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Australian Government Department of Foreign Affairs and Trade

ASSESSMENT OF WHO-CONVENED GLOBAL STUDY OF THE ORIGINS OF SARS-COV-2: CHINA PART REPORT

May 2021

# INTRODUCTION

In May 2020, the World Health Assembly resolution WHA73.1 requested the Director-General of the World Health Organization (WHO) to continue to work closely with the World Organization for Animal Health (OIE), the Food and Agriculture Organization (FAO), and member state countries, as part of the One Health approach, to identify the zoonotic source of SARS-CoV-2 (COVID-19 / the virus) and the route of introduction to the human population, including the possible role of intermediate hosts. This led to the establishment of the *WHO-convened Global Study of Origins of SARS-CoV-2* (the Study).

Two WHO officials completed a preliminary mission to China (11 July-3 August 2020) to inform the scope and terms of reference for a larger WHO-convened expert international mission (released 6 November 2020).

The expert mission team, comprised 17 Chinese and 17 international experts from other countries, including an Australian expert. The other experts included leading scientists and researchers from the US, Russia, Sudan, Denmark, Netherlands, Germany, Japan, Vietnam and the UK, as well as five WHO experts led by Dr Peter Ben Embarek; two OIE representatives and two observers from FAO.

Identifying the source of the virus is a complex scientific process. Phase 1 involved a team of international and Chinese experts undertaking in-depth analysis and studies on the first cases in Wuhan remotely. Phase 2 included an international field mission to China to conduct in-depth epidemiological and other assessments of humans and animal populations in specific geographic areas (informed by the outcome of Phase 1).

The international team of experts travelled to Wuhan on 14 January 2021. Following two weeks quarantine, the international mission visited: the Wuhan Institute of Virology; the Wuhan Wholesale Seafood Market (Huanan market); different hospitals around Wuhan where early patients presented; and the Hubei Provincial Center for Disease Control. The international team (and their Chinese counterparts) were divided into in three groups: epidemiology, animal studies and the environment and molecular and bioinformatics.

Member states received an embargoed copy of the WHO-convened Global Study of Origins of SARS-CoV-2: China Part joint report on 29 March (AEDT), ahead of its public release on 31 March (AEDT).

The Report outlined the four hypotheses for the introduction of the virus and the assessed likelihood of each (extract below):

The joint international team examined four scenarios for introduction:

- direct zoonotic transmission to humans (spillover);
- introduction through an intermediate host followed by spillover;
- introduction through the (cold) food chain;
- introduction through a laboratory incident.

For each of these possible pathways of emergence, the joint team conducted a qualitative risk assessment, considering the available scientific evidence and findings. It also stated the arguments against each possibility. The team assessed the relative likelihood of these pathways and prioritized further studies that would potentially increase knowledge and understanding globally.

The joint team's assessment of likelihood of each possible pathway was as follows:

- direct zoonotic spillover is considered to be a possible-to-likely pathway;
- introduction through an intermediate host is considered to be a likely to very likely pathway;

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- introduction through cold/ food chain products is considered a possible pathway;
- introduction through a laboratory incident was considered to be an extremely unlikely pathway.

The Report noted that that "the possible intermediate host of SARS-CoV-2 remains elusive" and further studies are recommended in China and the Southeast Asia region.

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## **ANALYSIS OF REPORT<sup>1</sup>**

# **HEADLINE REACTIONS**

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some important details contained in various parts of the report indicate a need for further studies to identify better epidemiological data for primary cases in the outbreak to strengthen conclusions about the role of markets. **The report provides strong evidence of the zoonotic origins of the virus,** and infers that it emerged in humans either directly from wildlife, or via an intermediary host that is yet to be identified (the latter being the hypotheses rated most likely in the report).

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Nor is this hypotheses relevant to the *origin event*; which was the focus of the study. While there is limited evidence that some *transmission* may have occurred via cold chain products/packaging when COVID-19 prevalence globally was already high, **there is no evidence to suggest imported cold chain is implicated in the origin event**. And at the time the origin event is approximated to have occurred, there is no corresponding evidence of large outbreaks in humans or animals outside of China. **s33**, **s47C** 

The conclusions drawn about the timing of the emergence of SARS-CoV-2 infection in humans (November – December 2019) were reasonable given the available data. The potential for undetected chains of transmission in the community prior to the 'explosive outbreak' in December was acknowledged by the authors.

<sup>1</sup> Analysis undertaken by experts from the Department of Foreign Affairs and Trade (including the Indo-Pacific Centre for Health Security), Department of Agriculture, Water and the Environment (including the Office of the Senior Veterinary Officer) and the Department of Health.

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# ALIGNMENT WITH TERMS OF REFERENCE

Overall, the Report did not arrive at a firm conclusion about the origin of the SARS-CoV-2 virus, s33, s47C . The Report's stated focus on how the virus was introduced into the human population in Wuhan is a slight nuance from the original TORs which were focused on tracing the origins. s33, s47C

The aims outlined in the Report do not refer to any role that the investigation could have had in potentially assisting with treatment and/or vaccine development. This is despite the fact that the TORs state that "understanding how the epidemic began is essential to preventing further SARS-CoV-2 virus introductions and help prevent introductions of new viruses in the future. It could also potentially assist with the development of treatments and vaccines".

# ADEQUACY OF METHODS AND DATA

There are useful studies outlined in the Report, but the **data presented is limited and interpretation largely inconclusive**. More detail in the Report around the methodology for identifying the four hypotheses would have been helpful.

