

Reply to Science Magazine

1. You have published many papers on coronaviruses and even warned of the possibility of a serious spillover event from animals to humans. Do you feel like your warnings were not appropriately heeded and how does what imagined compare to the scale of this pandemic?

A: The risk of cross-species infection always exists. With regards to this issue, China has undertaken scientific research projects, created facilities and equipment, and built teams of experts. With global environmental change and the expansion of human activity, the risk of infection continues to increase, which is confirmed and supported by our research. However, our study did not involve an investigation into the pandemic's scale.

2. How has the pandemic affected you, personally and professionally?

A: This pandemic has made me realize the importance of our work and the necessity of pursuing it. I think if we could do more basic research and technological development on vaccines and therapeutic drugs, we would do better in this regard.

3. What are your leading theories about the origin of SARS-CoV-2 and has your own research shed any light on the question?

A: According to the findings of our team and our international peers, SARS-CoV-2 is very likely to have originated from bats. It may have evolved in one or more intermediate hosts, become adapted to humans, and eventually spread among humans. However, it remains unclear which animals were the intermediate hosts and how it spilled over to humans.

4. Assuming this was natural event, a virus jumping species somewhere, how do you think this likely happened? Do you think it's possible that a bat in or close to Wuhan infected someone? If not, where could this have taken place? What do you think of the theory that infected people who lived near mines were the index cases and that they traveled to Wuhan?

A: There are two possibilities for the cross-species transmission from the natural host to humans. One is that the virus was transmitted directly from a bat to a human, while the other is that the virus spread to humans via one or more intermediate hosts. For SARS-CoV-2, though the first possibility cannot be ruled out, its likelihood is very low. I tend to support the second scenario.

When and where the earliest cross-species transmission of SARS-CoV-2 occurred from the intermediate host to humans has not been scientifically uncovered yet. We know from historical experience like HIV that the places where big emerging diseases first break out usually are not their place of origin (where the spillover originally happened). Tracing the origin of a virus is a very challenging scientific task. As for the origin and transmission routes

of SARS-CoV-2, it needs a pioneering vision, and the collective efforts of scientists all around the world, and it needs time as well.

We have done bat virus surveillance in Hubei Province for many years, but have not found that bats in Wuhan or even the wider Hubei Province carry any coronaviruses that are closely related to SARS-CoV-2. I don't think the spillover from bats to humans occurred in Wuhan or in Hubei Province.

I guess you are referring to the bat cave in Tongguan town in Mojiang county of Yunnan Province. To date, none of nearby residents is infected with coronaviruses. Thus the claim that the so-called "patient zero" was living near the mining area and then went to Wuhan is false.

5. An early cluster at the Huanan seafood market in Wuhan led many to think that an animal there somehow infected humans. How has your thinking about the seafood market's role evolved as it became clear that many of the earliest cases are not linked to it?

A: As you pointed out, some early patients do not have a history of Huanan seafood market exposure. We detected SARS-CoV-2 nucleic acids in environmental samples from sources such as rolling door handles, the ground and sewage in that market, but we did not detect any SARS-CoV-2 nucleic acids in frozen animal samples. The Huanan seafood market may just be a crowded location where a cluster of early novel coronavirus patients were found.

6. Do you know whether anyone tested animals from the market? If not, why not?

A: Under the deployment of Hubei Provincial Government, our team, alongside researchers from Huazhong Agricultural University, collected environmental samples and frozen animal samples in Huanan seafood market. We detected SARS-CoV-2 nucleic acids only in the environmental samples such as roller shutter door handles, the ground and sewage, but not in the animals.

7. Is there an attempt to use registries from the market to test farms that supplied animals to the market? Has your group or any other done testing of domesticated animals or wild animal farms at any farms for SARS-CoV-2 like viruses? If so, what has research found?

