Study on the origin of the coronavirus pandemic

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study for

Origin of the coronavirus pandemic

Head of the study and responsible for the content:

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University of Hamburg

period of the study

01/01/2020 – 12/31/2020

foreword

The present study on the origin of the coronavirus pandemic was carried out in the period from January 1st, 2020 to December 31st, 2020 at the University of Hamburg. First Interim results of this study were announced in a press release on May 5, 2020 announced. Since then, through the international exchange of information, more important findings and documents have been compiled.

The study is based on an interdisciplinary scientific approach, ie not one exclusively subject-specific perspective, as well as on an extensive research under Use of all conceivable sources of information. These include:

- interdisciplinary and subject-specific scientific literature based on scientific assessment (“peer review”),
- scientific literature without scientific review,
- Letters, correspondence and comments published in the scientific literature,
- Articles in print and online media,
- reports on the internet / in social media,
- Personal communication with international colleagues.

The references to this study have been structured accordingly to ensure a clear Distinction between scientific primary literature (with and without peer review) and to obtain published opinions.

This document was completed on January 6, 2021. It was first distributed and discussed exclusively in scientific circles. On February 12, 2021 the release for publication as the basis for a broad discussion in the Population that is to be given fact-based information given the importance of the topic and to be included in future decision-making processes.

Additional information and other documents can be requested from the head of the study will:

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1 Overview of the motivation and main results of the study

The current coronavirus pandemic is the greatest challenge for many people since the end of the Second World War. The global crisis is associated with the loss of many lives in connection with COVID-19 disease (according to statistics, around 1.8 million deaths within one year from Johns Hopkins University, USA).
Along with an unprecedented economic crisis, there are many, in some cases still unforeseeable, consequences for people's lives and prosperity - in many cases even for the most essential basis of life, especially in the poorest countries in the world.

Even if the current public discussion naturally focuses primarily on overcoming the consequences of the pandemic in the healthcare system, in the economy and in many areas of society, the question of the origin of the pandemic is of central importance: "Whenever a new type of virus occurs, it is very important to understand where the new virus came from, that is, to identify the source of the viruses and to study the details of the spread, in order to obtain important information on which to base current and future measures," says the World Health Organization (WHO). The science-based discussion of this important topic is the subject of the present study.

Since the beginning of the pandemic, there have been two different attempts to explain its cause:

1) The accidental transmission of corona viruses from the animal kingdom to humans ("zoonosis"), whereby a certain type of bat is considered as the original source of the virus. As a result of a virus mutation involving an intermediate host animal, transmission to humans then took place, with an animal market in the center of the city of Wuhan (China), the place of origin of the coronavirus pandemic, being of central importance.

2) Alternatively, since the beginning of the pandemic, a laboratory accident in a high-security biotechnology laboratory in the center of the city of Wuhan (not far from the suspected animal market) has been cited as a possible cause. This suspicion is based on the fact that for many years risky research and genetic manipulation of corona viruses have been the focus of the activities of the virological institute in Wuhan, which is documented by scientific publications in the specialist literature.

To date, there is no scientifically based rigorous evidence for either of the two theories mentioned. In such a situation, scientists - regardless of their discipline - should adopt a neutral stance and conduct an open-ended discussion until the crucial question of the origin of the pandemic has been finally clarified. Nevertheless, some well-known virologists committed themselves to the first theory, i.e. a zoonosis, in public statements very early on. This has led to leading representatives from politics and society increasingly speaking of a “natural disaster” in connection with the coronavirus pandemic.

But is this actually due to a natural disaster – comparable to an earthquake, a tsunami or a volcanic eruption? Is the current global crisis really the result of an accident of nature – a random mutation of a bat coronavirus with the help of an intermediate host animal – or the result of carelessness on the part of a scientist in conducting high-risk research with global pandemic potential?

Since there is no scientifically based evidence in the strict sense to answer this important question, only indications can be given that make one or the other theory appear more likely.

The present year-long study concludes that both the number and the quality of the evidence clearly point to a laboratory accident at the Wuhan City Virology Institute as the cause of the current pandemic. For this purpose, science-based analyzes of the existing specialist literature as well as independently verifiable relevant documents were used, which are not only quoted in the main part of this study, but also partly reflected in the original text, since the target audience of this study does not always have access to the relevant literature sources or does not find the time to call them all yourself.

Some of the main indicators that point to a laboratory accident as the cause of the current Pandemic and will be presented and discussed in detail in this study should be briefly summarized here at the beginning:

- Corona viruses, which originally go back to bats, do not easily lead to infectious diseases in humans with the severity we are experiencing in the current pandemic (very high transmission rate; virus infestation not only of the respiratory tract but also of other organs; etc.). In this context, virologists speak of an “adaptation barrier”.

- Coronavirus mutations may have occurred in intermediate host animals and eventually spread to humans at wildlife markets. However, no such intermediate host animal has been identified to date in connection with the current coronavirus pandemic.

- In addition, a key fact is that a significant proportion of the very first COVID-19 patients in Wuhan had no contact at all with the suspected wildlife market. This is proven by several original scientific publications in peer-reviewed journals.

- There are numerous independent indications that a young scientist from the "Wuhan Institute of Virology" was the first to become infected with the novel corona virus in the laboratory and was therefore at the beginning of the COVID-19 infection chain. Her entry on the institute's website was deleted and she has been considered missing since the end of 2019.

- According to numerous reports, bats were not offered at the suspected wildlife market in Wuhan. However, bat viruses have been collected from distant caves in a southern Chinese province for many years by scientists at the Wuhan Institute of Virology and shipped to Wuhan

brought. This is proven by several original scientific publications in peer-reviewed journals.

- A research group at the "Wuhan Institute of Virology" has not only studied naturally occurring corona viruses for many years, but also genetically manipulated them with the aim of making them more contagious and dangerous for humans. This so-called "gain-of-function" research at the "Wuhan Institute of Virology" is documented by several original scientific publications in peer-reviewed journals and has been critically assessed by many representatives of science for years.

- There were reports of significant security deficiencies at the Wuhan Institute of Virology even before the outbreak of the coronavirus pandemic. A look at the statistics of documented accidents in biotechnological high-security laboratories shows that the unwanted escape of highly infectious viruses from such laboratories was not uncommon in the past, both in China and in the USA. In addition, there are video recordings showing that laboratory waste was not properly disposed of at the "Wuhan Institute of Virology" and that the institute's employees were not wearing adequate protective clothing.

- An analysis of mobile phone usage activities in and around the "Wuhan Institute of Virology" in the second half of 2019 indicates that in the first half of October 2019 there was a temporary interruption in laboratory operations and cordoning off around the institute premises. At the same time, there were the first confirmed cases of fatal COVID-19 diseases in various hospitals in the city of Wuhan as early as October 2019. This also explains, among other things, why the very first cases of COVID-19 diseases were subsequently detected in Europe as early as November 2019 (such as through a detailed analysis of the lung images of a COVID-19 patient in France).

Based on this and many other indications presented in the present study and based on original scientific publications and verifiable documents, it may be all the more surprising that numerous virologists continue to propagate only one zoonosis as the cause of the current pandemic in all available media.
The present study therefore also deals with the role of science in connection with the question of the origin of the current coronavirus pandemic.

2 Central question about the origin of the coronavirus pandemic: natural disaster or laboratory accident?

In this time of restrictions on fundamental rights caused by the coronavirus pandemic, which is highly unusual for the post-war generation, every individual is increasingly asking the question: How dangerous is the corona virus really? Are we overestimating the danger? Are the civil liberties currently being unjustly restricted? Can the looming unprecedented economic slump be justified?
Are the current rules of conduct appropriate, or do they reflect an over-cautious state response in an unprecedented post-war situation?

Many people keep making comparisons with the well-known flu and point out that the 2017/18 flu season, for example, is estimated to have claimed around 25,000 lives in Germany and around 60,000 in the USA. Others, on the other hand, argue that without state intervention the number of fatalities as a result of COVID-19 disease would be significantly higher and that these days - despite all state protective measures - the global number of fatalities in this pandemic already exceeds 1.8 million (according to Statistics from Johns Hopkins University, USA).

But what distinguishes the novel coronavirus SARS-CoV-2 from all previously known types of corona virus and the multitude of other viruses to which we are constantly exposed throughout our lives? According to the current state of knowledge, the following properties of the new type of coronavirus are exceptional:

- Corona viruses have been known for a long time and can, among other things, trigger common colds in humans, which typically no longer appear after the end of April. Even with the flu caused by influenza viruses, the season flattens out significantly from the end of March, ie even if the flu season in the past was so severe, one could be sure that the flu wave would subside again in the spring. A "shutdown" of public life was not necessary. However, the novel corona virus obviously behaves differently and also spreads in those countries in the world where daylight saving time is in effect.

- Corona viruses also played an important role in more serious diseases in the past, such as the SARS epidemic in 2003. However, this type of corona virus was significantly less contagious for humans, so that the number of infected people was less than 10,000 and the number of deaths below 1,000 worldwide. New research indicates that the novel coronavirus SARS-CoV 2 can still be contagious up to three times the distance from an infected person compared to previous SARS coronaviruses. Furthermore, with the new type of corona virus, it is much easier to get infected when several people are in
  occur in an enclosed space, even if a minimum distance of two meters is maintained. The high risk of infection associated with the new

Coronavirus type is scientifically explained by the very good adaptation of the SARS CoV-2 virus to human cell receptors [I.1], so that the novel coronavirus finds much easier access to human cells and can very easily infect the people concerned.

- In fact, the adaptation of the SARS-CoV-2 virus to human cell receptors is so good that not only (upper) respiratory organs, but also other internal organs can be affected by this new virus type. In some cases, this leads to a very serious course of the disease in COVID-19 patients, caused by multi-organ failure.

Everyone can already see from the three characteristics of the new virus type listed above that we are not dealing with a viral disease that we are used to. "Whenever a new type of virus emerges, it is very important to understand where the new virus came from, that is, to identify the source of the viruses and to study the details of the spread, in order to obtain important information on which to base current and future measures win," according to the World Health Organization (WHO). The question of the origin of the current coronavirus pandemic is undoubtedly considered to be of particular importance with regard to future measures to reduce the likelihood of the outbreak of similar pandemics.

2.1 The wild animal market theory

Based on reports in scientific journals ([I.1]-[I.3]) and in various media, the coronavirus pandemic started at one point, the city of Wuhan in China, towards the end of 2019. A The wild animal market in the center of this city has been and still is the most frequently mentioned possible source of the novel corona viruses. The genetic analysis of the new SARS-CoV-2 viruses, which were taken from humans with a COVID 19 disease, shows a high degree of relationship to corona viruses in bats [I.1, I.3], similar to the case of the already known SARS viruses, which were responsible for the SARS epidemic in 2003. It is speculated that the coronaviruses could ultimately have been transmitted to humans via another wild animal as an intermediate host. In this context one speaks of a “zoonosis”. Since the beginning of the pandemic, the following animal species have been discussed as possible intermediate hosts: snakes, civets, pangolins and raccoon dogs [IV.1].

There are numerous scientifically based facts that speak against this theory:

1. Bats themselves were not found at the suspected wildlife market offered.

2. To date, none of the above-mentioned intermediate host animals have been proven to be carriers of the currently circulating coronavirus disease. However, one could still object at this point that it is also caused by previous illnesses

Corona viruses took a long time to identify the intermediate host animal.

3. A much more serious argument is that a significant proportion (34%) of the first documented COVID-19 patients had no contact with the suspected wildlife market [I.2, I.3]. In particular, the first patient documented in the original scientific literature had no contact with the wild animal market (more precisely: "Huanan seafood market"), which was officially declared by the Chinese government to be the cause of the COVID-19 diseases shortly after the outbreak of the pandemic. The authors of these studies included doctors from the clinics in Wuhan, who themselves treated the COVID-19 patients in the early phase of the pandemic and conducted epidemiologically relevant interviews.

   Below is an excerpt from the original scientific literature [I.2] with the essential diagram. The journal "LANCET" is one of the most respected journals in medical research:

LANCET VOLUME 395, ISSUE 10223, P. 497-506, FEBRUARY 15, 2020

Published online: January 24, 2020. DOI: https://doi.org/10.1016/S0140-6736(20)30183-5

Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China

Chaolin Huang, Yeming Wang, Xingwang Li, Lili Ren, Jianping Zhao, Yi Hu, Li Zhang, Guohui Fan, Jiuyang Xu, Xiaoying Gu, Zhenshun Cheng, Ting Yu, Jiaan Xia, Yuan Wei, Wenjuan Wu, Xuelei Xie, Wen Yin, Hui Li, Min Liu, Yan Xiao, Hong Gao, Li Guo, Jungang Xie, Guangfa Wang, Rongmeng Jiang, Zhancheng Gao, Qi Jin, Jianwei Wang, and Bin Cao

Summary

Background
A recent cluster of pneumonia cases in Wuhan, China, was caused by a novel betacoronavirus, the 2019 novel coronavirus (2019-nCoV). We report the epidemiological, clinical, laboratory, and radiological characteristics and treatment and clinical outcomes of these patients.

methods

All patients with suspected 2019-nCoV were admitted to a designated hospital in Wuhan. We prospectively collected and analyzed data on patients with laboratory-confirmed 2019-nCoV infection by real-time RT-PCR and next-generation sequencing. Data were obtained with standardized data collection forms shared by WHO and the International Severe Acute Respiratory and Emerging Infection Consortium from electronic medical records. Researchers also communicated directly with patients or their families to ascertain epidemiological and symptom data. Outcomes were also compared between patients who had been admitted to the intensive care unit (ICU) and those who had not.

findings

By Jan 2, 2020, 41 admitted hospital patients had been identified as having laboratory confirmed 2019-nCoV infection. Most of the infected patients were men (30 [73%] of 41); less than half had underlying diseases (13 [32%]), including diabetes (eight [20%]), hypertension (six [15%]), and cardiovascular disease (six [15%]). Median age was 49 0 years (IQR 41 0–58 0). 27 (66%) of 41 patients had been exposed to Huanan seafood market. One family cluster was found. Common symptoms at onset of illness were fever (40 [98%] of 41 patients), cough (31 [76%]), and myalgia or fatigue (18 [44%]); Less common symptoms were sputum production (11 [28%] of 39), headache (three [8%] of 38), haemoptysis (two [5%] of 39), and diarrhea (one [3%] of 38). Dyspnoea developed in 22 (55%) of 40 patients (median time from illness onset to dyspnoea 8 0 days [IQR 5 0–13 0]). 26 (63%) of 41 patients had lymphopenia. All 41 patients had pneumonia with abnormal findings on chest CT. Complications included acute respiratory distress syndrome (12 [29%]), RNAaemia (six [15%]), acute cardiac injury (five [12%]) and secondary infection (four [10%]). 13 (32%) patients were admitted to an ICU and six (15%) died. Compared with non-ICU patients, ICU patients had higher plasma levels of IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFÿ.

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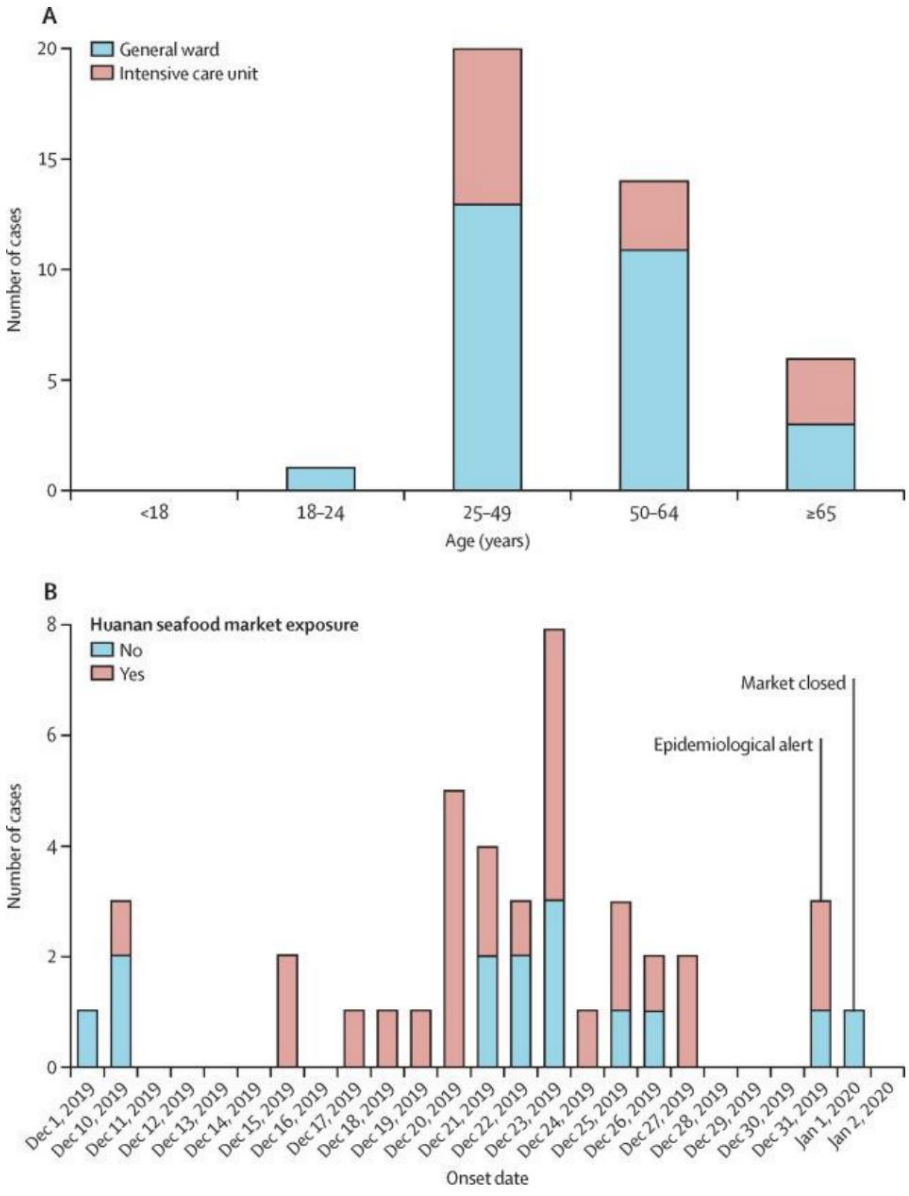
figures:

Date of illness onset and age distribution of patients with laboratory-confirmed 2019-nCoV infection.



It is also interesting in this context that the first confirmed patient in this publication had symptoms of COVID-19 disease as early as April 1.
December 2019 were detected. Due to the incubation period of up to 14 days in connection with the novel coronavirus disease, it must therefore be assumed that the first infections took place as early as November 2019. Among other things, this is compatible with a more recent report, according to which a very first case of COVID-19 disease in France was subsequently identified in November 2019 based on a detailed analysis of a patient's lung images. Recently, the treatment of the first COVID-19 patients in various hospitals in the city of Wuhan has even been reported as early as October 2019
(see eg [IV.2]). We will come back to this temporal aspect of the spread of the COVID-19 disease in the early phase of the pandemic later in the present study.

4. A scientific publication frequently cited in the media, which allegedly proves that the origin of the current coronavirus pandemic is zoonotic, turns out to be unsuitable on closer analysis to decide between the two alternative theories. Under the title "Researchers refute conspiracy theories" (see, for example, [IV.3]), reference was repeatedly made to a publication in the respected journal "Nature Medicine", which allegedly provided proof "that the pathogen SARS-CoV-2 spreads to natural Wisely developed and not created by genetic engineering in a laboratory".

   If you look at this publication in the journal "Nature Medicine" [III.1], you first have to recognize that this is not an original scientific publication, but a so-called **"Letter to the Editor",** in which five virologists present their personal view of the origin of the SARS-CoV-2 virus, see the following excerpt from the publication:

Nature Medicine 26, pages 450–452 (2020)
Correspondence, Published: 17 March 2020

The proximal origin of SARS-CoV-2

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To the Editor — Since the first reports of novel pneumonia (COVID-19) in Wuhan, Hubei province, China, there has been considerable discussion on the origin of the causative virus, SARS-CoV-2 (also referred to as HCoV-19). Infections with SARS-CoV-2 are now widespread, and as of 11 March 2020, 121,564 cases have been confirmed in more than 110 countries, with 4,373 deaths.

SARS-CoV-2 is the seventh coronavirus known to infect humans; SARS-CoV, MERS-CoV and SARS-CoV-2 can cause severe disease, whereas HKU1, NL63, OC43 and 229E are associated with mild symptoms. Here we review what can be deduced about the origin of SARS-CoV-2 from comparative analysis of genomic data. We offer a perspective on the notable features of the SARS-CoV-2 genome and discuss scenarios by which they could have arisen. Our analyzes clearly show that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus.

...

In the introduction, the authors write: "Our analyzes **clearly show** that SARS-CoV 2 is not a laboratory construct or a purposefully manipulated virus". Later in the text, completely different formulations are suddenly used: "It is **improbable** that SARS-CoV-2 emerged through laboratory manipulation of a related SARS-CoV 2-like coronavirus". "Instead, we **propose** two scenarios that can **plausibly** explain the origin of SARS-CoV-2". And finally in the final part: "Although the **evidence** shows that SARS-CoV-2 is not a purposefully manipulated virus, **it is currently impossible to prove or disprove the other theories of its origin described here". "More scientific data could swing the balance of evidence to favor one hypothesis over another".**

Scientific "proof" as seen by the media in this publication looks different. In this case, however, the misinterpretation is clearly to be attributed to the authors' extremely ambiguous opening statement, which is in clear contradiction to the concluding statement of this “Letter to the Editor”.

5. Another scientific original publication [I.4], which is repeatedly cited in scientific circles in the context of the theory of a zoonosis, comes from the research group of Zheng-Li Shi at the "Wuhan Institute of Virology", which has been intensive for many years conducted research on coronaviruses from different bat populations. The amazing thing about this publication in the famous journal "NATURE" is that there were only nine days between the submission date (01/20/2020) and the acceptance date (01/29/2020), which indicates in scientific circles that no well-founded critical expert assessment of this work was carried out here - as a rule - several experts may have taken place. The actual publication, which took place five days later, went even faster:

***Nature* 579, pages 270–273 (2020)**
article,
Received: 20 January 2020
Accepted: 29 January 2020
Published: 03 February 2020

A pneumonia outbreak associated with a new coronavirus of probable bat origin

Peng Zhou, Xing Lou Yang, Xian Guang Wang, Ben Hu, Lei Zhang, Wei Zhang, Hao Rui Si, Yan Zhu, Bei Li, Chao-Lin Huang, Hui-Dong Chen, Jing Chen, Yun Luo, Hua Guo, Ren Di Jiang, Mei-Qin Liu, Ying Chen, Xu-Rui Shen, Xi Wang, Xiao-Shuang Zheng, Kai Zhao, Quan-Jiao Chen, Fei Deng, Lin-Lin Liu, Bing Yan, Fa-Xian Zhan, Yan-Yi Wang, Geng Fu Xiao and Zheng-Li Shi

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Abstracts

Since the outbreak of severe acute respiratory syndrome (SARS) 18 years ago, a large number of SARS-related coronaviruses (SARSr-CoVs) have been discovered in their natural reservoir host, bats. Previous studies have shown that some bat SARSr-CoVs have the potential to infect humans. Here we report the identification and characterization of a new coronavirus (2019- nCoV), which caused an epidemic of acute respiratory syndrome in humans in Wuhan, China. The epidemic, which started on 12 December 2019, had caused 2,794 laboratory-confirmed infections including 80 deaths by 26 January 2020. Full-length genome sequences were obtained from five patients at an early stage of the outbreak. The sequences are almost identical and share 79.6% sequence identity to SARS-CoV. Furthermore, we show that 2019-nCoV is 96% identical at the whole-genome level to a bat coronavirus. Pairwise protein sequence analysis of seven conserved non-structural protein domains show that this virus belongs to the species of SARSr-CoV. In addition, 2019-nCoV virus isolated from the bronchoalveolar lavage fluid of a critically ill patient could be neutralized by sera from several patients. Notably, we confirmed that 2019-nCoV uses the same cell entry receptor—angiotensin converting enzyme II (ACE2)—as SARS-CoV.

