Study to

The Origin of the Coronavirus Pandemic

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Universität Hamburg

Period of the study

01.01.2020 – 31.12.2020

Preface

The present study of the origin of the coronavirus pandemic was conducted between 01.01.2020 and 31.12.2020 at the University of Hamburg (Universität Hamburg). Preliminary results of this study were published on May 5th, 2020 within the frame of a press release. Since this date, further crucial findings and documents were gathered through international knowledge sharing.

This study is based on an interdisciplinary scientific approach, meaning not exclusively a discipline-specific point of view, as well as comprehensive research with use of all possible information sources. These include:

- interdisciplinary as well as discipline-specific scientific literature based on scientific evaluation („peer review“),
- scientific literature without scientific evaluation,
- letters, correspondence and comments published in scientific literature,
- articles in print- and online media,
- reports on the internet/ social media,
- personal communication with international colleagues.

The references to the present study were structured accordingly, to obtain a clear distinction between scientific primary literature (with and without peer review) and published expressions of opinion.

The present document was completed on January 6th, 2021. It was initially distributed and discussed exclusively within the research community. On February 12th, 2021 approval for publication followed, serving as basis for a large-scaled discussion among the general public, which considering the importance of this matter, shall be informed fact-based and must be included in future decision processes.

Supplementary information and further documents can be obtained by contacting the study conductor:

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1 Motivation and Main Results of the Study - an Overview

The current coronavirus pandemic poses for many people the greatest challenge since the end of the Second World War. The global crisis is associated with the loss of many lives in relation to the COVID-19 disease (approximately 1.8 million deaths in one year according to statistics from Johns Hopkins University, USA). Along with an unprecedented economic crisis, there are many consequences, some of which remain to be seen, for people's lives and prosperity - in many cases affecting the most basic necessities of life, especially in the poorest countries of the world.

Even if the current public debate is naturally primarily focused on the consequences of the pandemic within the health sector, the economy, and many areas of society, the question of the origin of the pandemic is of central importance. Whenever a new type of virus emerges, it is very important to identify the source of the virus as well as to learn exactly how it spreads, as a basis for current and future action, according to the World Health Organization (WHO). The science-based approach to 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 coronaviruses from the animal kingdom to humans ("zoonosis"), whereby a certain type of bat has been suggested as the original source. Resulting from a virus mutation with the involvement of an intermediate host animal, transmission to humans may have taken place via an animal market in the centre of Wuhan (China), the city of origin of the coronavirus pandemic.

2) Alternatively, ever since the beginning of the pandemic a laboratory accident in a biotechnological high-security laboratory in the city of Wuhan (not far from the suspected animal market) has been mentioned as a possible cause. This suspicion is based on the fact that high-risk research and biotechnological manipulation of coronaviruses have been at the centre of the activities of the virology institute in Wuhan for many years, as is documented by numerous publications in the scientific literature.

To date, there is no scientifically based rigorous evidence for either of these two theories. In such a situation, scientists - irrespective of their field of expertise - should adopt a neutral stance and hold an open-ended discussion until the decisive question concerning the origin of the pandemic can be answered. However, very early on several renowned virologists adopted the first theory, hence a zoonotic origin, in public statements. This has led politicians and other leading public figures to increasingly speak of the coronavirus pandemic as a "natural disaster."

Is this however indeed a natural disaster, comparable to an earthquake, a tsunami or a volcanic eruption? Is the current global crisis in fact the result of an accident of nature, an accidental mutation of a bat coronavirus with the cooperation of an intermediate host animal? Or is it the result of carelessness on the part of scientists while conducting high-risk research with global pandemic potential?

Since there is yet no science-based evidence in the strict sense of the word to answer this important question, at present only circumstantial evidence can be cited which make one or the other theory more probable.

This year-long study concludes that both the quantity and quality of the evidence clearly point to a laboratory accident at the Wuhan Institute of Virology as the cause of the current pandemic. The author has used science-based literature as well as independently verifiable relevant documents, which are cited in the main part of the study and partly reported in its original form, as the target audience of this study may not have the means or time to access this literature directly.

Some of the key evidence pointing to a laboratory accident as the cause of the current pandemic, presented and discussed in detail in this study, is briefly summarized below:

- Coronaviruses that originated in bats do not lead as readily to infectious diseases in humans with the severity we are experiencing in the current pandemic (inter alia very high transmission rate and viral infestation not only of the respiratory tract but also other organs). In this context, virologists speak of an "adaptation barrier."

- Mutations of coronaviruses could have occurred in intermediate host animals and eventually been transmitted to humans in wildlife markets. However, such an intermediate host animal has not yet been identified in the context of 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 with the suspected wildlife market at all. This has been substantiated by several original scientific publications in peer-reviewed journals.

- There is ample independent evidence that a young female scientist at the Wuhan Institute of Virology was the first to be infected with the novel coronavirus in the laboratory, and thus standing at the beginning of the COVID-19 infection chain. Her entry on the institute's website has been deleted and her whereabouts since the end of 2019 is unknown.

- According to numerous reports, bats were not offered for sale at the suspected wildlife market in Wuhan. However, over many years, bat viruses from distant caves in a southern Chinese province have been collected and brought back to Wuhan by scientists of the Wuhan Institute of Virology. This has been substantiated by several original scientific publications in peer-reviewed scientific journals.

- Over many years, a group of researchers at the Wuhan Institute of Virology has not only studied naturally occurring coronaviruses, but also manipulated them genetically with the aim of making them more infectious and dangerous to humans. This so-called "gain-of-function" research at the Wuhan Institute of Virology has been documented in many peer-reviewed scientific journals for years and critically judged by many representatives of the scientific community.

- There were reports of significant safety deficiencies at the Wuhan Institute of Virology even before the outbreak of the coronavirus pandemic. The statistics of documented accidents in biotechnological high-security laboratories show that unintentional releases of highly infectious viruses from such laboratories has not been uncommon in the past, both in China and, for example, in the USA. In addition, video recordings exist which prove that laboratory waste at the Wuhan Institute of Virology was not properly disposed of and that the employees of the institute were not wearing adequate protective clothing.

- An analysis of cell phone usage in and around the Wuhan Institute of Virology in the second half of the year 2019 indicates that in the first half of October 2019 a temporary disruption of laboratory operations occurred, and barriers were erected around the institute premises. At this same time, initial confirmed cases of COVID-19 disease resulting in death, occurred 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 illness appeared in Europe in November 2019, detected retrospectively (for example, by detailed analysis of the lung images of a COVID-19 patient in France).

On the basis of these and many other findings presented in the present study, and based on scientific publications and verifiable documents, it is all the more surprising that numerous virologists continue to propagate only zoonosis as the cause of the current pandemic in all available media. Therefore, this study also addresses the role of science in connection with the question of the origin of the current coronavirus pandemic.

2 The Central Question of the Origin of the Coronavirus Pandemic: Natural Catastrophe or Laboratory Accident?

In this for the post-war generation highly exceptional time of restrictions of fundamental rights caused by the coronavirus pandemic, individuals more and more frequently wonder how dangerous the corona virus really is. Are we overestimating the danger? Is the right of freedom of all citizens currently unjustly restricted? Can the pending unprecedented collapse of the economy be justified? Are the currently asserted rules of conduct appropriate, or are they the manifestation of an overcautious reaction of the state within an unprecedented situation since the end of the war?

Many repeatedly make the comparison with the well-known influenza and point out that for instance the influenza season 2017/18 claimed the lives of an estimated 25.000 people in Germany and approximately 60.000 in the USA. Others in turn argue, that without governmental intervention, the number of fatalities following a COVID-19 infection would be noticeably higher and that to this day – despite governmental protective measures – the global number of deaths in this pandemic would already surpass 1.8 million (according to the statistics of the John Hopkins University, USA).

What is it that differentiates the new type of coronavirus SARS-CoV-2 from all previously known types of coronaviruses and the multitude of other viruses that we are constantly exposed to throughout our life? According to present knowledge, following aspects of the new coronavirus type are unusual:

- Coronaviruses have been known for a long time and can among others provoke common colds in humans, which however typically cease to occur after the end of April. Same applies to the flu, caused by influenza viruses, for which the season levels off significantly starting from the end of March, which means that even with a severe influenza season, in the past one could be sure that the wave of influenza would abate during spring. A “shutdown” of public life was hence not necessary. The new type of coronavirus apparently acts differently and also spreads in those countries of the world that are in summertime.

- Coronaviruses have also played an important role in severe illnesses in the past, for instance in the SARS-epidemic in 2003. However, this type of coronavirus was considerably less contagious for humans, so that the number of infections remained below 10.000 and the number of deaths below 1.000 worldwide. Various studies indicate that the new type of coronavirus SARS-CoV-2 can still be contagious with a distance to the infected three times bigger compared to the previous SARS coronaviruses. Moreover, with the new type of coronavirus, an infection among multiple people in a closed space can much easier occur even if a minimal distance of two meters is respected. The high risk of infection in relation to the new type of coronavirus is scientifically explained by a very good adaptation of the SARS-CoV-2 virus to the human cell receptors [I.1], so that the new type of coronavirus can much easier find access to human cells and easily infect the person in question.

- In fact, the adaptation of the SARS-CoV-2 virus to the human cell receptors is so good that not only the (upper) respiratory organs, but also other inner organs can be infested by this type of virus. In a few cases, this leads to a severe course of illness of COVID-19 patients, caused by multi-organ failure.

With these three particularities of the new virus type mentioned above, everyone can perceive that we are not dealing with a for us usual viral disease. The World Health Organization (WHO) states that whenever a new type of virus occurs, it is important to understand where this new virus comes from, which means to identify the source as well as to study the details of its spreading, in order to thus gather important information as a basis for current and future measures. The question of the source of the current coronavirus pandemic is without doubt particularly important with regard to future measures aiming to reduce the probability of an outbreak of a comparable or even worse pandemic.

2.1 The animal market theory

Based on reports in scientific journals ([I.1]-[I.3]) and various media, the coronavirus pandemic started at a specific place in the city of Wuhan in China towards the end of the year 2019. An animal market in the centre of this city has been and still is until today most often named as the possible source of the new types of coronaviruses. The genetic analysis of the new SARS-CoV-2 viruses, which were collected from humans with a COVID-19 infection, exhibit a high degree of relatedness to coronaviruses in bats [I.1, I.3], similar to the case of already known SARS viruses, which were responsible for the SARS-epidemic in 2003. It is speculated that coronaviruses could have been transmitted to the human via another wild animal serving as intermediate host. This is referred to as “zoonosis”. Since the beginning of the pandemic following animals have among others been suggested as intermediate hosts: snakes, civets, pangolins, and raccoon dogs [IV.1].