A: Under the deployment of the Hubei Provincial Government, our team and researchers from Huazhong Agricultural University collected samples of farmed animals and livestock from farms around Wuhan and in other places in Hubei Province. We did not detect any SARS-CoV-2 nucleic acids in these samples.

8. Were you ever given environmental or animal samples from the market to test yourself? If so, what did you find? If not, what do you know about the market samples tested?

A: We detected SARS-CoV-2 nucleic acids in environmental samples from the Wuhan seafood market, including on rolling door handles, on the ground and in sewage, but the detected numbers of viral genome copies were very low.

9. The earliest case report I'm aware of has a patient, unlinked to the seafood market, who had symptoms on December 1. One news story suggests there were cases in November. What is the earliest confirmed case you are aware of and what are the demographics?

A: I did not participate in the epidemiological survey and I don't know much about it. It was on December 30, 2019 that our Institute first received the clinical samples of a "pneumonia with unknown etiology".

10. Where do you think the zoonotic transmission most likely occurred? Wuhan? Hubei? Elsewhere?

A: I can't make any conclusions before we have solid evidence. Tracing the virus's origins is a scientific question, which ought to be answered by scientists based on solid data and scientific evidence. However, the historical experience I mentioned above is worth attention.

11. You have reported the existence of a bat coronavirus, RaTG13. It's clear that this is a distant ancestor of SARS-CoV-2 that differs by 1100 nucleotides. Using molecular clocks, other researchers have estimated that RaTG13 and SARS-CoV-2 shared a common ancestor at least 20 years ago. Have you made your own estimates? Do you think there was an intermediate species between bats and humans, and, if so, what do you think is most likely and why? What do you think of the pangolin data and suggestions that it might be an intermediary?

A: I am not an expert in bioinformatics, so I did not calculate the evolutionary distance between RaTG13 and SARS-CoV-2. I think the coronavirus carried by the natural host would have undergone transmission by at least one intermediate before it evolved into SARS-CoV-2.

The gene sequences of pangolin coronavirus, RaTG13 and SARS-CoV-2, are relatively close and they may have a common ancestor. But with the data currently available, I can't determine whether pangolins are the natural host or an intermediate host.

12. Have you or anyone else you're aware of contacted veterinarians about possible illnesses in animals that occurred that could be SARS-CoV-2 relatives? If so what have you learned?

A: No. I don't have any information on that.

13. Your group did a fascinating study with cats, showing that 15% of 102 stray and house cats in Wuhan had antibodies for SARS-CoV-2. A research group in Harbin separately showed cats could readily be infected and transmit the virus. Cats have been infected

presumably by infected humans in homes, and even big cats were infected, at a zoo in New York. How likely do you think it is that cats might be more involved in spread than is commonly thought?

A: The results of our tests of SARS-CoV-2 antibodies in cat sera, collected in Wuhan after the COVID-19 outbreak, revealed that the infection rate and antibody titers of SARS-CoV-2 in house cats was higher than in stray cats. So I think the SARS-CoV-2 infections in cats were very likely to have been transmitted by humans.

14. Could cats have been the intermediate species between bats and humans? Have you thought of this possibility? Has anyone studied it? I know in your study sera from cats pre-outbreak didn't harbor SARS-CoV-2 antibodies, but that was a small sample. Have you considered broadening that investigation?

A: Further research needs to be conducted to investigate whether cats are potential intermediate hosts of SARS-CoV-2. Currently our lab is not continuing with the studies on cats. Based on the current findings of our lab and other teams, it is probable that the viruses were transmitted from humans to cats.

15. Do you know which Chinese labs are investigating origin possibilities and what are they studying? Do you know why so few papers have been published that have new data?

A: Many groups in China are carrying out such studies. We are publishing papers and data, including those about the virus's origins. We are tracing the origin of the virus in different directions and through multiple approaches.

16. The Wuhan Institute of Virology has received worldwide attention as the possible source of SARS-CoV-2. President Donald Trump has said he has "high confidence" the virus came from the lab. What kind of an impact has this had on your lab? And on you personally?