This article contains the essential statement that the genetic fingerprint of the novel type of coronavirus (then called 2019-nCoV), which causes COVID-19 disease, corresponds to 96% with a type of coronavirus "RaTG13", which is found in horseshoe bats from the southern Chinese province of Yunnan originates. Since the genetic code of the novel type of coronavirus was not published until March 11.
January 2020 by the "China's National Center for Disease Control and Prevention", the research team led by Zheng-Li Shi only had nine days to compare the genetic fingerprint of the novel type of coronavirus with that of many other types of coronavirus in databases and to compare the virus type with to identify the highest similarity. Furthermore, the publication itself had to be written during this time and coordinated among all co-authors. Interestingly, the bat virus called "RaTG13" was isolated from horseshoe bats in Yunnan province by Zheng-Li Shi's research group in 2013, seven years earlier, without this being mentioned in previous publications by Zheng Li Shi's research team. Since the above-mentioned publication in the journal "NATURE" in February 2020, the virus with the designation "RaTG13" has been considered by many virologists as the "natural source" of the coronavirus pandemic.

However, there have been considerable doubts in scientific circles for several months regarding the truthfulness of the content of this NATURE publication from February 2020 (see for example [IV.4]). Here are three examples of the reservations expressed (for full versions see sources [II.1-II.3]):

Anomalies in BatCoV/RaTG13 sequencing and provenance

Daoyu Zhang

To this date, the most critical piece of evidence on the purposed "natural origin" theory of SARS-CoV-2, was the sequence known as RaTG13, allegedly collected from a single fecal sample from Rhinolophus Affinis. Understanding the provenance of RaTG13 is critical on the ongoing debate of the Origins of SARS-CoV-2. However, this sample is allegedly “used up” and therefore can no longer be accessed nor sequenced independently, and the only available data was the 3 related Genbank accessions: MN996532.1, SRX7724752 and SRX8357956.

We report these datasets possessed multiple significant anomalies, and the provenence of the promised claims of RaTG13 or it's role in proving a "probable bat origin" of SARS-CoV-2 can not be satisfied nor possibly be confirmed.

...

De novo Assembly of RaTG13 Genome Reveals Inconsistencies Further Obscuring SARS-CoV-2 Origins

Mohit Singla, Saad Ahmad, Chandan Gupta, and Tavpritesh Sethi

Received: August 25, 2020 / Approved: August 27, 2020 / Online: August 27, 2020

Abstracts

An intense scientific debate is ongoing as to the origin of SARS-CoV-2. An often-cited piece of information in this debate is the genome sequence of a bat coronavirus strain referred to as RaTG13 mentioned in a recent Nature paper showing 96.2% genome homology with SARS CoV-2. This is discussed as a fossil record of a strain whose current existence is unknown. The said strain is conjectured by many to have been part of the ancestral pool from which SARS CoV-2 may have evolved. Multiple groups have been discussing the features of the genome sequence of the said strain. In this paper, we report that the currently specified level of details are grossly insufficient to draw inferences about the origin of SARS-CoV-2. De novo assembly, KRONA analysis for metagenomic and re-examining data quality highlights the key issues with the RaTG13 genome and the need for a dispassionate review of this data. This work is a call to action for the scientific community to better collate scientific evidence about the origins of SARS-CoV-2 so that future incidences of such pandemics may be effectively mitigated.
...

All journal articles evaluating the origin or epidemiology of SARS-CoV-2 that utilize the RaTG13 bat strain genomics are potentially flawed and should be retracted

Dean Bengston

Recent SARS-CoV-2 epidemiological origin studies have made their conclusion based-in-part by analyzing a bat coronavirus strain that most closely matches SARS-CoV-2 called RaTG13. However, the origins of this strain are obfuscated and therefore the genomics of the strain cannot be trusted, especially in the context of determining the origin of SARS-CoV-2.

...

In summary, it can be stated that to date there is no scientifically sound basis for the claim that the current coronavirus pandemic was caused by a zoonosis. As a result, it is not scientifically appropriate to speak of a "natural disaster" at this time.

2.2 The Laboratory Accident Theory

It wasn't "conspiracy theorists", but two Chinese scientists, Lei and Botao Xiao from the South China University of Technology, who published a study on the international research online portal "Research Gate" in mid-February 2020, in which they were published for the first time after the outbreak of the epidemic publicly suspected that the biotechnology laboratory in central Wuhan could be the source of the novel coronaviruses. Shortly after the publication of this study, it disappeared from the online database of the "Research-gate" portal, but is still archived on the web [II.4].

In fact, the outbreak of the current coronavirus pandemic in the city of Wuhan raises the legitimate question of why this pandemic started in this city in 2019. Assuming a zoonosis that took place at a wildlife market in central Wuhan as the cause of the current pandemic, the first thing to note is that wildlife markets have existed in China for thousands of years, and until very recently there were thousands of such markets in China existed in all cities of China.

One therefore has to ask oneself why such a coronavirus pandemic emanated from the city of Wuhan in 2019?

In science, the city of Wuhan has primarily appeared in recent years through its research in the field of virology, not least through numerous publications in leading interdisciplinary scientific journals such as "NATURE" and "SCIENCE". The research group led by Zheng-Li Shi at the Wuhan Institute of Virology has played an important role in the field of virology for many years

Coronavirus Research. This began about 16 years ago - even before the establishment of the "Wuhan Institute of Virology" as part of a Chinese-French cooperation - and has been carried out for many years in close cooperation between the Chinese researchers and several American and Australian research groups [I.5 -I.10]. The source of the coronaviruses for virological research were different species of bats, which were collected by the Wuhan research team in caves in various Chinese provinces as part of numerous expeditions. The corona viruses were then isolated at the Wuhan Institute of Virology, multiplied and their interaction with animal and human cells examined (see e.g. [I.5, I.6, I.7, I.9]).

However, the research group led by Zheng-Li Shi at the "Wuhan Institute of Virology" not only examined naturally occurring corona viruses, but also specifically manipulated them with the aim of making them more contagious and dangerous for humans. This so-called **"gain-of-function" research** at the "Wuhan Institute of Virology" is documented by several original scientific publications in peer-reviewed journals (see e.g. [I.5, I.6, I.7, I.8] and was has been critically assessed by many representatives of science for years (see e.g. [III.2]).Due to its importance, two separate chapters following this introductory chapter are devoted to this background to the current coronavirus pandemic.In **particular, the dispute in scientific circles about the The potential danger of "gain-of-function" research, which is expressed in two letters to the President of the EU Commission in 2013 (see chapter: "Gain-of-function research"), shows how different the Opinions among scientists were even then and how great the need for discussion would actually be today - after the outbreak of a global pandemic.**

Although the "Wuhan Institute of Virology" operates a biotechnology laboratory with the highest security level, before the outbreak of the coronavirus pandemic, there were reports of significant safety deficiencies in the "Wuhan Institute of Virology" (see e.g. [IV.5]):

The Washington Post, April 14, 2020

State Department cables warned of safety issues at Wuhan lab studying bat coronaviruses

Josh Rogin

Two years before the novel coronavirus pandemic upended the world, US 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 coronaviruses from bats . The cables have fueled discussions inside the US 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 US Embassy in Beijing took the unusual step of repeatedly sending US science diplomats to the Wuhan Institute of Virology (WIV), 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 US 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 US 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, which I obtained, 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. (The State Department declined to comment on this and other details of the story.)

...

A look at the statistics of documented accidents in biotechnological high-security laboratories shows that the unwanted escape of highly infectious viruses from such laboratories was not uncommon in the past, both in China and in the USA. A separate chapter of this study is also devoted to this important topic.

But what do we really know about the early phase of the outbreak of the coronavirus pandemic in Wuhan? Unfortunately, very little from official sources, since China tried to cover up the true events from the start. This has already been extensively reported in the media (see for example [IV.6, IV.7, IV.8]). In the course of 2020, China even put pressure on the EU and countries like Australia - up to and including the threat of sanctions - if the Chinese handling of the pandemic is not praised as exemplary or even critical statements about the behavior of the Chinese government at the beginning of the pandemic would take place.

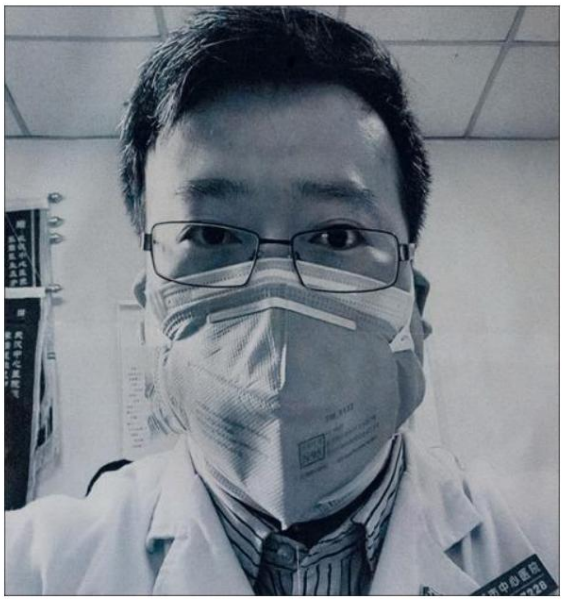
It is known from the scientific literature (see e.g. [III.3]) and from numerous media reports (see e.g. [IV.9]) that **the Chinese doctors in Wuhan were subjected to great pressure when they tried to convince other colleagues or even to inform the public truthfully about what is happening in connection with the new COVID-19 disease.** A particularly tragic example is the doctor Wenliang Li, whose fate was reported in the renowned magazine "LANCET" as follows:

THE LANCET, VOLUME 395, ISSUE 10225, P682, FEBRUARY 29, 2020

Li Wenliang

Andrew Green

On Dec 30, 2019, Li Wenliang sent a message to a group of fellow doctors warning them about a possible outbreak of an illness that resembled severe acute respiratory syndrome (SARS) in Wuhan, Hubei province, China, where he worked. Meant to be a private message, he encouraged them to protect themselves from infection. Days later, he was summoned to the Public Security Bureau in Wuhan and made to sign a statement in which he was accused of making false statements that disturbed the public order.



Ophthalmologist who warned about the outbreak of COVID-19. Born in Beizhen, China, on Oct 12, 1986, he died after becoming infected with SARS-CoV-2 in Wuhan, China, on Feb 7, 2020, aged 33 years.

In fact, Li was one of the first people to recognize the outbreak of 2019 novel coronavirus disease (COVID-19) in Wuhan that has now spread to 25 countries, killing 1669 people and infecting more than 51 800 people as of Feb 16, 2020 Li returned to work after signing the statement and contracted severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), apparently from a patient. His death sparked outrage in China, where citizens took to message boards to voice their gratitude for Li's dedicated front-line service and to criticize the initial response of Wuhan's security and medical officials to his warning. In the days before his death, Li said “If the officials had disclosed information about the epidemic earlier I think it would have been a lot better”, in an interview with *The New York Times.* "There should be more openness and transparency," he said.

Therefore, the only way to get information about the true events in the early phase of the pandemic - both inside China and from abroad - was to systematically analyze the reports on Chinese social media and

Online platforms, with much information only being available temporarily before being deleted again.

For example, the large discrepancy between the unofficial and official figures on infected people and deaths in China in the early phase of the pandemic was noticeable. This was also reported very early on in the media of neighboring Asian countries (see e.g. [IV.10], [IV.11]):

TAIWAN NEWS, 02/05/2020

Tencent may have accidentally leaked real data on Wuhan virus deaths

Tencent briefly lists 154,023 infections and 24,589 deaths from Wuhan coronavirus

Keoni Everington

TAIPEI (Taiwan News) — As many experts question the veracity of China's statistics for the Wuhan coronavirus outbreak, Tencent over the weekend appeared to inadvertently release what is potentially the actual number of infections and deaths — which are far higher than official figures, but eerily in line with predictions from a respected scientific journal.

As early as Jan. 26, netizens were reporting that Tencent, on its webpage titled "Epidemic Situation Tracker," briefly showed data on the novel coronavirus (2019 - nCoV) in China that was much higher than official estimates, before suddenly switching to lower numbers. Hiroki Lo, a 38-year-old Taiwanese beverage store owner, reported that day that Tencent and NetEase were both posting "unmodified statistics," before switching to official numbers in short order.

Lo told Taiwan News than on Jan. 26 he checked the numbers on both Tencent and NetEase and found them "really scary." He said he did not know whether the numbers were real or not, but did not have much time to think about it as he had a busy day of work ahead at his store.

Lo said he did not check the numbers again until he went home that evening, when he was shocked to see they had dropped dramatically and "something was wrong." He said he noticed individuals on a Hong Kong Facebook group also observed the same bizarre occurrence that day.

On late Saturday evening (Feb. 1), the Tencent webpage showed confirmed cases of the Wuhan virus in China as standing at 154,023, 10 times the official figure at the time. It listed the number of suspected cases as 79.808, four times the official figure.

The number of cured cases was only 269, well below the official number that day of 300. Most ominously, the death toll listed was 24,589, vastly higher than the 300 officially listed that day.

Moments later, Tencent updated the numbers to reflect the government's "official" numbers that day. Netizens noticed that Tencent has posted extremely high numbers, only to quickly lower them to government-approved statistics, on at least three occasions.

Feb. 1 chart showing higher numbers (left), chart showing "official" numbers (right). (web image)

Netizens also noticed that each time the screen with the large numbers appears, a comparison with the previous day's data appears above, which demonstrates a "reasonable" incremental increase, much like the official numbers. This has led some netizens to speculate that Tencent has two sets of data, the real data and "processed" data.

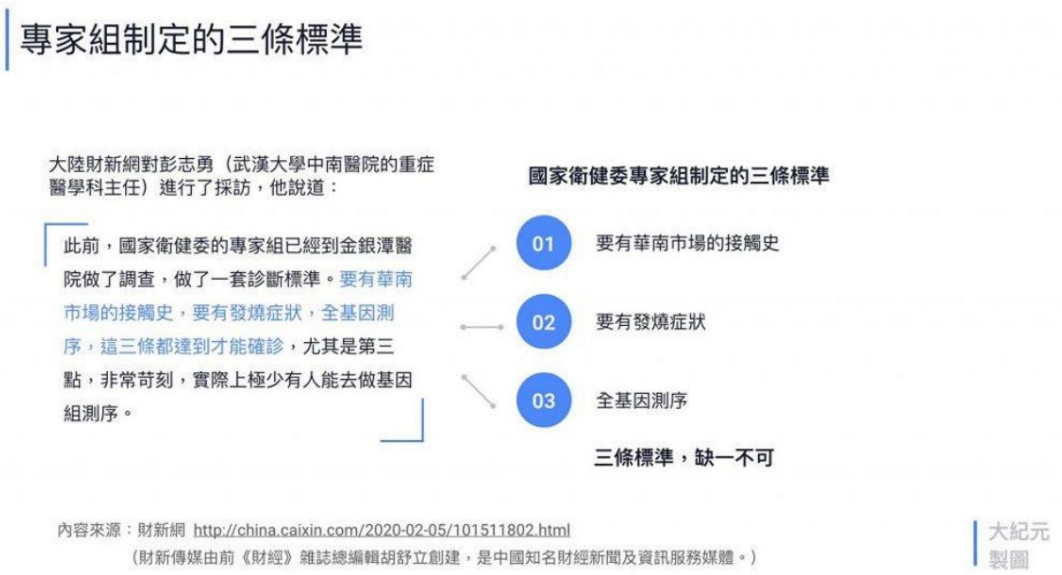
...

One of the reasons why the unofficial and official figures for diagnosed coronavirus infections and deaths in the early phase of the pandemic differed may be due to the strange definition of "official corona cases". For a positive diagnosis, three conditions had to be met [IV.12]:

1) The person concerned had to contact the "Huanan seafood market" have had.

2) The person concerned had to show symptoms of fever.

3) The diagnosis of a coronavirus infection had to be proven by gene sequencing.



The first criterion is particularly relevant in connection with the question of the origin of the coronavirus pandemic: **The Chinese government has therefore postulated from the outset that the origin of the COVID-19 disease should be the wild animal market in the center of the city of Wuhan,** which is known to be the same was closed by the Chinese government at the beginning of 2020. However, there was neither at that time nor to this day reliable scientific knowledge, so that the first of the three criteria mentioned above for the detection of a COVID-19 disease does not make sense from a medical diagnostic point of view, but should rather be understood as a politically motivated definition .

Of course, one has to ask oneself why the Chinese government at this early point in time declared the wild animal market as the origin of the coronavirus pandemic as the only possible explanation and has since done everything possible to propagate the zoonosis theory both within its own country and to other countries .

The background to this is that numerous indications were given and published very early on in the Chinese social media that "patient zero" of the COVID-19 infection chain was a young scientist from the "Wuhan Institute of Virology". Her name is Yanling Huang, born on October 20, 1988. She has been on the staff of the "Wuhan Institute of Virology" since 2012 and has published at least six scientific papers at this institute's address. Since the end of 2019 she has been considered missing and her photo and profile have been deleted from the institute's website (as well as her personal website):

However, evidence that Yanling Huang was a staff member of the "Wuhan Institute of Virology" can still be found on the following website, which lists the Institute's doctoral students including student IDs (the original website is in Chinese; here is an in the German language translated version is reproduced):

20140923 The completion status of 2012 PhD opening report system
gd.whiov.cas.cn/zxpy/yjsswgg/201409/t20140923_258008.html 1/2

Chinese Academy of Science
Wuhan Institute of Virology

Your current position: Home >> Education >> Company announcement

20140923 The completion status of the opening reporting system for PhD students 2012

Source: Published: 09/23/2014

serial number	student ID	Surname	degree type	Name of teacher	
1	201218012415001	chai fan	Ph.D	Xiao Gengfu	Passed the valuation
2	201218012415002	He Xuan	Ph.D	Yan Huimin	Passed the valuation
3	201218012415003	Feng Lipeng	Ph.D	Chen Shiyun	Passed the valuation
4	201218012415004	Ge Sai	Ph.D	Yuan Zhiming	Passed the valuation
5	201218012415005	Xie Jumin	Ph.D	Guan Wuxiang	Passed the valuation
6	201218012415006	Kangzhenyu	Ph.D	Wang Hualin	Passed the valuation

7	201218012415007	Kuang Wenhua	Ph.D	Hu Zhihong	Passed the valuation
8	201218012415008	Li Xiaojun	Ph.D	Luo Minhua	Passed the valuation
9	201218012415009	Li Xiaodan	Ph.D	Zhang Bo	Passed the valuation
10	201218012415010	Peng Qin	Ph.D	Gao Meiying	Passed the valuation
11	201218012415011	Qiao Jinjuan	Ph.D	Wei Hongping	Passed the valuation
12	201218012415012	Shang Yu	Ph.D	Hu Zhihong	Passed the valuation
13	201218012415013	Su Lan	Ph.D	Sun Xiulian	Passed the valuation
14	201218012415014	Sun Manluan	Ph.D	Zhang Xianen	Passed the Rating none
15	201218012415015	Tan Bing	Ph.D	Shi Zhengli	records At the Submit review team
16	201218012415016	Teng Tieshan	Ph.D	Wei Hongping	At the Submit review team
17	201218012415017	Wang Jinpei	Ph.D	Zhou Ningyi	Passed the valuation
18	201218012415018	Yan Liming	Ph.D	Fang Qin	Passed the valuation
19	201218012415019	poetry	Ph.D	Zhang Xianen	Passed the valuation
20	201218012415020	Jae Junjie	Ph.D	Yuan Zhiming	At the Submit review team
21	201218012415021	Zou Jing	Ph.D	Yuan Zhiming	Passed the valuation
22	201218012415022	Bi Peng	Ph.D	gong bang	Passed the valuation
23	201218012415023	Chen Jungang	Ph.D	Chen Xulin	Passed the valuation
24	201218012415024	Hao Sujuan	Ph.D	Guan Wuxiang	Passed the valuation
25	201218012415025	Li Qian	Ph.D	Wang Hanzhong	Passed the Rating none
26	201218012415026	Li Xingguang	Ph.D	Yang Rongge	records
27	201218012415028	Liu Shuhui	Ph.D	Chen Xinwen	Passed the valuation
28	201218012415029	Wu Guiru	Ph.D	Li Chaoyang	At the Submit review team
29	201218012415030	Yan Yan	Ph.D	Hu Qinxue	Passed the valuation
30	201218012415031	Yao Yongxuan	Ph.D	Chen Xinwen	Passed the valuation
31	201218012415032	Yu Jie	Ph.D	Yan Huimin	Passed the valuation
32	201218012415033	Zhang Mudan	Ph.D	Hu Qinxue	
33	201218012415034	Zheng Caishang	Ph.D	Wang Hanzhong	Passed the valuation
34	201218012415035	Zhou Ming	Ph.D	Hu Kanhong	Passed the valuation
35	201218012415036	Wang Zhilong	Ph.D	Tang Hong	Passed the valuation
36	201228012415001	Chen Xiuxiu	master's degree	Zhang Xianen	Passed the valuation
37	201228012415002	Shichenyan	master's degree	Yuan Zhiming	Passed the valuation
38	201228012415003	Wang Mingxiu	master's degree	Cui Zongqiang	Passed the valuation
39	201228012415005	Yan Shicui	master's degree	Fang Qin	Passed the valuation
40	201228012415007	Zhou Yu	master's degree	Zhou Ningyi	Passed the valuation
41	201228012415009	Chen Yajun	master's degree	Gao Meiying	Passed the valuation
42	201228012415010	Feng Lianwei	master's degree	Yang Rongge	Passed the valuation
43	201228012415012	He woo	master's degree	Zhou Ningyi	Passed the valuation
44	201228012415013	Huberdan	master's degree	Hu Qinxue	Passed the valuation
45	201228012415014	Huang Yanling	master's degree	Wei Hongping	Passed the valuation
46	201228012415015	Jiang Liangyu	master's degree	Chen Xulin	Passed the valuation
47	201228012415016	Liu Lili	master's degree	Wang Yanyi	Passed the valuation
48	201228012415019	Mengxiangzheng	master's degree	Dengjiaoyu	Passed the valuation

49	201228012415021	Shi Jing	master's degree	Li Chaoyang	Passed the valuation
50	201228012415023	Wang Bo	master's degree	Shi Zhengli	Passed the valuation
51	201228012415028	Xu Hao	master's degree	Wang Hualin	Passed the valuation
52	201228012415029	Yang Bo	master's degree	Luo Minhua	
53	201228012415031	ZhangWeihong	master's degree	Tang Hong	At the Submit review team
54	2012E8012461033	Gao Yutao	master's degree	Shi Zhengli	Passed the valuation
55	2012E8012461034	Hou Shoucai	master's degree	Sun Xiulian	Passed the valuation
56	2012E8012461035	Wang Jing	master's degree	Wei Hongping	Passed the valuation
57	2012E8012461036	Wang Yifei	master's degree	Chen Shiyun	In Review
58	2012E8012461037	phase star	master's degree	Hu Xiaomin	Passed the valuation
59	2012E8012461038	Xiong Chaochao	master's degree	Chen Jianjun	At the Submit review team
60	2012E8012461039	Yao Weitong	master's degree	Yang Rongge	Passed the valuation
61	2012E8012461040	Zhao Bali	master's degree	Yan Huimin	Passed the valuation
62	2012E8012461042	Zhu Zheng	master's degree	Hu Zhihong	Passed the valuation
63	2012E8012461043	Wen Lei	master's degree	Simon Rayner	Passed the valuation
64	2012E8012461044 Ma Ruipeng		master's degree	Sun Xiulian	Passed the valuation
65	2012E8012461045	Mei Xiaofen	master's degree	Yuan Zhiming	In Review
66	2012E8012461046	Xu Ting	master's degree	Gong Rui	Passed the valuation
67	2012E8012461049	Zhao Kaitao	master's degree	Chen Xinwen	At the Submit review team

In 2018, Yanling Huang was still at the Wuhan Institute of Virology, as a group photo from that year shows:

A comprehensive report on the fate of Yanling Huang and the background to her disappearance as well as numerous other documentary evidence can be accessed via the following link [IV.13]:

https://www.youtube.com/watch?v=bpQFCcSI0pU

There is also **a website** on the topic "Where is Huang Yan Ling?", which provides much more information and background information:

https://twitter.com/whereisyanling

Despite the seriousness of the allegations, which have been repeatedly raised in both Chinese and international social media and online platforms, neither the laboratory manager responsible, Zheng-Li Shi, nor an official representative of the "Wuhan Institute of Virology" have been willing to comment on the whereabouts by Yanling Huang to provide information. The Chinese government has officially denied the "rumors" about Yanling Huang, but on the other hand refuses to provide any information about the whereabouts of the young scientist.