Several scientifically based facts contradict this theory:

1. Bats themselves were not offered on the suspected animal market.
2. Until today none of the above-mentioned intermediate host animals have been proven to be the transmitter of the currently circulating coronavirus disease. One could however argue, that in previous cases of diseases caused by coronaviruses in the past, it also took a while to identify the intermediate host animal.
3. A substantially weightier argument is that a significant amount (34%) of the first documented COVID-19 patients had no contact to the suspected animal market [I.2, I.3]. In particular the first in scientific original literature documented patient had no contact to the animal market (more specifically “Huanan seafood market”) that was officially declared by the Chinese government as the cause of the COVID-19 disease, shortly after the outbreak of the pandemic.

Below follows an excerpt from the scientific original literature [I.2] with the essential diagram. The journal “LANCET” represents one of the most renowned journals of 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 analysed data on patients with laboratory-confirmed 2019-nCoV infection by real-time RT-PCR and next-generation sequencing. Data were obtained with standardised data collection forms shared by WHO and the International Severe Acute Respiratory and Emerging Infection Consortium from electronic medical records. Researchers also directly communicated 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 diarrhoea (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 TNFα.

…

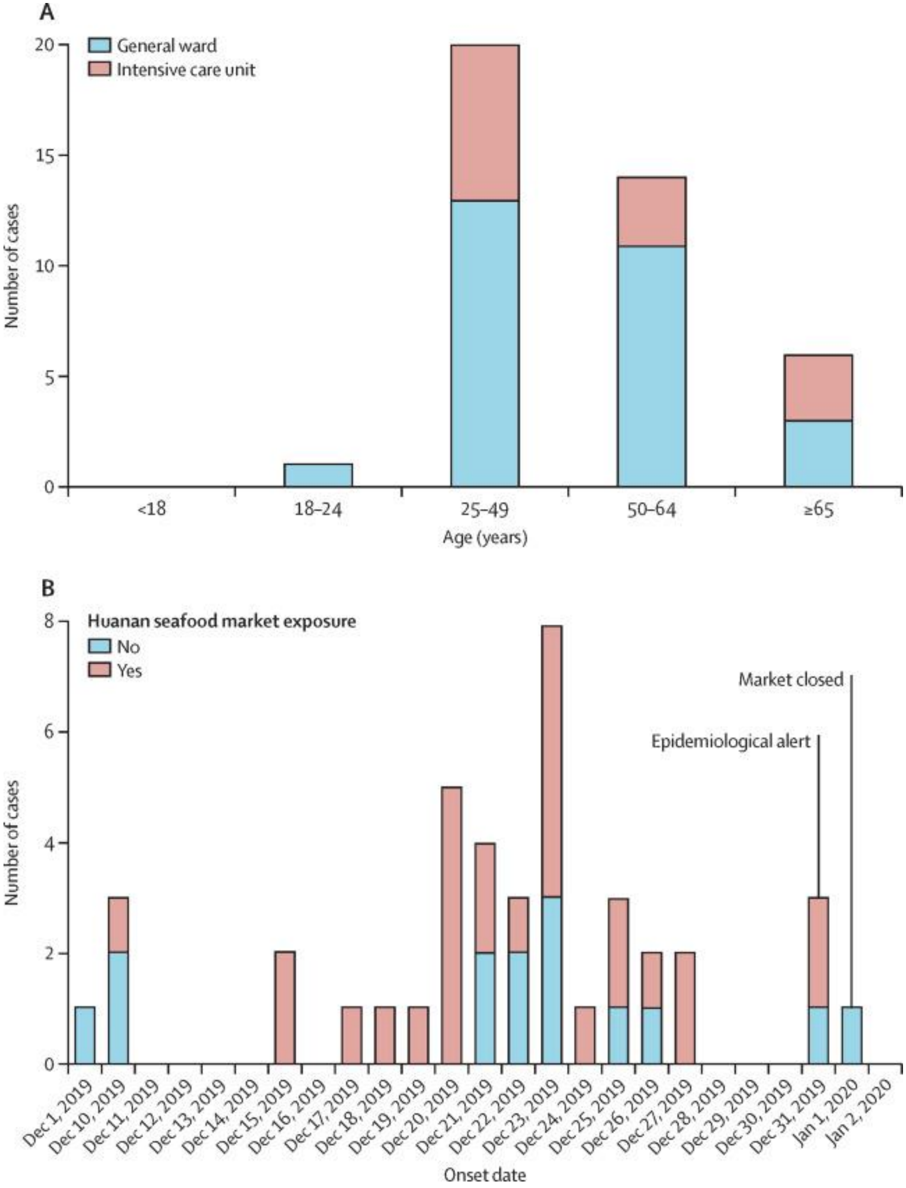

Figure:

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

Interesting in this context is further, that for the first patient in this publication, the symptoms of a COVID-19 infection were determined as early as December 1st, 2019. Due to an incubation period of up to 14 days in relation to the new type of coronavirus disease, consequently one must expect that first infections have occurred in November 2019. This is among others, compatible with a more recent report according to which a very first case of a COVID-19 infection was retrospectively determined based on a detailed analysis of lung recordings of a patient in France. Recently treatment of first COVID-19 patients in different hospitals of the city of Wuhan taking place already in October 2019 are being reported (see for example [IV.2]). We will return to the time-related aspects regarding the spreading of the COVID-19 disease during the early phase of the pandemic later on in the current study.

4. After closer analyses, one scientific publication frequently cited in the media, which supposedly proves that the origin of the current coronavirus pandemic is a zoonosis, turns out unsuitable in order to decide between the two alternative theories. With the title “Scientists disprove conspiracy theories” [see for instance [IV.3]) it was repeatedly referred to a publication of the reputed journal “Nature Medicine” which supposedly presented proof “that SARS-CoV-2 developed naturally and not by means of biotechnological modification in a laboratory”. Accessing the publication in the journal “Nature Medicine” [III.1] one must initially see that it is not an original publication, but a so-called “**Letter to the Editor**” in which five virologists present their personal view about the origin of the SARS-Cov-2 virus, see following extract of 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 analyses clearly show that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus.

…

In the introduction the authors are writing: „Our analyses **clearly show** that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus“. Later in the text suddenly another wording is 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 ultimately in the concluding 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**“. A scientific "proof" as media saw in this publication certainly looks different. The misinterpretation in this case however, is clearly caused by the misleading opening statement of the authors, which stands in clear discrepancy to the final statement of this "Letter to the Editor".

5. A further scientific original publication [I.4] that is repeatedly mentioned in the context of the theory of zoonosis within the scientific community originates from the research team of Zheng-Li Shi at the Wuhan Institute of Virology which has been conducting intensive research on coronaviruses of different bat populations for many years:

***Nature* 579, pages 270–273 (2020)**
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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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Lin-Lin Liu & Fa-Xian Zhan

Abstract

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 proteins 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 information that the genetic fingerprint of the new coronavirus type (at the time called 2019-nCoV), which is causing a COVID-19 disease, corresponds to 96% to a coronavirus type “RaTG13” originating from horseshoe bats from the province of Yunnan in southern China. Due to the fact that the genetic code of the new coronavirus type was published by “China’s National Center for Disease Control Prevention” only on January 11th, 2020, the research team around Zheng-Li Shi consequently solely had nine days to collate the genetic fingerprint of this new coronavirus type with many other types of coronaviruses from the database and to identify the virus type with the highest similarity. Moreover, within this time, the publication had to be written and agreed upon by all co-authors. Interestingly, the bat virus denominated “RaTG13” has been isolated from horseshoe bats from the Yunnan province as early as January 2013, hence seven years before, by the research team around

Zheng-Li Shi, without having been mentioned in previous publications of the research team around Zheng-Li Shi. The virus denominated “RaTG13” is held as “natural source” of the coronavirus pandemic by many virologists since the above-mentioned publication in the journal “NATURE” in February 2020.

However, for a few months now there has been serious doubt within the scientific community concerning the veracity of the content of this NATURE-publication from February 2020 (see for instance [IV.4]). At this point, three examples of expressed reservations shall be presented (for the complete 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, Tavpritesh Sethi

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

Abstract

An intense scientific debate is ongoing as to the origin of SARS-CoV-2. An oft-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 incidence 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 context of determining the origin of SARS-CoV-2.

…

In summary, it can be noted that until this present day there is no scientific and well-sound basis for the claim that the present coronavirus pandemic was caused by zoonosis. Consequently, based on scientific reasons, it is not appropriate to speak of a "natural catastrophe" at the present time.

2.2 The Laboratory Accident Theory

Not "conspiracy theorists", but rather two Chinese scientists Lei and Botao Xiao of the South China University of Technology, were the ones who published a study on the international research online portal "Research Gate" in mid-February 2020, ich which they speculated for the first time publicly that one of the two biotechnological laboratories in the centre of Wuhan could be the source of the new type of coronaviruses. Shortly after the publication of this study it disappeared from the online database "Research Gate", however it is archived on the web [II.4].

Indeed, the outbreak of the current coronavirus pandemic in the city of Wuhan leads to the justified question, why this pandemic started in specifically this city in the year 2019. Assuming that zoonosis taking place at the animal market in the centre of Wuhan is the cause of the current pandemic, one has to take into account that animal markets have been existing for thousands of years in China, and that thousands of markets exist in all cities of China. Therefore, one has to ask the question, why especially in 2019 such a coronavirus pandemic originates from the city of Wuhan?

Within the scientific community, the city of Wuhan is primarily known for its research in the field of virology, not lastly through numerous publications in leading interdisciplinary scientific journals such as “NATURE” and “SCIENCE”. In this context, the research team around Zheng-Li Shi working at the Wuhan Institute of Virology has been playing an important role in the field of coronavirus research for many years. The research began approximately 16 years ago – prior to the creation of the “Wuhan Institute of Virology” within the frame of a Franco-Chinese cooperation – and has since then been conducted for many years partially in tight cooperation between the Chinese researchers and multiple American and Australian research teams [I.5- I.10]. The source of coronaviruses for the virological research were thereby different bat species that were collected from caves in various Chinese provinces by the Wuhan research team during numerous expeditions. The coronaviruses were then isolated at the Wuhan Institute of Virology, multiplied, and their interaction with animal and human cells investigated (see for instance [I.5, I.6, I.7, I.9].

However, the research team around Zheng-Li Shi at the Wuhan Institute of Virology has not solely investigated naturally occurring coronaviruses, but in addition systematically manipulated them with the aim of making them more contagious and more dangerous for humans. This so-called **“gain-of-function” research** at the Wuhan Institute of Virology is documented through various scientific original publications in peer-reviewed journals [see for instance [I.5, I.6, I.7, I.8], and has been critically judged by many representatives of the scientific community for many years [see for example [III.2]). Due to their importance in respect to the current coronavirus pandemic, two individual chapters following this introductory chapter are dedicated to the previous history of the coronavirus pandemic. **Especially the dispute within scientific circles focussing on the pandemic potential of “gain-of-function” research that has - among others - been expressed in two letters to the president of the EU-commission in 2013, reveals clearly how divergent the opinions were already back then among researchers, and how great the need for discussion – especially after the outbreak of a worldwide pandemic – would in fact be today.**

Even though the Wuhan Institute of Virology is operating a biotechnical laboratory of highest security level, reports existed before the outbreak of the pandemic indicating **substantial safety flaws at this Institute** (see for instance [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, U.S. Embassy officials visited a Chinese research facility in the city of Wuhan several times and sent two official warnings back to Washington about inadequate safety at the lab, which was conducting risky studies on coronaviruses from bats. The cables have fueled discussions inside the U.S. government about whether this or another Wuhan lab was the source of the virus — even though conclusive proof has yet to emerge.