Much of the work of the joint mission seemed to be reviewing and understanding work and analysis already done by Chinese experts, rather than reviewing primary data afresh. Chinese experts provided presentations of their work, e.g. types and sources of animal products in Huanan market and surveillance of SARS-COV-2 in animals.s33, s47C

The data provided was often poor quality and lacking detail. For example, review of animal testing data did not provide information on dates and locations, meaning it appeared non-systematic. Hospital sentinel surveillance for influenza-like illness (ILI) appeared to not accurately reflect what we would expect to see in this population. There are questions about the availability and arrangements pertaining to sequencing data, which can help to understand: relationships between virus strains; trace cases and clusters; and how long ago spillover from animals to humans may have occurred.

Further **qualitative epidemiological data** may also have assisted in providing context for interpretation of other data, particularly in relation to the human-animal interface of the original event (identified as a knowledge gap in the *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19),* 16-24 February 2020. For example, information about social, cultural, and economic drivers of human behaviour and systems would be particularly important for understanding the global lessons for preventing pandemics.

At times it is clear that **full access to data was requested and expected but appears to have not been provided in the end** (e.g. in section 2B page 8 of the annex, in relation to surveillance data and health facility records). However, we also recognise that expectations about data from the early stage of the pandemic need to be realistic.

While some convenience sampling of biobank specimens was conducted, there has been no sampling of sewage or blood bank samples (as noted on page 57 – "given the outstanding questions and the potential for limited clusters that would not be detected through the studies done so far, access to systematically collected historical samples, including routinely stored blood bank samples, would be of great added value"). However, a serosurvey of stored blood specimens may not be sensitive enough for identifying the timing of the original transmission event.

#### Surveillance data-morbidity

While analysis of the ILI and severe acute respiratory infection (SARI) data aimed to identify a rise in presentations of respiratory syndromes that may have indicated the timing of the beginning of the outbreak and related outbreaks in other areas, the existing surveillance systems were unable to identify the beginning of the outbreak or if related outbreaks were occurring in other areas in late 2019.

Several limitations of the surveillance systems used to collect ILI and SARI data may have influenced how accurately this was measured in the population, for example:

- the systems are designed for national reporting and so are not necessarily representative at the local level;
- the data provided by the two participating hospitals in Wuhan for ILI surveillance are unlikely to be representative of the 11 million residents of Wuhan;
- one of the two Wuhan hospitals was a paediatric hospital; these data may not have signalled increased ILI presentations due to SARS-COV-2 in the wider (adult) population given the generally mild disease experienced by children;
- for SARI surveillance, data was not available at all for Wuhan, which leaves a gap in the evidence of severe disease presentations due to SARS-COV-2 early in the outbreak;
- while the upward trend in ILI presentations in sentinel surveillance in Wuhan at the end of 2019 was reasonably attributed to influenza, the percentage of those testing positive should be interpreted with caution due to the low number of persons (20) with ILI tested each week; and
- the data on retrospective testing of ILI specimens from October to December 2019 from Wuhan showed that the surveillance was insensitive to early indications of the outbreak.

In addition, the analysis of antipyretics, cold remedies and cough medications did not appear useful given the way the data were presented by month. The analysis of mass gatherings to identify if there was evidence of potential cases occurring in well-defined populations was a sensible approach, although this did not identify any likely clusters of fever or severe respiratory disease.

### Surveillance data-mortality

It was encouraging to see that mortality surveillance covered the population in Wuhan and the investigation team's conclusions seem appropriate for mortality surveillance.

The Report indicates mortality from SARS-COV-2 increased in January 2020 but was not detected earlier. This is expected for fatal cases where there is a 2–6 week lag between a person acquiring SARS-CoV-2 infection and dying. Deaths are not a good way to detect the beginning of an epidemic due to the proportion of cases that are mild or asymptomatic and the lag between infection and death. The Report notes that SARS-COV-2 must have been widespread in the community by the first week of January 2020 to have caused the observed large jump in mortality.

# Clinical review of surveillance data and National Notifiable Disease Reporting System (NNDRS) data

The investigation of early cases of SARS-COV-2 reported in Wuhan is an important aspect of the investigation, although there were several gaps. In particular, the review of the descriptive epidemiology of 174 SARS-COV-2 cases reported to the NNDRS was inadequate as it did not provide statistical information

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about the likely contribution of key risk factors for infection, which analytic studies aim to do. **Ideally, the international team and Chinese investigations should conduct a case control study of cases in December 2019 to identify risk factors for infection.** This is particularly important given the primary case (ie, the earliest known case) with an onset of illness of 8 December 2019 did not report exposure to the Huanan Market (as a risk factor). A retrospective case control study would have confirmed the key role of the Huanan Market and other important risk factors for infection. Risks were also poorly defined, possibly due to an unstated proportion of cases where information was unavailable.

SARS-COV-2 is a disease that is strongly clustered, in that infections occur together wherever people live or work together. In the descriptive review the investigation team report that 8% (15/174) of cases were part of seven recognised clusters. The high proportion of cases that were unlinked is indicative of many unreported and undiscovered clusters. This gives a clear indication that widespread community transmission was occurring at this time. Ideally, the investigation team should identify when these clusters occurred over time relative to sporadic cases. As such, we support the investigation team's recommendation to re-interview early cases and re-analyse data.