In view of the fact that in the early phase of the pandemic, scientists, doctors, journalists and private individuals in China were pressured by the Chinese government to provide false information about the background of the COVID-19 disease (see e.g [III.3], [IV.14]) or have even disappeared without a trace (see for example [IV.6], [IV.15]), it is incomprehensible to a large number of scientists that some virologists in the context of a joint statement [III.4] have praised "the fast, open and transparent" information policy on the part of the Chinese side. In truth, not only people like Yanling Huang [IV.13] and Fang Bin [IV.15] have disappeared, but important research samples have also been withheld (see eg [IV.16], [II.1]) or per Order canceled by the Health and Medical Commission of Hubei Province in early January 2020.

The statement of the group of virologists was as follows [III.4]:

THE LANCET 395, ISSUE 10226, E42-E43, MARCH 07, 2020

CORRESPONDENCE

Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19

Charles Calisher, Dennis Carroll, Rita Colwell, Ronald B. Corley, Peter Daszak, Christian Drosten, Luis Enjuanes, Jeremy Farrar, Hume Field, Josie Golding, Alexander Gorbalenya, Bart Haagmans, James M Hughes, William B Karesh, Gerald T Keusch, Sai Kit Lam, Juan Lubroth, John S Mackenzie, Larry Madoff, Jonna Mazet, Peter Palese, Stanley Perlman, Leo Poon, Bernard Roizman, Linda Saif, Kanta Subbarao, and Mike Turner

We are public health scientists who have closely followed the emergence of the 2019 novel coronavirus disease (COVID-19) and are deeply concerned about its impact on global health and wellbeing. We have watched as the scientists, public health professionals, and medical professionals of China, in particular, have worked diligently and effectively to rapidly identify the pathogen behind this outbreak, put in place significant measures to reduce its impact, and share their results transparently with the global health community. This effort has been remarkable.

We sign this statement in solidarity with all scientists and health professionals in China who continue to save lives and protect global health during the challenge of the COVID-19 outbreak. We are all in this together, with our Chinese counterparts in the forefront, against this new viral threat.

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. Scientists from multiple countries have published and analyzed genomes of the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and they overwhelmingly conclude that this coronavirus originated in wildlife, as have so many other emerging pathogens. This is further supported by a letter from the presidents of the US National Academies of Science, Engineering, and Medicine and by the scientific communities they represent. Conspiracy theories do nothing but create fear, rumors, and prejudice that jeopardize our global collaboration in the fight against this virus. We support the call from the Director-General of WHO to promote scientific evidence and unity over misinformation and conjecture.

We want you, the science and health professionals of China, to know that we stand with you in your fight against this virus.

We invite others to join us in supporting the scientists, public health professionals, and medical professionals of Wuhan and across China. Stand with our colleagues on the front line!

We speak in one voice. To add your support for this statement, sign our letter online. LM is editor of ProMED-mail. We declare no competing interests.

It should already be noted at this point that people from the circle of authors - as in the case of Peter Daszak - were themselves personally involved in "gain-of-function" experiments in the past and worked for years with the group around Zheng-Li Shi on the "Wuhan Institute of Virology" have researched and published together. This is discussed in more detail in the later chapter on "Gain-of-function research".

It should also be noted that the statement: “Scientists from multiple countries have published and analyzed genomes of the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and they overwhelmingly conclude that this coronavirus originated in wildlife, as have so many other emerging pathogens” in this form cannot stand without pointing out that there are now at least as many scientists from many countries, including Nobel laureate, gives that on the basis of analyzes of genetic fingerprints

of the new SARS-CoV-2 viruses have come to the opposite conclusion (see for example: [I.11], [II.5], [II.6], [II.7], [II.8]).

In summary, it can be said that there are many indications that a laboratory accident at the "Wuhan Institute of Virology" appears to be by far the most likely cause of the corona pandemic. In this case it would not not a "natural disaster" but a man-made tragedy. There is a very great danger in declaring the cause of the current pandemic "as resolved", as in the statement [III.4] of some virologists. For policymakers, there is an undeniable difference between banning wildlife markets globally and banning high-risk research using genetically engineered viruses. This question must come to the fore more, otherwise corona and other types of viruses could develop a much greater potential for danger, not only in the present but also in the future.

3 History of the coronavirus pandemic: Research and genetic engineering of bat coronaviruses at the Institute of Virology in Wuhan, China

In previous coronavirus-related diseases, such as SARS (2003), mutations of bat-derived coronaviruses have occurred in intermediate host animals, making subsequent transmission to humans possible. A direct transmission of coronaviruses from bats to humans was not previously known. In this context, virologists speak of an “adaptation barrier”. It was therefore of great importance to identify the intermediate host animals in question for various coronavirus-related diseases through intensive research.

Striking in the current pandemic compared to previous outbreaks of Coronavirus-related diseases is:

1) In the current pandemic we are dealing with a pathogen that is associated with a **hitherto unknown efficiency attacks human cells.**

2) Not only the (upper) respiratory tract, but **also internal organs are attacked and their function is sometimes severely damaged.**

It is therefore necessary to ask the question of how such an **almost perfect adaptation of corona viruses to human cell receptors** could have come about in order to be able to identify future pandemic risk potential.

The history of the coronavirus pandemic is examined in more detail below. As has been proven by numerous publications in scientific journals, the research group led by Zheng-Li Shi at the "Wuhan Institute of Virology" has been collecting bat viruses in caves in various southern Chinese provinces for many years and brought them to Wuhan. However, the research group not only studied the naturally occurring corona viruses scientifically, but also specifically manipulated them with the aim of making the corona viruses more contagious and dangerous for people. This so-called "gain of function" research at the "Wuhan Institute of Virology" is documented by several original scientific publications in refereed journals and has been viewed very critically by many representatives of science for years.

In a 2013 publication in the journal "NATURE" [I.7], the research team led by **Zheng-Li Shi** and **Peter Daszak** reports on the successful docking of the prongs of the coronavirus crown to human ACE2 cell receptors. So-called horseshoe bats from the Chinese province of Yunnan were used as a source of SARS-like corona viruses. The main part of this publication is reproduced below:

***Nature* 503,** pages 535–538 (2013), Published: 30 October 2013

Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor

Xing-Yi Ge, Jia-Lu Li, Xing-Lou Yang, Aleksei A Chmura, Guangjian Zhu, Jonathan H. Epstein, Jonna K. Mazet, Ben Hu, Wei Zhang, Cheng Peng, Yu-Ji Zhang, Chu-Ming Luo, Bing Tan, Ning Wang, Yan Zhu, Gary Crameri, Shu-Yi Zhang, Lin-Fa Wang, Peter Daszak & Zheng-Li Shi

affiliations

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Jonna K Mazet

CSIRO Australian Animal Health Laboratory, Geelong, 3220, Victoria, Australia

Gary Crameri & Lin-Fa Wang

College of Life Sciences, East China Normal University, Shanghai 200062, China

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Emerging Infectious Diseases Program, Duke-NUS Graduate Medical School, Singapore 169857

Lin Fa Wang

Abstracts

The 2002-3 pandemic caused by severe acute respiratory syndrome coronavirus (SARS-CoV) was one of the most significant public health events in recent history. An ongoing outbreak of Middle East respiratory syndrome coronavirus suggests that this group of viruses remains a key threat and that their distribution is wider than previously recognized. Although bats have been suggested to be the natural reservoirs of both viruses, attempts to isolate the progenitor virus of SARS-CoV from bats have been unsuccessful. Various SARS-like coronaviruses (SL-CoVs) have now been reported from bats in China, Europe and Africa, but none is considered a direct progenitor of SARS-CoV because of their phylogenetic disparity from this virus and the

inability of their spike proteins to use the SARS-CoV cellular receptor molecule, the human angiotensin converting enzyme II (ACE2). Here we report whole-genome sequences of two novel bat coronaviruses from Chinese horseshoe bats (family: Rhinolophidae) in Yunnan, China: RsSHC014 and Rs3367. These viruses are far more closely related to SARS-CoV than any previously identified bat coronaviruses, particularly in the receptor binding domain of the spike protein. Most importantly, we report the first recorded isolation of a live SL-CoV (bat SL-CoV-WIV1) from bat faecal samples in Vero E6 cells, which has typical coronavirus morphology, 99.9% sequence identity to Rs3367 and uses ACE2 from humans, civets and Chinese horseshoe bats for cell entry. Preliminary *in vitro* testing indicates that WIV1 also has a broad species tropism. Our results provide the strongest evidence to date that Chinese horseshoe bats are natural reservoirs of SARS-CoV, and that intermediate hosts may not be necessary for direct human infection by some bat SL-CoVs. They also highlight the importance of pathogen discovery programs targeting high-risk wildlife groups in emerging disease hotspots as a strategy for pandemic preparedness.

Main

The 2002-3 pandemic of SARS1 and the ongoing emergence of the Middle East respiratory syndrome coronavirus (MERS-CoV) demonstrate that CoVs are a significant public health threat. SARS-CoV was shown to use the human ACE2 molecule as its entry receptor, and this is considered a hallmark of its cross-species transmissibility. The receptor binding domain (RBD) located in the amino-terminal region (amino acids 318-510) of the SARS-CoV spike (S) protein is directly involved in binding to ACE2. However, despite phylogenetic evidence that SARS-CoV evolved from bat SL-CoVs, all previously identified SL-CoVs have major sequence differences from SARS-CoV in the RBD of their S proteins, including one or two deletions. Replacing the RBD of one SL-CoV S protein with SARS-CoV S conferred the ability to use human ACE2 and replicate efficiently in mice. However, to date, no SL-CoVs have been isolated from bats, and no wild-type SL-CoV of bat origin has been shown to use ACE2.

We conducted a 12-month longitudinal survey (April 2011–September 2012) of SL-CoVs in a colony of *Rhinolophus sinicus* at a single location in Kunming, Yunnan Province, China. A total of 117 anal swabs or faecal samples were collected from individual bats using a previously published method. A one-step reverse transcription (RT)-nested PCR was conducted to amplify the RNA-dependent RNA polymerase (RdRP) motifs A and C, which are conserved among alphacoronaviruses and betacoronaviruses.

Twenty-seven of the 117 samples (23%) were classified as positive by PCR and subsequently confirmed by sequencing. The species origin of all positive samples was confirmed to be *R. sinicus* by cytochrome b sequence analysis, as described previously16. A higher prevalence was observed in samples collected in October (30% in 2011 and 48.7% in 2012) than those in April (7.1% in 2011) or May (7.4% in 2012). Analysis of the S protein RBD sequences indicated the presence of seven different strains of SL-CoVs. In addition to RBD sequences, which closely matched previously described SL-CoVs (Rs672, Rf1 and HKU3), two novel strains (designated SL-CoV RsSHC014 and Rs3367) were discovered. Their full-length genome sequences were determined, and both were found to be 29,787 base pairs in size (excluding the poly(A) tail). The overall nucleotide sequence identity of these two genomes with human SARS-CoV (Tor2 strain) is 95%, higher than that observed previously for bat SL CoVs in China (88–92%) or Europe (76%). Higher sequence identities were observed at the protein level between these new SL-CoVs and SARS-CoVs. To understand the evolutionary origin of these two novel SL-CoV strains, we conducted recombination analysis with the

Recombination Detection Program 4.0 package using available genome sequences of bat SL CoV strains (Rf1, Rp3, Rs672, Rm1, HKU3 and BM48-31) and human and civet representative SARS-CoV strains (BJ01, SZ3, Tor2 and GZ02). Three breakpoints were detected with strong *P* values (<10ÿ20) and supported by similarity plot and bootscan analysis. Breakpoints were located at nucleotides 20,827, 26,553 and 28,685 in the Rs3367 (and RsSHC014) genome, and generated recombination fragments covering nucleotides 20,827–26,533 (5,727 nucleotides) (including partial open reading frame (ORF) 1b, full-length S, ORF3, E and partial M gene) and nucleotides 26,534-28,685 (2,133 nucleotides) (including partial ORF M, full length ORF6, ORF7, ORF8 and partial N gene). Phylogenetic analysis using the major and minor parental regions suggested that Rs3367, or RsSHC014, is the descendent of a recombination of lineages that ultimately lead to SARS-CoV and SL-CoV Rs672.

The most notable sequence differences between these two new SL-CoVs and previously identified SL-CoVs is in the RBD regions of their S proteins. First, they have higher amino acid sequence identity to SARS-CoV (85% and 96% for RsSHC014 and Rs3367, respectively). Second, there are no deletions and they have perfect sequence alignment with the SARS-CoV RBD region. Structural and mutagenesis studies have previously identified five key residues (amino acids 442, 472, 479, 487 and 491) in the RBD of the SARS-CoV S protein that have a pivotal role in receptor binding. Although all five residues in the RsSHC014 S protein were found to be different from those of SARS-CoV, two of the five residues in the Rs3367 RBD were conserved.

Despite the rapid accumulation of bat CoV sequences in the last decade, there has been no report of successful virus isolation. We attempted isolation from SL-CoV PCR-positive samples. Using an optimized protocol and Vero E6 cells, we obtained one isolate which caused cytopathic effect during the second blind passage. Purified virions displayed typical coronavirus morphology under electron microscopy. Sequence analysis using a sequence independent amplification method to avoid PCR-introduced contamination indicated that the isolate was almost identical to Rs3367, with 99.9% nucleotide genome sequence identity and 100% amino acid sequence identity for the S1 region. The new isolate was named SL-CoV WIV1.

To determine whether WIV1 can use ACE2 as a cellular entry receptor, we conducted virus infectivity studies using HeLa cells expressing or not expressing ACE2 from humans, civets or Chinese horseshoe bats. We found that WIV1 is able to use ACE2 of different origins as an entry receptor and replicated efficiently in the ACE2-expressing cells. This is, to our knowledge, the first identification of a wild-type bat SL-CoV capable of using ACE2 as an entry receptor.

To assess its cross-species transmission potential, we conducted infectivity assays in cell lines from a range of species. Our results indicate that bat SL-CoV-WIV1 can grow in human alveolar basal epithelial (A549), pig kidney 15 (PK-15) and Rhinolophus sinicus kidney (RSKT) cell lines, but not in human cervix (HeLa), Syrian golden Hamster kidney (BHK21), Myotis davidii kidney (BK), Myotis chinensis kidney (MCKT), Rousettus leschenaulti kidney (RLK) or Pteropus alecto kidney (PaKi) cell lines. Real-time RT–PCR indicated that WIV1 replicated much less efficiently in A549, PK-15 and RSKT cells than in Vero E6 cells.

To assess the cross-neutralization activity of human SARS-CoV sera against WIV1, we conducted serum-neutralization assays using nine convalescent sera from SARS patients collected in 2003. The results showed that seven of these were able to completely neutralize 100 tissue culture infectious dose 50 (TCID50) WIV1 at dilutions of 1:10 to 1:40, further confirming the close relationship between WIV1 and SARS-CoV.

Our findings have important implications for public health. First, they provide the clearest evidence yet that SARS-CoV originated in bats. Our previous work provided phylogenetic evidence of this, but the lack of an isolate or evidence that bat SL-CoVs can naturally infect human cells, until now, had cast doubt on this hypothesis. Second, the lack of capacity of SL CoVs to use of ACE2 receptors has previously been considered as the key barrier for their direct spillover into humans, supporting the suggestion that civets were intermediate hosts for SARS CoV adaptation to human transmission during the SARS outbreak. However, the ability of SL CoV-WIV1 to use human ACE2 argues against the necessity of this step for SL-CoV-WIV1 and suggests that direct bat-to-human infection is a plausible scenario for some bat SL-CoVs. This has implications for public health control measures in the face of potential spillover of a diverse and growing pool of recently discovered SARS-like CoVs with a wide geographic distribution.

Our findings suggest that the diversity of bat CoVs is substantially higher than that previously reported. In this study we were able to demonstrate the circulation of at least seven different strains of SL-CoVs within a single colony of R. sinicus during a 12-month period. The high genetic diversity of SL-CoVs within this colony was mirrored by high phenotypic diversity in the differential use of ACE2 by different strains. It would therefore not be surprising if further surveillance reveals a broad diversity of bat SL-CoVs that are able to use ACE2, some of which may have even closer homology to SARS-CoV than SL-CoV-WIV1. Our results—in addition to the recent demonstration of MERS-CoV in a Saudi Arabian bat, and of bat CoVs closely related to MERS-CoV in China, Africa, Europe and North America—suggest that bat coronaviruses remain a substantial global threat to the public health.

Finally, this study demonstrates the public health importance of pathogen discovery programs targeting wildlife that aim to identify the 'known unknowns'—previously unknown viral strains closely related to known pathogens. These programs, focused on specific high-risk wildlife groups and hotspots of disease emergence, may be a critical part of future global strategies to predict, prepare for, and prevent pandemic emergence.

This work was commented on, among others, by colleagues from the "Wuhan Institute of Virology" as follows [I.12]:

COMMENT on this article in:
Virol. sin 28(6), 315 (2013), doi: 10.1007/s12250-013-3402-x.

Bats as animal reservoirs for the SARS coronavirus: hypothesis proved after 10 years of virus hun

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Abstracts

Recently, the team led by Dr. Zhengli Shi from Wuhan Institute of Virology, Chinese Academy of Sciences, and Dr. Peter Daszak from Ecohealth Alliance identified SL-CoVs in Chinese horseshoe bats that were 95% identical to human SARS-CoV and were able to use human angiotensin-converting enzyme 2 (ACE2) receptor for docking and entry. Remarkably, they isolated the first known live bat SL-CoV that replicates in human and related cells. Their findings provide clear evidence that some SL-CoVs circulating in bats are capable of infecting and replicating in humans (Ge XY, et al., 2013). The severe acute respiratory syndrome (SARS) was the first pandemic of the new millennium. It started in November 2002 in Southern China and had spread over 33 countries, causing 8096 infections and 774 dead cases (fatality rate of 9.6%), along with huge economic losses. The etiological agent of SARS was identified as a novel coronavirus (SARS-CoV) (Drosten C, et al., 2003; Ksiazek TG, et al., 2003). However, the origin of SARS-CoV remains elusive. Although it is suggested that bats are the natural reservoirs for SARS-CoV, isolation of a SARS like virus (SL-CoV) from bats have been unsuccessful. To trace the origin of the sudden emerging SARS-CoV, molecular epidemiological studies have been conducted by different research groups. In 2003, Guan et al. isolated SARS-CoVs from Himalayan palm civets and two other species in a live-animal market in Guangdong, China (Guan Y, et al, 2003). The Chinese SARS molecular epidemiology consortium suggested that the early-phase human SARS-CoV strains may have originated from wild animals (The Chinese SARS Molecular Epidemiology Consortium, 2004). These and other evidences suggested that palm civets were the direct source since the isolates from civets were highly related to human isolates from 2002-3 and 2003-4 SARS pandemic (Guan Y, et al, 2013; Song HD, et al., 2005 ; Wang M, et al, 2005). Since 2004, SL-CoVs have been identified from bats by several research groups including Dr. Shi's lab (Li W, 2005; Lau SK, et al, 2005). These bat isolates are more genetically diverse and share an overall nucleotide identity of 88% to 92% to the SARSCoVs from humans or civets, resulting in the hypothesis that bats may be the natural hosts of SARS-CoV. However, there are still some missing links between previously characterized SL-CoVs from bats and SARS-CoV that precipitated the 2002-3 outbreaks. 1) Although the overall genome sequence similarity, there are significant differences in spike (S) protein between the previously known SL-CoVs and SARS-CoVs. The sequence identity of S1 fell to 64%, accompanying with insertions and (or) mutations in this region. S1 contains the receptor binding domain (RBD), which plays a key role in receptor recognition and is a major determinant of host range and cross-species infection of SARSCoV. It was suggested that the previously known bat SL-CoV stains cannot jump from bats to civets or humans owing to the significant differences between their RBDs (Li F, 2013); 2) although SL-CoVs have been identified from different bat species, isolation of a live SL-CoVs from bats never succeed; 3) no native SL-CoV from bats could use ACE2 as receptors and infected human cells, only when its RBD is replaced with the counterpart from a human SARS-CoV strain (Li W, et al, 2003; Becker MM, et al, 2008; Ren W, et al, 2008). Therefore, these SL-CoVs seem unlikely to be the immediate precursors of civet or human SARS-CoVs (Li F, 2013).

Two years later, another article by the research group led by **Zheng-Li Shi** and **Ralph Baric** in the journal "NATURE MEDICINE", which proves that **genetic engineering**

Changes in corona viruses from horseshoe bats lead to new, artificially generated "hybrid viruses" which can couple to human airway cells in a particularly efficient manner [I.8]. The researchers created a "chimeric" virus composed of the surface protein of a bat virus called SHC014 and the backbone of a SARS coronavirus. The chimeric virus infected human airway cells and provided evidence that the surface protein of SHC014 has the necessary structure to very efficiently bind to and infect a key human cell receptor. The main part of this publication is reproduced below:

***Nature Medicine* 21,** pages 1508–1513 (2015), Published: 09 November 2015

A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence

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Abstracts

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 populations. Using the SARS-CoV reverse genetics system, 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.

These experiments build on work published in 2008 and 2010 by the Wuhan research group led by Zheng-Li Shi in the "Journal of Virology" ([I.5], [I.6]), in which it was already possible to show **how to use genetic engineering can cause viruses to specifically infect human cells using an HIV-based pseudovirus.** The essential parts of these two publications are reproduced below:

JOURNAL OF VIROLOGY, Feb. 2008, p. 1899-1907 Vol. 82, No. 4, DOI: 10.1128/JVI.01085-07

Difference in Receptor Usage between Severe Acute Respiratory Syndrome (SARS) Coronavirus and SARS-Like Coronavirus of Bat Origin

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ABSTRACT

Severe acute respiratory syndrome (SARS) is caused by the SARS-associated coronavirus (SARS-CoV), which uses angiotensin-converting enzyme 2 (ACE2) as its receptor for cell entry. A group of SARS-like CoVs (SL-CoVs) has been identified in horseshoe bats. SL-CoVs and SARS-CoVs share identical genome organizations and high sequence identities, with the main exception of the N terminus of the spike protein (S), known to be responsible for receptor binding in CoVs. In this study, we investigated the receptor usage of the SL-CoV S by combining a human immunodeficiency virus-based pseudovirus system with cell lines expressing the ACE2 molecules of human, civet, or horseshoe bat. In addition to full-length S of SL-CoV and SARS-CoV, a series of S chimeras was constructed by inserting different sequences of the SARS-CoV S into the SL-CoV S backbone. Several important observations were made from this study. First, the SL-CoV S was unable to use any of the three ACE2 molecules as its receptor. Second, the SARS-CoV S failed to enter cells expressing the bat ACE2. Third, the chimeric S covering the previously defined receptor-binding domain gained its ability to enter cells via human ACE2, albeit with different efficiencies for different constructs. Fourth, a minimal insert region (amino acids 310 to 518) was found to be sufficient to convert the SL-CoV S from non-ACE2 binding to human ACE2 binding, indicating that the SL-CoV S is largely compatible with SARS-CoV S protein both in structure and in function.
The significance of these findings in relation to virus origin, virus recombination, and host switching is discussed.