In January 2018, the U.S. Embassy in Beijing took the unusual step of repeatedly sending U.S. science diplomats to the Wuhan Institute of Virology (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 U.S. delegation was led by Jamison Fouss, the consul general in Wuhan, and Rick Switzer, the embassy's counselor of environment, science, technology and health. Last week, WIV erased that statement from its website, though it remains archived on the Internet.

What the U.S. officials learned during their visits concerned them so much that they dispatched two diplomatic cables categorized as Sensitive But Unclassified back to Washington. The cables warned about safety and management weaknesses at the WIV lab and proposed more attention and help. The first cable, 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.)

…

Looking at the statistics of documented accidents in biotechnological high-safety laboratories reveals, that unintended escape of highly infectious viruses from such laboratories has not been rare in the past, in China as well as for instance in the USA. An entire individual chapter is dedicated to this important subject within the current study.

What do we in fact know about the early phase of the outbreak of the coronavirus pandemic in Wuhan? Unfortunately little is known from official sources, as China has tried covering up real occurrences since the very beginning. This has already been intensively reported in the media (see for instance [IV.6, IV.7, IV.8]). China even put pressure on the EU and countries such as Australia throughout the year 2020 – up to the point of threatening with sanctions – in case the Chinese handling of the pandemic would not be praised or in case critical comments concerning the behaviour of the Chinese government at the beginning of the pandemic would arise.

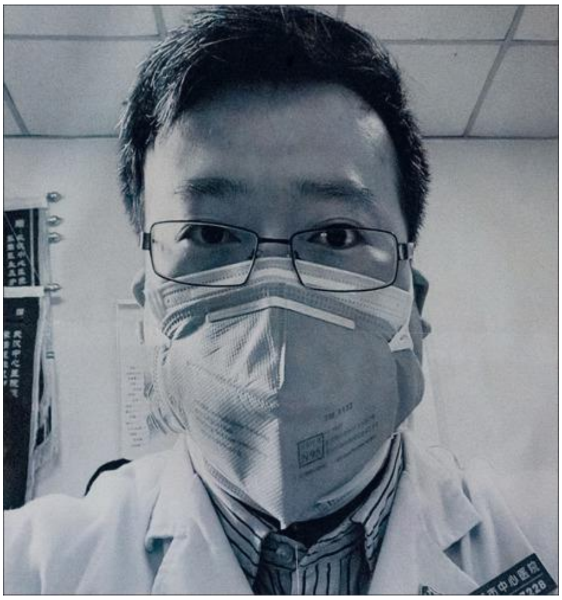
From scientific literature (see for instance [III.3]) as well as numerous media reports (see for example [IV.9]) it is known **that Chinese Medicals in Wuhan were exposed to high pressure when they tried to truthfully inform other colleagues or even the public of procedures in relation to the new COVID-19 disease**. A particularly tragic example is the doctor Wenliang Li; his fate was reported in the renowned journal "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 recognise 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 criticise 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.

The only way to access information about the true occurrences in the early phase of the pandemic – within China as well as from foreign countries – was therefore the systematic analysis of reports in Chinese social media and online platforms, whereby many pieces of information were accessible only temporarily, before they were deleted again.

In this context the great discrepancy between unofficial and official numbers regarding the infected persons and number of deaths in China in the early phase of the pandemic were striking. Very early on media in neighbouring Asian countries reported about this among other things [see for instance [IV.10], [IV.11]):

TAIWAN NEWS, 05.02.2020

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, that day reported 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 on at least three occasions posted extremely high numbers, only to quickly lower them to government-approved statistics.

新型冠状病毒肺炎
疫情实时追踪
疫情严峻，请您尽量不要聚会，避免出入公共场所
统计截至 2020-02-01 23:39:04
较昨日+20979 154023 全国确诊
较昨日+20750 79808 疑似病例
较昨日+72 269 治愈人数
24589 死亡人数

新型冠状病毒肺炎
疫情实时追踪
数据来源：全国及各地卫健委每日信息发布
数据说明 统计截至 2020-02-02 16:03:42 更新于11分钟前
较上日+2655 14446 全国确诊
较上日+1556 19544 疑似病例
较上日+108 351 治愈人数
较上日+45 304 死亡人数

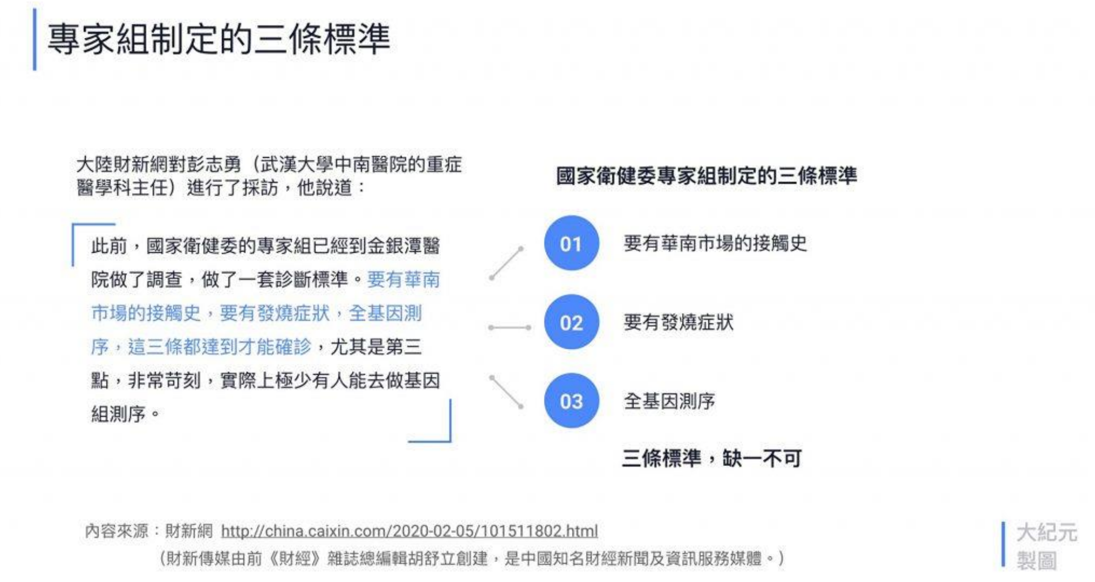
Feb. 1 chart showing higher numbers (left), chart showing "official" numbers (right). (Internet image)

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 unofficial and official numbers regarding the diagnosed coronavirus-infected and -deaths in the early phase were divergent may among others be due to the odd definition of “official coronavirus cases”. For a positive diagnosis, three requirements had to be met [IV.12]:

1) **The concerned person had to have had contact with the “Huanan seafood market”.**
2) The concerned person had to have displayed fever symptoms.
3) The diagnosis of a coronavirus infection had to be proven through gene sequencing.



專家組制定的三條標準

大陸財新網對彭志勇（武漢大學中南醫院的重症醫學科主任）進行了採訪，他說道：

此前，國家衛健委的專家組已經到金銀潭醫院做了調查，做了一套診斷標準。要有華南市場的接觸史，要有發燒症狀，全基因測序，這三條都達到才能確診，尤其是第三點，非常苛刻，實際上極少有人能去做基因組測序。

國家衛健委專家組制定的三條標準

01 要有華南市場的接觸史

02 要有發燒症狀

03 全基因測序

三條標準，缺一不可

內容來源：財新網 http://china.caixin.com/2020-02-05/101511802.html
（財新傳媒由前《財經》雜誌總編輯胡舒立創建，是中國知名財經新聞及資訊服務媒體。）

大紀元製圖

Especially the first criterion is relevant in relation to the question of the origin of the coronavirus pandemic: According to this, since the very beginning the Chinese government has postulated that the origin of the COVID-19 disease were the animal market in the centre of the city of Wuhan, that, as is well known, had been closed directly at the beginning of the year 2020 by the Chinese government. For this, however, no ensured scientific findings existed then nor until this present day, so that the first of the three above-mentioned criteria as proof of a COVID-19 infection does not make any sense from a medical perspective, however, is to be understood as a politically motivated definition.

Clearly one has to wonder, why the Chinese government declared the animal market as the unique possible explanation to the origin of the coronavirus pandemic and has since then done everything to propagate the zoonosis theory within their own country as well as to foreign countries.

The background to this is, that very early on Chinese social media provided hints and made public that "patient zero" of the COVID-19 chain of infection was a young scientist from the Wuhan Institute of Virology. Her name is Yanling Huang, born on October 20th, 1988. She has been a staff member of the Wuhan Institute of Virology since 2012 and published at least six scientific studies using this institutional address. Since the end of 2019 she is deemed as disappeared and her photograph and profile were deleted from the institutional website (as well as her personal website):

The proof that Yangling Huang was a staff member of the Wuhan Institute of Viroology can however be found on the following website which lists doctoral students of the institute including student-ID (the original website is written in Chinese; reported here is a translated version into German):

20140923 Der Abschlussstatus des Eröffnungsberichtssystems für Doktoranden 2012
gd.whiov.cas.cn/zxpy/yjsswgg/201409/t20140923_258008.html 1/2

Chinesische Akademie der Wissenschaft
Wuhan Institut für Virologie

Ihre derzeitige Position: Startseite >> Schulausbildung >> Unternehmensmitteilung

20140923 Der Abschlussstatus des Eröffnungsberichtssystems für Doktoranden 2012

Quelle: Veröffentlicht: 23.09.2014

Ordnungsnummer	Studenten ID	Name	Abschlussart	Name des Lehrers	
1	201218012415001	Chai Fan	PhD	Xiao Gengfu	Bestanden die Bewertung
2	201218012415002	Er Xuan	PhD	Yan Huimin	Bestanden die Bewertung
3	201218012415003	Feng Lipeng	PhD	Chen Shiyun	Bestanden die Bewertung
4	201218012415004	Ge Sai	PhD	Yuan Zhiming	Bestanden die Bewertung
5	201218012415005	Xie Jumin	PhD	Guan Wuxiang	Bestanden die Bewertung
6	201218012415006	Kang Zhenyu	PhD	Wang Hualin	Bestanden die Bewertung
7	201218012415007	Kuang Wenhua	PhD	Hu Zhihong	Bestanden die Bewertung