#### Retrospective search for potential cases of SARS-CoV-2

Chinese authorities conducted a search for initial cases of SARS-CoV-2 between 1–31 December 2019, recognising this would have been very challenging being 14 months later. The search was reasonably extensive, involving 234 institutions from 15 districts in Wuhan. The search identified 76,253 episodes of fever, ILI, Acute Respiratory Illness (ARI) or pneumonia. Review identified 92 clinically compatible cases; all of which were ultimately rejected as cases of SARS-COV-2. The nature of review of these 92 cases was unclear, although it did involve serological testing over one year after the original outbreak. It is difficult to validate the results of this element of the investigation and recommendation of further analysis is desirable.

#### **Review of Stored Biological Samples Testing**

The report documents a search for stored specimens that could be tested for SARS-CoV-2 nucleic acid and antibodies. All samples were negative and little conclusions can be drawn from this analysis.

#### Wuhan Blood Center presentation

Testing of stored samples from blood donors is a viable way of examining the presence of antibodies for SARS-CoV-2. We support the investigation team's recommendation to test stored blood donor samples from prior to December 2019 for SARS-CoV-2 antibodies.

#### Animal testing and sampling

More than 80 000 wildlife, livestock and poultry samples were collected from 31 provinces in China and no positive result was identified for SARS-CoV-2 antibody or nucleic acid before and after the SARS-CoV-2 outbreak in China. The testing and surveillance data from China were presented at a very high level, and much of the data was not particularly relevant, including species that were subsequently not shown to be susceptible. However, it is acknowledged that at the time of testing it may have been useful to test a large range of species, when confronting unknowns around species' susceptibility.

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Specimen types were also vaguely described. For example in one study, blood and faeces were tested from 2,328 animals representing 69 species. All were negative for SARS-COV-2, however, testing of respiratory specimens would have been more sensitive. The testing was done before, during and after the height of the outbreak, including in animals in contact with confirmed cases, and all again were PCR and ELISA negative. This conflicts with a separate study finding 15% seropositivity in cats in Wuhan in March 2020.<sup>2</sup>

The report contains contradictions around whether live animals were sold in the Huanan market. For example, page 101 states 'according to sales records (provided by the Market Authority), in late December 2019, 10 animal stalls sold animals or products from snakes, avian species (chickens, ducks, gooses, pheasants and doves), Sika deer, badgers, rabbits, bamboo rats, porcupines, hedgehogs, salamanders, giant salamanders, bay crocodiles and Siamese crocodiles, among which snakes, salamanders and crocodiles were traded as live animals (Annex F, Table 3). Other products sold were frozen goods or bai tiao (remaining parts of poultry or livestock after removal of hair and viscera). Snakes and salamanders were slaughtered before being sold, but crocodiles were alive when sold.' However, the Market Manager (Annex D4) 'stated that no live animals were sold and no animals were butchered on the premises.' And yet the team saw 'evidence of the sale of live aquatic animals in the presence of fish tanks and shallow tanks that would have been used for turtles or amphibians.'

There is some evidence of Chinese Authorities trying to trace back to suppliers. For example, page 102 states 'samples from animals raised by some Huanan market suppliers in Hubei were also sampled and tested between February and March 2020 (Table 5.1)' and 'SARS-CoV-2 surveillance within wild animals were also done in some other provinces (Table 5.2)'. Full and comprehensive trace back to wildlife farms supplying Huanan market is the most logical and pressing course of action, consistent with the most likely hypothesis and what is known about the series of events and their geography, which in our view is most likely to yield meaningful insights into the origins of SARS-COV-2.

#### Molecular epidemiology

The report focuses on two questions which molecular epidemiology can help explain: seeking evidence of circulation in a wildlife reservoir or intermediate hosts (listing bats, mink and pangolin as possibilities) and also using molecular epidemiology to decode clusters in humans and estimate the number of generations of transmission from the original spillover event(s).

The number and diversity of coronaviruses in wild animals is known to be vast. The most closely related viruses in wildlife (to SARS-COV-2) have been found in bats and pangolins, but no viruses have been found from these species that are closely enough related to be thought to be the direct progenitor of human infections. The report noted surveys of wildlife have not been systematic, and potential reservoir hosts are massively under-sampled. The report recommended further systematic sampling. It concludes that there is sufficient evidence to include both bats and pangolins in the search for the reservoirs and/or intermediate hosts.

The report decodes clusters in humans and estimates the number of generations of transmission from the original spillover event(s). Combining epidemiological and viral sequence data allows reconstruction of how and where the virus evolved – which helps in finding infection clusters and tracing chains of transmission. It is important to note that early in this outbreak Chinese laboratories had only sequenced 4.2% (494/11821) of strains from SARS-COV-2 cases.

<sup>2</sup> Zhang Q, Zhang H, et al. A serological survey of SARS-CoV-2 in cats in Wuhan. Emerg Microbes Infect. 2020; 1:2013-9.

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For the analyses of molecular epidemiology, it was unclear if the reanalysed raw sequence data with epidemiological data from questionnaires was from the report team or from Chinese scientists. It appears that the case questionnaires themselves were made available, and sequence data had been lodged on international data-sharing platforms. They were also given access to the viruses and re-did some of the sequencing to improve consistency for analysis where sequencing had been done by different laboratories. This highlights that there are many different subsets of data mentioned in the report which is confusing, but overall access to data related to molecular epidemiology seems to have been good. However, it is unclear if relevant information about the movements and interactions of these earliest confirmed cases was collected or provided to the international team. If not, it could be useful to re-evaluate data associated with all early cases as well as cases excluded from analysis.