The outbreaks of severe acute respiratory syndrome (SARS) in 2002-2003, which resulted in over 8,000 infections and close to 800 deaths, which was caused by a novel coronavirus (CoV), now known as the SARS-associated CoV (SARS-CoV) . The association of SARS-CoV with animals was first revealed by the isolation and identification of very closely related viruses in several Himalayan palm civets *(Paguma larvata)* and a raccoon dog *(Nyctereutes procyonoides)* at a

live animal market in Guangdong, China. A very high genome sequence identity (more than 99%) exists between the SARS-CoV-like virus from civets and SARS-CoV from humans, supporting the notion that SARS-CoV is of animal origin. However, subsequent studies showed that palm civets on farms and in the field were largely free from SARS-CoV infection. These results suggested that palm civets played a role as an intermediate host rather than as a natural reservoir. Subsequent surveillance studies among different bat populations revealed the presence in several horseshoe bat species (genus *Rhinolophus)* of a diverse group of CoVs, which are very similar to SARS-CoV in genome organization and sequence. These viruses are designated SARS-like CoVs (SL-CoVs) or SARS-CoV-like viruses. Such discoveries raised the possibility that bats are the natural reservoirs of SARS-CoV and triggered a surge in the search for CoVs in different bat species in different geographic locations. Phylogenetic analysis based on different protein sequences suggested that SL-CoVs found in bats and SARS-CoVs from humans and civets should be placed in a separate subgroup (group b) in CoV group 2 (G2b) to differentiate them from other group 2 CoVs in the genus *Corona virus.* G2b CoVs display major sequence differences in the N-terminal regions of their S proteins. The S proteins of CoVs play a key role in virus entry into host cells, including binding to host cell receptors and membrane fusion. Angiotensin-converting enzyme 2 (ACE2) has been identified as the functional receptor of SARS-CoV, and the molecular interaction between ACE2 and the SARS-CoV S protein has been well characterized. A 193-residue fragment (amino acids [aa] 318 to 510) in the SARS-CoV S protein was demonstrated to be the minimal receptor-binding domain (RBD) which alone was able to efficiently bind to ACE2. Furthermore, it was shown that minor changes in amino acid residues of the receptor-binding motif (RBM) of SARS-CoV S protein could abolish the entry of SARS-CoV into cells expressing human ACE2 (huACE2). In the corresponding RBD region of the SL-CoV S proteins, there is significant sequence divergence from those of the SARS-CoV S proteins, including two deletions of 5 and 12 or 13 aa. From crystal-structural analysis of the S-ACE2 complex, it was predicted that the S protein of SL-CoV is unlikely to use huACE2 as an entry receptor, although this has never been experimentally proven due to the lack of live SL-CoV isolates . Whether it is possible to construct an ACE2-binding SL-CoV S protein by replacing the RBD with that from SARS-CoV S proteins is also unknown.

In this study, a human immunodeficiency virus (HIV)-based pseudovirus system was employed to address these issues. Our results indicated that the SL-CoV S protein is unable to use ACE2 proteins of different species for cell entry and that SARS-CoV S protein also failed to bind the ACE2 molecule of the horseshoe bat, Rhinolophus pearsonii. However, when the RBD of SL CoV S was replaced with that from the SARS-CoV S, the hybrid S protein was able to use the huACE2 for cell entry, implying that the SL-CoV S proteins are structurally and functionally very similar to the SARS-CoV S. These results suggest that although the SL-CoVs discovered in bats are so far unlikely to infect humans using ACE2 as a receptor, it remains to be seen whether they are able to use other surface molecules of certain human cell types to gain entry. It is also conceivable that these viruses may become infectious to humans if they undergo N terminal sequence variation, for example, through recombination with other CoVs, which in turn might lead to a productive interaction with ACE2 or other surface proteins on human cells.

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Angiotensin-converting enzyme 2 (ACE2) proteins of different bat species confer variable susceptibility to SARS-CoV entry

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Abstracts

The discovery of SARS-like coronavirus in bats suggests that bats could be the natural reservoir of SARS-CoV. However, previous studies indicated the angiotensin-converting enzyme 2 (ACE2) protein, a known SARS-CoV receptor, from a horseshoe bat was unable to act as a functional receptor for SARS-CoV. Here, we extended our previous study to ACE2 molecules from seven additional bat species and tested their interactions with human SARS-CoV spike protein using both HIV-based pseudotype and live SARS-CoV infection assays. The results show that ACE2s of *Myotis daubentoni* and *Rhinolophus sinicus* support viral entry mediated by the SARS-CoV S protein, albeit with different efficiency in comparison to that of the human ACE2. Further, the alteration of several key residues either decreased or enhanced bat ACE2 receptor efficiency, as predicted from a structural modeling study of the different bat ACE2 molecules. These data suggest that *M. daubentoni* and *R. sinicus* are likely to be susceptible to SARS-CoV and may be candidates as the natural host of the SARS-CoV progenito Furthermore, our current study also demonstrates that the genetic diversity of ACE2 among bats is greater than that observed among known SARS-CoV susceptible mammals, highlighting the possibility that there are many more uncharacterized bat species that can act as a reservoir of SARS-CoV or its progenitor viruses. This calls for continuation and expansion of field surveillance studies among different bat populations to eventually identify the true natural reservoir of SARS-CoV.

Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV) is the aetiological agent responsible for the SARS outbreaks during 2002–2003, which had a huge global impact on public health, travel and the world economy [4, 11]. The host range of SARS-CoV is largely determined by the specific and high-affinity interactions between a defined receptor-binding domain (RBD) on the SARS-CoV spike protein and its host receptor, angiontensin-converting enzyme 2 (ACE2) [6 , 7, 9]. It has been hypothesized that SARS-CoV was harbored in its natural reservoir, bats, and was transmitted directly or indirectly from bats to palm civets and then to humans [10]. However, although the genetically related SARS-like coronavirus (SL-CoV) has been identified in horseshoe bats of the genus *Rhinolophus* [5, 8, 12, 18], its spike protein was not able to use the human ACE2 (hACE2) protein as a receptor [13]. Close examination of the crystal structure of human SARS-CoV RBD complexed with hACE2 suggests that truncations in the receptor-binding motif (RBM) region of SL-CoV spike protein abolish its hAC

binding ability [7, 10], and hence the SL-CoV found recently in horseshoe bats is unlikely to be the direct ancestor of human SARS-CoV. Also, it has been shown that the human SARS CoV spike protein and its closely related civet SARS-CoV spike protein were not able to use a horseshoe bat *(R. pearsoni)* ACE2 as a receptor [13], highlighting a critical missing link in the bat-to-civet/human transmission chain of SARS-CoV.

There are at least three plausible scenarios to explain the origin of SARS-CoV. First, some unknown intermediate hosts were responsible for the adaptation and transmission of SARS CoV from bats to civets or humans. This is the most popular theory of SARS-CoV transmission at the present time [10]. Second, there is an SL-CoV with a very close relationship to the outbreak of SARS-CoV strains in a non-bat animal host that is capable of direct transmission from reservoir host to human or civet. Third, ACE2 from yet to be identified bat species may function as an efficient receptor, and these bats could be the direct reservoir of human or civet SARS CoV. Unraveling which scenario is most likely to have occurred during the 2002–2003 SARS epidemic is critical for our understanding of the dynamics of the outbreak and will play a key role in helping us to prevent future outbreaks. To this end, we have extended our studies to include ACE2 molecules from different bat species and examined their interaction with the human SARS-CoV spike protein. Our results show that there is great genetic diversity among bat ACE2 molecules, especially at the key residues known to be important for interacting with the viral spike protein, and that ACE2s of *Myotis daubentoni* and *Rhinolophus sinicus* from Hubei province can support viral entry.

In the period that followed, a heated debate ignited **among scientists as to whether the knowledge gained from such experiments justified the potential risk of a pandemic.** A prominent virologist from the Institut Pasteur in Paris noted that the Wuhan Institute researchers created a novel virus that replicates remarkably well in human cells, adding: **"If the virus escaped, no one could predict its spread".** A molecular biologist added: **"The only significance of this study is the creation of a laboratory-based, new, non-natural hazard."** The debate at that time was taken up and commented on in numerous articles in specialist journals and in the media. Two examples of this are given below ([III.2], [III.5]):

Nature (2015), doi:10.1038/nature.2015.18787

***NATURE* | NEWS**

Engineered bat virus stirs debate over risky research

Lab-made coronavirus related to SARS can infect human cells.

Declan Butler

An experiment that created a hybrid version of a bat coronavirus — one related to the virus that causes SARS (severe acute respiratory syndrome) — has triggered renewed debate over whether engineering lab variants of viruses with possible pandemic potential is worth the risks.

In an article published in *Nature Medicine* on 9 November, scientists investigated a virus called SHC014, which is found in horseshoe bats in China. The researchers created a chimaeric virus, made up of a surface protein of SHC014 and the backbone of a SARS virus that had been adapted to grow in mice and to mimic human disease. The chimaera infected human airway cells — proving that the surface protein of SHC014 has the necessary structure to bind to a key receptor on the cells and to infect them. It also caused disease in mice, but did not kill them.

Although almost all coronaviruses isolated from bats have not been able to bind to the key human receptor, SHC014 is not the first that can do so. In 2013, researchers reported this ability for the first time in a different coronavirus isolated from the same bat population.

The findings reinforce suspicions that bat coronaviruses capable of directly infecting humans (rather than first needing to evolve in an intermediate animal host) may be more common than previously thought, the researchers say.

But other virologists question whether the information gleaned from the experiment justifies the potential risk. Although the extent of any risk is difficult to assess, Simon Wain-Hobson, a virologist at the Pasteur Institute in Paris, points out that the researchers have created a novel virus that “grows remarkably well” in human cells. "If the virus escaped, nobody could predict the trajectory," he says.

Creation of a chimaera

The argument is essentially a rerun of the debate over whether to allow lab research that increases the virulence, ease of spread or host range of dangerous pathogens — what is known as 'gain-of-function' research. In October 2014, the US government imposed a moratorium on federal funding of such research on the viruses that cause SARS, influenza and MERS (Middle East respiratory syndrome, a deadly disease caused by a virus that sporadically jumps from camels to people).

The latest study was already under way before the US moratorium began, and the US National Institutes of Health (NIH) allowed it to proceed while it was under review by the agency, says Ralph Baric, an infectious-disease researcher at the University of North Carolina at Chapel Hill, a co-author of the study. The NIH eventually concluded that the work was not so risky as to fall under the moratorium, he says.

But Wain-Hobson disapproves of the study because, he says, it provides little benefit, and reveals little about the risk that the wild SHC014 virus in bats poses to humans.

Other experiments in the study show that the virus in wild bats would need to evolve to pose any threat to humans — a change that may never happen, although it cannot be ruled out. Baric

and his team reconstructed the wild virus from its genome sequence and found that it grew poorly in human cell cultures and caused no significant disease in mice.

"The only impact of this work is the creation, in a lab, of a new, non-natural risk," agrees Richard Ebright, a molecular biologist and biodefence expert at Rutgers University in Piscataway, New Jersey. Both Ebright and Wain-Hobson are long-standing critics of gain-of function research.

In their paper, the study authors also concede that funders may think twice about allowing such experiments in the future. "Scientific review panels may deem similar studies building chimeric viruses based on circulating strains too risky to pursue," they write, adding that discussion is needed as to "whether these types of chimeric virus studies warrant further investigation versus the inherent risks involved".

But Baric and others say the research did have benefits. The study findings "move this virus from a candidate emerging pathogen to a clear and present danger", says Peter Daszak, who co-authored the 2013 paper. Daszak is president of the EcoHealth Alliance, an international network of scientists, headquartered in New York City, that samples viruses from animals and people in emerging-diseases hotspots across the globe.

Studies testing hybrid viruses in human cell culture and animal models are limited in what they can say about the threat posed by a wild virus, Daszak agrees. But he argues that they can help indicate which pathogens should be prioritized for further research attention.

Without the experiments, says Baric, the SHC014 virus would still be seen as not a threat. Previously, scientists had believed, on the basis of molecular modeling and other studies, that it should not be able to infect human cells. The latest work shows that the virus has already overcome critical barriers, such as being able to latch onto human receptors and efficiently infect human airway cells, he says. "I don't think you can ignore that." He plans to do further studies with the virus in non-human primates, which may yield data more relevant to humans.

The Scientist, November 16 (2015)

Lab-Made Coronavirus Triggers Debate

The creation of a chimeric SARS-like virus has scientists discussing the risks of gain-of-function research.

Jeff Akst

Ralph Baric, an infectious-disease researcher at the University of North Carolina at Chapel Hill, last week (November 9) published a study on his team's efforts to engineer a virus with the surface protein of the SHC014 coronavirus, found in horseshoe bats in China , and the backbone of one that causes human-like severe acute respiratory syndrome (SARS) in mice. The hybrid

virus could infect human airway cells and caused disease in mice, according to the team's results, which were published in *Nature Medicine.*

...

Despite this sometimes very heated debate and the warnings of a global pandemic by numerous scientific representatives, the group led by Zheng-Li Shi at the "Wuhan Institute of Virology" continued their high-risk research work on genetically modified corona viruses **in cooperation with Peter Daszak** the two following works from the years 2017 and 2018 prove ([I.9], [I.10]). The methods of genetic manipulation that have been established for years were used, as can be seen from the work [1.10]:

PLoS Pathog 13(11): e1006698. https://doi.org/10.1371/journal.ppat.1006698

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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

Ben Hu, Lei-Ping Zeng, Xing-Lou Yang, Xing-Yi Ge, Wei Zhang, Bei Li, Jia-Zheng Xie, Xu Rui Shen, Yun Zhi Zhang, Ning Wang, Dong Sheng Luo, Xiao Shuang Zheng, Mei Niang Wang, Peter Daszak, Lin-Fa Wang, Jie Cui and Zheng-Li Shi

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Abstracts

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.

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 various 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.

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Nature **volume 556,** pages 255–258 (2018)

Fatal swine acute diarrhea syndrome caused by an HKU2-related coronavirus of bat origin

Peng Zhou, Hang Fan, Tian Lan, Xing-Lou Yang, Wei-Feng Shi, Wei Zhang, Yan Zhu, Ya Wei Zhang, Qing-Mei Xie, Shailendra Mani, Xiao-Shuang Zheng, Bei Li, Jin-Man Li, Hua Guo, Guang-Qian Pei, Xiao-Ping An, Jun-Wei Chen, Ling Zhou, Kai-Jie Mai, Zi-Xian Wu, Di Li, Danielle E. Anderson, Li-Biao Zhang, Shi-Yue Li, Zhi -Qiang Mi, Tong-Tong He, Feng Cong, Peng-Ju Guo, Ren Huang, Yun Luo, Xiang-Ling Liu, Jing Chen, Yong Huang, Qiang Sun, Xiang-Li-Lan Zhang, Yuan-Yuan Wang, Shao -Zhen Xing, Yan-Shan Chen, Yuan Sun, Juan Li, Peter Daszak, Lin-Fa Wang, Zheng-Li Shi, Yi-Gang Tong & Jing-Yun Ma

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Abstracts

Cross-species transmission of viruses from wildlife animal reservoirs poses a marked threat to human and animal health. Bats have been recognized as one of the most important reservoirs for emerging viruses and the transmission of a coronavirus that originated in bats to humans via intermediate hosts was responsible for the high-impact emerging zoonosis, severe acute respiratory syndrome (SARS). Here we provide virological, epidemiological, evolutionary and experimental evidence that a novel HKU2-related bat coronavirus, swine acute diarrhea syndrome coronavirus (SADS-CoV), is the aetiological agent that was responsible for a large scale outbreak of fatal disease in pigs in China that has caused the death of 24,693 piglets across four farms. Notably, the outbreak began in Guangdong province in the vicinity of the origin of the SARS pandemic. Furthermore, we identified SADS-related CoVs with 96–98% sequence identity in 9.8% (58 out of 591) of anal swabs collected from bats in Guangdong province during 2013–2016, predominantly in horseshoe bats *(Rhinolophus* spp.) that are known reservoirs of SARS-related CoVs. We found that there were striking similarities between the SADS and SARS outbreaks in geographical, temporal, ecological and aetiological settings. This study highlights the importance of identifying coronavirus diversity and distribution in bats to mitigate future outbreaks that could threaten livestock, public health and economic growth.

methods

sample collection

Bats were captured and sampled in their natural habitat in Guangdong province as described previously. Faecal swab samples were collected in viral transport medium (VTM) composed of Hank's balanced salt solution at pH 7.4 containing BSA (1%), amphotericin (15 ÿg mlÿ1), penicillin G (100 units mlÿ1) and streptomycin (50 ÿg mlÿ1). Stool samples from sick pigs were collected in VTM. When appropriate and feasible, intestinal samples were also taken from deceased animals. Samples were aliquoted and stored at -80 °C until use. Blood samples were collected from recovered sows and workers on the farms who had close contact with sick pigs.
Serum was separated by centrifugation at 3,000g for 15 min within 24 h of collection and preserved at 4 °C. Human serum collection was approved by the Medical Ethics Committee of the Wuhan School of Public Health, Wuhan University and Hummingbird IRB. Human, pigs and bats were sampled without gender or age preference unless indicated (for example, piglets or sows). No statistical methods were used to predict sample size.

...

Amplification, cloning and expression of human and swine genes

Construction of expression clones for human *ACE2* in pcDNA3.1 has been described previously (Ge, XY et al.: Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503, 535–538 (2013) and Ren , W. et al.: Difference in receptor usage between severe acute respiratory syndrome (SARS) coronavirus and SARS-like coronavirus of bat origin J Virol 82, 1899-1907 (2008)). Human *DPP4* was amplified from human cell lines. Human *APN* (also known as *ANPEP)* was commercially synthesized. Swine *APN* (also known as *ANPEP), DPP4* and *ACE2* were amplified from piglet intestine. Full-length gene fragments were amplified using specific primers (provided upon request). Human *ACE2* was cloned into pCDNA3.1 fused with a His tag. Human *APN* and *DPP4,* swine *APN, DPP4* and *ACE2* were cloned into pCAGGS fused with an S tag. Purified plasmids were transfected into HeLa cells. After 24 h, expression of human or swine genes in HeLa cells was confirmed by immunofluorescence assay using mouse anti-His tag or mouse anti-S tag monoclonal antibodies (produced in house) followed by Cy3-labelled goat anti-mouse/rabbit IgG (Proteintech groups).

pseudovirus preparation

The codon-humanized *S* genes of SADS-CoV or MERS-CoV cloned into pcDNA3.1 were used for pseudovirus construction as described previously (Ge, XY et al.: Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor Nature 503, 535–538 (2013) and Ren W et al.: Difference in receptor usage between severe acute respiratory syndrome (SARS) coronavirus and SARS-like coronavirus of bat origin J Virol 82, 1899–1907 (2008)). In brief, 15 ÿg of each pHIV-Luc plasmid (pNL4.3.Luc.RE-Luc) and the S protein-expressing plasmid (or empty vector control) were co-transfected into 4 × 106 HEK293T cells using Lipofectamine 3000 (Thermo Fisher Scientific). After 4 h, the medium was replaced with fresh medium. Supernatants were collected 48 h after transfection and clarified by centrifugation at 3,000g, then passed through a 0.45-ÿm filter (Millipore). The filtered supernatants were stored at ÿ80 °C in aliquots until use. To evaluate the incorporation of S proteins into the core of HIV virions, pseudoviruses in supernatant (20 ml) were concentrated by ultracentrifugation through a 20% sucrose cushion (5 ml) at 80,000 g for 90 min using a SW41 rotor (Beckman). Pelleted pseudoviruses were dissolved in 50 ÿl phosphate buffered saline (PBS) and examined by electron microscopy.

pseudovirus infection

HeLa cells transiently expressing APN, ACE2 or DPP4 were prepared using Lipofectamine 2000 (Thermo Fisher Scientific). Pseudoviruses prepared above were added to HeLa cells overexpressing APN, ACE2 or DPP4 24 h after transfection. The unabsorbed viruses were removed and replaced with fresh medium at 3 h after infection. The infection was monitored by measuring the luciferase activity conferred by the reporter gene carried by the pseudovirus, using the Luciferase Assay System (Promega) as follows: cells were lysed 48 h after infection, and 20 ÿl of the lysates was taken for determining luciferase activity after the addition of 50 ÿl of luciferase substrate.

reviewer information

Nature thanks C Drosten, G Palacios and L Saif for their contribution to the peer review of this work.

In fact, it was not only the research activities of the group led by Zheng-Li Shi at the "Wuhan Institute of Virology" on corona viruses, but also research activities of other groups on other types of viruses, which pursued the goal of making **naturally occurring viruses more contagious, dangerous and harmful to humans through genetic manipulation to make it deadlier.** This **"gain-of-function" research** and the associated heated debate between various representatives of science will be presented in more detail in the following chapter.

4 "Gain-of-function research": International debate about this Risk of research into the manipulation of viruses with regard to higher transmission capacity, dangerousness and mortality rates

The debate about the possible benefits, but also the dangers associated with research into manipulating viruses to make them more contagious, dangerous and ultimately deadly for humans, started in 2011. This debate was primarily triggered by two scientific papers international research groups, which showed how genetic modifications can make H5N1 viruses (bird flu pathogens) more contagious for humans [I.13, I.14]. These two works by the research groups led by Yoshihiro Kawaoka and Ron Fouchier, which were published in the journals "NATURE" and "SCIENCE" in 2012, are reproduced here in excerpts:

***Nature* 486,** 420-428 (2012)

Published: 02 May 2012

Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

Masaki Imai, Tokiko Watanabe, Masato Hatta, Subash C. Das, Makoto Ozawa, Kyoko Shinya, Gongxun Zhong, Anthony Hanson, Hiroaki Katsura, Shinji Watanabe, Chengjun Li, Eiryo Kawakami, Shinya Yamada, Maki Kiso, Yasuo Suzuki, Eileen A. Maher, Gabriele Neumann and Yoshihiro Kawaoka

Abstracts

Highly pathogenic avian H5N1 influenza A viruses occasionally infect humans, but currently do not transmit efficiently among humans. The viral haemagglutinin (HA) protein is a known host-range determinant as it mediates virus binding to host-specific cellular receptors. Here we assess the molecular changes in HA that would allow a virus possessing subtype H5 HA to be transmissible among mammals. We identified a reassortant H5 HA/H1N1 virus—comprising H5 HA (from an H5N1 virus) with four mutations and the remaining seven gene segments from a 2009 pandemic H1N1 virus—that was capable of droplet transmission in a ferret model. The transmissible H5 reassortant virus preferentially recognized human-type receptors, replicated efficiently in ferrets, caused lung lesions and weight loss, but was not highly pathogenic and did not cause mortality. These results indicate that H5 HA can convert to an HA that supports efficient viral transmission in mammals; however, we do not know whether the four mutations in the H5 HA identified here would render a wholly avian H5N1 virus transmissible. The genetic origin of the remaining seven viral gene segments may also critically contribute to transmissibility in mammals. Nevertheless, as H5N1 viruses continue to evolve and infect humans, receptor-binding variants of H5N1 viruses with pandemic potential, including a

human reassortant viruses as tested here, may emerge. Our findings emphasize the need to prepare for potential pandemics caused by influenza viruses possessing H5 HA, and will help individuals conducting surveillance in regions with circulating H5N1 viruses to recognize key residues that predict the pandemic potential of isolates, which will inform the development, production and distribution of effective countermeasures.

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Science 336, Issue 6088, pp. 1534-1541, 22 Jun 2012:
DOI: 10.1126/science.1213362

SCIENCE REPORT

Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

Sander Herfst, Eefje JA Schrauwen, Martin Linster, Salin Chutinimitkul, Emmie de Wit, Erin M Sorrell, Vincent J Munster, Theo M Bestebroer, David F Burke, Derek J Smith, Guus F. Rimmelzwaan, Albert DME Osterhaus, Ron AM Fouchier

Abstracts

Highly pathogenic avian influenza A/H5N1 virus can cause morbidity and mortality in humans but thus far has not acquired the ability to be transmitted by aerosol or respiratory droplet (“airborne transmission”) between humans. To address the concern that the virus could acquire this ability under natural conditions, we genetically modified A/H5N1 virus by site-directed mutagenesis and subsequent serial passage in ferrets. The genetically modified A/H5N1 virus acquired mutations during passage in ferrets, ultimately becoming airborne transmissible in ferrets. None of the recipient ferrets died after airborne infection with the mutant A/H5N1 viruses. Four amino acid substitutions in the host receptor-binding protein hemagglutinin, and one in the polymerase complex protein basic polymerase 2, were consistently present in airborne-transmitted viruses. The transmissible viruses were sensitive to the antiviral drug oseltamivir and reacted well with antisera raised against H5 influenza vaccine strains. Thus, avian A/H5N1 influenza viruses can acquire the capacity for airborne transmission between mammals without recombination in an intermediate host and therefore constitute a risk for human pandemic influenza.

