been implicated

Approximately 70% of all initial cases are reported to have had a relationship to the Huanan Seafood Wholesale Market in Wuhan.⁵ In noting this fact Andersen et al. wrote:

“As many early cases of COVID-19 were linked to the Huanan market in Wuhan, it is possible that an animal source was present at this location. For a precursor virus to acquire both the polybasic cleavage site and mutations in the spike protein suitable for binding to human ACE2, an animal host would probably have to have a high population density (to allow natural selection to proceed efficiently) and an ACE2-encoding gene that is similar to the human ortholog.”

In an effort to find the intermediate host predicted by Andersen “to have a high population density,” an extensive investigation¹⁸ was conducted. 457 animals from 18 species from the Huanan market, warehouses related to the market, and other markets were tested for the virus. 616 animals (10 species) from suppliers to the market were tested. 1864 wild animals from 27 species (including pangolins, civet cats and *Rhinolophus affinis* bats) from Southern China of the type found in the market were tested. Domestic animals from 31 provinces were tested for antibodies (11,708 samples) or nucleic acid by PCR (12,092 samples). In total, over 80,000 specimens from 209 different species were tested, and all were negative for evidence of infection by antibody serology and/or nucleic acid PCR. Each of these sampling exercises is sufficiently large to permit

¹⁷ Reference 3B, p. 49.

¹⁸ Reference 2B, p. 92.

a conclusion that the population of SARS-CoV-2 infected animals in each population must be significantly less than 1.0% to produce the results observed.

A study of 1055 imported cold chain food products to the Huanan market were also all negative by PCR testing.¹⁹

For SARS-CoV-1 and MERS, when human cases were found to be associated with markets, animal testing in those markets found a high level of infection (about 80%) in the respective intermediate hosts and a detectable level (about 10%) in farms in the supply chain.²⁰ Market workers were seropositive at high levels (10-40%) and the virus could be recovered from multiple markets for many months.²¹

Furthermore, given the known high susceptibility of felines to infection with SARS-CoV-2 if live animals being sold in the market had been infected with SARS-CoV-2 then this would be expected to have transmitted just as readily to local cat populations around the market as human populations. No such evidence of feline infections was found.

Holmes et al.⁴ describe the situation concerning SARS-CoV-2 and market emergence here:

“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 and the Xinyuan market in Guangzhou.”

While we agree with Holmes et al.⁴ in their characterization of SARS-CoV, for the SARS-CoV-2 outbreak the lack of evidence of animal infection, lack of seroprevalence in market workers or anyone in the community pre-epidemic, and the genetic purity (*infra*) of the virus isolated is in fact the mirror opposite situation to the first SARS outbreak. We can find no scientific justification within the Holmes et al.⁴ pre-print for what they say about SARS-CoV-2.

The Holmes et al.⁴ review emphasizes that the search for an intermediate animal host needs to be focused on the Wuhan markets and their supply chains. This search of the Wuhan markets and their supply chains has been completed and no intermediate host was found, nor even peripheral evidence that an intermediate host may have existed.

Holmes et al.⁴ list many potential animal hosts for SARS-CoV-2 in their review. As documented in the WHO Report, all of the animal hosts they propose were among the 209 species tested and all were found to be negative.

¹⁹ Reference 2B, p. 107.

²⁰ Tu C, Crameri G, Kong X, et al. Antibodies to SARS coronavirus in civets. *Emerg Infect Dis.* 2004 Dec;10(12):2244-8. doi: 10.3201/eid1012.040520. [SARS Antibodies in Civets](#).

²¹ Hu S, Shi Z-L. Investigation of Animal Reservoir(s) in SARS-CoV. Y. Lu et al. (eds.), *Emerging Infections in Asia*. Springer Science+Business Media, LLC 2008, pp 57-73.

Circulation in an intermediate animal host could not have occurred

The time to Most Recent Common Ancestor (tMRCA), determined by a phylogenetic analysis, is one method in zoonotic research to estimate the time when a virus was first transmitted into the intermediate host population and began to develop a background of genetic diversity. This is typically well before the human epidemic spread and even before the first animal-to-human transmission. For example, with MERS it was determined that the virus had entered the camel populations of the Middle East about two years before the first human clinical cases became known.²²

The SARS-CoV-2 phylogeny is of course different. A universal observation of the SARS-CoV-2 genome was its lack of mutational diversity at the beginning. The WHO and Drs. Baric, Rambaut, Holmes, and even Shi²³ have all commented on this. The WHO has written:

“All the published genetic sequences of SARS-CoV-2 isolated from human cases are very similar, suggesting that the start of the outbreak resulted from a single point introduction in the human population around the time that the virus was first reported in humans in Wuhan, China. The analyses of the published genetic sequences further suggest that the spillover from an animal source to humans happened during the last quarter of 2019.

“Current findings show that the virus has been remarkably stable since it was first reported in Wuhan, with sequences well conserved in different countries, suggesting that the virus was well adapted to human transmission from the moment it was first detected.”