Further analysis was conducted for the total of 348 high quality sequences of SARS-CoV-2 from China and 142 from elsewhere in the world (Mexico, Thailand and Spain), case and environmental samples. Two major clusters were observed, but there is currently no consensus on which case is likely to be the closest to that which emerged from the original source. In total, all 13 early human cases with symptom onset before 31 December 2019 were identified in the sequence dataset. This data was combined with epidemiological data from case databases and from questionnaires. Of the 13 cases, 11 had connections to the Huanan market, including 7 vendors at the market, three customers and one visitor. The two remaining people had visited other markets.

Among 11 sequences obtained from samples related to the Huanan market, eight had no mutations, two had the same single mutation and one sequence showed two mutations. Sequences from the two patients not linked with Huanan market had one and three mutations, respectively. Two sequences were from isolates obtained from environmental samples collected from Huanan market on 1 January 2020; these had zero and two mutations, respectively. The report concludes that the diversity of viruses in the early cases suggests unrecognised transmission was occurring.

Sequences analyses by multiple authors outside of the mission, and included in the report, suggest that the outbreak started between mid-November and mid-December 2019. s33, s47C

Notably the other two of the four were clustered with most of the others from the Huanan market. Also of note, there are a large number of sequences of viruses relating to the cold chain from August 2020 onwards, but none prior to that. The time period of these specimens makes them irrelevant to the hypothesis that the virus was introduced to Wuhan via cold chain (or indeed that the virus' origins were linked to cold chain).

#### Evidence of earlier transmission in other countries:

Several pieces of evidence were cited in the report as possibly providing evidence of earlier presence of SARS-CoV-2 elsewhere in the world:

- In Spain, detection of SARS-CoV-2 in sewage in March 2019, which was never sequenced and is thought a possible false positive;
- In Italy, detection of SARS-CoV-2 in sewage in mid-December 2019, and in a throat swab from a child in early December 2019, some suggestive evidence of a woman being SARS-CoV-2 in November 2019, and serological evidence in October 2019;
- In France, serological evidence of an increase in prevalence of SARS-CoV-2 in mid-December, suggesting earlier transmission;
- In Brazil, positive sewage results in November 2019.

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The report concludes that the SARS-CoV-2 circulation preceded the initial detection of cases by several weeks. Some of the suspected positive samples were detected even earlier than the first case in Wuhan, suggesting that circulation of the virus in other regions had been missed. So far, however, the study findings were not confirmed, methods used were not standardized, and serological assays may lack specificity.

#### Possible pathways of emergence

The international team considered four different scenarios for SARS-CoV-2 introduction: (1) direct zoonotic transmission, (2) introduction through an intermediate host, (3) introduction through the cold/food chain, and (4) introduction through a laboratory incident. The international team considered the relative likelihood based on a Likert scale from extremely likely through to very likely. The 'qualitative risk assessment' was done in an informal style of an expert elicitation. The nature of evidence that the team relied on was not detailed, nor was the consensus-making mechanism. Ideally, the international team would take a more structured and detailed approach to identifying the most likely source of introduction, as this was a main aim of the mission.

#### Direct zoonotic transmission

The international team considered that direct zoonotic transmission from an animal (wild or domesticated) was possible to likely. The report discusses that the SARS-CoV-2 virus is a member of a clade (a 'natural group') of coronaviruses that is almost exclusively found in bats. One area of the investigation that is lacking is a systematic approach to reporting the animals sold at the Huanan Market and the results of trace back investigations of Chinese authorities. Ideally, a case control study of cases early in the outbreak when the cluster centred around Huanan Market in Wuhan would have identified if exposure to live animals were a risk factor for infection. The report refers to other countries, such as Japan, Cambodia and Thailand, which in addition to China, are home to the species of bat known to have viruses most closely related to SARS-CoV-2. The meaning of this information in the context of the origin event for SARS-CoV-2 is not well extrapolated.

#### Intermediate host followed by zoonotic transmission

The international team considered that introduction through an intermediate host followed by zoonotic transmission was considered likely to very likely. Many areas of the report reference the high likelihood that the origins of this pandemic "are thought to have its ecological niche in an animal reservoir" (pg. 95), and that "circumstantial evidence supports a range of potential spillover pathways", being spillover from bats to humans directly or via an intermediate host. The report highlights that the closest evolution between bat viruses and SARS-CoV-2 is likely to be several decades, and that there are other animals, such as mink, that are readily infected and able to transmit to humans. The report implies here, and elsewhere, that it is important the international community investigate sources further outside of China for the origin event including in the recommendations for future studies.

#### Introduction through the cold/food chain

The international team considered that introduction through the food-chain or cold chain is considered possible. The Report does not provide any new evidence that would change the Australian Government's view that SARS-COV-2 is not a foodborne disease. The Report does not also present strong evidence in relation to respiratory transmission as a result of contact with contaminated food packaging. The Report

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states imported cold chain products from the market were examined (**no domestic cold-chain products could be located**) and all 1,055 samples tested negative. **s33**, **s47C** 

The Report further strengthens the evidence that SARS-COV-2 is a respiratory disease spreading primarily from person to person. There is no definitive evidence presented in the Report that SARS-COV-2 is transmitted to humans through food or food packaging, nor that the imported commercial cold food chain is in any way linked to the origin event. While traces of SARS-CoV-2 genetic material and possibly live virus were detected on food products or packaging in China, the Report does not provide substantiated evidence of SARS-COV-2 cases contracting the virus from food or food packaging. s33, s47C

The four hypotheses presented in the Report do not equate to only four risk pathways for the origin of the pandemic, again highlighting the need for clarity around the rationale and methods behind selection of these four hypotheses for examination. Specifically, the cold chain hypothesis, equates to three separate, distinct hypotheses or pathways. These are viral transmission within Wuhan originating from:

- a) frozen bodies of SARS-COV-2 susceptible wildlife species from farms within China;
- b) frozen bodies of SARS-COV-2 susceptible wildlife species imported from Southeast Asian countries;
- c) commercial frozen food products imported from some other country anywhere in the world (noting there was no concurrent evidence of a human outbreak in any other country around the time of the origin event).