...

Even before these two publications were officially published, there was a very intensive discussion and **extremely controversial debate among scientists and politicians as to** whether such research results should be made public at all and whether "gain-of-function" research activities should be completely prohibited in the future. At that time there were already fears associated with the **nightmare of a possible pandemic caused by the accidental escape of artificially produced viruses from genetic engineering laboratories, with an unforeseeable risk potential for mankind.**

A few examples from scientific journals [III.6-III.9], which provide a good insight into the discussion at the time, are given below:

Nature 480, 421-422 (22 December 2011) doi:10.1038/480421a

***NATURE*| NEWS**

Fears grow over lab-bred flu

Scientists call for stricter biosafety measures for dangerous avian influenza variants.

Declan Butler

It is a nightmare scenario: a human pandemic caused by the accidental release of a man-made form of the lethal avian influenza virus H5N1.

Yet the risk is all too real. Since September, news has been circulating about two groups of scientists who have reportedly created mutant H5N1 variants that can be transmitted between ferrets merely breathing the same air, generally an indicator that the virus could also spread easily among humans.

The work raises the specter of a disease that spreads as nearly as ordinary seasonal flu, but with a fatality rate akin to wild-type H5N1 — an order of magnitude greater than the mortality rate of roughly 2.5% seen during the catastrophic flu pandemic of 1918 .

Until now, debate about the new variants has focused on whether the research poses too great a security risk to be published — even if partially redacted — a question currently under consideration by the US National Science Advisory Board for Biosecurity (NSABB).

A number of scientists argue, however, that the NSABB's deliberations have come far too late. Because further research on the new variants now seems inevitable, a far more important question, they say, is whether the labs that hold samples of the virus — and those who will seek to work with them in the future — have sufficient biosafety protection to make sure it cannot escape.

“This horse is out of the barn,” says Richard Ebright, a molecular biologist and biodefence expert at Rutgers University in Piscataway, New Jersey. "At this point, it is utterly futile to be discussing restricting the publication of this information," he adds, pointing out that the results have already been seen by many flu scientists, including referees, and are probably spreading through the flu grapevine faster than a speeding neutrino.

Sources say that one of the studies, led by Ron Fouchier of Erasmus Medical Center in Rotterdam, the Netherlands, has been submitted to *Science,* and that the other, led by Yoshihiro Kawaoka of the University of Wisconsin, Madison, has been sent to *Nature . (Nature's* journalists do not have access to submitted manuscripts or the journal's confidential deliberations on them.) Fouchier also presented his results in September at the annual European Scientific Working Group on Influenza conference in Malta.

The mutant strains were not born out of a reckless desire to push the boundaries of high-risk science, but to gain a better understanding of the potential for avian H5N1 to mutate into a form that can spread easily in humans through coughing or sneezing. Some virologists have suggested that any genetic changes that made it more transmissible would probably blunt its deadliness. The new work seems to contradict that comforting idea. The studies should also

help boost surveillance for similar changes in wild-type strains, and to develop diagnostics, drugs and vaccines.

Both experiments were conducted in labs rated at 'biosafety level 3 (BSL-3) enhanced' (see 'Safety by degrees'). Such labs require scientists to shower and change clothes when leaving the lab, and include other safety features such as negative air pressure and passing exhaust air through high-efficiency particulate air filters. This should be quite sufficient to provide protection against an accidental release of the virus, some virologists say.

"Current biosafety rules are adequate for safely doing such transmission experiments with H5N1 viruses or any other influenza virus," says Peter Palese, a virologist at Mount Sinai School of Medicine in New York.

Requiring the more stringent protocols of BSL-4 facilities would hamper the research needed to develop countermeasures against an H5N1 pandemic, says Masato Tashiro, a virologist at the National Institute of Infectious Diseases in Tokyo, because it would limit the number of researchers able to work with the virus. As such, he believes that the work should be done in BSL-3 enhanced facilities.

High security

But others say that to protect not only the researchers working on the viruses, but also society at large, the new H5N1 variants must be restricted to BSL-4 labs. These labs have far tougher safety and security measures, such as requiring workers to wear positive air pressure suits and undergo more rigorous decontamination; some also have additional security measures, such as video surveillance and bomb-proofing. Corralling this research in BSL-4 facilities would also immediately limit the proliferation of the viruses in labs, because only a few dozen such facilities exist worldwide, says Ebright. Indeed, one regulatory official, who requested anonymity, says that he is most concerned about the H5N1 mutants being handled in BSL-3 labs in countries with weak biosafety cultures or competences.

Deborah Middleton, an H5N1 researcher at the high-containment facilities at the Australian Animal Health Laboratory in Geelong, says that the characteristics of the new variants "fulfil the criteria of a BSL-4 pathogen", adding that she believes they would probably be handled as such in her institution. Indeed, the original experiments to create the viruses should also have been conducted in a BSL-4 facility, argues Hervé Raoul, director of the Jean Meriéux-INSERM BSL-4 lab in Lyons, France.

Past experience suggests that the risk of the new variant H5N1 escaping from a lab is far from negligible. Over the past decade, severe acute respiratory syndrome (SARS) has accidentally infected staff at four high-containment labs in mainland China, Taiwan and Singapore, variously rated as BSL-3 and BSL-4. A US National Research Council report released in September detailed 395 biosafety breaches during work with select agents in the United States between 2003 and 2009 — including seven laboratory-acquired infections — that risked accidental release of dangerous pathogens from high-containment labs.

And the rapid spread of an escaped flu virus would make it more dangerous than other deadly pathogens. “When SARS or BSL-4 agents get out, their potential for transmission on a global basis is quite limited,” says Michael Osterholm, who heads the University of Minnesota's Center for Infectious Disease Research and Policy in Minneapolis, and is a member of the NSABB. "Influenza presents a very difficult challenge because if it ever were to escape, it is one that would quickly go round the world."

Fouchier declined to comment on these biosafety issues, saying only that his experiments had been reviewed by authorities in the Netherlands and the United States where “H5N1 virus is a

class-3 agent because antivirals and vaccines are available". Kawaoka did not respond to interview requests.

Some scientists say that they are looking to the World Health Organization (WHO) to provide timely leadership in this biosafety debate. But Gregory Hartl, a spokesman for the WHO in Geneva, Switzerland, says the agency is unable to comment because it has not yet seen the written studies. Meanwhile, the NSABB has not said when it will publish its advice. In a statement to *Nature,* the US Department of Agriculture said that it (and the US Department of Health and Human Services) will conduct any appropriate technical review of the new H5N1 variants.

Ebright laments that important questions of biosafety and biosecurity are largely left to the discretion of individual researchers. "In the United States, there is only voluntary oversight for biosafety, and with the exception of the select agents rule, there is no oversight of biosecurity," he says. Given the choice, says Middleton, flu researchers often resist working in higher biocontainment levels simply because they would no longer have the convenience of doing their research in BSL-3 labs at their own institutes, and because working in a BSL-4 lab is inherently more difficult.

The situation contrasts sharply with the barrage of legislation to regulate research that involves placing human subjects at risk, notes Ebright, where proposed projects are rigorously reviewed before they can start. "What's remarkable," says Ebright, is that for dual-use research of this type on H5N1, "which puts at risk not one individual but potentially hundreds, thousands or millions of individuals, there is no oversight whatsoever".

On 20 December, the US National Science Advisory Board for Biosecurity (NSABB) released a statement outlining its recommendations to the authors of the two flu studies under review, and to the editors of the journals that are considering publishing them. The statement says:

"Due to the importance of the findings to the public health and research communities, the NSABB recommended that the general conclusions highlighting the novel outcome be published, but that the manuscripts not include the methodological and other details that could enable replication of the experiments by those who would seek to do harm. The NSABB also recommended that language be added to the manuscripts to better explain the goals and potential public health benefits of the research, and to detail the extensive safety and security laboratory workers and the public."
measures taken to protect

In response, Science's Editor-in-Chief Bruce Alberts said:

"Science editors will be evaluating how best to proceed. Our response will be heavily dependent upon the further steps taken by the US government to set forth a written, transparent plan to ensure that any information that is omitted from the publication will be provided to all those responsible scientists who request it, as part of their legitimate efforts to improve public health and safety."

In response, Nature's Editor-in-Chief Philip Campbell said:

"We have noted the unprecedented NSABB recommendations that would restrict public access to data and methods and recognize the motivation behind them. It is essential for public health that the full details of any scientific analysis of flu viruses be available to researchers. We are discussing with interested parties how, within the scenario recommended by NSABB, appropriate access to the scientific methods and data could be enabled."

Nature 481, 417-418 (26 January 2012), doi:10.1038/481417a
***NATURE*| NEWS**

Caution urged for mutant flu work

Public-health benefits of controversial research questioned.

Declan Butler

Why would scientists deliberately create a form of the H5N1 avian influenza virus that is probably highly transmissible in humans? In the growing debate about research that has done precisely that, a key question is whether the public-health benefits of the work outweigh the risks of a potential pandemic if the virus escaped from the lab.

For the scientists who have created the mutated strains of the H5N1 virus, the justifications are clear. Surveillance of flu viruses could, they argue, allow health organizations to monitor birds and other animals for the mutations that would provide an early warning of a pandemic and enable authorities to act quickly to contain the virus.

That claim is meeting with skepticism, however. More than a dozen flu experts contacted by *Nature* say they believe that the work opens up important vistas in basic research, and that it sends a valuable warning about the potential for the virus to spark a human pandemic. But they caution that virus surveillance systems are ill-equipped to detect such mutations arising in flu viruses. As such, work on the viruses is unlikely to offer significant, immediate public health benefits, they say.

That tips the balance of risk–benefit assessment in favor of a cautious approach, says Michael Osterholm, who heads the University of Minnesota's Center for Infectious Disease Research and Policy in Minneapolis, and who is a member of the US National Science Advisory Board for Biosecurity (NSABB).

In a paper submitted to *Science,* Ron Fouchier's team at Erasmus Medical Center in Rotterdam, the Netherlands, found that just five mutations allowed avian H5N1 to spread easily among ferrets, which are a good proxy for how flu behaves in other mammals, including humans. All five mutations have been spotted individually — although not together — in wild viruses. Yoshihiro Kawaoka of the University of Wisconsin-Madison and his colleagues have submitted similar work to *Nature,* which is partially described in an online Comment published this week.

Acting on advice from the NSABB, the US government last month asked *Science* and *Nature* to publish only the broad conclusions of the two studies, and not to reveal the scientific details, in order to limit the risk that uncontrolled proliferation of such research might lead to accidental or intentional release of similar mutant viruses. The journals and the authors have agreed to this redaction, provided that a mechanism is established to disseminate the data to flu researchers and public-health officials on a need-to-know basis. The US government, the World Health Organization (WHO) and other bodies are now trying to put this mechanism together, along with a framework for international oversight of such research.

Last week, in a statement jointly published in *Nature* and *Science,* 39 flu researchers declared a 60-day pause in the creation of lab mutant strains of the H5N1 avian flu virus. the hiatus,

they hope, should give scientists and policy-makers time to debate how such research might best proceed, and what safety measures should be required of labs that handle the virus. The signatories to the statement, including the key authors behind the controversial research, plan to bring together some 50 experts at a WHO-hosted meeting in Geneva, Switzerland, next month to discuss these thorny issues.

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Nature 485, 431-434 (24 May 2012), doi:10.1038/485431a
***NATURE*| NEW FEATURE**

Bird-flu research: The biosecurity oversight

The fight over mutant flu has thrown the spotlight on a little-known government body that oversees dual-use research. Some are asking if it was up to the task.

Brendan Maher

The packages that started arriving by FedEx on 12 October last year came with strict instructions: protect the information within and destroy it after review. Inside were two manuscripts showing how the deadly H5N1 avian influenza virus could be made to transmit between mammals. The recipients of these packages — eight members of the US National Science Advisory Board for Biosecurity (NSABB) — faced the unenviable task of deciding whether the research was safe to publish.

...

Nature 493, 460 (24 January 2013) doi:10.1038/493460a
***NATURE*| NEWS**

Work resumes on lethal flu strains

Study of lab-made viruses a 'public-health responsibility'.

Declan Butler

An international group of scientists this week ended a year-long moratorium on controversial work to engineer potentially deadly strains of the H5N1 avian flu virus in the lab.

Researchers agreed to temporarily halt the work in January 2012, after a fierce row erupted over whether it was safe to publish two papers reporting that the introduction of a handful of mutations enabled the H5N1 virus to spread efficiently between ferrets, a model of flu in mammals . Both papers were eventually published, one in *Nature* and one in *Science.*

Now, in a letter simultaneously published on 23 January by *Nature* and *Science,* the 40 scientists involved say that the moratorium has served its purpose: allowing time for authorities to review the conditions under which the research could be safely conducted and for scientists to explain the public-health benefits of the work. Scientists who now have official approval in their countries to conduct such research “have a public-health responsibility to

resume this important work", the letter states, "because the risk exists in nature that an H5N1 virus capable of transmission in mammals may emerge".

The move follows a large international workshop convened on December 17-18 by the US National Institutes of Health in Bethesda, Maryland, to discuss 'gain-of-function research' — that intended to increase the transmissibility, host range or virulence — in H5N1 viruses, and the development of US rules for stricter oversight of research in this area. The proposed rules require an assessment of, for example, whether the scientific aims of such studies could be addressed using alternative, less-risky approaches, and whether biosafety and biosecurity risks can be adequately mitigated. They are expected to enter into force soon, allowing scientists working in the United States or on US-funded grants to restart such research.

The groups that published the original research have outlined a suite of possible follow-up experiments, including a search for other combinations of mutations that would allow H5N1 to transmit between mammals — which could answer basic-science questions and, they argue, aid efforts to watch for dangerous mutations in the wild. The researchers also suggest extending the studies in ferrets to other mammals, such as guinea pigs, because further evidence of transmission within mammalian species would increase confidence that the mutated virus would transmit between humans.

But the scientific community remains divided on whether the practical benefits of the research outweigh the risks of an accidental or deliberate release of a lab-created flu strain. Ian Lipkin, a specialist on emerging infectious diseases at Columbia University in New York, believes that the risks are high and worse, that such research may end up being done in labs with insufficient biosafety standards.

The World Health Organization (WHO) posted general biosafety guidelines for such work on its website last July, but Lipkin says such guidelines need to be extended and given more teeth before work restarts. He suggests that this could be done by including them in the WHO's international legally binding treaty on global threats to health — the 2005 International Health Regulations. Ron Fouchier at Erasmus Medical Center in Rotterdam, the Netherlands, who led the research behind last year's *Science* paper, disagrees. He says that national and institutional procedures have long proved adequate. "If we have to wait until all national governments in the world agree on terms and conditions, we can wait for years if not forever," he says. "That is unacceptable."

But even some who support the lifting of the moratorium have misgivings about the future. Ilaria Capua, a flu researcher at the Veterinary Public Health Institute in Legnaro, Italy, who signed the letter, says that she is less concerned about current work, which is limited to a handful of labs with high biosafety standards than about the risk of proliferation of such research in the longer term. "This is not a decision for scientists," she says, "it's a decision for policy-makers; do we want to continue to invest public funds in this type of work?"

In 2012 there were numerous international workshops dealing with the risks of gain-of-function research. A **moratorium on this type of research** initially existed for one year (from January 2012 to January 2013). In October 2014, the American government under Barack Obama imposed a **ban on "gain-of-function" research in the USA** due to security concerns [III.10]:

***NATURE*| NEWS**
22 October 2014

US suspends risky disease research

Government to cease funding gain-of-function studies that make viruses more dangerous, pending a safety assessment.

Sarah Reardon

The US government surprised many researchers on 17 October when it announced that it will temporarily stop funding new research that makes certain viruses more deadly or transmissible. The White House Office of Science and Technology Policy is also asking researchers who conduct such 'gain-of-function' experiments on influenza, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) to stop their work until a risk assessment is completed — leaving many uncertain of how to proceed.

"I think it's really excellent news," says Marc Lipsitch, an epidemiologist at the Harvard School of Public Health in Boston, Massachusetts, who has long called for more oversight for gain-of function research. "I think it's common sense to deliberate before you act."

Critics of such work argue that it is unnecessarily dangerous and risks accidentally releasing viruses with pandemic potential — such as an engineered H5N1 influenza virus that easily spreads between ferrets breathing the same air. In 2012, such concerns prompted a global group of flu researchers to hold gain-of-function experiments for a year (see *Nature* http://doi.org/wgx; 2012). The debate reignited in July, after a series of lab accidents involving mishandled pathogens at the US Centers for Disease Control and Prevention in Atlanta, Georgia.

The White House's abrupt move seems to be a response to renewed lobbying by gain-of function critics who wanted such work suspended and others who sought to evaluate its risks and benefits without disrupting existing research.

Arturo Casadevall, a microbiologist at the Albert Einstein College of Medicine in New York City, calls the plan “a knee-jerk reaction”. “There is really no evidence that these experiments are in fact such high risk,” he says. "A lot of them are being done by very respectable labs, with lots of precautions in place."

Some researchers are confused by the moratorium's wording. Viruses are always mutating, and Casadevall says that it is difficult to determine how much mutation deliberately created by scientists might be “reasonably anticipated” to make a virus more dangerous — the point at

which the White House states research must stop. The government says that this point will be determined for individual grants in discussions between funding officers and researchers.

One of the most prominent laboratories conducting gain-of-function studies is run by Yoshihiro Kawaoka, a flu researcher at the University of Wisconsin-Madison. In 2012, Kawaoka published a controversial paper reporting airborne transmission of engineered H5N1 flu between ferrets. He has since created an H1N1 flu virus using genes similar to those from the 1918 pandemic strain, to show how such a dangerous flu could emerge. The engineered H1N1 was transmissible in mammals and much more harmful than the natural strain.

Kawaoka says that he plans to comply with the White House directive to halt current research once he understands which of his projects it affects. “I hope that the issues can be discussed openly and constructively so that important research will not be delayed indefinitely,” he says.

But it seems that the freeze could be lengthy. The White House says that it will wait for recommendations from the US National Science Advisory Board for Biosecurity (NSABB) and the National Research Council before deciding whether and how to lift the ban. The groups are expected to finish their work within a year. As *Nature* went to press, the NSABB was set to convene on 22 October, its first meeting in two years. Lipsitch, who will speak at the event, says that he will advocate for the development of an objective risk-assessment tool to evaluate individual research projects. In particular, he says, decision-makers should consider whether a gain-of-function study makes a contribution to a public-health goal, such as the prevention and treatment of flu, that could justify both the risk and the use of money that could be spent on safer research.

"There clearly are going to be instances where gain-of-function research is necessary and appropriate, and there are others where the opposite applies," says Ian Lipkin, a virologist at Columbia University in New York City. The need to understand the ongoing Ebola outbreak in West Africa and control its spread, for instance, emphasizes the importance of infectious disease research — as well as the regulation of such work, Lipkin says. Although public worry about Ebola being transferred through the air is unfounded, researchers could make a case for the need to determine how the virus could evolve in nature by engineering a more dangerous version in the lab. “I think we should have some sort of guidelines in place before such experiments are even proposed,” says Lipkin. Yet Ebola is not included in the White House's research-funding ban, and a spokesperson says that there are no plans to include it on the list.

Shortly before this ban, the NIAID (National Institute of Allergy and Infectious Disease) under the director Dr. Anthony Fauci jointly with the NIH (National Institutes of Health) a 5-year, $3.7 million project entitled "Understanding the Risk of Bat Coronavirus Emergence" to Peter Daszak (Ecohealth Alliance, Inc.).

The information on this from the website of the third-party funder is listed below:

Project Information
2R01AI110964-06

Project Number: 2R01AI110964-06 **Contact PI / Project Leader:** DASZAK, PETER **Title:** UNDERSTANDING THE RISK OF BAT
Awardee Organization: ECOHEALTH ALLIANCE, CORONAVIRUS EMERGENCE INC.

Total project funding amount for **6** projects is **$3,748,715***

** Only NIH, CDC, and FDA funding data.*

Page 1 of 1

Project Number	sub #	ProjectTitle	Contact PI / Project leaders	Organization FY	admin IC	funding IC	FY total cost by IC
2R01AI110964-06		UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2019 NIAID	NIAID	$661,980
5R01AI110964-05		UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2018 NIAID	NIAID	$581,646
5R01AI110964-04		UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2017 NIAID	NIAID	$597,112
5R01AI110964-03		UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2016 NIAID	NIAID	$611,090
5R01AI110964-02		UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2015 NIAID	NIAID	$630,445
1R01AI110964-01		UNDERSTANDING THE RISK OF BAT CORONAVIRUS EMERGENCE	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.	2014 NIAID	NIAID	$666,442

Project Information

2R01AI110964-06

Project Number: 2R01AI110964-06 **Contact PI / Project Leader:** DASZAK, PETER **Title:** UNDERSTANDING THE RISK OF BAT **Awardee Organization:** ECOHEALTH CORONAVIRUS EMERGENCE ALLIANCE, INC.

AbstractText:

Project Summary: Understanding the Risk of Bat Coronavirus Emergence Novel zoonotic, bat-origin CoVs are a significant threat to global health and food security, as the cause of SARS in China in 2002, the ongoing outbreak of MERS, and of a newly emerged Swine Acute Diarrhea Syndrome in China. In a previous R01 we found that bats in southern China harbor an extraordinary diversity of SARSr-CoVs, some of which can use human ACE2 to enter cells, infect humanized mouse models causing SARS-like illness, and evade available therapies or vaccines. We found that people living close to bat habitats are the primary risk groups for spillover, that at one site various SARSr-CoVs exist that contain every genetic element of the SARS-CoV genome, and identified serological evidence of human exposure among people living nearby. These findings have led to 18 published peer-reviewed papers, including two papers in Nature, and a review in Cell. Yet salient questions remain on the origin, diversity, capacity to cause illness, and risk of spillover from these viruses. In this R01 renewal we will address these issues through 3 specific aims: Aim 1. Characterize the diversity and distribution of high spillover-risk SARSr-CoVs in bats in southern China. We will use phylogeographic and viral discovery curve analyzes to target additional bat sample collection and molecular CoV screening to fill in gaps in our previous sampling and fully characterize natural SARSr-CoV diversity in southern China. 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). Aim 2. Community, and clinic-based syndromic, surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences. We will conduct biological-behavioral surveillance in high-risk populations, with known bat contact, in community and clinical settings to 1) identify risk factors for serological and PCR evidence of bat SARSr-CoVs; & 2) assess possible health effects of SARSr-CoVs infection in people. We will analyze bat CoV serology against human-wildlife contact and exposure data to quantify risk factors and health impacts of SARSr-CoV spillover. Aim 3. In vitro and in vivo characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyzes to identify the regions and viruses of public health concern. 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. We will combine these data with bat host distribution, viral diversity and phylogeny, human survey of risk behaviors and illness, and serology to identify SARSr-CoV spillover risk hotspots across southern China. Together these data and analyzes will be critical for the future development of public health interventions and enhanced surveillance to prevent the re-emergence of SARS or the emerg

Public health relevance statement:

Program Director/Principal Investigator: Daszak, Peter Renewal: Understanding the Risk of Bat Coronavirus Emergence Project Narrative Most emerging human viruses come from wildlife, and these represent a significant threat to public health and biosecurity in the US and globally, as was demonstrated by the SARS coronavirus pandemic of 2002-03. This project seeks to understand what factors allow coronaviruses, including close relatives to

SARS, to evolve and jump into the human population by studying viral diversity in their animal reservoirs (bats), surveying people that live in high-risk communities in China for evidence of bat-coronavirus infection, and conducting laboratory experiments to analyze and predict which newly discovered viruses pose the greatest threat to human health.