8	201218012415008	Li Xiaojun	PhD	Luo Minhua	Bestanden die Bewertung
9	201218012415009	Li Xiaodan	PhD	Zhang Bo	Bestanden die Bewertung
10	201218012415010	Peng Qin	PhD	Gao Meiying	Bestanden die Bewertung
11	201218012415011	Qiao Jinjuan	PhD	Wei Hongping	Bestanden die Bewertung
12	201218012415012	Shang Yu	PhD	Hu Zhihong	Bestanden die Bewertung
13	201218012415013	Su Lan	PhD	Sun Xiulian	Bestanden die Bewertung
14	201218012415014	Sun Manluan	PhD	Zhang Xianen	Bestanden die Bewertung
15	201218012415015	Tan Bing	PhD	Shi Zhengli	keine Aufzeichnungen
16	201218012415016	Teng Tieshan	PhD	Wei Hongping	Beim Bewertungsteam einreichen
17	201218012415017	Wang Jinpei	PhD	Zhou Ningyi	Beim Bewertungsteam einreichen
18	201218012415018	Yan Liming	PhD	Fang Qin	Bestanden die Bewertung
19	201218012415019	Dichtung	PhD	Zhang Xianen	Bestanden die Bewertung
20	201218012415020	Jae Junjie	PhD	Yuan Zhiming	Beim Bewertungsteam einreichen
21	201218012415021	Zou Jing	PhD	Yuan Zhiming	Bestanden die Bewertung
22	201218012415022	Bi Peng	PhD	Gong Peng	Bestanden die Bewertung
23	201218012415023	Chen Jungang	PhD	Chen Xulin	Bestanden die Bewertung
24	201218012415024	Hao Sujuan	PhD	Guan Wuxiang	Bestanden die Bewertung
25	201218012415025	Li Qian	PhD	Wang Hanzhong	Bestanden die Bewertung
26	201218012415026	Li Xingguang	PhD	Yang Rongge	keine Aufzeichnungen
27	201218012415028	Liu Shuhui	PhD	Chen Xinwen	Bestanden die Bewertung
28	201218012415029	Wu Guiru	PhD	Li Chaoyang	Beim Bewertungsteam einreichen
29	201218012415030	Yan Yan	PhD	Hu Qinxue	Bestanden die Bewertung
30	201218012415031	Yao Yongxuan	PhD	Chen Xinwen	Bestanden die Bewertung
31	201218012415032	Yu Jie	PhD	Yan Huimin	Bestanden die Bewertung
32	201218012415033	Zhang Mudan	PhD	Hu Qinxue	
33	201218012415034	Zheng Caishang	PhD	Wang Hanzhong	Bestanden die Bewertung
34	201218012415035	Zhou Ming	PhD	Hu Kanghong	Bestanden die Bewertung
35	201218012415036	Wang Zhilong	PhD	Tang Hong	Bestanden die Bewertung
36	201228012415001	Chen Xiuxiu	Master-Studium	Zhang Xianen	Bestanden die Bewertung
37	201228012415002	Shi Chenyan	Master-Studium	Yuan Zhiming	Bestanden die Bewertung
38	201228012415003	Wang Mingxiu	Master-Studium	Cui Zongqiang	Bestanden die Bewertung
39	201228012415005	Yan Shicui	Master-Studium	Fang Qin	Bestanden die Bewertung
40	201228012415007	Zhou Yu	Master-Studium	Zhou Ningyi	Bestanden die Bewertung
41	201228012415009	Chen Yajun	Master-Studium	Gao Meiying	Bestanden die Bewertung
42	201228012415010	Feng Lianwei	Master-Studium	Yang Rongge	Bestanden die Bewertung
43	201228012415012	Er Hui	Master-Studium	Zhou Ningyi	Bestanden die Bewertung
44	201228012415013	Huberdan	Master-Studium	Hu Qinxue	Bestanden die Bewertung
45	201228012415014	Huang Yanling	Master-Studium	Wei Hongping	Bestanden die Bewertung
46	201228012415015	Jiang Liangyu	Master-Studium	Chen Xulin	Bestanden die Bewertung
47	201228012415016	Liu Lili	Master-Studium	Wang Yanyi	Bestanden die Bewertung
48	201228012415019	Meng Xiangzheng	Master-Studium	Deng Jiaoyu	Bestanden die Bewertung
49	201228012415021	Shi Jing	Master-Studium	Li Chaoyang	Bestanden die Bewertung

50	201228012415023	Wang Bo	Master-Studium	Shi Zhengli	Bestanden die Bewertung
51	201228012415028	Xu Hao	Master-Studium	Wang Hualin	Bestanden die Bewertung
52	201228012415029	Yang Bo	Master-Studium	Luo Minhua	
53	201228012415031	Zhang Weihong	Master-Studium	Tang Hong	Beim Bewertungsteam einreichen
54	2012E8012461033	Gao Yutao	Master-Studium	Shi Zhengli	Bestanden die Bewertung
55	2012E8012461034	Hou Shoucai	Master-Studium	Sun Xiulian	Bestanden die Bewertung
56	2012E8012461035	Wang Jing	Master-Studium	Wei Hongping	Bestanden die Bewertung
57	2012E8012461036	Wang Yifei	Master-Studium	Chen Shiyun	In Bewertung
58	2012E8012461037	Phasenstern	Master-Studium	Hu Xiaomin	Bestanden die Bewertung
59	2012E8012461038	Xiong Chaochao	Master-Studium	Chen Jianjun	Beim Bewertungsteam einreichen
60	2012E8012461039	Yao Weitong	Master-Studium	Yang Rongge	Bestanden die Bewertung
61	2012E8012461040	Zhao Bali	Master-Studium	Yan Huimin	Bestanden die Bewertung
62	2012E8012461042	Zhu Zheng	Master-Studium	Hu Zhihong	Bestanden die Bewertung
63	2012E8012461043	Wen Lei	Master-Studium	Simon Rayner	Bestanden die Bewertung
64	2012E8012461044	Ma Ruipeng	Master-Studium	Sun Xiulian	Bestanden die Bewertung
65	2012E8012461045	Mei Xiaofen	Master-Studium	Yuan Zhiming	In Bewertung
66	2012E8012461046	Xu Ting	Master-Studium	Gong Rui	Bestanden die Bewertung
67	2012E8012461049	Zhao Kaitao	Master-Studium	Chen Xinwen	Beim Bewertungsteam einreichen

Wuhan Institut für Virologie, Chinesische Akademie der Wissenschaften Alle Rechte vorbehalten Seriennummer des Datensatzes: Hubei ICP-Datensatz 05001977 Adresse: Nr. 44 Mittlerer Distrikt Xiaohongshan, Distrikt Wuchang, Stadt Wuhan, Provinz Hubei Postleitzahl: 430071 E-Mail: wiv@wh.iov.cn

Also in the year of 2018 Yanling Huang was still at the Wuhan Institute of Virology, as a group photograph from this year is proving:

Using the following link [IV.13] a comprehensive report concerning the fate of Yanling Huang and the background of her disappearance as well as numerous further supporting documents of evidence can be found:

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

Further, an existing website concerning the subject "Where is Huang Yan Ling?" provides additional information and backgrounds:

https://twitter.com/whereisyanling

Despite the severity of accusations that were made in Chinese as well as international social media and online platforms, neither the laboratory head Zheng-Li Shi, nor an official representative of the Wuhan Institute of Virology were willing to provide information concerning the stay of Yanling Huang. Though the Chinese government officially denied the "rumours" around Yanling Huang, it refused any type of statement regarding the remaining of the young scientist.

Given the fact that in the early phase of the pandemic scientists, doctors, journalists as well as private persons in China were pressed by the Chinese government to make false statements concerning the backgrounds of the COVID-19 disease (see for instance [III.3], [IV.14]) or even disappeared without leaving any trace (see for instance [IV.6], [IV.15]), it is incomprehensible for numerous scientists, that a few virologists have praised "the fast, open and transparent" information policy from the Chinese side within the frame of a joint statement [III.4]. In actual fact, not only people like Yanling Huang [IV.13] and Fang Bin [IV.15] disappeared, but also important samples from the research have been withheld [see for example [IV.16], [II.1]), or more specifically been destroyed by order of the "Health and Medical Commission of Hubei Province" in the beginning of January, 2020.

The statement of the group of virologists reads 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, Mike Turner

We are public health scientists who have closely followed the emergence of 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 rumours 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 analysed 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, rumours, and prejudice that jeopardise 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 frontline!

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 needs to be pointed out here, that people from this group of authors – as is the case with Peter Daszak – have themselves personally been involved in “gain-of-function”-experiments in the past and have been researching and publishing together with the group around Zheng-Li Shi at the Wuhan Institute of Virology for years. This will be discussed in more detail in the later chapter regarding “gain-of-function research”.

Furthermore, it is worth mentioning that the statement: “Scientists from multiple countries have published and analysed 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” cannot remain as it stands without the indication that in the meantime at least as many scientists from many different countries, among which Nobel Prize winners exist, who - based on the genetic fingerprints of the new SARS-CoV-2 virus - came to contrary conclusions [see for instance: : [I.11], [II.5], [II.6], [II.7], [II.8]).

In conclusion, it can be noted that there are many indications that suggest a laboratory accident at the Wuhan Institute of Virology seems by far the most likely cause of the coronavirus pandemic. In this case, it would not be a “natural catastrophe”, however a manmade tragedy. A great danger consists in declaring the question of the origin of the pandemic as “solved”, as for instance in the statement [III.4] of a few virologists. For decision-makers it indisputably makes a difference whether they shall forbid animal markets or high-risk research that includes biotechnologically manipulated viruses with a pandemic potential, worldwide. This question must be brought to the fore, otherwise corona- and other types of viruses could develop a by far greater potential of danger to mankind, not only in the present, but also in the future.

3 Pre-History of the Coronavirus Pandemic: Research and Biotechnological Manipulation of Coronaviruses from Bats at the Institute of Virology in Wuhan, China

In earlier coronavirus-caused diseases, as for instance SARS (2003), mutations of coronaviruses originating from bats have taken place in an intermediate host animal, so that a subsequent transmission to the human became possible. A direct transmission of coronaviruses from bats to humans has to date not been known of. In this context, virologists are speaking of an "adaptation barrier". It was therefore of utmost importance to identify the intermediate host animal in question for various coronavirus related diseases through intensive research.

Striking in this current pandemic is the comparison to previous outbreaks of coronavirus diseases:

1) In this current pandemic we are dealing with a coronavirus **that attacks human cells with a previously unknown efficiency**.
2) Thereby not only the (upper) respiratory tract is **attacked, but also inner organs and their functions are partially severely damaged**.

One must necessarily ask the question how such a **nearly perfect adaptation of coronaviruses to human receptor cells** could take place, in order to identify future danger potential for pandemics.

In the following, a closer look will be taken at the pre-history of the coronavirus pandemic. As documented through numerous publications in scientific journals, the research team around Zheng-Li Shi at the Wuhan Institute of Virology collected bat viruses from caves in various southern Chinese provinces over many years and brought these to Wuhan. The research team did not only study the naturally occurring coronaviruses scientifically, but deliberately manipulated these with the aim of making the coronaviruses more contagious and dangerous for humans. This so-called "gain-of-function" research at the Wuhan Institute of Virology is proven through multiple scientific original publications in peer-reviewed journals and has been seen critically by many representatives of the scientific community for many years.