These three different origin possibilities are completely different pathways with completely different likelihood ratings. Referring to these different risk pathways interchangeably or as one pathway, has potential to impact trade in food animal products and divert attention away from the public health response and prevention of pandemics emerging from human interaction with animals.

It would have been helpful for the summary of the different hypotheses to be presented in a simple qualitative risk assessment format, with risk ratings categorised more as 'highly likely' (wildlife spillover) through to 'highly improbable', rather than just 'more likely' and 'less likely' and all needing further exploration. This has led to an overall impression from the WHO investigation media presentation and the report that all hypotheses (apart from the laboratory escape) are of similar validity and plausibility.

#### Introduction through a laboratory incident

The hypothesis is not dealt with in any detail in the Report but is directly addressed by the Director of the Wuhan Institute of Virology in Annex D7 on page 133. It is mentioned in the key findings on page 9 and on page 121 after the conclusions. s33, s47C

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Director-General of the WHO has indicated that further work will be undertaken across all hypotheses.

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# COMMENTS ON RECOMMENDATIONS AND FURTHER STUDIES:

The recommendations provide some useful insight into where data was lacking, as they point specifically to where further information would be most helpful for phase 2 studies. Australia supports further studies to better understand the origin event and virus transmission, though does not support the proposition that the imported food cold chain warrants further investigation as a potential origin event. **s33**, **s47C** 

(note the following recommendation on page 113: Analyse virus persistence and viability at different temperatures to simulate the freeze-thaw cycle that would happen naturally as products are shipped from one port to another, then through the supply chain.)

The recommendations include (page 112-113) further studies in bats, pangolins and other wild species in China and the Southeast Asia region; and in potential wildlife and livestock hosts linked by value chains to the Huanan and other Wuhan markets. Australia agrees that further wildlife studies in China that include trace back and testing of wildlife farms supplying markets in China are essential and a priority for future work. Increasing testing of bats and pangolins in Southeast Asia is a good strategy for future spillover prevention but is less likely to be a source for the current pandemic than many other potential pathways in China.

While the international expert team has made progress, and the report provides further information about the COVID-19 outbreak, we are concerned that the mission was significantly delayed and lacked access to complete, original data and samples. Australia joined a statement with 13 other countries, including the US, UK, Canada, Republic of Korea and Japan in expressing these shared concerns (31 March). The joint statement also calls for the need for clear timeframes for follow up actions and next steps for further investigations, as well as the adequate resourcing for them. The 27 member countries of the European Union also expressed concerns in a separate EU statement (31 March), and India issued a separate statement on 1 April.

Australia wants to see phase 2 studies prioritise the mostly likely hypotheses [direct and indirect zoonotic spillover] in China, and data standards should be set up front.

Australia considers the following recommendations and closely related work as high priorities:

- Australia agrees that further wildlife studies in China that include trace back and testing of wildlife farms supplying markets or livestock that have potential to be infected in China are essential and a priority for future work. Increasing testing of bats and pangolins in Southeast Asia is a good strategy for future spillover prevention but is less likely to be a source for the current pandemic than many other potential pathways in China;
- analytical studies, particularly a retrospective case control study using cases from December 2019 in order to further investigate transmission pathways at the beginning of the pandemic;
- identification of when early clusters occurred over time relative to sporadic cases, as part of the investigation team's recommendation to re-interview early cases and re-analyse these data;
- presentation of additional data on the search for retrospective SARS-CoV-2 cases and further analysis;
- further investigation of mild and asymptomatic cases in Wuhan;
- testing of stored blood donor samples from prior to December 2019 for SARS-CoV-2 antibodies, as recommended;

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- sequencing of early cases or samples collected in future SARS-CoV-2 global tracing studies using multi-platforms and high-depth sequencing;
- further trace-back at the wildlife farms that previously supplied Huanan market and other Wuhan
  markets linked to positive cases, including interviews and serological testing of farmers and their
  workers, vendors, delivery staff, cold-chain suppliers, and other relevant people and their close
  contacts;
- DNA barcoding of the meat product samples from Huanan market, to identify more precisely species involved and potential intermediate hosts or wildlife reservoirs of CoVs that might have been involved in the food chain.