NIH Donation Category:

biodefense; biotechnology; clinical research; emerging infectious diseases; Infectious diseases; lungs; pneumonia; pneumonia & influenza; prevention; Rare Diseases

Project Terms:

acute; acute diarrhea; Address; Amino Acid Sequence; animals; base; behavior; behavioral; Biological; biosecurity; cells; China; chiroptera; clinic; clinic visits; clinical; communities; community clinic; coronavirus; coronavirus infections; coupled; Data; data analysis; development; disease outbreaks; epidemiological data; epithelial cells; experimental study; exposed human population; exposure route; exposure to; Family suidae; follow-up; food safety; Future; genetic elements; genomes; geographical Distribution; geography; global health; habitats; Health; high-risk; high-risk population; human; human population study; humanized mouse; in vitro; in vivo; individual; infection; influenza; investigation; laboratory experiment; leads; maps; Middle East respiratory syndrome coronavirus; modelling; molecular; monoclonal antibodies; mouse model; Nature; novel; pandemic disease; Paper; patients; Phylogenetic Analysis; phylogeny; prevalence; prevent; principal investigator; programs; proteins; public health; public health intervention; publishing peer reviews; questionnaires; readiness; reagent; receptor binding; recombinant virus; respiratory; risks; Risk Behaviors; risk factors; sample collection; sampling; SARS coronavirus; screening; serologic tests; serological; seropositive; Severe Acute Respiratory Syndrome; site; surveys; syndromes; syndrome surveillance; Technologies; testing; therapeutic intervention; Therapeutic monoclonal antibodies; therapeutic vaccines; time; traits transgenic organisms; vaccines; viral; virology; Virus; work; zoonoses

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These research activities of Peter Daszak were not stopped during the period of the ban on "gain-of-function" research by the Barack government, but largely through the cooperation with the research group led by Zheng-Li Shi at the "Wuhan Institute of Virology". outsourced [IV.17]. This was done in the knowledge and agreement of NIAID Director Dr. Anthony Fauci.

In fact, much more money went to Peter Daszak and his "EcoHealth Alliance" for "gain-of-function" experiments, as recently became public [IV.18]:

Independent Science News
FOR FOOD AND AGRICULTURE

BIOTECHNOLOGY, HEALTH, NEWS DECEMBER 16, 2020

Peter Daszak's EcoHealth Alliance Has Hidden Almost $40 Million In Pentagon Funding And Militarized Pandemic Science

Sam Husseini

“Pandemics are like terrorist attacks: We know roughly where they originate and what's responsible for them, but we don't know exactly when the next one will happen. They need to be handled the same way — by identifying all possible sources and dismantling those before the next pandemic strikes.”

This statement was written in the *New York Times* earlier this year by Peter Daszak. Daszak is the longtime president of the EcoHealth Alliance, a New York-based non-profit whose claimed focus is pandemic prevention. But the EcoHealth Alliance, it turns out, is at the very center of the COVID-19 pandemic in many ways.

To depict the pandemic in such militarized terms is, for Daszak, a common place. In on Oct. 7 online talk organized by Columbia University's School of International and Public Affairs, Daszak presented a slide titled “Donald Rumsfeld's Prescient Speech”: “There are known knowns; there are things we know that we know. There are known unknowns; that is to say, there are things that we know we don't know. But there are also unknown unknowns — there are things we don't know we don't know.” (This Rumsfeld quote is in fact from a news conference).

In the subsequent online discussion, Daszak emphasized the parallels between his own crusade and Rumsfeld's, since, according to Daszak, the "potential for unknown attacks" is "the same for viruses".

Daszak then proceeded with a not terribly subtle pitch for over a billion dollars. This money would support a fledgling virus hunting and surveillance project of his, the Global Virome Project — a “doable project” he assured watchers — given the cost of the pandemic to governments and various industries.

Also on the video was Columbia University professor Jeffrey Sachs. Sachs is a former special advisor to the UN, the former head of the Millennium Villages Project, and was recently appointed Chair of the newly-formed EAT Lancet Commission on the pandemic. In September, Sachs' commission named Daszak to head up its committee on the pandemic's origins. Daszak is also on the WHO's committee to investigate the pandemic's origin. He is the only individual on both committees.

These leadership positions are not the only reason why Peter Daszak is such a central figure in the COVID-19 pandemic, however. His appointment dismayed many of those who are aware that Daszak's EcoHealth Alliance funded bat coronavirus research, including virus collection, at the Wuhan Institute for Virology (WIV) and thus could themselves be directly implicated in the outbreak.

For his part, Daszak has repeatedly dismissed the notion that the pandemic could have a lab origin. In fact, a recent FOIA by the transparency group US Right To Know revealed that Peter Daszak drafted an influential multi-author letter published on February 18 in the Lancet. That letter dismissed lab origin hypothesis as “conspiracy theory.” Daszak was revealed to have orchestrated the letter such as to “avoid the appearance of a political statement.”

...

As can be seen from the article reproduced above, **Peter Daszak was appointed a member of the commission of inquiry set up by the WHO to clarify the question of the origin of the coronavirus pandemic.** This has caused a lack of understanding in scientific circles, since there is a **clear conflict of interest** here , especially since Peter Daszak himself was involved in the "gain-of-function" research at the "Wuhan Institute of Virology" for years (see e.g. [III. 11]).

In Europe, there was also an intensive debate between scientists who advocated and wanted to continue "gain-of-function" experiments and those who saw them as too great a risk with regard to the possibility of a global pandemic. The following two articles give an impression of the discussion in Europe at the time ([III.12], [III.13]):

Nature 503, 19 (07 November 2013), doi:10.1038/503019a

***NATURE*| NEWS**

Pathogen research laws queried

Scientists fear EU biosafety rules could complicate publication of work on infectious diseases.

Declan Butler

Leading virologists have written to the president of the European Commission to urge him to clarify how laws designed to curb the proliferation of biological weapons apply to the publication of research on dangerous pathogens. The move by the European Society for Virology (ESV) comes after a Dutch court in September upheld a government order that scientists who engineered forms of H5N1 avian influenza to make them transmissible between mammals needed to seek an export permit before publishing such work.

The ESV's five-page letter to José Manuel Barroso, dated 16 October, warns that the court ruling sets an unwelcome precedent. H5N1 is just one of more than 100 dangerous human, animal and plant pathogens and toxins that fall under European Union (EU) export-control legislation from 2009. This means, say the virologists, that any EU scientist who works on one of the listed pathogens could be forced to apply for an export permit before publishing their research.

They write that to better inform courts and policy-makers on scientific issues related to biosecurity laws, the European Commission should consider creating an equivalent of the US National Science Advisory Board for Biosecurity — an independent committee in Bethesda, Maryland, that advises on issues of biosecurity and dual-use research (findings that could be adapted for harmful purposes). ...

***NATURE*| NEWS**

Nature doi:10.1038/nature.2013.14429, 20 December 2013

Scientists call for urgent talks on mutant-flu research in Europe

Benefits and risks of 'gain-of-function' work must be evaluated, they say.

Heidi Ledford

A group of over 50 researchers has called on the European Commission to hold a scientific briefing on research that involves engineering microbes to make them more deadly.

In an 18 December letter to European Commission president José Manuel Barroso, the scientists — including representatives from the non-profit Foundation for Vaccine Research in Washington DC — urged the commission to organize the briefing, and to formally evaluate the risks and benefits of such ' gain-of-function' research.

"Gain-of-function research into highly pathogenic microbes with pandemic potential has global implications for public health," says Ian Lipkin, an infectious disease researcher at Columbia University in New York, who is one of the signatories of the letter. "We are not seeking to shut down all gain-of-function research, but asking that stakeholders meet to establish guidelines for doing it."

The recent controversy over gain-of-function studies began in 2011 when Ron Fouchier, a virologist at the Erasmus Medical Center in Rotterdam, the Netherlands, sought to publish a study detailing how his team had engineered H5N1 avian influenza strains that could infect ferrets in separate cages through the air. Avian flu infections can be deadly for humans, but presently circulating strains of the virus are specific to birds and rarely infect mammals.

Proponents of the work say that it provides insight into how avian flu strains could naturally evolve to become more dangerous — results that could inform flu surveillance as well as vaccine and drug development. Opponents say that the work is too risky, because it involves engineering a deadly form of flu that could escape from research facilities or, in the wrong hands, could be intentionally released to cause a pandemic.

In October, the European Society for Virology (ESV) wrote its own letter to the European Commission, voicing concern that the Dutch government had used European export regulations to regulate the dissemination of Fouchier's research results, pushing him to apply

for an export license to publish his study in the journal *Science.* This approach to regulating sensitive research is inappropriate, argued ESV president Giorgio Palù, a virologist at the University of Padua in Italy, on behalf of society. The letter urged the commission to evaluate alternative means of overseeing such work.

Although the 18 December statement from scientists and the Foundation for Vaccine Research is framed as a response to the ESV's October letter, it explicitly does not tackle the issue of export controls; instead, it argues against some of the purported benefits of Fouchier's research. The work does not aid vaccine or drug development, says virologist Simon Wain-Hobson of the Pasteur Institute in Paris, who is chair of the foundation and a co-author of the letter, in part because flu outbreaks are impossible to predict. He also disputes claims that viruses similar to those engineered by Fouchier's laboratory are already appearing in the field.

Palù says that the letter from Wain-Hobson and signatories misses the crux of the ESV's concerns. “We don't want to enter the scientific quarrel,” says Palù. "Our intent was just to say that the export legislation is not the proper way to deal with this research."

But Wain-Hobson says that it is important for regulators to be informed about the scientific debate. "We're not against the science, and we're not against working on deadly pathogens," he explains. “But this is different — this research is making something new.”

And although most of the discussion so far has centered on flu, Wain-Hobson argues that it is time for regulators to think ahead to similar studies of other pathogens. "Flu was just the match that set off the barrel of gunpowder," he says. “This research has been going on for more than ten years — the technology is powerful now.”

...

According to the report reproduced above, on December 18, 2013, a group of 56 scientists addressed the then President of the European Commission, José Manuel Barroso, with a request to understand the dangers associated with genetically modified viruses, which are more deadly to humans than naturally occurring viruses, to be evaluated. **Due to the importance of this letter for the political discussion about "gain-of-function" research in Europe, this letter is reproduced in full below:**

The FOUNDATION for VACCINE RESEARCH

WORKING TO SECURE OUR CHILDREN'S FUTURE

December 18, 2013

Mr. José Manuel Barroso
President of the European Commission
Berlaymont Building
200 Rue de la Loi, 13th Floor
1049 Brussels, Belgium

cc:
Mrs. Viviane Reding, Vice President of the European Commission
Mrs. Máire Geoghegan-Quinn, Commissioner for Research, Innovation and Science
Mr. Tonio Borg, Commissioner for Health and Consumer Policy
Mr. Neven Mimica, Commissioner for Consumer Protection

RESPONSE TO LETTER BY THE EUROPEAN SOCIETY FOR VIROLOGY
ON "GAIN-OF-FUNCTION" INFLUENZA RESEARCH
AND
PROPOSAL TO ORGANIZE A SCIENTIFIC BRIEFING
FOR THE EUROPEAN COMMISSION &
CONDUCT A COMPREHENSIVE RISK-BENEFIT ASSESSMENT

Dear President Barroso,

We are writing to you on behalf of the Foundation for Vaccine Research and the 56 undersigned scientists to express our concern about a recent letter sent to you by the European Society for Virology (ESV). Several members of our group and the undersigned are members of the ESV.

We would like to correct some of the scientific misstatements in that letter. We would also like to propose: (1) a scientific briefing for the European Commission on so-called "gain-of-function" research, more properly defined as research to increase the pathogenicity, transmissibility, or alter the host range of highly pathogenic microbes with pandemic potential, including, but not limited to, influenza A viruses such as H5N1 and H7N9, and (2) consideration of a comprehensive risk-benefit assessment of this type of research. It is overdue that the risks associated with gain-of-function research be rigorously assessed and quantified. Researchers stand poised to conduct gain-of-function experiments with the SARS coronavirus and a host of other microbes with pandemic potential.

Misstatements
We would like to rebut some of the misleading scientific statements contained in ESV's letter of October 16 about EU laws, rules, and regulations governing the submission of manuscripts to international scientific journals, especially the need for export licenses for papers describing the results of so-called "gain-of-function" transmission experiments with highly pathogenic avian influenza H5N1 viruses conducted by Dr. Ron Fouchier at the Erasmus Medical Center in Rotterdam (*1*).

We do not take a position on the issue of export licenses, although we do understand the Dutch government's concern.

Regarding the scientific misstatements in ESV's letter, we take particular exception to the following sentence:

it's time. Campaign for an HIV, TB and Malaria Vaccine
601 Pennsylvania Avenue NW, Suite 900, South Building, Washington, DC 20004
Tel +1 202 220 3008 • Fax +1 202 639 8238 • www.vaccinefoundation.org • www.itstimecampaign.org
THE IT'S TIME CAMPAIGN IS A PROGRAM OF THE FOUNDATION *for* VACCINE RESEARCH

"However, it has to be mentioned that, in this specific case, **the "gain of function" was used to reproduce what nature already selected** (as demonstrated by sequencing of field mutants) with the variation that the aim of the study was to predict/anticipate biological evolution and to provide us with critical information to specify preventive and therapeutic measures, e.g., the improved surveillance and proper evaluation of candidate vaccines and drugs."

First, the statement that gain-of-function was used "to reproduce what nature already selected" is incorrect. Nature has *not* already selected an H5N1 virus that is readily transmissible between mammals. Highly pathogenic avian influenza H5N1 viruses are primarily transmitted between birds, not between mammals, and are only inefficiently transmitted between humans, if at all.

Fouchier *et al.* created novel mutant strains of H5N1 viruses that are genetically different from *any* known H5N1 virus strain found in nature, and that, importantly, have a specific property that makes them more dangerous than *any* known natural H5N1 virus, i.e., they are efficiently transmitted between mammals via respiratory droplets. Using ferrets, the preferred animal model for research with influenza A viruses, Fouchier and colleagues employed laboratory techniques that do *not* exist in nature, notably laboratory-directed, so-called "forced evolution," to see "what it would take" for H5N1 viruses to become transmissible via the aerosol route. Naturally occurring H5N1 viruses are highly virulent for humans – killing as many as 60% of those with known infections – but are not readily transmissible between mammals, including between humans. The sole purpose of the experiments in question was to generate H5N1 viruses that could be transmitted between mammals as readily as seasonal flu via respiratory droplets, i.e., by coughing or sneezing.

Despite intensive field surveillance conducted by national health authorities, government agencies, local and regional disease surveillance networks in Southeast Asia and elsewhere over a period of 16 years, *there is no evidence that efficiently mammalian-transmissible H5N1 viruses have ever emerged naturally in the wild*. Whereas it is correct that some individual mutations and some subsets of mutations identified by Fouchier *et al*., after repeated passage of H5N1 viruses between ferrets, have been found in nature, these mutations in different genetic backgrounds do *not suffice* to confer efficient binding to mammalian receptors. Additional mutations are necessary (*2*). The only unambiguous way to find out whether a field isolate is capable of aerosol transmission between ferrets is to perform a transmission experiment. Furthermore, whether the results of such experiments could extend to humans is unknown. Mapping mutations is *not* a surrogate marker for transmission. In summary, the statement that "gain-of-function" was used to reproduce "what nature already selected (as demonstrated by sequencing of field mutants)" is simply untrue.

Second, there is no compelling evidence or scientific basis for the assertion that gain-of-function research conducted by Fouchier *et al.* – or, indeed, by any other group (*3,4*) – can help us "predict or anticipate biological evolution and provide us with critical information to specify preventive and therapeutic measures, e.g., the improved surveillance and proper evaluation of candidate vaccines and drugs."

Given the highly unpredictable nature of influenza viruses, it is not possible to predict or anticipate biological evolution with any certainty and thereby to predict or anticipate the next influenza outbreak (*5-13*). Indeed, the track record in this domain is extremely poor. Evolutionary pressures result in multiple reassortment and mutational events that follow no clear pathway and are impossible to predict or associate with a specific outcome in any population (*11,14*). The experimental design of these influenza gain-of-function experiments is such that the outcome is strongly influenced by the experimenter. Hence, the probability of anticipating nature is very low indeed.

Third, there is no scientific basis for the claim that gain-of-function research may lead to the development of more effective vaccines, a major argument advanced by proponents of gain-of-function research, by providing "critical information for the proper evaluation of candidate vaccines."

Such a claim fails to appreciate the complexities of how influenza vaccines are developed (*14*). Gain-of-function studies on highly pathogenic avian influenza H5N1 viruses conducted to date in Europe, North America and Asia have contributed nothing so far to the development of new vaccines or prophylactic measures. The choice of H5N1 virus with which to make a vaccine is based on immunogenicity, not on virulence. Vaccine developers will need the actual H5N1 pandemic strain that is spreading in order to make that selection, rather than one obtained via gain-of-function experiments. Influenza vaccines have been manufactured for many decades based on the isolation of a virus with a specific pandemic potential or seasonal prevalence. It has so far been necessary to produce a new vaccine to protect against every influenza virus suspected of pandemic or seasonal threat, irrespective of the structure of the viral hemagglutinin or detected mutations in its amino acid sequence. Moreover, it is unlikely that any manufacturer would start epidemic vaccine production without knowing with certainty which strain to use. In this context, it is difficult to see how gain-of-function research can lead to more effective vaccines, at least in the near future.

Fourth, there is little evidence for the claim that gain-of-function research can provide "critical information for the proper evaluation of candidate drugs." Our 25 years of experience with HIV-1, another virus with a high propensity to mutate, has taught us that the only way to evaluate the efficacy of candidate antiviral drugs for RNA viruses is to conduct clinical trials. If ever H5N1 influenza went pandemic, we could only hope that the strain would be sensitive to some of the existing anti-influenza drugs. It would take several years to evaluate and get a new antiviral drug to market.

Taken together, these bold yet misleading claims made by the European Society for Virology are claims that have been repeatedly refuted (*14,15*). These misstatements weaken their case and should be corrected.

The power of synthetic biology has received considerable attention in recent years. Synthetic biologists do not deliberately try to increase the danger level of pathogens, toxins or the environment in which we live. It would be of the utmost concern if they did. By contrast, the influenza gain-of-function transmission experiments conducted by Fouchier *et al.* are notable for their *deliberate intent* to make a pathogen more dangerous for humanity. To justify such experiments, there must be extraordinary practical benefits that outweigh the risk of accidental release.

Despite significant improvements in safety conditions in research laboratories during the last decade, there is no such thing as "zero" risk. In this context, the potential for accidental release of a hazardous pathogen is real, not hypothetical, as demonstrated by an alarming increase in the number of potential and actual release events in laboratories working with high-threat pathogens (*16*). The number of potential and actual release events in Europe has not been recorded. However, between 2003 and 2009 the United States Centers for Disease Control and Prevention (CDC) recorded 395 domestic potential release events in laboratories working with high-threat pathogens (*17*). In Asia, three cases of laboratory-acquired SARS infections were reported in 2003, one in Singapore, one in Taiwan, and one in Beijing (*18-20*). These laboratory-acquired infections occurred after the WHO declared the end of the SARS outbreak. Moreover, the Beijing SARS infections spread beyond the laboratory into the community before the infections were detected and stopped.

Accidents do happen even in high-containment laboratories. The accidental release of even an attenuated virus strain can have global consequences. We need look no further than the re-emergence of the H1N1 influenza virus in 1977, after a 20-year hiatus. Most scientists who have investigated the 1977 outbreak concluded that the re-emergence was the result of an accidental release from a laboratory source (*21*), most likely from a laboratory in the former Soviet Union that was working on a live-attenuated H1N1 virus vaccine. Although the virus was an attenuated strain, it was nevertheless highly transmissible and went global, causing an epidemic, albeit a mild one.

For this reason, we are primarily concerned about the safety of gain-of-function research and the consequences of an accidental release. We are in a situation where the probabilities of a laboratory accident that leads to global spread of an escaped mutated virus are small but finite, while the impact of global spread could be catastrophic. Many other types of research on the biology of influenza viruses are possible that could provide crucial scientific information without creating a virus capable of transmission in mammals – that is, without the risk entailed by the experiments of Fouchier *et al*. In contrast to the substantial risks of gain-of-function research, the benefits of such research are hypothetical at best. There is little to no pre-existing immunity in the general population to the H5N1 virus, and none to the H7N9 virus discovered earlier this year in China. Moreover, there are only limited quantities of H5N1 vaccines readily available and stockpiled (vaccines which may not be a good match), and there is no licensed H7N9 vaccine. As a result, the accidental or deliberate release of an artificial, laboratory-generated, human-transmissible H5N1 or H7N9 virus into the community could be difficult or impossible to contain. There are few situations where a small but finite risk could, in the event of an accidental release, have such far-reaching consequences.

Proposals

1. A scientific briefing for the European Commission

Since the controversy surrounding H5N1 – and now H7N9 (*22*) – gain-of-function research is a complex scientific issue, and since the consequences of an accidental release affect the entire population of the European Union, we would like to propose that a scientific briefing be organized for the European Commission.

Such a briefing could be prepared at relatively short notice. The purpose of the briefing would be to inform Commissioners and their staff – and Members of the European Parliament, if desired – about gain-of-function research, presenting arguments in favour of and against the research. Given this information, Commissioners and MEPs would be in a better position to determine whether the risks are outweighed by the potential benefits, e.g., in predicting a pandemic or developing more effective vaccines. The National Academy of Sciences in Washington will shortly be debating these topics in a symposium. It is vitally important that European voices be heard and that Europeans participate in this debate. Indeed, there is an opportunity for Europe to take the lead on this issue.

The Foundation for Vaccine Research has the experience and the expertise to organize such a briefing, as one of the organizers and the moving force behind a 2-day international symposium, "H5N1 Research: Biosafety, Biosecurity and Bioethics," held at the Royal Society in London on April 3-4, 2012. The symposium was open to the public and webcast live. It was the first and remains the largest meeting organized to date on this topic. We would be happy to follow up with a detailed proposal regarding how such a scientific briefing could be organized for the European Commission.

2. A comprehensive risk-benefit assessment of gain-of-function research

Despite two years of controversy surrounding gain-of-function research and the lack of a scientific consensus, we still do not have a comprehensive risk-benefit analysis, as we would have hoped for on such an important topic. Many organizations, groups and individuals in Europe and the United States, including the journal *Nature*, have called for an independent risk-benefit assessment, but so far without success (*9,23*). A rigorous, comprehensive risk-benefit assessment could help determine whether the unique risks to human life posed by these sorts of experiments are balanced by unique public health benefits which could not be achieved by alternative, safe scientific approaches. Since scientists do not agree on the scientific merits of gain-of-function research, it will be hard to quantify the benefits. However, the risks *can* be quantified, as has been suggested in several preliminary studies (*24-28*). A comprehensive risk assessment would be able to quantify the risks of a release of a mutated virus into the community in terms of the loss of human life, the cost to health care systems, the financial and socio-economic costs, and the liability costs. These are man-made viruses and so liability becomes a novel issue, absent in the case of a naturally occurring epidemic.

Given your position as President of the European Commission, the combined experience and expertise of Commissioners and their staff, and the resources at your command, the Commission could make an important and immediate contribution by calling for a rigorous, comprehensive risk-benefit assessment of gain-of-function research to inform decision makers in Europe and worldwide. We have explored the feasibility of conducting such an assessment and would be happy to follow up with your staff with a detailed proposal regarding how an assessment could be undertaken.

Next steps

We would be honoured to follow up directly with Science Commissioner, Máire Geoghegan-Quinn, and her staff, on how a scientific briefing for the European Commission could be organized at short notice, as well as how a comprehensive risk-benefit analysis could be conducted.

We look forward to hearing from you,

Sincerely,

Professor Simon Wain-Hobson, D.Phil.
Chief, Molecular Retrovirology Unit
Department of Virology
Institut Pasteur, Paris
FVR Board Chair

This letter impressively shows how different the assessment of the risk potential of "gain-of-function" research was, even among virologists. Among the 56 people who signed the letter were the **three Nobel Prize winners Harald zur Hausen, Richard Ernst and Sir Richard Roberts.**

Regardless of one's point of view, the coronavirus research program has NOT prevented the current pandemic.
One has to legitimately ask oneself what sense this high-risk research actually has in addition to the fact that this research itself poses a very great risk potential for the world population.