A: We first received the clinical samples of SARS-CoV-2 on December 30 2019, which were called back then samples of "pneumonia with unknown etiology". Subsequently, we rapidly conducted research in parallel with other domestic institutions, and quickly identified the pathogen. The complete genome sequence of SARS-CoV-2 was submitted and published via WHO on January 12. Before that, we had never been in contact with or studied this virus, nor did we know of its existence.

Scientists from around the world have overwhelmingly concluded that SARS-CoV-2 originated naturally rather than from any institution. The US President Trump's claim that SARS-CoV-2 was leaked from our institute totally contradicts the facts. It jeopardizes and affects our academic work and personal life. He owes us an apology.

17. Many scientists who have analyzed the sequence of SARS-CoV-2 have concluded that it does not have the signatures of a lab-engineered virus. But even some of these researchers say it remains possible that SARS-CoV-2 existed in your lab and accidentally infected a lab worker. They note that several labs had accidental infections with the virus that causes SARS. So how can you rule out this possibility?

A: We have isolated three closely-related bat coronaviruses over the last 15 years (here an isolated virus is a live virus which can grow in cultured cells in the laboratory) and all of them are SARS-related coronaviruses. These bat viruses share 79.8% sequence identity and are distantly related to SARS-CoV-2. On February 3, we published a paper in Nature and reported that SARS-CoV-2 is 96.2% identical at the whole-genome level to a bat coronavirus named RaTG13 (I would like to emphasize that we have only the genome sequence and didn't isolate this virus). With about 30,000 nucleotides, coronaviruses have a larger genome size than most animal RNA viruses. The 3.8% difference in genome sequence is a significant difference for coronaviruses. Five renowned virologists from Scripps Research Translational Institute, Columbia University, Tulane University, the University of Edinburgh and the University of Sydney published a paper titled "The proximal origin of SARS-CoV-2" in Nature Medicine on March 18. The authors stated that "although RaTG13 is 96% identical overall to SARS-CoV-2, its spike diverges in the receptor binding domain." On April 23, the US news site "VOX" quoted opinions from Prof. Edward Holmes, an expert in virus evolution at the University of Sydney. "The level of genome sequence divergence between SARS-CoV-2 and RaTG13 is equivalent to an average of 50 years (and at least 20 years) of evolutionary change," said Professor Holmes. The genomes of RaTG13 carried by bats and SARS-CoV-2 differ in 1,177 nucleotide positions. It would have taken a very long time to accumulate sufficient numbers of mutations through natural evolution. The probability is extremely tiny that the mutations occurred exactly in these 1,100-plus positions to be identical to SARS-CoV-2. Therefore, RaTG13 evolving into SARS-CoV-2 in nature is only theoretically possible.

Meanwhile, the research and experiments in our institute are in strict accordance with the international and national management requirements of biosafety laboratories and experimental activities, which are conducted in the required biosafety laboratories. Both the facilities and management of P3 and P4 laboratories are very strict. For example, personal protective equipment must be worn by the research staff. The air in the laboratory can only be discharged after highly efficient filtration. Waste water and solid waste must be sterilized under high temperatures and high pressure. The entire process of the experimental activities is video-monitored by biosafety management personnel. Every year, the lab's facilities and equipment must be tested by a third-party institution authorized by the government. Only after passing the test can the lab continue to run. The high-level biosafety laboratories at our institute have been operated safely and stably. To date, no pathogen leaks or personnel infection accidents have occurred.

18. The people who have floated these theories have proposed several ways in which the virus could have escaped from the Wuhan Institute of Virology. I'd like to ask some detailed, factual questions about the work at your lab that could shed more light on those scenarios:

(1) Are bat coronaviruses grown at the institute?