In a publication of the journal "NATURE" in 2013 [I.7] the research team around **Zheng-Li Shi** and **Peter Daszak** report about the successful docking of the spikes of the coronavirus-crown to human ACE2-cell receptors. Hereby, the so-called horseshoe bats from the Chinese province of Yunnan were used as source for the SARS-like coronaviruses. The essential part of this publication is reported 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

Center for Emerging Infectious Diseases, State Key Laboratory of Virology, Wuhan Institute of Virology of the Chinese Academy of Sciences, Wuhan, 430071, China

Xing-Yi Ge, Jia-Lu Li, Xing-Lou Yang, Ben Hu, Wei Zhang, Cheng Peng, Yu-Ji Zhang, Chu-Ming Luo, Bing Tan, Ning Wang, Yan Zhu & Zheng-Li Shi

EcoHealth Alliance, New York, 10001, New York, USA

Aleksei A. Chmura, Guangjian Zhu, Jonathan H. Epstein & Peter Daszak

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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

Shu-Yi Zhang

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

Lin-Fa Wang

Abstract

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. Diverse 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 SARS[1] 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 classed as positive by PCR and subsequently confirmed by sequencing. The species origin of all positive samples was confirmed to be *R. sinicus* by cytochrome b sequence analysis, as described previously[16]. A higher prevalence was observed in samples collected in October (30% in 2011 and 48.7% in 2012) than those in April (7.1% in 2011) or May (7.4% in 2012). 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 ($<10^{-20}$) 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 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 study has among others been commented by colleagues of 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 hunting

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Abstract

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 human (Ge X Y, 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 T G, 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 H D, 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 S K, 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) albeit 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 infect human cells, only when its RBD is replaced with the counterpart from a human SARS-CoV strain (Li W, et al, 2003; Becker M M, 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 publication from the research team around **Zheng-Li Shi** and **Ralph Baric** was released in the journal “NATURE MEDICINE”, documenting that **biotechnological modification of coronaviruses from horseshoe bats are leading to new artificially created “hybrid viruses”, which can extremely efficiently dock to human airway cells [I.8].** The researchers created a chimeric virus consisting of the surface protein of a bat virus called SHC014 and the backbone of a SARS-coronavirus. The chimeric virus infects human airway cells and provides proof that the surface protein of SHC014 has the structure needed to very efficiently dock to human key receptor cells and to infect these. The essential part of this publication is provided 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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Abstract

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 upon studies published as early as 2008 and 2010 by the Wuhan research group around Zheng-Li Shi in the “Journal of Virology” ([I.5], [I.6]) in which it was shown how **by using biotechnological manipulation, viruses can be changed to specifically infect human cells by using an HIV-based pseudovirus**. The essential part of these two publications are documented 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, 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 *Coronavirus*. 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 so far are 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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Abstract

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 progenitor viruses. 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 hACE2-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 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 following, a **heated debate sparked among scientists regarding whether knowledge gained through such experiments justifies the potential risk of a pandemic**. A well-known virologist of the “Institut Pasteur” in Paris stated that the researchers of the Wuhan institute had created a new type of virus that reproduces remarkably well in human cells and added: **“If the virus escaped, nobody could predict the trajectory”.** A molecular biologist from Rutgers University added: **“The only impact of this work is the creation, in a lab, of a new, non-natural risk”**. The debate of that time was picked up and commented on in numerous publications of scientific journals and in the media. Two examples are reported 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 modelling 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.

Jef 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 quite heavily conducted debate and the warning of a worldwide pandemic through numerous representatives of the scientific community, the group around Zheng-Li Shi at the Wuhan Institute of Virology continued their highly risky research regarding biotechnologically modified coronaviruses in cooperation with Peter Daszak, as documented in both following studies from the years 2017 and 2018 ([I.9], [I.10]). Thereby, methods of biotechnological manipulation established for many years already, were applied, as is apparent in the study [I.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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Yunnan Institute of Endemic Diseases Control and Prevention, Dali, China;
Dali University, Dali, China;
EcoHealth Alliance, New York, New York, United States of America;
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Abstract

A large number of SARS-related coronaviruses (SARSr-CoV) have been detected in horseshoe bats since 2005 in different areas of China. However, these bat SARSr-CoVs show sequence differences from SARS coronavirus (SARS-CoV) in different genes (S, ORF8, ORF3, *etc*) and are considered unlikely to represent the direct progenitor of SARS-CoV. Herein, we report the findings of our 5-year surveillance of SARSr-CoVs in a cave inhabited by multiple species of horseshoe bats in Yunnan Province, China. The full-length genomes of 11 newly discovered SARSr-CoV strains, together with our previous findings, reveals that the SARSr-CoVs circulating in this single location are highly diverse in the S gene, ORF3 and ORF8. Importantly, strains with high genetic similarity to SARS-CoV in the hypervariable N-terminal domain (NTD) and receptor-binding domain (RBD) of the S1 gene, the ORF3 and ORF8 region, respectively, were all discovered in this cave. In addition, we report the first discovery of bat SARSr-CoVs highly similar to human SARS-CoV in ORF3b and in the split ORF8a and 8b.

Moreover, SARSr-CoV strains from this cave were more closely related to SARS-CoV in the non-structural protein genes ORF1a and 1b compared with those detected elsewhere. Recombination analysis shows evidence of frequent recombination events within the S gene and around the ORF8 between these SARSr-CoVs. We hypothesize that the direct progenitor of SARS-CoV may have originated after sequential recombination events between the precursors of these SARSr-CoVs. Cell entry studies demonstrated that three newly identified SARSr-CoVs with different S protein sequences are all able to use human ACE2 as the receptor, further exhibiting the close relationship between strains in this cave and SARS-CoV. This work provides new insights into the origin and evolution of SARS-CoV and highlights the necessity of preparedness for future emergence of SARS-like diseases.

Author summary

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

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

Fatal swine acute diarrhoea 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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Abstract

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 diarrhoea 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 μg ml^{-1}), penicillin G (100 units ml^{-1}) and streptomycin (50 μg ml^{-1}). 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 3,000*g* 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 predetermine 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, X. Y. 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 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 Group).

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, X. Y. 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 μg of each pHIV-Luc plasmid (pNL4.3.Luc.R-E-Luc) and the S-protein-expressing plasmid (or empty vector control) were co-transfected into 4 × 10^6 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 3,000*g*, then passed through a 0.45-μm filter (Millipore). The filtered supernatants were stored at −80 °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 μl 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 μl of the lysates was taken for determining luciferase activity after the addition of 50 μl 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 were not only the research activities of the group around Zheng-Lin Shi at the Wuhan Institute of Virology regarding coronaviruses, but also research activities of other groups regarding other types of viruses, who aimed to make **naturally occurring viruses more contagious, more dangerous and more lethal for humans through biotechnological manipulation**. This **“gain-of-function” research** and the related heavy dispute between different representatives of the scientific community shall be described in more detail in the following chapter.

4 „Gain-of-Function Research“: International Debate About the Risk of Research Concerning Manipulation of Viruses with Regard to Higher Transmissibility, Danger for Humans and Death Rates

The debate about the possible gain but also the danger associated with research aiming at virus manipulation in order to make the virus more contagious, more dangerous, and – in the end - more lethal, started in 2011. This debate was primarily triggered by two scientific studies of international research groups that demonstrated, how biotechnological manipulation of H5N1-viruses (pathogen of the avian flu) can be made more contagious for humans [I.13, I.14]. These two studies from the research teams around Yoshihiro Kawaoka and Ron Fouchier, published in the journals “NATURE” and “SCIENCE” in 2012, shall be displayed in parts below:

***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

Abstract

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 avian–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.

…

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 J. A. Schrauwen, Martin Linster, Salin Chutinimitkul, Emmie de Wit , Vincent J. Munster , Erin M. Sorrell, Theo M. Bestebroer, David F. Burke, Derek J. Smith , Guus F. Rimmelzwaan, Albert D. M. E. Osterhaus, Ron A. M. Fouchier

Abstract

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.

…

Already before the official release of these two publications, an intensive discussion and **extremely controversially conducted debate among scientists and politicians** existed regarding whether such reports of research findings and “gain-of-function” research activities themselves should be entirely banned in the future. Already then, apprehensions related to the **nightmare of a possible pandemic caused by accidental leaking of artificially created viruses from biotechnological laboratories and with incalculable danger potential for humanity**, existed.

A few examples from scientific journals [III.6- III.9] that are giving a good insight into the discussion at that time, are presented in the following:

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 spectre of a disease that spreads as fast 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 explain better the goals and potential public health benefits of the research, and to detail the extensive safety and security measures taken to protect laboratory workers and the public."

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 recognise 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 scepticism, 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 favour 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.

…

Nature 485, 431–434 (24 May 2012), doi:10.1038/485431a
***NATURE* | NEWS 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 17–18 December 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 Centre 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 the year 2012 numerous international workshops addressing the risks of "gain-of-function" research were held. A **moratorium for this type of research** existed initially for one year (from January 2012 until January 2013). In October 2014 the American government under Barack

Obama announced a **ban of “gain-of-function” research in the USA**, based on 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.

Sara 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 unsure 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 halt 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 director Dr. Anthony Fauci, together with the NIH (National Institute of Health), awarded a 5-year project amounting to 3.7 million USD under the title “Understanding the Risk of Bat Coronavirus Emergence“ to Peter Daszak (Ecohealth Alliance, Inc.).

In the following, information from the official website about the third-party funds is provided:

Project Information

2R01AI110964-06

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

Abstract Text:

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 diverse 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 of 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 analyses 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 analyses 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 analyses will be critical for the future development of public health interventions and enhanced surveillance to prevent the re-emergence of SARS or the emergence of a novel SARSr-CoV.

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 Spending Category:

Biodefense; Biotechnology; Clinical Research; Emerging Infectious Diseases; Infectious Diseases; Lung; 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 Analyses; Development; Disease Outbreaks; epidemiologic data; Epithelial Cells; experimental study; exposed human population; exposure route; Exposure to; Family suidae; follow-up; food security; Future; genetic element; Genome; Geographic 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; Lead; Maps; Middle East Respiratory Syndrome Coronavirus; Modeling; 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; Risk; Risk Behaviors; Risk Factors; sample collection; Sampling; SARS coronavirus; screening; Serologic tests; Serological; seropositive; Severe Acute Respiratory Syndrome; Site; Surveys; Syndrome; syndromic surveillance; Technology; Testing; Therapeutic Intervention; Therapeutic Monoclonal Antibodies; therapeutic vaccine; Time; trait; Transgenic Organisms; Vaccines; Viral; virology; Virus; Work; Zoonoses

These research activities of Peter Daszak were not ceased during the period of the ban of “gain-of-function” research through the Obama-government, but were at least partially outsourced to the Wuhan Institute of Virology through a cooperation with the research team around Zheng-Li Shi [IV.17]. This happened with knowledge and in agreement with the NIAID-director Dr. Anthony Fauci.

In fact, probably much more money has flowed to Peter Daszak and his “EcoHealth Alliance” for “gain-of-function” experiments, as has recently been published [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 centre of the COVID-19 pandemic in many ways.