How justified the concerns of the signatories to this letter were is impressively demonstrated by the high number of accidents in biotechnological laboratories, even at the highest safety level. This will be the subject of the following chapter.

5 How safe are high-security laboratories for research on dangerous pathogens?

In fact, the danger posed by biotechnological laboratories, even with the highest level of security, cannot be underestimated, as evidenced by numerous past and recent reports in different countries. Two examples of such reports are given below ([III.14], [IV.19]):

Nature 510, 443 (26 June 2014), doi:10.1038/510443a

***NATURE* | EDITORIAL**

Biosafety in the balance

An accident with anthrax demonstrates that pathogen research always carries a risk of release — and highlights the need for rigorous scrutiny of gain-of-function flu studies.

The news last week of an accident involving live anthrax bacteria at the US Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia is troubling. Some 84 workers were potentially exposed to the deadly Ames strain at three CDC labs. But the incident will cause much wider ripples: it highlights the risks of the current proliferation of biocontainment labs and work on dangerous pathogens. If an accident can happen at the CDC, then it can happen anywhere.

Details are sparse, but it seems that the anthrax was being inactivated in a biosafety-level-3 (BSL-3) high-containment lab so that it could be studied at the three BSL-2 labs. But live bacteria survived the inactivation step, and were not detected before samples were sent out. The CDC considers the risk that the exposed workers have been infected to be low, and all have been offered protective antibiotics.

Such lab accidents are fortunately not common place. A CDC analysis in 2012 reported, for example, that there were 727 incidents of theft, loss or release of Select Agents and Toxins in the United States between 2004 and 2010, resulting in 11 laboratory-acquired infections and no secondary transmission (RD Henkel *et al. Appl. Biosafety* **17,** 171-180; 2012). Anthrax is contracted by direct exposure to spores, and does not spread between people. Much more potentially dangerous are lab accidents involving agents that do. It is impossible to read about the CDC incident and not breathe a large sigh of relief that it did not involve a novel engineered pandemic influenza strain.

Groups led by Ron Fouchier of the Erasmus Medical Center in Rotterdam, the Netherlands, and Yoshihiro Kawaoka of the University of Wisconsin–Madison created a storm in late 2011 when they artificially engineered potentially pandemic forms of the H5N1 avian flu virus. In January last year, researchers ended a voluntary 12-month moratorium on such gain-of-function flu research, which can increase the host range, transmissibility or virulence of viruses (see *Nature* **493,** 460; 2013), and work resumed.

This month, Kawaoka's group reported that it had engineered a *de novo* flu virus from wild avian-flu-strain genes that coded for proteins similar to those in the 1918 pandemic virus (T. Watanabe *Cell Host Microbe* **15,** 692-705; 2014). The researchers were able to make a virulent version that could transmit between ferrets, and they concluded that a 1918-like virus could therefore emerge from wild avian flu viruses.

In the century since the 1918 flu hit, no similar pandemic variant has emerged despite wild animal flu viruses mutating and reassorting incessantly. The 1918 H1N1 virus was reconstructed in 2005, but human immunity to it became widespread following the 2009 H1N1 pandemic. There are no mammalian-transmissible 1918-like avian flus in the wild; the only ones that exist are Kawaoka's team's engineered strains.

“The idea of an accidental release of a potentially pandemic flu virus cannot be completely written off.”

Researchers such as Kawaoka and Fouchier argue that by engineering mutant viruses in the lab, they can identify mutations and traits that allow the pathogens to spread between mammals. This in turn, they argue, allows assessment of the pandemic potential of animal-flu viruses. In the long term, such experiments could help to elucidate the mechanisms of virus transmissibility and pathogenicity. But their shorter-term public-health benefits have been overstated. The risks and benefits must therefore be carefully weighed, and rigorous oversight is needed to ensure that such work is done only at facilities with the highest standards of biosafety.

Other scientists argue that the concept of predicting the pandemic potential of flu viruses from mutations, although appealing, is simplistic. They say that the identified mutations are but a handful out of millions of possible combinations, many of which might also allow mammalian transmission. They argue that mutations in specific proteins cannot reliably predict traits, and that outcomes depend on interactions between various other background genetic changes throughout the virus.

These points were highlighted in a paper in *PLoS Medicine* last month (M. Lipsitch and AP Galvani *PLoS Med.* **11,** e1001646; 2014), and in a letter by 56 leading virologists, infectious disease specialists and public-health experts to European Commission president José Manuel Barroso last December (see *Nature* http://doi.org/tdb; 2013). They also question the claimed public-health benefits of such research, and argue that similar information could be obtained through safer experiments. Opponents of gain-of-function flu research call, in particular, for more rigorous risk-benefit assessments. The CDC accident shows that, should such research proliferate, the idea of an accidental release of a potentially pandemic flu virus cannot be completely written off. This demands that such research proposals receive the utmost scrutiny.

A US Government Accountability Office report released in February last year expressed concern that the proliferation of US high-containment labs following the terrorist attacks of 11 September 2001 and the anthrax-letter attacks the same year was proceeding without a rigorous assessment of the nation's real needs across all government agencies, universities and private companies. "Increasing the number of laboratories also increases the aggregate national risk," it noted. No one keeps track, for example, of how many BSL-3 labs there are in the United

States alone, although their number is thought to be in the thousands. The number of such labs is increasing in China and elsewhere.

After smallpox was eradicated in 1980, there was a concerted international effort to reduce the number of labs holding stocks to just two: one at the CDC and one at the Russian State Research Center of Virology and Biotechnology in Koltsovo. All research at these centers must be approved by the World Health Organization. The fewer the labs that perform experiments, the smaller is the risk of an accidental release. But as the CDC accident reminds us, should gain of function flu research proliferate, in particular at facilities with less than exemplary biosafety standards, the risks of an accidental release of a potentially pandemic flu virus will be multiplied.

The New York Times, August 5th (2019)

Deadly Germ Research Is Shut Down at Army Lab Over Safety Concerns

Problems with disposal of dangerous materials led the government to suspend research at the military's leading biodefense center.

By Denise Grady

Safety concerns at a prominent military germ lab have led the government to shut down research involving dangerous microbes like the Ebola virus.

"Research is currently on hold," the United States Army Medical Research Institute of Infectious Diseases, in Fort Detrick, Md., said in a statement on Friday. The shutdown is likely to last months, Caree Vander Linden, a spokeswoman, said in an interview.

The statement said the Centers for Disease Control and Prevention decided to issue a "cease and desist order" last month to halt the research at Fort Detrick because the center did not have "sufficient systems in place to decontaminate wastewater" from its highest-security labs .

But there has been no threat to public health, no injuries to employees and no leaks of dangerous material outside the laboratory, Ms. Vander Linden said.

In the statement, the CDC cited “national security reasons” as the rationale for not releasing information about its decision.

The institute is a biodefense center that studies germs and toxins that could be used to threaten the military or public health, and also investigates disease outbreaks. It carries out research projects for government agencies, universities and drug companies, which pay for the work. It has about 900 employees.

The shutdown affects a significant portion of the research normally conducted there, Ms. Vander Linden said.

The suspended research involves certain toxins, along with germs called select agents, which the government has determined have determined “the potential to pose a severe threat to public, animal or plant health or to animal or plant products.” There are 67 select agents and toxins; Examples include the organisms that cause Ebola, smallpox, anthrax and plague, and the poison ricin.

In theory, terrorists could use select agents as weapons, so the government requires any organization that wants to handle them to pass a background check, register, follow safety and security procedures, and undergo inspections through a program run by the CDC and the United States Department of Agriculture. As of 2017, 263 laboratories — government, academic, commercial or private — had registered with the program.

The institute at Fort Detrick was part of the select agent program until its registration was suspended last month, after the CDC ordered it to stop conducting the research.

The problems date back to May 2018, when storms flooded and ruined a decades-old steam sterilization plant that the institute had been using to treat wastewater from its labs, Ms. Vander Linden said. The damage halted research for months, until the institute developed a new decontamination system using chemicals.

Two years before the outbreak of the corona pandemic, warnings were also given of security risks at the Wuhan Institute of Virology, according to reports by US diplomats in China. A corresponding comment on this is reproduced below [IV.5]:

THE WASHINGTON POST, April 14, 2020

State Department cables warned of safety issues at Wuhan lab studying bat coronaviruses

Josh Rogin

Two years before the novel coronavirus pandemic upended the world, US 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 coronaviruses from bats . The cables have fueled discussions inside the US 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 US Embassy in Beijing took the unusual step of repeatedly sending US science diplomats to the Wuhan Institute of Virology (WIV), 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 US delegation was led by Jamison Fouss, the consul general in

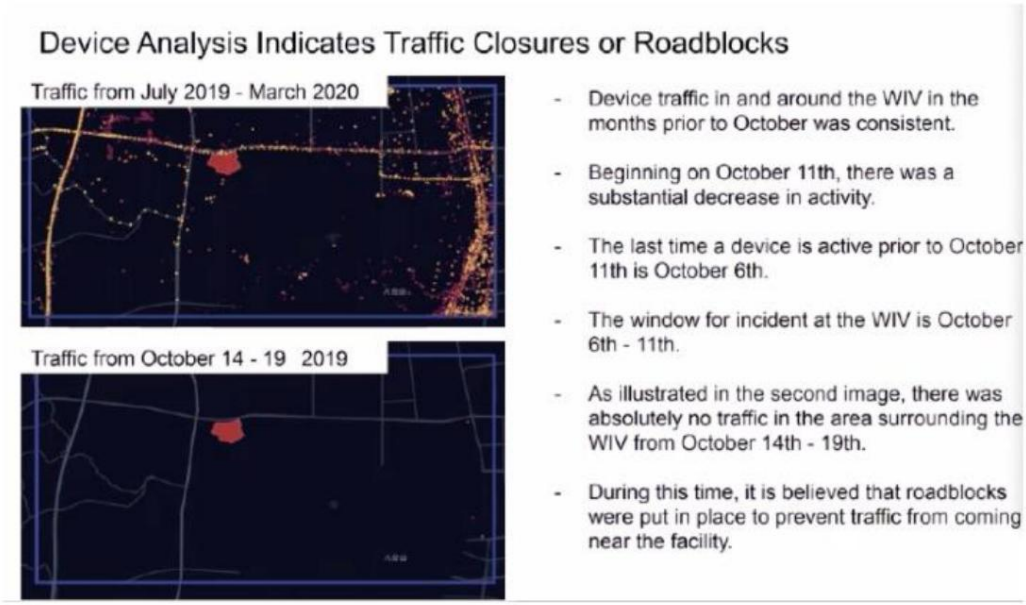
Wuhan, and Rick Switzer, the embassy's counselor of environment, science, technology and health. Last week, WIV erased that statement from its website, though it remains archived on the Internet.

Even after the outbreak of the corona pandemic, evidence of serious safety deficiencies at the "Wuhan Institute of Virology" became public. Chinese journalists, for example, made films from the institute premises and put them online, which prove the improper disposal of laboratory waste (see, for example, [IV.20], especially the film section from time 8:15):

https://www.youtube.com/watch?v=qbUgF_mQy90

Furthermore, photos and video recordings by researchers from the "Wuhan Institute of Virology" have become public, showing that they wore **insufficient or no protective clothing** when collecting bat samples and when examining them in the laboratory (see, for example, [IV.21]).

An analysis of mobile phone usage activities in and around the "Wuhan Institute of Virology" in the second half of 2019 indicates that in the first half of October 2019 there was a **temporary interruption in laboratory operations and cordoning off around the institute** premises [IV.22], see graphic below:



At the same time, there were the first confirmed cases of fatal COVID-19 diseases in various hospitals in the city of Wuhan as early as October 2019 [IV.2]. It is therefore reasonable to assume that the barriers around the "Wuhan Institute of Virology" with

Investigations into the origin of these cases of illness were carried out, especially since at that time there were already indications circulating in Chinese social media that the first COVID-19 patient was an employee of this institute (see chapter: "Key question about the origin of the coronavirus pandemic: natural disaster or laboratory accident ?).

The question naturally arises as to why the “Wuhan Institute of Virology”, as the most likely point of origin of the coronavirus pandemic, should be taken out of suspicion by the Chinese government under all circumstances. There are now many representatives from science and politics (see for example [II.9], [IV.23]) who see a **connection between scientific high-risk research with bat viruses and military interests .** In fact, the **"dual use" possibility of "gain-of-function" research** has been discussed for years in the scientific and political arena.

The fact that there are close connections between this type of scientific research and military interests is not a “conspiracy theory”, but is evidenced by a large number of co-authorships in the scientific specialist literature. Two examples of this are given below [I.15], [I.16]:

Journal of Virology, Volume 88, Number 12, p. 7070-7082, June 2014

Identification of Diverse Alphacoronaviruses and Genomic Characterization of a Novel Severe Acute Respiratory Syndrome-Like Coronavirus from Bats in China

Biao He, Yuzhen Zhang, Lin Xu, Weihong Yang, Fanli Yang, Yun Feng, Lele Xia, Jihua Zhou, Weibin Zhen, Ye Feng, Huancheng Guo, Hailin Zhang, and Changchun Tu

Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Institute of Military Veterinary, Academy of Military Medical Sciences, Changchun, Jilin Province, China;
Yunnan Institute of Endemic Diseases Control and Prevention, Dali, Yunnan Province, China;
Baoshan Prefecture Center for Diseases Control and Prevention, Baoshan, Yunnan Province, China;
Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, Jiangsu Province, China

DOI: 10.1128/JVI.00631-14

ABSTRACT

Although many severe acute respiratory syndrome-like coronaviruses (SARS-like CoVs) have been identified in bats in China, Europe, and Africa, most have a genetic organization significantly distinct from human/civet SARS CoVs in the receptor-binding domain (RBD) ,

which mediates receptor binding and determines the host spectrum, resulting in their failure to cause human infections and making them unlikely progenitors of human/civet SARS CoVs. Here, a viral metagenomic analysis of 268 bat rectal swabs collected from four counties in Yunnan Province has identified hundreds of sequences relating to alpha- and betacoronaviruses. Phylogenetic analysis based on a conserved region of the RNA-dependent RNA polymerase gene revealed that alphacoronaviruses had diversities with some obvious differences from those reported previously. Full genomic analysis of a new SARS-like CoV from Baoshan (LYRa11) showed that it was 29,805 nucleotides (nt) in length with 13 open reading frames (ORFs), sharing 91% nucleotide identity with human/civet SARS CoVs and the most recently reported SARS-like CoV Rs3367, while sharing 89% with other bat SARS-like CoVs. Notably, it showed the highest sequence identity with the S gene of SARS CoVs and Rs3367, especially in the RBD region. Antigenic analysis showed that the S1 domain of LYRa11 could be efficiently recognized by SARS-convalescent human serum, indicating that LYRa11 is a novel virus antigenically close to SARS CoV. Recombination analyzes indicate that LYRa11 is likely a recombinant descended from parental lineages that had evolved into a number of bat SARS like CoVs.

IMPORTANCE

Although many severe acute respiratory syndrome-like coronaviruses (SARS-like CoVs) have been discovered in bats worldwide, there are significantly different genetic structures, particularly in the S1 domain, which are responsible for host tropism determination, between bat SARS like CoVs and human SARS CoVs, indicating that most reported bat SARS-like CoVs are not the progenitors of human SARS CoV. We have identified various alphacoronaviruses and a close relative (LYRa11) to SARS CoV in bats collected in Yunnan, China. Further analysis showed that alpha- and beta-coronaviruses have different circulation and transmission dynamics in bat populations. Notably, full genomic sequencing and antigenic study demonstrated that LYRa11 is phylogenetically and antigenically closely related to SARS CoV. Recombination analyzes indicate that LYRa11 is a recombinant from certain bat SARS-like CoVs circulating in Yunnan Province.

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Emerging Microbes & Infections 7(1), 154 (2018). doi: 10.1038/s41426-018-0155-5.

Genomic characterization and infectivity of a novel SARS-like coronavirus in Chinese bats

Dan Hu [1 2], Changqiang Zhu [2], Lele Ai [2], Ting Hey [2], Yi Wang [3], Fuqiang Ye [2], Lu Yang [2], Chenxi thing [2], Xuhui Zhu [2], Ruicheng Lv [2], Jin Zhu [2], Bachar Hasan [4], Youjun feng [5], Weilong Tan [6], Changjun Wang [7 8]

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Abstracts

SARS coronavirus (SARS-CoV), the causative agent of the large SARS outbreak in 2003, originated in bats. Many SARS-like coronaviruses (SL-CoVs) have been detected in bats, particularly those that reside in China, Europe, and Africa. To further understand the evolutionary relationship between SARS-CoV and its reservoirs, 334 bats were collected from Zhoushan city, Zhejiang province, China, between 2015 and 2017. PCR amplification of the conserved coronaviral protein RdRp detected coronaviruses in 26.65% of bats belonging to this region, and this number was influenced by seasonal changes. Full genomic analyzes of the two new SL-CoVs from Zhoushan (ZXC21 and ZC45) showed that their genomes were 29,732 nucleotides (nt) and 29,802 nt in length, respectively, with 13 open reading frames (ORFs). These results revealed 81% shared nucleotide identity with human/civet SARS CoVs, which was more distant than that observed previously for bat SL-CoVs in China. Importantly, using pathogenic tests, we found that the virus can reproduce and cause disease in suckling rats, and further studies showed that the virus-like particles can be observed in the brains of suckling rats by electron microscopy. Thus, this study increased our understanding of the genetic diversity of the SL-CoVs carried by bats and also provided a new perspective to study the possibility of cross-species transmission of SL-CoVs using suckling rats as an animal model.

...

The topic of **"biosecurity"** has become increasingly important in recent years, particularly due to the fact that high-risk research and the development of bioweapons often go hand in hand and represent a **substantial threat to the health of the world population** (see for example [II.10]):

Biosecurity and the Risk to Global Health

Christian Enmark

The Oxford Handbook of Global Health Politics

Edited by Colin McInnes, Kelley Lee, and Jeremy Youde
Online Publication Date: Jan
2018 Print Publication Date: Mar
2020 DOI: 10.1093/oxfordhb/9780190456818.013.12

Global health is potentially diminished by practices of biosecurity aimed at safeguarding the health of human populations against selected infectious disease risks. Some diseases inspire so much government concern that they are accorded the status of security issues, and adopting a security-based rationale for prevention and response efforts can garner extra resources and stronger powers for risk-reduction purposes. However, such an approach can result in practices that are counterproductive from a health perspective. This chapter shows that biosecurity can endanger global health in at least four areas of policy concern: the development of defenses against biological weapons, the management of security risks arising from laboratory research on pathogenic microorganisms, the prioritization of disease risks and response mechanisms as part of an agenda of global health security, and the use of national borders to contain transnational contagion.

As devastating as the effects of atomic bombings, nuclear reactor accidents, or the use of chemical warfare agents have been in the past, the effects have ultimately been regionally localized. However, the current coronavirus pandemic shows us what dangers posed by released dangerous pathogens actually exist globally for the entire world population.
Future international agreements must therefore focus more on B (in addition to A and C) hazard potentials.

6 The Role of Science in the Question of the Origin of the Coronavirus Pandemic

Scientific findings, analyzes and predictions play a central role in the coronavirus pandemic. The great importance of science for society in times of the Corona crisis is also emphasized in statements by numerous scientific societies [IV.24].

In the current pandemic, the serious communication of scientific knowledge is essential for the acceptance of necessary measures to contain the spread of the virus and for the protection of risk groups. In science communication, it is particularly important to reduce the complexity of scientific facts in such a way that their essential content is not lost and can be understood by the public.

Various avenues of disseminating information to the general public have been used by scientists since the beginning of the pandemic. This includes science programs on television, radio podcasts, talk shows, but also articles in newspapers and magazines as well as in online media. The success of these extensive efforts in science communication in recent months can be seen, among other things, from the results of surveys among the population [IV.25]: 77 percent of those surveyed in Germany say they feel well informed about the coronavirus pandemic, and 73 percent of those surveyed accept the government-imposed measures to contain the coronavirus pandemic.

The general trust of the German population in science and research increased significantly during the coronavirus pandemic: from around 50 percent before the pandemic to 73 percent in May 2020 [IV.25]. Almost 90 percent of those surveyed are of the opinion that scientific knowledge is important in order to slow down the spread of the coronavirus pandemic in Germany. And finally, 81 percent of those surveyed believe that political decisions in dealing with the coronavirus pandemic should be based on scientific knowledge [IV.25].

Every representative of the scientific system is currently delighted with this development and takes the opportunity to point out the need for further expansion of scientific education and research [IV.24].

However, the question that arises in this context is to what extent this positive development could be endangered from a scientific point of view if the origin of the coronavirus pandemic is not a zoonosis (and thus comparable to a natural disaster), but a biotechnological laboratory of a scientific institute for Virology of the city of Wuhan in China would be as presented and justified in this present study as the most likely scenario. How would the mood of the population in Germany, but also worldwide, change if the current global crisis were not the result of a coincidence in nature - a random mutation of a coronavirus in a bat with the involvement of an intermediate host animal - but the result of the carelessness of a scientist conducting high-risk research with global pandemic potential [IV.26]? Wouldn't there be more questions about the responsibility of science in view of the dimensions of the current worldwide

catastrophe arise? Wouldn't calls be made for an immediate cessation of this type of research? How many scientific laboratories around the world would have to fear being closed as a result of the enormous public and political pressure? Would this be a scenario that science itself might have to rule out? **How would this affect the need to clarify the important question of the origin of the coronavirus pandemic? Can science itself remain open-ended on this question? Are there any signs that it hasn't been for quite some time?**

It is undoubtedly amazing to what extent some well-known virologists identified the animal market in Wuhan as the source of the SARS-CoV-2 pathogen very early on in public statements (see [IV.1], [IV.3], among others), whereby again and again new suspicions about the possible intermediate host animal (including snakes, civets, pangolins, raccoon dogs) were expressed. So far, however, it has not been possible to prove scientifically that a zoonosis actually took place. That the laboratory of the Wuhan Institute of Virology, where it has been proven - i.e. supported by the existing scientific literature - for many years high-risk research on corona viruses including genetically modified variants, could also be considered as the source of the SARS-CoV-2 pathogen , was ruled out by some virologists right from the start, without there being a scientifically comprehensible reason for this to this day. Without having proof for one or the other theory, it would be imperative for science to adopt a neutral, ie open-ended, position on this question. Surprisingly, however, this is not the case.

The media spoke very early on of a “conspiracy theory” in connection with the thesis of the laboratory origin of the coronavirus pandemic, without however explaining why the scientifically plausible assumption regarding the origin of the pandemic had the character of a “conspiracy”.

The statement by 27 scientists [III.4], published in the journal "The Lancet", also sounds strange, in which the signatories explain the following: "We have watched as the scientists, public health professionals, and medical professionals of China, in particular, have worked diligently and effectively to rapidly identify the pathogen behind this outbreak, put in place **significant measures to reduce its impact,** and **share their results transparently with the global health community".** "The **rapid, open, transparent sharing of data on this outbreak** is now being threatened by rumors and misinformation around its origin". **"We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin".** Apart from the fact that this publication does not provide any scientific evidence that the SARS-CoV-2 pathogen does not originate in the Wuhan Laboratory for Virology, the confirmation of a "transparent" information policy on the Chinese side is in obvious contradiction to the facts (see inter alia [III.3], [IV.6]-[IV.12], [IV.14], [IV.15]).

