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Subcommittee on Investigations and Oversight
Principles for Outbreak Investigation: COVID-19 and Future Infectious Diseases.

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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 asymptomatically 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.

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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.