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# WHO-convened Global Study of Origins of SARS-CoV-2: China Part

Joint WHO-China Study 14 January-10 February 2021

Joint Report

#### LIST OF ABBREVIATIONS AND ACRONYMS

ARI	acute respiratory illness
cDNA	complementary DNA
China CDC	Chinese Center for Disease Control and Prevention
CNCB	China National Center for Bioinformation
CoV	coronavirus
Ct values	cycle threshold values
DDBJ	DNA Database of Japan
EMBL-EBI	European Molecular Biology Laboratory and European Bioinformatics Institute
FAO	Food and Agriculture Organization of the United Nations
GISAID	Global Initiative on Sharing Avian Influenza Database
GOARN	Global Outbreak Alert and Response Network
Hong Kong SAR	Hong Kong Special Administrative Region
Huanan market	Huanan Seafood Wholesale Market
IHR	International Health Regulations (2005)
ILI	influenza-like illness
INSD	International Nucleotide Sequence Database
MERS	Middle East respiratory syndrome
MRCA	most recent common ancestor
NAT	nucleic acid testing
NCBI	National Center for Biotechnology Information
NMDC	National Microbiology Data Center
NNDRS	National Notifiable Disease Reporting System
OIE	World Organisation for Animal Health (Office international des Epizooties)
PCR	polymerase chain reaction
PHEIC	public health emergency of international concern
RT-PCR	real-time polymerase chain reaction
SARI	severe acute respiratory illness
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SARSr-CoV-2	Severe acute respiratory syndrome coronavirus 2-related virus
tMRCA	time to most recent common ancestor
WHO	World Health Organization
WIV	Wuhan Institute of Virology

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WHO also gratefully acknowledges the technical, administrative and logistics support of many agencies and offices in the preparations and conduct of the joint mission. Staff at WHO Country Office in Beijing and at WHO Headquarters worked closely with Chinese counterparts and colleagues and with partner organizations and agencies on detailed practical arrangements and logistics and provided staff to support the joint mission. Further, the WHO rapid review team and OIE provided a database of relevant literature on SARS-CoV-2 potential origins to complement the technical working groups during the joint study.

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WHO-convened global study of origins of SARS-CoV-2: China Part

Joint WHO-China Study Team report

14 January-10 February 2021

#### Summary

In May 2020, the World Health Assembly in resolution WHA73.1 requested the Director-General of the World Health Organization (WHO) to continue to work closely with the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO) and countries, as part of the One Health approach, to identify the zoonotic source of the virus and the route of introduction to the human population, including the possible role of intermediate hosts. The aim is to prevent both reinfection with the virus in animals and humans and the establishment of new zoonotic reservoirs, thereby reducing further risks of the emergence and transmission of zoonotic diseases.

In July 2020, WHO and China began the groundwork for studies to better understand the origins of the virus. Terms of Reference (TORs) were agreed that defined a phased approach, and the scope of studies, the main guiding principles and expected deliverables. The TORs envisaged an initial Phase 1 of short-term studies to better understand how the virus might have been introduced and started to circulate in Wuhan, China.

WHO selected an international multidisciplinary team of experts to work closely with a multidisciplinary team of Chinese experts in the design, support and conduct of these studies and to conduct a follow-up visit to review progress and agree upon a series of further studies.

The joint international team comprised 17 Chinese and 17 international experts from other countries, the World Health Organization (WHO), the Global Outbreak Alert and Response Network (GOARN), the World Organisation for Animal Health (OIE). (Annex B) The Food and Agriculture Organization of the United Nations (FAO) participated as an observer. Following initial online meetings, a joint study was conducted over a 28-day period from 14 January to 10 February 2021 in the city of Wuhan, People's Republic of China.

The team agreed a workplan and established working groups to review the progress made in Phase 1 studies in the areas of: epidemiology; animals and the environment; and molecular epidemiology and bioinformatics. During the course of the discussions, the international experts gained deeper understanding of the methods used and data obtained. In response to requests during the visit, further data and analyses were generated, reflecting a productive iterative approach to refining the design and interpretation of complex studies in all areas.

In addition to group work, the team shared scientific and thematic presentations on relevant topics to help inform its work, undertook a series of site visits to important locations and conducted interviews with key informants.

The epidemiology working group closely examined the possibilities of identifying earlier cases of COVID-19 through studies from surveillance of morbidity due to respiratory diseases in and around Wuhan in late 2019. It also drew on national sentinel surveillance data; laboratory confirmations of disease; reports of retail pharmacy purchases for antipyretics, cold and cough medications; a convenience subset of stored samples of more than 4500 research project samples from the second half of 2019 stored at various hospitals in Wuhan, the rest of Hubei Province and other provinces. In

none of these studies was there evidence of an impact of the causative agent of COVID-19 on morbidity in the months before the outbreak of COVID-19.

Furthermore, surveillance data on all-cause mortality and pneumonia-specific mortality from Wuhan city and the rest of Hubei Province were reviewed. The documented rapid increase in all-cause mortality and pneumonia-specific deaths in the third week of 2020 indicated that virus transmission was widespread among the population of Wuhan by the first week of 2020. The steep increase in mortality that occurred one to two weeks later among the population in the Hubei Province outside Wuhan suggested that the epidemic in Wuhan preceded the spread in the rest of Hubei Province.

Both surveillance data and cases reported to the National Notifiable Disease Reporting System (NNDRS) in China were subjected to clinical review. The NNDRS was notified of 174 COVID-19 cases with onset of symptoms in December 2019. In an extensive exercise by 233 health institutions in Wuhan, some 76,253 records of cases of respiratory conditions in the two months of October and November before the outbreak in late 2019 were scrutinized clinically. Although 92 cases were considered to be compatible with SARS-CoV-2 infection after review, subsequent testing and further external multidisciplinary clinical review determined that none was in fact due to SARS-CoV-2 infection. Based on the analysis of this and other surveillance data, it is considered unlikely that any substantial transmission of SARS-CoV-2 infection was occurring in Wuhan during those two months.