It is even stranger that scientific publications by the research group led by Zheng-Li Shi from the "Wuhan Institute of Virology", which have appeared in journals of the "NATURE" group and the targeted genetic manipulation of corona viruses with regard to higher Contagion rates and danger to humans prove, as well as commentary articles that

refer to this, were subsequently provided by SpringerNature-Verlag with the following note:

30 March 2020 *Editors' note, March 2020: We are aware that this article is being used as the basis for unverified theories that the novel coronavirus causing COVID-19 was engineered. There is no evidence that this is true; Scientists believe that an animal is the most likely source of the coronavirus.*

This statement by the hitherto highly respected scientific publishing group **SpringerNature** caused a lack of understanding in scientific circles in several ways:

- The sentence "scientists believe..." is untenable in this form, since there is a proven **plurality of opinions among scientists ,** which has been documented by many publications, as far as the origin of the coronavirus pandemic is concerned. At best, the sentence should have been “some scientists believe…”.
- Furthermore, the wording "scientists believe..." is inappropriate for a scientific journal, if only because **science is based on verifiable facts and not on what a subset of scientists believe.**

Unfortunately, this is not the first time SpringerNature has given in to pressure from the Chinese government, as evidenced by the following article [IV.27]:

The New York Times, **Nov. 1, 2017**

Leading Western Publishers Bows to Chinese censorship

Javier C. Hernandez

BEIJING — One of the world's largest academic publishers was criticized on Wednesday for bowing to pressure from the Chinese government to block access to hundreds of articles on its Chinese website.

Springer Nature, whose publications include Nature and Scientific American, acknowledged that at the government's request, it had removed articles from its mainland site that touch on topics the ruling Communist Party considers sensitive, including Taiwan, Tibet, human rights and elite politics.

The publisher defended its decision, saying that only 1 percent of its content was inaccessible in mainland China.

Under President Xi Jinping, China has grown increasingly confident in using its vast market as a bargaining chip, forcing foreign firms to acquiesce to strict demands on free speech.

Academic publishers have become a popular target, part of Mr. Xi's efforts to restrict the flow of ideas at universities.
...

In the science magazine **"Scientific American",** which is also published by SpringerNature Verlag, the head of the coronavirus research program at the "Wuhan Institute of Virology", Zheng-Li Shi, is presented by the Chinese author as a scientific pioneer and heroine [IV.28]. There is no reference to the history of the critical discussion about the risk and dangers associated with the "gain-of-function" research carried out at the Wuhan Institute.

The article ends with the statement: The "team has estimated that there are as many as 5,000 coronavirus strains waiting to be discovered in bats globally". The team "is planning a national project to sample viruses in bat caves - with much greater scope and intensity than the team's previous attempts". However, the question remains whether the global community would accept a 5,000-fold risk for further coronavirus-related pandemics, regardless of the origin of the SARS-CoV-2 virus.

While only the animal market version has been propagated as the source of the SARS-CoV-2 viruses in the scientific literature for months, contrary results from scientific studies with different strategies are suppressed at the same time. A research team from New Delhi reported in a preprint of a publication [II.8] that the scientists had found HIV RNA sequences during the genetic analysis of the SARS CoV-2 virus, indicating an artificial origin of this novel type of coronavirus . The authors were then vehemently criticized by well-known virologists and asked to withdraw the publication.

Interestingly, the French Nobel Prize winner and discoverer of the HIV virus, Luc Montagnier, together with a colleague, found RNA sequences of HIV viruses during the genetic engineering of SARS-CoV-2 viruses that had not naturally become part of these novel corona viruses could be [II.7]. In an interview with French television, Montagnier said: "In order to insert an HIV sequence into the genome, molecular tools are needed and that can only be done in a laboratory". The reaction to this statement by the French Nobel Prize winner were not scientific arguments from the other side, but exclusively defamatory comments that either referred to Montagnier's age [IV.29] or pointed towards the fact that the Nobel Prize winner was now "controversial" [IV .30]. In fact, HIV-based pseudoviruses were used for gene manipulation experiments by the Wuhan research group led by Zheng-Li Shi, as evidenced by several publications in the scientific literature (see eg [I.6], [I.10]).

Based on detailed analyzes of the gene sequence of SARS-CoV-2 viruses, which cause the COVID-19 disease, the Chinese virologist Li-Meng Yan has also found clear indications of a non-natural origin of these novel viruses [II.5]. After her work was published on the Zenodo online portal in September 2020, she was heavily criticized by several virologists. She found that the SARS-CoV 2 virus was a laboratory product using bat viruses named ZC45 and

ZXC21 as a template or backbone. However, these same types of coronaviruses were also identified by the group of Chinese scientists and doctors when analyzing the gene sequences of pathogens from the very first COVID-19 patients in Wuhan. This work was published in February 2020 in the highly regarded journal "THE LANCET" [I.3]. Excerpts from both works are reproduced below:

Unusual features of the SARS-CoV-2 genome Suggesting Sophisticated Laboratory Modification Rather Than Natural Evolution and Delineation of Its Probable Synthetic Route

Yan, Li Meng; Kang, Shu; Guan, Jie; Hu, Shanchang

The COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has led to over 910,000 deaths worldwide and unprecedented decimation of the global economy. Despite its tremendous impact, the origin of SARS-CoV-2 has remained mysterious and controversial. The natural origin theory, although widely accepted, lacks substantial support. The alternative theory that the virus may have come from a research laboratory is, however, strictly censored on peer-reviewed scientific journals. Nonetheless, SARS-CoV-2 shows biological characteristics that are inconsistent with a naturally occurring, zoonotic virus. In this report, we describe the genomic, structural, medical, and literature evidence, which, when considered together, strongly contradicts the natural origin theory. The evidence shows that SARS-CoV-2 should be a laboratory product created by using bat coronaviruses ZC45 and/or ZXC21 as a template and/or backbone. Building upon the evidence, we further postulate a synthetic route for SARS-CoV-2, demonstrating that the laboratory-creation of this coronavirus is convenient and can be accomplished in approximately six months. Our work emphasizes the need for an independent investigation into the relevant research laboratories. It also argues for a critical look into certain recently published data, which, although problematic, was used to support and claim a natural origin of SARS-CoV-2. From a public health perspective, these actions are necessary as knowledge of the origin of SARS-CoV-2 and of how the virus entered the human population are of pivotal importance in the fundamental control of the COVID-19 pandemic as well as in preventing similar , future pandemics.

...

LANCET VOLUME 395, ISSUE 10224, P565-574, FEBRUARY 22, 2020

Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding

Roujian Lu, Xiang Zhao, Juan Li, Peihua Niu, Bo Yang, Honglong Wu, Wenling Wang, Hao Song, Baoying Huang, Na Zhu, Yuhai Bi, Xuejun Ma, Faxian Zhan, Liang Wang, Tao Hu, Hong Zhou, Zhenhong Hu, Weimin Zhou, Li Zhao, Jing Chen, Yao Meng, Ji Wang, Yang Lin, Jianying Yuan,

Zhihao Xie, Jinmin Ma, William J Liu, Dayan Wang, Wenbo Xu, Edward C Holmes, George F Gao, Guizhen Wu, Weijun Chen, Weifeng Shi, and Wenjie Tan

Summary

Background

In late December, 2019, patients presenting with viral pneumonia due to an unidentified microbial agent were reported in Wuhan, China. A novel coronavirus was subsequently identified as the causative pathogen, provisionally named 2019 novel coronavirus (2019-nCoV). As of Jan 26, 2020, more than 2000 cases of 2019-nCoV infection have been confirmed, most of which involved people living in or visiting Wuhan, and human-to-human transmission has been confirmed.

methods

We did next-generation sequencing of samples from bronchoalveolar lavage fluid and cultured isolates from nine inpatients, eight of whom had visited the Huanan seafood market in Wuhan. Complete and partial 2019-nCoV genome sequences were obtained from these individuals. Viral contigs were connected using Sanger sequencing to obtain the full-length genomes, with the terminal regions determined by rapid amplification of cDNA ends. Phylogenetic analysis of these 2019-nCoV genomes and those of other coronaviruses was used to determine the evolutionary history of the virus and help infer its likely origin. Homology modeling was done to explore the likely receptor-binding properties of the virus.

findings

The ten genome sequences of 2019-nCoV obtained from the nine patients were extremely similar, exhibiting more than 99 98% sequence identity. Notably, 2019-nCoV was closely related (with 88% identity) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in 2018 in Zhoushan, eastern China , but were more distant from SARS-CoV (about 79%) and MERS-CoV (about 50%). Phylogenetic analysis revealed that 2019-nCoV fell within the subgenus Sarbecovirus of the genus Betacoronavirus, with a relatively long branch length to its closest relatives bat-SL CoVZC45 and bat-SL-CoVZXC21, and was genetically distinct from SARS-CoV. Notably, homology modeling revealed that 2019-nCoV had a similar receptor-binding domain structure to that of SARS-CoV, despite amino acid variation at some key residues.

The dispute over the authority to interpret the question of the origin of the coronavirus pandemic culminated in the statement by a well-known virologist in Germany in the course of 2020 that scientists who are not in the field of virology, even in the special field of Corona viruses work, it is better not to comment on the topics related to the coronavirus pandemic [IV.29]. This statement is obviously closely linked to the question of today's understanding of science: **should science only be understood as the totality of specific disciplines with clear demarcations of the "responsibilities" of individual scientific disciplines, or aren't there also overriding questions of science that we can't deal with lastly, the critical, self-reflecting consideration of processes in the**

Science, but also questions about the responsibility of science for the well-being of mankind should count?

There are quite a few scientists who are currently speaking of the worst case scenario of a coordinated misleading of the general public as to the origin of the coronavirus pandemic (see e.g. [II.9]).

A group of “Concerned People of the World” has meanwhile written an open letter to the members of the WHO commission of inquiry into the origin of the coronavirus pandemic [IV.31], which begins by saying:

"Every human being is entitled to know the truth of the origins of the COVID-19 pandemic".

There is really nothing more to add, except for referring to the content of the questions formulated by a group of scientists, which indicate the tasks to be performed in the investigation of what is happening in Wuhan, especially in the last quarter of 2019 are [IV.31]:

Open Letter to the WHO COVID-19 International investigation team

Prof. Dr. Thea Fisher, MD, DMSc(PhD) (Nordsjællands Hospital, Denmark)
Prof John Watson (Public Health England, United Kingdom)
Prof. Dr. Marion Koopmans, DVM PhD (Erasmus MC, Netherlands)
Prof. Dr. Dominic Dwyer, MD (Westmead Hospital, Australia)
Vladimir Dedkov, Ph.D (Institut Pasteur, Russia)
dr Hung Nguyen, PhD (International Livestock Research Institute (ILRI), Vietnam)
PD. dr med vet. Fabian Lendertz (Robert Koch Institute, Germany)
dr Peter Daszak, Ph.D (EcoHealth Alliance, USA)
dr Farag El Moubasher, Ph.D (Ministry of Public Health, Qatar)
Prof. Dr. Ken Maeda, PhD, DVM (National Institute of Infectious Diseases, Japan)
Copy to: Peter K. Ben Embarek Scientist - Program Manager at World Health Organization.

Dear Fellow Scientists,

The COVID-19 pandemic has been ravaging the world for over a year now and it is showing no sign of easing in many countries, with infection cases and death tolls continuing to climb. Millions of our brothers and sisters have lost their loved ones, their jobs, businesses, livelihoods and education opportunities. The economies of many nations have been severely compromised, resulting in great tribulation for many sectors, with many closed or bankrupt businesses and millions of unemployed.

Sadly today, we are all still as clueless as to the origins of COVID-19 as we were 10 months ago, despite numerous scientific studies and research conducted around the world since then.

We are glad that the WHO is able to form an investigation team of 10 international experts sitting in the East to undertake the task of unraveling these mysteries and take us from darkness to light.

We, the concerned people around the world, on behalf of all those who have died, widowers, widows, distressed sons, daughters and orphans, therefore call on you to conduct the investigation with transparency, impartiality and bravery without bowing to any pressure or national interest.

Such an investigation, to be both credible and successful must take into consideration all scenarios in a scientific way without giving preference to any default hypothesis, however disturbing this may be.

In support of this investigation, a dedicated group of researchers in various parts of the world have spent months unearthing documents, web pages, papers, and reports to compile a list of relevant and as yet unanswered questions about the origins of COVID-19.

We therefore call on the WHO investigation team to answer the following questions which we feel are of paramount importance to a successful investigation into the origins of SARS-COV 2.

We wish you success and thank you sincerely for your endeavors in search of the truth!

From Concerned People of the World

"Every human being is entitled to know the truth of the origins of the COVID-19 pandemic"

Questions for the WHO January 2021 mission

A. Questions about the positive samples from the market

1. What animals in the Wuhan Huanan Seafood Market were tested, what types of specimens were obtained (apart from frozen animal carcasses), and what were all the results?

2. Were samples gathered from the Huanan market prior to it being sanitized? If so, have these samples been shared with the WHO and what do they reveal?

3. Recently, a floor plan map of the Huanan Seafood Market was “leaked” to the public.

Breakout at the Huanan Seafood Wholesale Market

An SCMP reproduction of a leaked floorplan from the Chinese CDC's investigations into the early spread of the novel coronavirus (Study from January 2020)

Why did it take 10 months for this map to be published and then only via a “leak”?

4. What does this “One Health” blueprint map of the market reveal in terms of

   a. the 33 positive & 552 negative “environmental samples”

   b. the 27 + persons epidemiologically linked to the Market

   c. all the negative & any positive specimens from specific animals

   i.e. the role of sewage and drainage in the market outbreak.

5. Why were a further 70 environmental samples obtained on Jan 12 from the market, after the 515 samples obtained on Jan 1st, and what did these later samples reveal?

6. How many of the samples collected on Jan 12th tested positive for SARS-CoV-2?

7. What are the results of testing in other markets in Wuhan such as the North Hankou Seafood Market, and those outside Wuhan in Hubei province, and outside Hubei province?

8. What animal species were tested? For example, those species now known to be susceptible to the virus, such as: ferrets, cats, mink, tigers, dogs and others?

9. What animals were sold on the 22 stalls in the Western Section of the Wuhan Seafood Market where 14 of the 31 positive samples came from?

10. What were the sources and types of wildlife species sold at this market and why has China still not disclosed this information nearly one year after the events?

11. What information on the investigation of the purported animal source of the virus at the Wuhan Seafood Market was provided in the WHO mission report?

12. Why have antibody tests (IgM & IgG) used to identify infected humans & animals in Wuhan between Sep-Dec 2019 not been made public?

13. What was the destination of the animals after the market was closed?

14. Why has China not published results of their investigation into the 4 key data streams identified by Dr. Alyward in Annex D of the WHO-China Joint Mission on Coronavirus Disease 2019 Report (28-02-2020)?

1. Vendor records of animal sales
2. Samples kept from swabbing including gutters where urine & faeces collect.
3. Freezers full of animal parts.
4. Tracking of earliest patients

B. Questions about the alleged November 17th patient

15. In light of the confirmed report of the November 17th Covid-19 patient published in the SCMP, why is that patient not officially acknowledged?

16. What has been ascertained from the CCDC regarding contact tracing of that patient?

C. Questions about February 20th data collection of suspected early Covid-19 cases in Wuhan

reference materials: https://gillesdemaneuf.medium.com/early-cases-of-suspected-covid-19-in-wuhan-feb-20-data-collection-b7740ed1436f

17. What the WHO actually shown this data?

18. Was the WHO team directed to hospitals with early cases during their one-day visit to Wuhan in February?

19. Given that the very rushed request for medical and admission data still returned some candidates for early Covid-19 cases (going back to the very beginning of October or earlier), did China take the time to do a more thorough and coherent data collection exercise? If not, why not? If yes, where are the results?

20. Were these early cases followed up to refine their diagnostics, especially in the cases of deaths (for instance by testing any available sample for antibodies), and were early patients' work unit, location, and residence all recorded? If not, why not? If yes, where are the results?

21. Was that data collection exercise eventually extended to suspected cases prior to the 1st October 2019?

22. How should we interpret the cluster of imaging cases with similarities to Covid-19 pathology at Wuhan Puren Riverside Hospital with admission dates of 1st and 2nd October 2019, in that same collected data?

23. Will the WHO team have access to patient details and files and be able to interview selected cases?

D. Questions about the official national database of Covid-19 managed by Pr. Yu Chanhua

24. Did the official national database of actual and suspected cases managed by Pr. Yu Chanhua (ÿÿÿ) and his team contain any suspected October or November cases prior to the Wuhan data collection exercise in February?

25. Were the results of the above data collection added to that national database managed by Pr. Yu Chuanhua, even if starting first as suspected cases (especially for Form 2 and Form 3 cases) before further checks?

26. Were the suspected pre-December cases - such as the 29th Sep CT-imaging case and some November cases he mentioned as being present in the national database - confirmed?

27. Were these conclusions of that verification work eventually shared with the WHO?

E. Questions about the NUDT "War Epidemic Resumption Big Data" platform and related data

28. Were the "War Epidemic Resumption Big Data" platform (ÿÿÿÿÿÿÿÿ) developed at the NUDT (National University of Defense Science and Technology) and its corresponding epidemic data shown to the WHO mission?

29. What Pr. Yu Chuanhua's data work fed into the "War Epidemic Resumption Big Data platform"?

30. Why was a version of the "War Epidemic Resumption Big Data platform" with limited data resolution available only for a while at the web portal of the NUDTy (https://nudtdata.com.cn), before being taken offline?

F. Questions about the proceedings of the WHO February 2020 mission

31. Did the WHO consider the implications on public trust of the inclusion of Pr. Dong Xioaping (ÿ ÿÿ) in a prominent role on the Chinese side of the February 2020 WHO mission,

given that he had been sanctioned for his role in the multiple SARS leaks at the Beijing CDC P3 lab in 2004??

32. Why was the WHO visit of Wuhan delayed until after the rushed completion of the Data Collection (point C above)?

G. Questions about deleted Wuhan Institute of Virology Viral pathogen databases

33. Why are all the Wuhan Institute of Virology databases (including the 61.5 Mb SQL version) still offline? Pr. Zhengli Shi claimed they were offline for cybersecurity issues and would be made available “when they felt safe”. This was 5 months ago. There are at least 100 unpublished sequences of bat betacoronaviruses on these databases which need to be sequenced by international scientists.

a. WIV Database 1: http://batvirus.whiov.ac.cn/ (Archive seems to be unavailable)

b. WIV SQL online Database 2: http://csdata.org/p/308/

Archived:https://web.archive.org/web/20200507214518/http://csdata.org/p/308/

and: http://archive.is/HLuio

c. WIV Database 3: http://www.viruses.nsdc.cn/vri.jsp

- Archived: https://web.archive.org/web/20200125203943/http://www.viruses.nsdc.cn/vri.jsp
- Discussion of significance here:

Guoke Faji 2019/236 and the SARS-CoV-2 Outbreak http://archive.is/uHqSw#selection-29.0-29.47

i.e. WIV Database 4: http://www.viruses.nsdc.cn/chinavpi

Archived: https://web.archive.org/web/20200404100024/http://www.viruses.nsdc.cn/chinavpi

Referenced in a paper by Zhiming Yuan of the Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, (+86-27-87197242, email: yzm@wh.iov.cn)

"Investigation of Viral Pathogen Profiles in Some Natural Hosts and Vectors in China", https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6178075/

e. WIV Database 5: http://www.wfcc.info/ccinfo/collection/col_by_country/c/86/

- Archived: https://web.archive.org/web/20200515223251/http://www.wfcc.info/ccinfo/collection/col_by_country/c/86/ which links to: http://wfcc.info/ccinfo/collection/by_id/613
- Archived: https://web.archive.org/web/20200108181714/http://wfcc.info/ccinfo/collection/by_id/613 links to: http://www.virus.org.cn/ (404 for the database in question)
- Archived: https://web.archive.org/web/20191230091754/http://www.virus.org.cn/

- Other on archived description of the WIV database : https://web.archive.org/web/20200117011358/http://www.whiov.ac.cn/xwdt_105286/zhxw/201804/t20180423_5000795.html

In order to clarify the deletion of these databases, please note that these are under the management of:

Prof. Fei Deng and Prof. Zhihong Hu:

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tel: (86) 27-87168465 facsimile: (86) 27-87168465

e-mails: Prof. Fei Deng: df@wh.iov.cn and Prof. Zhihong Hu huzh@wh.iov.cn

34. Why were the description and many keywords in the online SQL version of the WIV database altered by Professor Zhengli Shi on Dec 30th while she was returning from Shanghai to Wuhan on the night train?

- Version 1 of the SQL database description: ”Wildlife-borne Viral Pathogen Database”

(Release time: July 17th, 2019) Originally available here: http://csdata.org/p/308/2/

Can be seen here: https://web.archive.org/web/20200507214437/http://csdata.org/p/308/2/

- Version 2 of the same SQL database: “Bat and rodent-borne viral pathogen database”

(Updated on December 30th 2019 from Shanghai to Wuhan night train by Pr. Shi)

Originally available here: http://csdata.org/p/308/4/

Can be seen here:https://web.archive.org/web/20200507214519/http://csdata.org/p/308/4/

H. Question about Chinese BatCoV vaccine development programs

35. Can China provide details about any specific strategy followed to prepare for Disease X (a combination of pre-emergent BatCoV features which would represent the most threatening evolutionary front)?

I. Questions about RaTG13 and the 8 SARSr of the Ra7896 clade

36. Was RaTG13 a consensus sequence as recently claimed by Peter Daszak in an interview (TWiV 623) with Vincent Racaniello?

37. Some RaTG13 amplicons include a "7896" label. So what Ra7896 in fact used for sequencing RaTG13?

38. Why did WIV not fully sequence the 8 SARSr of the 7896-clade further than their RdRp when they were the second closest viruses to SARS-CoV-2?

39. Were these 8 remaining SARSr from the 7896 clade collected from the same Tongguan mine as RaTG13?

40. Will Ecohealth publish the initial draft of Latinne et al. (2020)

41. There is a correlative series of isolates from WIV but two are missing from the series. Specifically, why were the WIV6 and WIV15 isolates never disclosed? See numbered series.

J. Mojiang Miners Pneumonia Cases

42. Can WIV clarify the full details of the 2012 pneumonia outbreak among the Mojiang miners, especially regarding the subsequent samplings and all blood and BALF results?

43. Can WIV clarify what happened to the samples collected from the Mojiang miners between 2012 and 2019 and whether they are still available for independent analysis?

44. Did WIV culture any virus from the Tongguan mineshaft pneumonia cases in animals or cell lines? If so, were the sequences used as “backbones” for creating other viruses?

K. Laboratory Questions

45. Professor Zhengli Shi recently stated that she would welcome any kind of visit to her Laboratory in order to clarify the origins of SARS-COV-2 (BBC 2020). In light of this declaration, the WHO investigation team will therefore inspect or organize inspections of the following laboratories in Wuhan:

a. WCDC Pathogen BSL-2 at 288 Machang Road

b. Wuhan University Institute of Model Animal ABSL-3 at 115 Donghu Road

c. Huazhong Agricultural University ABSL-3

i.e. Hubei CDC BSL-3 and Hubei Animal CDC ABSL-3 (in Wuhan)

e. Wuhan Institute of Virology BSL-2 and BSL-3 in Xiaohongshan park

f. Wuhan Institute of Virology BSL-2, BSL-3, ABSL-3, BSL-4 at Zhengdian park

G. Wuhan Institute of Biological Products (vaccine development & production platform) Zhengdian park and its former location (see map)

46. Will the WHO have access to the laboratory records which are supposed to be exhaustive and kept for 20 years at least? Specifically:

1.Lab notebooks

2. Safety procedures, safety audit reports and safety incident reports,

3. Project proposals, status updates and project reports,

4. Environmental audit reports and environmental incident reports

5. Facility improvement projects and monthly reports

6. Purchasing records by department for supplies and new equipment

7. Facility and equipment maintenance logs and records

L. Miscellaneous Questions

47. Are any of the 10 members of the WHO investigation team fluent in Mandarin?

48. Has the CCDC shared primary isolates of SARS-CoV-2 with the WHO and the international community? If not, why not?

49. Why was the WIV unable to transfer samples to the University of Texas Medical Laboratory in Galveston in line with their request? (House Foreign Affairs Committee Report on the Origins of the COVID-19)

50. In light of the "leak" of hospital data which revealed an investigation by the Chinese health authorities into early cases of covid-19 in Wuhan & Hubel province, the WHO team will query the patient details & files to further clarify the putative cases of covid-19 in October at Wuhan Hospitals.

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