Holmes and Rambaut wrote:

“Because of the early sampling and genome sequencing of COVID-19 cases in China, especially in Hubei province, it appears that the ‘root sequence’ of SARS-CoV-2 is known. Many of the genomes from the earliest sampled cases are genetically identical and hence also probably identical to the most recent common ancestor of all sampled viruses. This occurrence is different to previous viruses and epidemics.” [Emphasis added].

²² Lau SKP, Wong ACP, Lau TCK, Woo PCY. Molecular Evolution of MERS Coronavirus: Dromedaries as a Recent Intermediate Host or Long-Time Animal Reservoir? *Int J Mol Sci.* 2017 Oct 16;18(10):2138. doi: 10.3390/ijms18102138. PMID: 29035289; PMCID: PMC5666820. [Molecular Evolution of MERS Coronavirus](#)

²³ WHO statements: [WHO Statement 23 Apr 2020](#); Baric R: “early strains identified in Wuhan, China, showed limited genetic diversity, which suggests that the virus may have been introduced from a single source.” Emergence of a Highly Fit SARS-CoV-2 Variant. *N Engl J Med* 2020; 383:2684-2686 DOI: 10.1056/NEJMcibr2032888. [NEJM](#); Shi, Z-L: On January 23, 2020 Dr. Shi wrote in the draft of her paper: “The almost identical sequences of this virus in different patients imply a probably recent introduction in humans...” [23 Jan 2020 draft pre-print](#). By February 3, 2020, when the final version of this paper was published, this sentence had been deleted. Zhou, P., Yang, X.L., Wang, X.G. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273 (2020). [Final paper](#); Rambaut, A., Holmes, E.C., O’Toole, Á. et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* 5, 1403–1407 (2020). [Rambaut & Holmes et al paper](#)

Holmes et al.⁴ updated the early genomic clade analysis in their recent review, establishing that two clades were circulating early. They fail to point out, however, that these clades differ by only two base substitutions. All SARS-CoV-2 samples taken from any source collapse phylogenetically into a tMRCA in approximately November 2019. This includes all human clinical cases, regardless of their relationship to any market, all environmental specimens from any market tested, any surface specimens from any market, etc.

The WHO Report states: “the time to most recent common ancestor (tMRCA) inferred by more than 10 groups using different approaches is similar: between mid-November and mid-December 2019.” This is also the timing of the clinical outbreak, meaning this data establishes that SARS-CoV-2 was unlikely to have been circulating in an intermediate host for any measurable time before the clinical outbreak.

For SARS-CoV-2, the observation that the timing of the first human cases and the tMRCA are the same means there is no measurable temporal period of intermediate host infection. All human cases are traced to a presumed single animal-to-human transmission.²⁴ For MERS and SARS-CoV 50% or more of early cases were independent animal-to-human transmissions, with the posterior diversity and a tMRCA years before as the evidence for a natural zoonosis.

In the laboratory, experiments using stored, aliquoted virus samples makes the use of molecular clock estimates unreliable; for example, a year’s worth of experiments could be performed with no apparent base substitutions by using aliquots of the virus, and codon re-optimization when a virus is grown in cells of a different species to the original host may also accelerate virus evolution compared to its mutation rate in a stable host.

Two additional genomic signatures of SARS-CoV-2 are worth noting at this point. The polybasic cleavage site and its unusual -CGG-CGG- coding sequence, and the evidence of pre-adaption to the human host, whether looking at the entire genome or just the receptor binding domain of the Spike Protein.²⁵

Because the subgenera of sarbecovirus to which SARS-CoV-2 belongs has neither furin cleavage sites (of any sequence) or -CGG-CGG- dimer codons, a recombination with a non-sarbecovirus needs to be proposed. Field data from Drs. Shi and Daszak documenting a lack of betacoronavirus co-infection that includes sarbecoviruses²⁶ and furin site containing viruses from other subgenera

²⁴ The earliest SARS-CoV-2 sequences in GISAID from China were collected (n=259) and compared to the GenBank Reference Sequence ([Ref Seq 1798174254](#)). The substitution rate per year was calculated using the number of changes from the reference sequence and the time between sampling dates. The established human-to-human intrinsic rate of SARS-CoV-2 nt substitution was taken to be 17.9 with a confidence interval of 12.0 to 20.9 nt per year. [Emergence of genetic diversity and recurrent mutations in SARS-CoV-2](#). No sequences were found that had a substitution rate greater than 20.9 nt per year, as would be characteristic of an independent animal-to-human transmission with posterior diversity.

²⁵ Zhan SH, Deverman BE, Chan YA. SARS-CoV-2 is well adapted for humans. What does this mean for re-emergence? *bioRxiv* 2020.05.01.073262; doi: [SARS1 vs. SARS-CoV-2 whole genome SNV evolution](#); Starr et al. Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding 2020, Cell 182, 1295–1310 September 3, 2020. [Bloom laboratory yeast display](#)

²⁶ Latine, A., Hu, B., Olival, K.J. et al. Origin and cross-species transmission of bat coronaviruses in China. *Nat Commun* 11, 4235 (2020). [Daszak & Shi Co-Infection paper](#) They sampled 187 *Rhinolophidae Sp.* bats and

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and the transcription regulatory site incompatibility between subgenera reduce significantly the already low likelihood of SARS-CoV-2 acquiring this critical feature in nature.²⁷ Extensive prior work in many laboratories synthetically inserting furin cleavage sites with the effect of changing the pathogenesis of the virus show a clear historical precedent for a laboratory route to this SARS-CoV-2 feature. Serial passage in cells of different species is also a well-established method for host adaptation to humans in coronavirus biology.²⁸

The evidence of pre-adaption to a human or human-like host must be juxtaposed with the failure of finding any evidence of pre-epidemic circulation in humans or in any of 209 different animal species.