Many of the early cases were associated with the Huanan market, but a similar number of cases were associated with other markets and some were not associated with any markets. Transmission within the wider community in December could account for cases not associated with the Huanan market which, together with the presence of early cases not associated with that market, could suggest that the Huanan market was not the original source of the outbreak. Other milder cases that were not identified, however, could provide the link between the Huanan market and early cases without an apparent link to the market. No firm conclusion therefore about the role of the Huanan market in the origin of the outbreak, or how the infection was introduced into the market, can currently be drawn.

The molecular epidemiology and bioinformatics working group examined the genomic data of viruses collected from animals. Evidence from surveys and targeted studies so far have shown that the coronaviruses most highly related to SARS-CoV-2 are found in bats and pangolins, suggesting that these mammals may be the reservoir of the virus that causes COVID-19. However, neither of the viruses identified so far from these mammalian species is sufficiently similar to SARS-CoV-2 to serve as its direct progenitor. In addition to these findings, the high susceptibility of mink and cats to SARS-CoV-2 suggests that additional species of animals may act as a potential reservoir.

To analyse the viral genomes and epidemiological data from the early phase of the outbreak, the team reviewed data collected through the China National Centre for Bioinformation integrated database on all available coronaviruses sequences and their metadata. All sequence data from samples collected in December 2019 and January 2020 were subjected to deeper analysis to see the diversity of viruses in the first phases of the outbreak. For the cases detected in Wuhan, data on samples from cases with illness onset before 31 December 2019 were linked with epidemiological background data. Several samples from patients with exposure to the Huanan market had identical virus genomes, suggesting that they may have been part of a cluster. However, the sequence data also showed that some diversity of viruses already existed in the early phase of the outbreak in Wuhan, suggesting unsampled chains of transmission beyond the Huanan market cluster. There was no obvious clustering by the epidemiological parameters of exposure to raw meat or furry animals.

In addition, the time to the most recent common ancestor of the SARS-CoV-2 sequences in the final data set was estimated and compared with results from previous studies. Such analyses can be considered estimates but do not provide definitive proof of time of origins. Based on molecular sequence data, the results suggested that the outbreak may have started some time in the months before the middle of December 2019. The point estimates for the time to the most recent ancestor

ranged from late September to early December, but most estimates were between mid-November and early December.

Finally, the team reviewed data from published studies from different countries suggesting early circulation of SARS-CoV-2. The findings suggest that circulation of SARS-CoV-2 preceded the initial detection of cases by several weeks. Some of the suspected positive samples were detected even earlier than the first case in Wuhan, suggesting the possibility of missed circulation in other countries. So far, however, the quality of the studies is limited. Nonetheless, it is important to investigate these potential early events.

The animal and environment working group reviewed existing knowledge on coronaviruses that are phylogenetically related to SARS-CoV-2 identified in different animals, including horseshoe bats (*Rhinolophus* spp) and pangolins. However, the presence of SARS-CoV-2 has not been detected through sampling and testing of bats or of wildlife across China. More than 80 000 wildlife, livestock and poultry samples were collected from 31 provinces in China and no positive result was identified for SARS-CoV-2 antibody or nucleic acid before and after the SARS-CoV-2 outbreak in China. Through extensive testing of animal products in the Huanan market, no evidence of animal infections was found.

Environmental sampling in Huanan market from right at the point of its closing showed out of 923 environmental samples in Huanan market, 73 samples were positive. This revealed widespread contamination of surfaces with SARS-CoV-2, compatible with introduction of the virus through infected people, infected animals or contaminated products.

The supply chains to Huanan market included cold-chain products and animal products from 20 countries, including those where samples have been reported as positive for SARS-CoV-2 before the end of 2019 and those where close relatives of SARS-CoV-2 are found. There is evidence that some domesticated wildlife the products of which were sold in the market are susceptible to SARS-CoV, but none of the animal products sampled in the market tested positive in this study. In the early phase of pandemic, due to lack of awareness of the potential role of cold chain in virus introduction and transmission, the cold-chain products were not tested. These findings however, do raise the possibility of different potential pathways of introduction. Preliminary sampling and testing of other markets in Wuhan and upstream suppliers to the Huanan market taken during 2020 did not reveal evidence of SARS-CoV-2 circulating in animals.

SARS-CoV-2 has been found to persist in conditions found in frozen food, packaging and cold-chain products. Index cases in recent outbreaks in China have been linked to the cold chain; the virus has been found on packages and products from other countries that supply China with cold-chain products, indicating that it can be carried long distances on cold-chain products.

Further analysis will examine spatial and temporal correlations and correct for underlying biases in sampling, and also to trace frozen products back to the Huanan market from suppliers.

The team suggested next-phase studies to help tracing the origin of SARS-CoV-2 and the closest common ancestor to this virus, including analysis of trade and history of trade in animals and products in other markets, particularly in markets epidemiologically linked to early human cases or sequence data, surveys of susceptible animals in farms in South-East Asia and further afield for viruses related to SARS-CoV-2, livestock farms where coronavirus-susceptible animals are present, and continued, targeted surveys of fur farms for SARS-CoV-2 and related viruses. Farmers, suppliers and their contacts could be followed up, and cohorts of workers who have an occupational risk of exposure to animals and cold-chain products could be serologically tested for unusually high antibody titres that might suggest a risk for SARS-Cov-2 emergence.

The joint international team made a series of recommendations for each area (see details in the report) and in doing so assessed the likelihood of different possible pathways for the introduction of the virus.

The joint international team examined four scenarios for introduction:

- direct zoonotic transmission to humans (spillover);
- introduction through an intermediate host followed by spillover;
- introduction through the (cold) food chain;
- introduction through a laboratory incident.