A: We have only isolated three strains of live SARS-related coronaviruses (SARSr-CoV) from bats, which shared 95-96% genome sequence similarity with SARS-CoV and less than 80% similarity with SARS-CoV-2. These results were published in Nature [2013, 593(7477):535-538], the Journal of Virology [2016, 90(6), 3253-3256] and PLoS Pathogens [2017, 13(11):e1006698], respectively.

(2) Does your group extract viruses from biological samples and do the sequencing or does that take place elsewhere?

A: We isolated viruses or extracted virus RNA from biological samples in the lab. The sequencing was done mostly in Wuhan.

(3) Has your lab done any animal experiments with SARS-related viruses recently? If so, can you provide any details?

A: We performed in vivo experiments in transgenic (human ACE2 expressing) mice and civets in 2018 and 2019 in the Institute's biosafety laboratory. The viruses we used were bat SARSr-CoV close to SARS-CoV. Operation of this work was undertaken strictly following the regulations on biosafety management of pathogenic microbes in laboratories in China. The results suggested that bat SARSr-CoV can directly infect civets and can also infect mice with human ACE2 receptors. Yet it showed low pathogenicity in mice and no pathogenicity in civets. These data are being sorted and will be published soon.

(4) Is it possible that someone associated with the institute became infected in some other way, for instance while collecting, sampling, or handling bats?

A: Such a possibility did not exist. Recently we tested the sera from all staff and students in the lab and nobody is infected by either bat SARSr-CoV or SARS-CoV-2. To date, there is "zero infection" of all staff and students in our institute.

(5) Is it possible that you have biological samples from bats in your lab that you have yet to test for viruses? If so, how many samples have you tested and how many remain untested? If some remain untested, how do you know for certain that none contain SARS-CoV-2 or a close relative?

A: We tested all bat samples that we collected, including bat anal swabs, oral swabs and fecal samples, and 2,007 samples were positive for coronavirus. We did not find any viruses whose gene sequence is more similar to SARS-CoV-2 than RaTG13.

(6) Your lab was one of the first to sequence and isolate the virus. When and where did you first sequence it?

A: We received the first batch of samples from seven patients on December 30 2019. Using pan-coronavirus RT-PCR and quantitative RT-PCR, which can detect all SARS-related coronaviruses, we found samples from five patients were positive. On December 31, when analyzing the sequencing result of the RT-PCR product, we identified that it was a novel SARS-related coronavirus. We then confirmed the result via different methods and performed full-length genome sequencing as well as virus isolation. We released the genome sequence to the global public on January 12 via WHO.

(7) What about the cave at Mojiang in 2013? When did you first isolate RaTG13? When did you complete the full sequencing of it?

A: We detected the virus by pan-coronavirus RT-PCR in a bat fecal sample collected from Tongguan town, Mojiang county in Yunnan province in 2013, and obtained its partial RdRp sequence. Because the low similarity of this virus to SARS-CoV, we did not pay special attention to this sequence. In 2018, as the NGS sequencing technology and capability in our lab was improved, we did further sequencing of the virus using our remaining samples, and obtained the full-length genome sequence of RaTG13 except the 15 nucleotides at the 5' end. As the sample was used many times for the purpose of viral nucleic acid extraction, there was no more sample after we finished genome sequencing, and we did not do virus isolation and other studies on it. Among all the bat samples we collected, the RaTG13 virus was detected in only one single sample. In 2020, we compared the sequence of SARS-CoV-2 and our unpublished bat coronavirus sequences and found it shared a 96.2% identity with RaTG13. RaTG13 has never been isolated or cultured.

(8) Some people who suspect a lab accident occurred have suggested that BtCoV/4991, a bat virus you described in 2016, is SARS-CoV-2. When you published, you only had the sequence of one protein, RNA dependent RNA polymerase (RdRp). A blast analysis on GenBank shows that the RdRp of BtCoV/4991 and RaTG13 are 100% homologous. Is BtCoV/4991 actually RaTG13, which would be consistent with your 2020 report that described how you did the full sequence of a virus you only had done the RdRp sequence for earlier? If so, why did you rename the virus? What does "TG" stand for in RaTG13?