To depict the pandemic in such militarized terms is, for Daszak, a commonplace. In an 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 U.S. 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 hypothesese as “conspiracy theory.” Daszak was revealed to have orchestrated the letter such as to “avoid the appearance of a political statement.”

…

As can be extracted from the partially provided article above, Peter Daszak was appointed member of the investigative commission of the WHO for clarification of the question regarding the origin of the coronavirus pandemic. This was met with incomprehension among the scientific community, as an **unequivocal conflict of interest** exists, particularly because Peter Daszak himself had been involved in the “gain-of-function” research activities at the Wuhan Institute of Virology for many years (see for instance [III.1]).

In Europe as well, an **intensive dispute among scientists endorsing and wanting to continue “gain-of-function” experiments, and those who saw a far too great risk potential regarding the possibility of a worldwide pandemic,** existed. The two following articles exemplify an impression of the discussion in Europe at this 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/nature2013.14429, 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 seperate 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 licence 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 the 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.

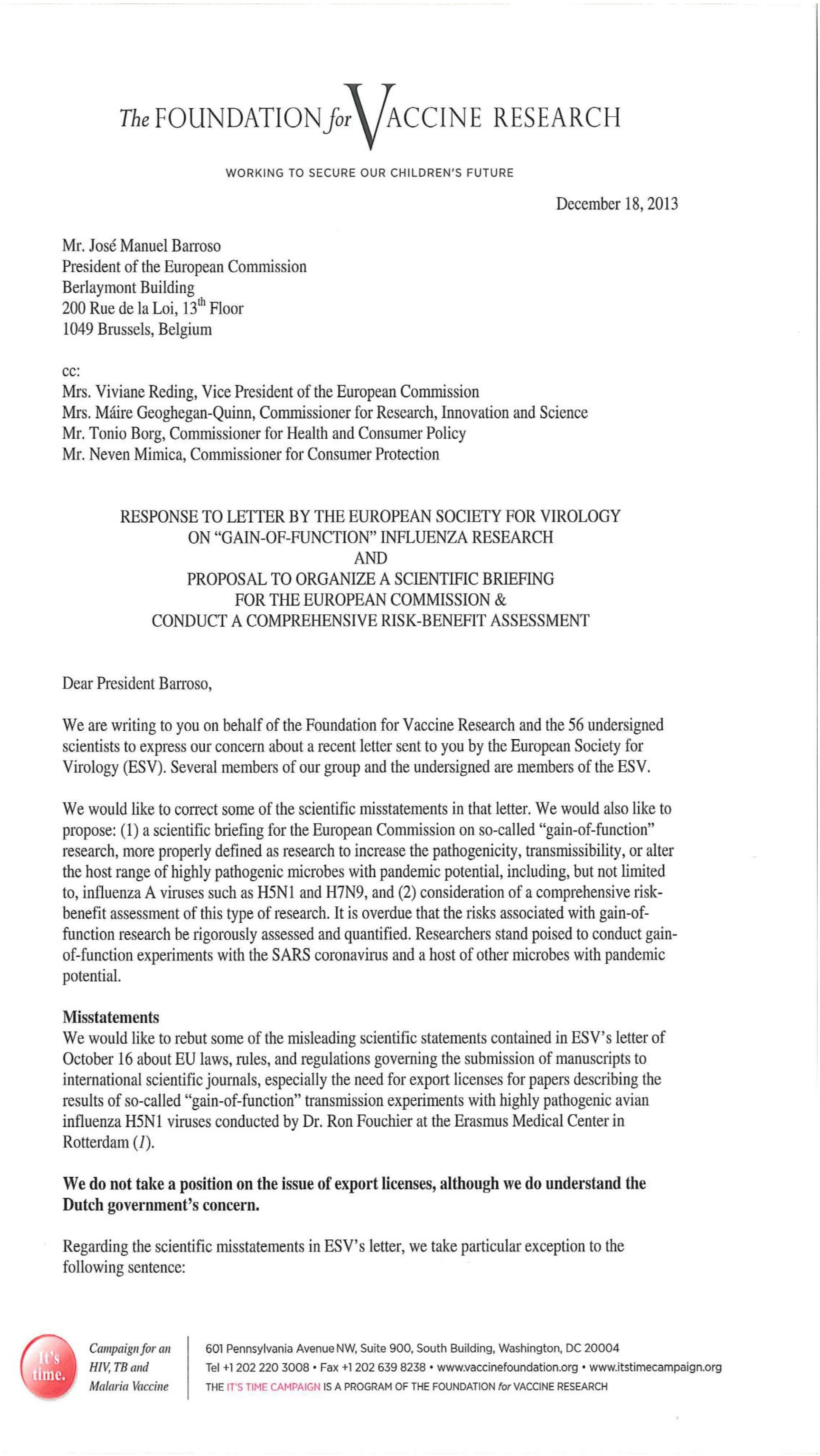
Palù says that the letter from Wain-Hobson and signatories misses the crux of the ESV's concerns. "We don't want to enter into the scientific quarrel," says Palù. "Our intent was just to say that the export legislation is not the proper way to deal with this research."

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 centred 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."

…

As is apparent from the above-inserted report, a group of 56 scientists had turned to the president of the European Commission of that time José Manuel Barroso, on December 18th, 2013, requesting him to evaluate the risks associated with biotechnologically modified viruses that can be more lethal to humans than naturally occurring viruses. **Due to the importance of this writing for the political discussion concerning "gain-of-function" research in Europe, this letter shall be reported in its full length:**



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.

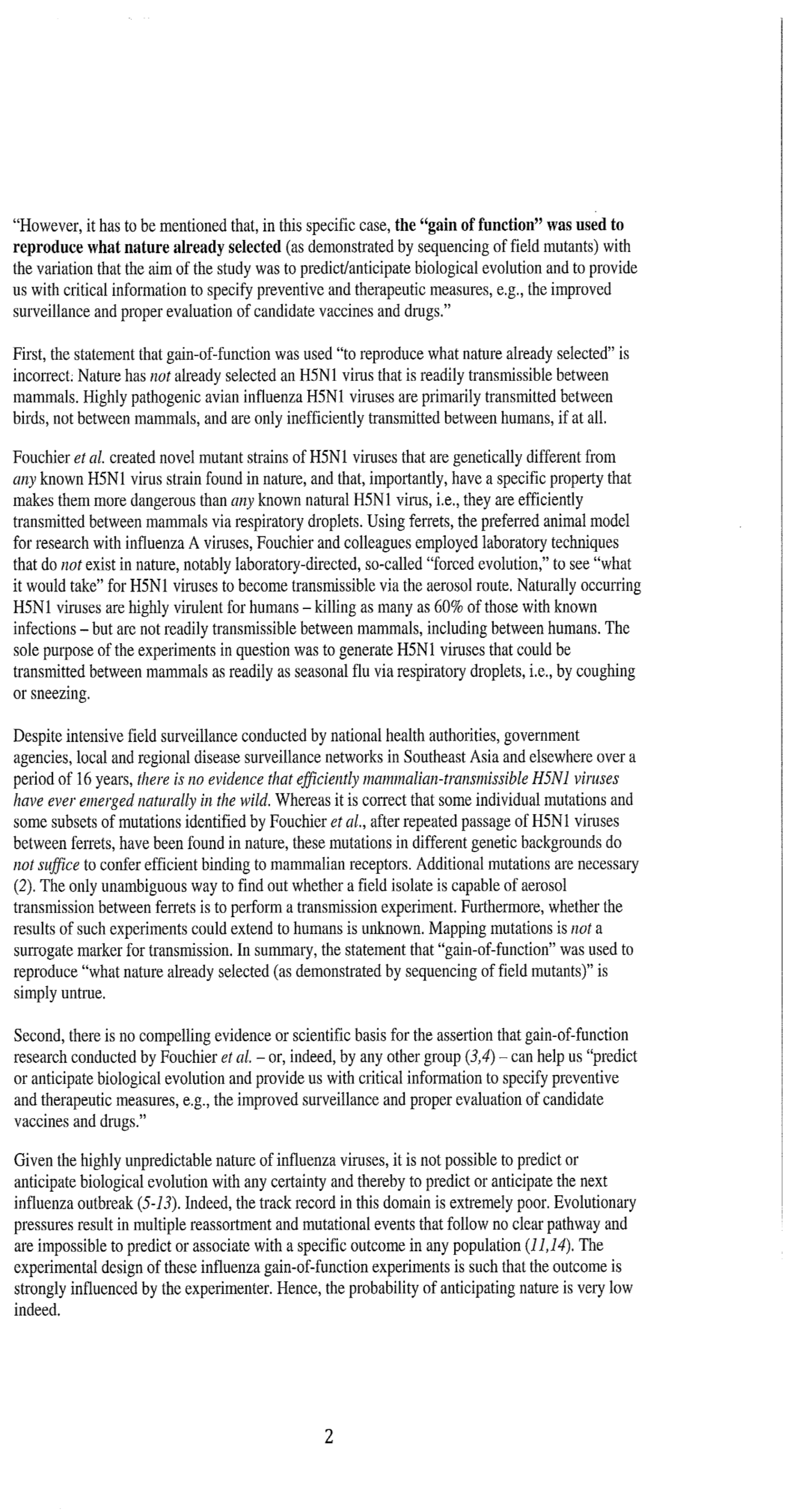
Misstatements

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.