Despite a plea to not speculate, the Holmes et al. note contains many arguments that are just that. One for example, is the notion that because wildtype mice are difficult to infect with SARS-CoV-2 one could speculate the virus was not derived from a laboratory. This ignores many published papers, including by Baric and Shi in early 2020,²⁹ of the use of humanized ACE2 transgenic mice for SARS-CoV-2 research, mice which the virus infects so well it is often lethal, and displays CNS infection.

Many scientists and the leadership of the WIV have claimed that the WIV was not conducting research on SARS-CoV-2 or any related coronavirus. They have also asserted that no one from the WIV became ill in the fall of 2019 or at any later time. This claim is challenged by an analysis of how very unlikely it would be for a facility in Wuhan with up to 590 employees to have no seroconversions, given the incidence of seroconversions of the Wuhan residents at the time was over four percent.³⁰ In any case, these are personal assertions, not scientific observations. To be

determined subgenera beta coronavirus infections. All 187 were infected with sarbecoviruses but there were no co-infections of other beta coronavirus subgenera. [Supplemental material, pp. 17-8.]

²⁷Yiyang Yang, Wei Yan, A Brantley Hall, Xiaofang Jiang, Characterizing Transcriptional Regulatory Sequences in Coronaviruses and Their Role in Recombination, Molecular Biology and Evolution, V. 38, Issue 4, April 2021, pp. 1241–1248, [Subgenera TRS & Recombination](#) ; Baric et al. use bespoke TRS changes and create recombination-resistant vaccine candidates: Graham, R.L., Deming, D.J., Deming, M.E. et al. Evaluation of a recombination-resistant coronavirus as a broadly applicable, rapidly implementable vaccine platform. Commun Biol 1, 179 (2018). [Recombination resistant vaccine candidate via TRS modification.](#)

²⁸ Cockrell AS, Leist SR, Douglas MG, Baric RS. Modeling pathogenesis of emergent and pre-emergent human coronaviruses in mice. Mamm Genome. 2018 29: (7-8):367-383. doi: 10.1007/s00335-018-9760-9.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6132729/>

²⁹ Jiang RD, Liu MQ, Chen Y, et al. Pathogenesis of SARS-CoV-2 in Transgenic Mice Expressing Human Angiotensin-Converting Enzyme 2. Cell. 2020 182:50-58.e8. doi: 10.1016/j.cell.2020.05.027. [Baric & Shi SARS-CoV-2 Transgenic Mouse Model](#)

³⁰ Quay S, Demaneuf G. An analysis of the results of routine employee testing for SARS-like infections within the WIV and other Wuhan labs raises serious issues about their validity. Researchgate, 2021. [WIV COVID-19 Seroconversion Analysis](#)

²⁶ Rahalkar MC and Bahulikar RA (2020) Lethal Pneumonia Cases in Mojiang Miners (2012) and the Mineshaft Could Provide Important Clues to the Origin of SARS-CoV-2. Front. Public Health 8:581569. doi: 10.3389/fpubh.2020.581569

included in a science-based analysis, independent verification is required. Such claims should not have been relied upon by Calisher et al (2021) and the review by Holmes et al.

There was no mention in Holmes et al 2021 and Calisher et al 2021 about an important clue which connects the Mojiang mineshaft, Yunnan with a lethal pneumonia in six miners and the bat coronavirus, RaTG13, the closest genomic relative of SARS-CoV-2. In 2012, six men went to clean an abandoned mineshaft infested with six species of bats, including horseshoe bats. All of them suffered from pneumonia, including four deaths, and their complications on retrospective analysis were similar to COVID-19.³¹ The coincidence between the 2012 illness in Mojiang miners, the subsequent samplings, and finding the nearest SARS-CoV-2 relative from this same mine warrants further inquiry.

Conclusion

An investigation of the origin of the COVID-19 pandemic needs to be conducted to a science-based resolution of competing hypotheses. There is currently no evidence to support the hypothesis this was a natural zoonotic infection of the type previously seen in for example the SARS CoV outbreak. Until new evidence is found, an investigation of the pathway for the origin of COVID-19 should proceed with the assumption that a laboratory-acquired infection or laboratory accident is at least as likely, if not more likely, than a natural zoonotic transmission.

³¹Rahalkar MC and Bahulikar RA (2020) Lethal Pneumonia Cases in Mojiang Miners (2012) and the Mineshaft Could Provide Important Clues to the Origin of SARS-CoV-2. *Front. Public Health* 8:581569. doi: 10.3389/fpubh.2020.581569 [Mojiang Miners Pneumonia](#)