A: Ra4991 is the ID for a bat sample while RaTG13 is the ID for the coronavirus detected in the sample. We changed the name as we wanted it to reflect the time and location for the sample collection. 13 means it was collected in 2013, and TG is the abbreviation of Tongguan town, the location where the sample was collected.

(9) Why do you have RdRp sequences for some viruses and not their full sequences? How many full-length sequences are there of the samples you've tested and how many are just RdRp?

A: Due to financial and manpower constraints, it is impossible for us to do the whole genome sequencing of all samples. We hope to conduct further full-length coronavirus

genome sequencing in some other samples within the next two years. However, for some samples, it is impossible to obtain the whole virus genome sequences because of the low quantity of the viral nucleic acids in them.

(10) Were you ever instructed to destroy any viruses after the outbreak surfaced?

A: No.

(11) Is it possible that there was an accidental release at another lab in Wuhan? The Wuhan Center for Disease Control has been mentioned. If you have ruled this out as a possibility, why?

A: Based on daily academic exchanges and discussion, I can rule out such a possibility.

(12) What haven't I asked you that you would like people to know?

A: With the continuing occurrences of emerging infectious diseases all over the world, scientists are beginning to study the viruses carried by wild animals, which is not only the key to having early warnings of the diseases from those origins, but also an important scientific basis for disease prevention. In this context, I have conducted collaborative research with Dr. Peter Daszak, President and PI of EcoHealth Alliance. We have established a good relationship in the fields of virus surveillance and pathogen discovery. Our research team has found a variety of coronaviruses with different sequences in bat populations. Some of them have the potential to spread to humans and animals, such as SARS-related coronavirus, MERS-related coronavirus, SADS-related coronavirus, etc. The findings provide important clues for the prevention and control of infectious diseases.

We don't understand the NIH termination of funding support for our collaborative project and feel it is absolutely absurd. This project should be an international cooperative work aiming to gather scientists from different countries to jointly explore early warnings and predictions of infectious diseases, which will help vaccine design and drug development to protect us from coronavirus threats.

Over the past 20 years, coronaviruses have been disrupting and impacting human lives and economies. Here, I would like to make an appeal to the international community to strengthen international cooperation on research into the origins of emerging viruses. I hope scientists around the world can stand together and work together. The purpose of the search for the origin of a virus is to prevent the recurrence of similar outbreaks which will harm human society, and in this way, we can respond more effectively when an outbreak happens.

Note: *Science* asked two follow-up questions after receiving the replies above.

Q: Did you do or collaborate on any gain-of-function experiments with coronaviruses that were not published, and, if so what are the details?

A: No.

Q: Given that coronavirus research in most places is done in BSL-2 or BSL-3 labs--and indeed, you WIV didn't even have an operational BSL-4 until recently--why would you do any coronavirus experiments under BSL-4 conditions?

A: The coronavirus research in our laboratory is conducted in BSL-2 or BSL-3 laboratories.

After the BSL-4 laboratory in our institute has been put into operation, in accordance with the management regulations of BSL-4 laboratory, we have trained the scientific researchers in the BSL-4 laboratory using the low-pathogenic coronaviruses as model viruses, which aims to prepare for conducting the experimental activities of highly pathogenic microorganisms.

After the COVID-19 outbreak, our country has stipulated that the cultivation and the animal infection experiments of SARS-CoV-2 should be carried out in BSL-3 laboratory or above. Since the BSL-3 laboratories in our institute do not have the hardware conditions to conduct experiments on non-human primates, and in order to carry out the mentioned research, our institute had applied to the governmental authorities and obtained the qualification to conduct experiments on SARS-CoV-2 for Wuhan P4 laboratory, in which the rhesus monkey animal model, etc. have been carried out.

The experimental activities are supervised by our institute's biosafety committee and complied with the biosafety regulations.