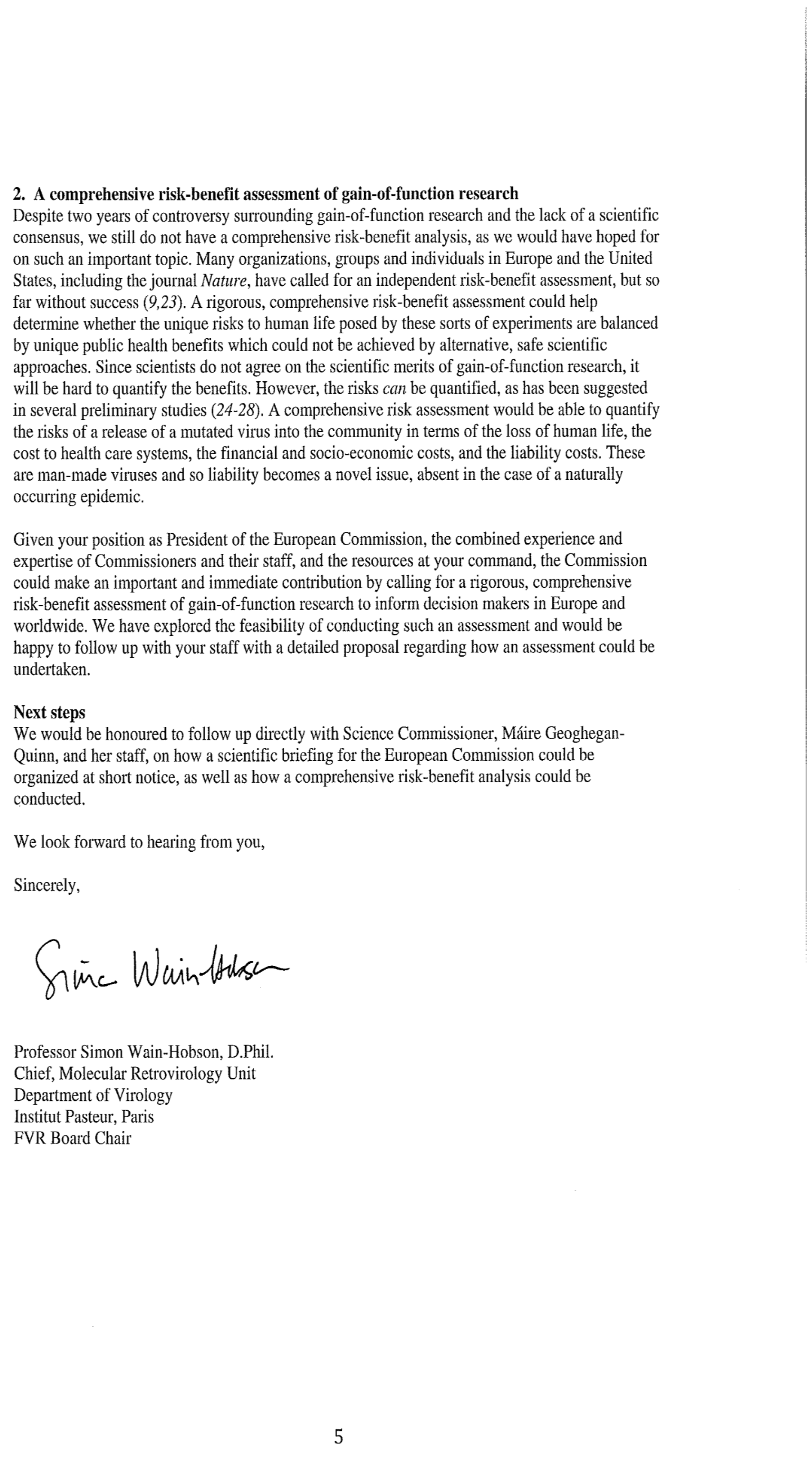
For this reason, we are primarily concerned about the safety of gain-of-function research and the consequences of an accidental release. We are in a situation where the probabilities of a laboratory accident that leads to global spread of an escaped mutated virus are small but finite, while the impact 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,

Simon Wain-Hobson

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

This letter clearly reveals how different the estimation of the risk potential of "gain-of-function" research was among virologists already back then. Among the 56 signatories of the writing were the **three Nobel laureates Harald zur Hausen, Richard Ernst und Sir Richard Roberts.**

It needs to be noted, that – independent from each standpoint – the coronavirus research program did NOT prevent the current pandemic. Justifiably, one may ask what the sense of this high-risk research really is, next to the fact that the research itself poses a danger potential for the global population.

How justified the concerns of the signatories were, is convincingly proved by the high number of accidents occurring in biotechnological laboratories even of highest security level. This shall be the point of focus in the next chapter.

5 How Safe are High-Safety Laboratories Researching Dangerous Pathogens?

De facto, the danger coming from biotechnological laboratories even from those with the highest security level, is not to be underestimated, as numerous reports have proven in the past as well as presently in various countries. Two examples of such reports are provided 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 commonplace. 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 (R. D. 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 A. P. 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 centres 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 C.D.C. 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 “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 C.D.C. 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 C.D.C. 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.

…

Already two years prior to the outbreak of the coronavirus pandemic, warnings regarding the security risks of the Wuhan Institute of Virology existed, as apparent from reports by US diplomats in China. A corresponding commentary is displayed 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, U.S. Embassy officials visited a Chinese research facility in the city of Wuhan several times and sent two official warnings back to Washington about inadequate safety at the lab, which was conducting risky studies on coronaviruses from bats. The cables have fueled discussions inside the U.S. government about whether this or another Wuhan lab was the source of the virus — even though conclusive proof has yet to emerge.

In January 2018, the U.S. Embassy in Beijing took the unusual step of repeatedly sending U.S. science diplomats to the Wuhan Institute of Virology (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 U.S. delegation was led by Jamison Fouss, the consul general in Wuhan, and Rick Switzer, the embassy’s counselor of environment, science, technology and health. Last week, WIV erased that statement from its website, though it remains archived on the Internet.

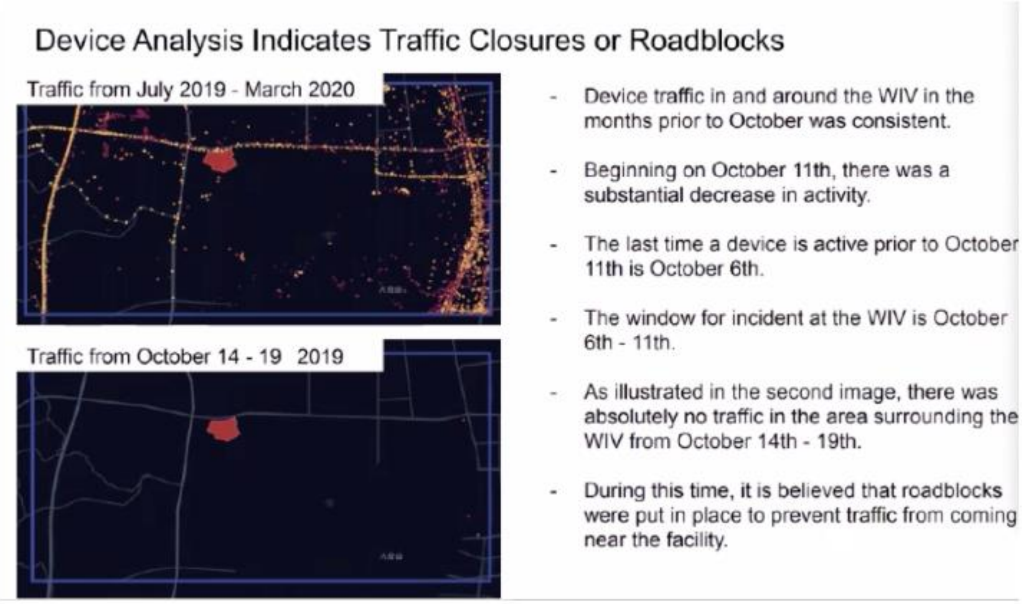
…

Even after the outbreak of the coronavirus pandemic, evidence of severe safety deficiencies at the Wuhan Institute of Virology became public. Chinese journalists for instance have video recorded and uploaded recordings of the institute premises, evidencing inappropriate disposal of laboratory waste (see for instance [IV.20] in particular the film segment starting at 8:15):

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

Further, photographs and video recordings of researchers of the Wuhan Institute of Virology became public, which show that no or insufficient protective gear was worn during the collection of bat samples as well as during analyses in the laboratory (see for instance [IV.21]).

An analysis of the activity of mobile phone use in and around the Wuhan Institute of Virology in the second half of the year 2019 indicates that a temporary interruption of the laboratory operations as well as a blocking around the institutional site took place in the first half of October 2019 [IV.22], see following figure:



Device Analysis Indicates Traffic Closures or Roadblocks

Traffic from July 2019 - March 2020

Traffic from October 14 - 19 2019

- Device traffic in and around the WIV in the months prior to October was consistent.
- Beginning on October 11th, there was a substantial decrease in activity.
- The last time a device is active prior to October 11th is October 6th.
- The window for incident at the WIV is October 6th - 11th.
- As illustrated in the second image, there was absolutely no traffic in the area surrounding the WIV from October 14th - 19th.
- During this time, it is believed that roadblocks were put in place to prevent traffic from coming near the facility.

Simultaneously, first confirmed cases of COVID-19 infections leading to death existed in various hospitals of the city of Wuhan as early as in October 2019 [IV.2]. Which therefore suggests itself, that the barriers around the Wuhan Institute of Virology are related to the investigation of the source of these cases of disease, particularly as indications that the first

COVID-19 afflicted was an employee of this institute, circulated within Chinese social media at this time (see chapter "The central question of the origin of the coronavirus pandemic: natural catastrophe or laboratory accident?").

Naturally the question arises, why the Wuhan Institute of Virology as most probable source of origin of the coronavirus pandemic should be out of focus by all means through the Chinese government. By now, many representatives from science and politics (see for instance [II.9], [IV.23]) suppose a **connection between scientific high-risk research of bat viruses and military interest**. As a matter of fact, this **"dual-use" possibility of "gain-of-function" research** has been discussed in scientific and political committees for many years. That there is a connection between this type of research and military interest is not a "conspiracy theory", but in contrast documented through numerous co-authorships within scientific literature. Two examples for this are presented 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, 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 analyses 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 significant different genic 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 diverse alphacoronaviruses and a close relative (LYRa11) to SARS CoV in bats collected in Yunnan, China. Further analysis showed that alpha- and betacoronaviruses 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 analyses indicate that LYRa11 is a recombinant from certain bat SARS-like CoVs circulating in Yunnan Province.

…

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 He [2], Yi Wang [3], Fuqiang Ye [2], Lu Yang [2], Chenxi Ding [2], Xuhui Zhu [2], Ruicheng Lv [2], Jin Zhu [2], Bachar Hassan [4], Youjun Feng [5], Weilong Tan [6], Changjun Wang [7 8]

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Abstract

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 analyses 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 **„Biosecurity“** has gained increasing importance in previous years particularly due to the fact that high-risk research and the development of biological weapons often go hand in hand, and represent a **substantial danger for the health of the global population** (see for example [II.10]):

Biosecurity and the Risk to Global Health

Christian Enemark

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 defences 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 disastrous as the impacts of atomic bombing, accidents in atomic reactors, or the use of chemical weapons have been in the past, the impacts ultimately were regionally restricted. The current coronavirus pandemic, however, shows us, which sort of dangers related to released pathogenic agents really exist globally for the entire world population. Future international conventions must therefore focus more intensely on risk potentials of B-type (next to A- and C-types).

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

Scientific findings, analyses and predictions play a central role in the coronavirus pandemic. The high importance of science for the society in times of the corona-crisis is emphasised among others in statements of numerous scientific expert associations [IV.24].

In the current pandemic, the trustful mediation of scientific findings is essential for the acceptance of necessary measures to contain the spreading of the virus, as well as the protection of risk groups. Thereby, regarding the science communication, what matters and is important, is to reduce the complexity of scientific circumstances in a way that their essential contents do not get lost and are understandable to the population.

Various ways of information dissemination from the side of science to the broad public have been used since the beginning of the pandemic. Included are science programmes in the television, radio broadcasts, talk-shows, as well as articles in newspapers, magazines, and online media. The success of this extensive efforts of scientific communication in the past months can among others be seen in results of surveys conducted among the general public [IV.25]: 77 percent of the interviewed in Germany declared feeling well-informed regarding the coronavirus pandemic, and 73 percent of the interviewed accept the state-imposed measures for the containment of the coronavirus pandemic.

The general trust of the German population into science and research has considerably increased during the time of the coronavirus pandemic: from approximately 50 percent before the pandemic, to 73 percent in May 2020 [IV.25]. Almost 90 percent of the interviewed believe that scientific findings are important in order to slow down the spreading of the coronavirus pandemic. And finally, 81 percent of the interviewed think that political decisions when dealing with the coronavirus pandemic should be based on scientific findings [IV.25].

Each representative of the scientific community currently appears delighted and uses the opportunity of the moment, to point out the necessity of further expansion of scientific education and research [IV.24].

The question in this regard however is, how much this positive development from the viewpoint of science could be endangered, if the origin of the coronavirus pandemic is not a zoonosis (and therefore comparable to a natural catastrophe), but in contrast the result of an accident in a biotechnological laboratory of a scientific institute of virology in the city of Wuhan in China. How would the general mood of the population change in Germany, but also worldwide, if the current worldwide crisis were not the result of a coincidence of nature – a mutation of a coronavirus from a bat by means of an intermediate host animal – but rather the result of negligence of a scientist while conducting high-risk research with worldwide pandemic-potential [IV.26]? What kind of questions regarding the responsibility of science would come up in light of the dimension of the current worldwide catastrophe? Would there not be demands for immediate cease of such type of research? How many scientific laboratories worldwide would have to risk being closed due to an immense public and political pressure? Would this be a scenario that if necessary, ought to be excluded by scientists themselves? **What kind of**

influence would this have onto the necessary clarification of the important question about the origin of the coronavirus pandemic? Can science itself remain unbiased faced to this question? Are there any indications that this may not have been the case anymore for a considerable time already?

It is undoubtedly astonishing to what extent some well-known virologists have made public statements (see among others [IV.1], [IV.3]) very early on determining the animal market of Wuhan as the source of the SARS-CoV-2 pathogen, while repeatedly new assumptions were expressed regarding the possible intermediate host animal (among others snakes, civets, pangolins, raccoon dogs). Until now a zoonosis could not scientifically be proven. That the Wuhan Institute of Virology in which verifiably – this means documented through existing scientific literature – high-risk "gain-of-function" research involving biotechnological manipulation of coronaviruses was conducted for years, could also be a possible source of the SARS-CoV-2 pathogen, was precluded by a few virologists since the very beginning despite any existing scientifically sound reason. Without having proof for one or the other theory, it would be a law of science to take a neutral, which means unbiased position. Astonishingly this is not the case.

In relation to the hypothesis of the laboratory as origin of the coronavirus pandemic, the media spoke of a "conspiracy theory", however, without justifying why this from scientific perspective absolutely plausible assumption regarding the origin of the pandemic, has the character of a "conspiracy".

Strange is also the statement of 27 scientists [III.4] published in the journal "The Lancet" in which the signatories declare 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 rumours 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 in this publication no scientific proof that the SARS-CoV-2 pathogen does not have its origin in the Wuhan Institute of Virology is provided, the affirmation of a "transparent" information policy from the Chinese side obviously contradicts the evidence base (see among others [III.3], [IV.6]-[IV.12], [IV.14], [IV.15]).

Even more curious is, that the scientific publications of the research team around Zheng-Li Shi from the Wuhan Institute of Virology which appeared in journals of the NATURE publishing group and which document deliberate biotechnological manipulation of coronaviruses with regard to an increased transmissibility and dangerousness for humans, as well as commentary articles that refer to this, were later labeled with the following remark:

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 of the hitherto highly regarded scientific publishing group **SpringerNature** has triggered incomprehension in the scientific community in many ways:

- The sentence "scientists believe…" is unsustainable in this form, as the existence of **plurality of opinions among scientists** with regard to the origin of the coronavirus pandemic, has been proven and documented through many publications. The sentence could at best have been worded „some scientists believe…".
- Further, alone the reason that **science is based on verifiable facts and not on what a subset of scientists believe,** the wording "scientists believe…" is inappropriate for a scientific journal.

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

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

Leading Western Publisher Bows to Chinese Censorship

Javier C. Hernández

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 scientific magazine "Scientific American", which is also published by the SpringerNature publishing house, the head of the coronavirus research program at the Wuhan Institute of Virology Zheng-Li Shi is presented as scientific pioneer and hero by the Chinese author [IV.28]. No indication regarding the pre-history of critical discussions concerning the risk and the dangers accompanying the "gain-of-function" research conducted at the Wuhan institute can be found. The article concludes 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 systematically sample viruses in bat caves – with much greater scope and intensity than the team’s previous attempts“. An unanswered question however remains, asking whether the global community wants to accept a danger for further coronavirus-caused pandemics that is 5.000 times greater, regardless of the origin of the SARS-CoV-2 virus.

While scientific literature has been propagating intensively the version of the animal market as source of the SARS-CoV-2 virus for months, deviating results of scientific studies have been simultaneously suppressed by the use of various strategies. A research team from New Delhi reported in a preprint [II.8] that the scientists had found HIV-RNA sequences during the genetic analysis of the SARS-CoV-2 virus, which suggests an artificial origin of this new coronavirus type. The authors were thereupon vehemently criticized by well-known virologists and urged to withdraw the publication.

Interestingly, the French Nobel laureate and discoverer of the HIV-virus Luc Montagnier and his colleague also found RNA-sequences of HIV-viruses during genetic investigations of the SARS-CoV-2 viruses, which could not have become part of this new type of coronaviruses in a natural way [II.7]. In an interview of the French television, Montagnier said: “In order to introduce an HIV-sequence in the genome, molecular tools are necessary, and this can only be done in a laboratory” The reaction to this statements of the French Nobel laureates were no scientific arguments from the opposition, however in contrast defamatory commentaries that either aimed at the age of Montagnier [IV.29] or aimed into the direction that the Nobel laureate had become “controversial” in the meantime [IV.30]. As a matter of fact, HIV-based pseudoviruses were utilized for genetic manipulation experiments by the Wuhan research team around Zheng-Li Shi, as a number of publications in the scientific literature are documenting (see for instance [I.6], [I.10]).

The Chinese virologist Li-Meng Yan as well has found clear indications of a non-natural origin of the SARS-CoV-2 virus based on detailed analysis of its genetic sequence [II.5]. After publishing of her work on the online-portal Zenodo in September 2020, she was heavily criticized by a number of virologists. She found out that SARS-CoV-2 viruses are a lab-based product under use of bat viruses called ZC45 and ZXC21 as template and/or backbone. However, exactly these types of coronaviruses were identified by the group of Chinese scientists and doctors during the analysis of the genetic sequences of pathogens of the very early COVID-19 patients. This work was published in February 2020 in the prestigious Journal “THE LANCET” [I.3]. Both works are inserted in extracts 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, albeit 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 characterisation 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 modelling 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 modelling revealed that 2019-nCoV had a similar receptor-binding domain structure to that of SARS-CoV, despite amino acid variation at some key residues.

…

In the course of 2020, the dispute concerning the prerogative of interpretation regarding the question of the origin of the coronavirus pandemic culminated with the statement of a well-known virologist in Germany saying that scientists who are not working within the field of virology or indeed even in the specific area of coronaviruses, should better not express themselves regarding subjects related to the coronavirus pandemic [IV.29]. This statement is evidently closely linked to the question of the present understanding of science: **Should science solely be understood as a whole of the specific individual disciplines with clear demarcations of the "responsibility" of individual scientific disciplines, or are there not also superordinate questions of science including not lastly the critical, self-reflective view of procedures within science, as well as the question regarding the responsibility of science concerning the well-being of all humans on our planet?**

There are some scientists who presently speak about the worst case of a coordinated misleading of the broad public concerning the question of the origin of the coronavirus pandemic (see for example [II.9]).

In the meantime, a group of "Concerned People of the World" has written an open letter to the members of the WHO-investigation commission exploring the origin of the coronavirus pandemic, with the opening:

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

This group of scientists has summarized which tasks are to be completed during the investigation of the processes in Wuhan, especially during the final quarter of the year 2019 [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 - Programme 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 unravelling 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 endeavours 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

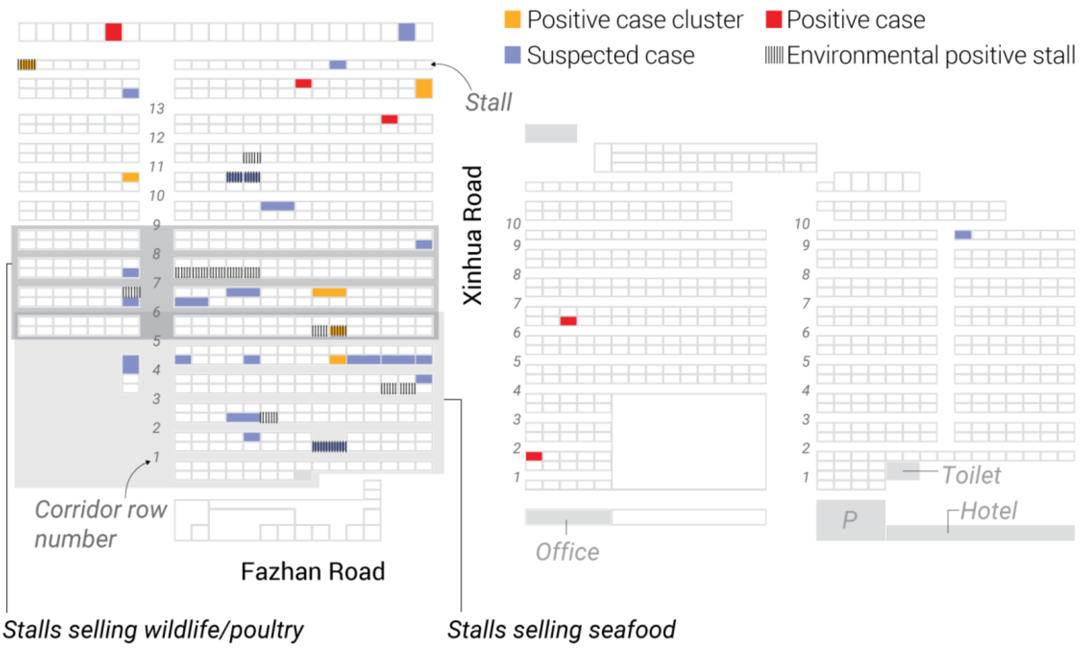
1. What animals in the Wuhan Huanan Seafood Market were tested, what types of specimens were obtained (apart from frozen animal carcasses), and what were all the results?

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)



SCMP

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

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

17. Was 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 (宇传华) 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 (战疫复工大数据) developed at the NUDT (National University of Defense Science and Technology) and its corresponding epidemic data shown to the WHO mission?

29. Was 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 (董小平) 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

d. 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 in turn 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/

- And an 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:

address: Xiaohongshan NO. 44, Wuhan, Hubei, 430071

tel: (86) 27-87168465 Fax: (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, was 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, will the WHO investigation team therefore inspect or organise 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

d. 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, will the WHO team query the patient details & files to further clarify the putative cases of covid-19 in October at Wuhan Hospitals.

7 References

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