For each of these possible pathways of emergence, the joint team conducted a qualitative risk assessment, considering the available scientific evidence and findings. It also stated the arguments against each possibility. The team assessed the relative likelihood of these pathways and prioritized further studies that would potentially increase knowledge and understanding globally.

The joint team's assessment of likelihood of each possible pathway was as follows:

- direct zoonotic spillover is considered to be a possible-to-likely pathway;
- introduction through an intermediate host is considered to be a likely to very likely pathway;
- introduction through cold/ food chain products is considered a possible pathway;
- introduction through a laboratory incident was considered to be an extremely unlikely pathway.

#### Background

The emergence of SARS-CoV-2 was first observed when cases of unexplained pneumonia were noted in the city of Wuhan, China. (1) During the first weeks of the epidemic in Wuhan, an association was noted between the early cases and the Wuhan Huanan Seafood Wholesale Market (hereafter referred to as the "Huanan market"); cases were mainly reported in operating dealers and vendors. (1) The authorities closed the market on 1 January 2020 for environmental sanitation and disinfection. The market, which predominantly sold aquatic products and seafood as well as some farmed wild animal products, was initially suspected to be the epicentre of the epidemic, suggesting an event at the human-animal interface. Retrospective investigations identified additional cases with onset of disease in December 2019, and not all the early cases reported an association with the Huanan Market.(2)

Although the role of civets as intermediate hosts in the outbreak of severe acute respiratory syndrome (SARS) in 2002-2004 had been favoured and a role for pangolins in the outbreak of COVID-19 was initially posited, subsequent epidemiological and epizootic studies have not substantiated the contribution of these animals in transmission to humans. The possible intermediate host of SARS-CoV-2 remains elusive.

Bats have been identified as the hosts of a series of important zoonotic viruses (for example, Nipah virus, Hendra virus and SARS-CoV), including coronaviruses with considerable genetic diversity. (3, 4) Of particular relevance with regard to COVID-19 are those coronaviruses that were found to be associated with the outbreaks in humans of SARS in 2002 and the Middle East respiratory syndrome (MERS) in 2013.(5)

The causative virus of COVID-19 was rapidly isolated from patients and sequenced, with the results from China subsequently being shared and published in January 2020. (6) The findings showed that it was a positive-stranded RNA virus belonging to the *Coronaviridae* family (a subgroup B betacoronavirus) and was new to humans. In the early work, analysis of the genomic sequence of the new virus (SARS-CoV-2) showed high homology with that of the coronavirus that caused SARS in 2002-2004, namely SARS-CoV (another subgroup B betacoronavirus). (5) Over the next year extensive work globally on sequences and phylogeny followed and the results have been shared internationally and stored through the GISAID platform.

SARS-CoV-2 also shares a 96.2% homology with a sequence of a strain of coronavirus (RaTG13) previously identified by genetic sequencing from a horseshoe bat sample (*Rhinolophus* species) and to a lesser extent with a strain isolated from pangolins. The RaTG13 virus sequence is the closest known sequence to SARS-CoV-2.

As with the coronaviruses that cause SARS and MERS, human-to-human transmission of SARS-CoV-2 was soon established, (7) but the virus demonstrated much greater in fectivity than these other two coronaviruses. (8) SARS-CoV-2 shows a broad tissue tropism, in particular binding through its spike protein to angiotensin-converting enzyme 2 (ACE2). It also directly infects endothelial cells lining the blood vessels, unusually for a human respiratory virus. Other novel pathological features of the virus are hypercoagulability and the excessive multi-organ immune system response and long-term sequelae. People infected with SARS-CoV-2 appear to be most infectious at the time of onset of symptoms but were also infectious in the days before onset. Infections can be asymptomatic, cause a mild illness or result in severe disease and death.

In February 2020 the joint WHO-China mission on COVID-19 (9) was convened to inform planning in China and internationally on the next steps in the response to the ongoing outbreak of COVID-19. Its major objectives were:

- to enhance understanding of the evolving COVID-19 outbreak in China and the nature and impact of ongoing containment measures;
- to share knowledge on the COVID-19 response and preparedness measures being implemented in countries affected by or at risk of importations of COVID-19;
- to generate recommendations for adjusting COVID-19 containment and response measures in China and internationally; and
- to establish priorities for a collaborative programme of work, research and development to address critical gaps in knowledge and response and readiness tools and activities.

In May 2020, the Seventy-third World Health Assembly adopted resolution WHA73.1 on the COVID-19 response. Through the resolution, Members States requested the Director-General "to continue to work closely with the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO) and countries, as part of the One-Health Approach to identify the zoonotic source of the virus and the route of introduction to the human population, including the possible role of intermediate hosts, including through efforts such as scientific and collaborative field missions, which will enable targeted interventions and a research agenda to reduce the risk of similar events occurring, as well as to provide guidance on how to prevent infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in animals and humans and prevent the establishment of new zoonotic reservoirs, as well as to reduce further risks of emergence and transmission of zoonotic diseases".

In July 2020, building on the recommendations of the Seventy-third World Health Assembly, the WHO sent an advance team to China to agree on a way forward to better understand the origins of the virus. The agreed Terms of Reference (10) defined the scope of studies, the main guiding principles and the main expected deliverables. These ToRs envisaged two phases of studies: short-term studies (Phase 1) to better understand how the virus started to circulate in Wuhan; and, building on the findings and the published scientific literature, longer-term studies (Phase 2). The ToRs included the setting up of a joint international team of experts that would help analyse Phase 1 studies outcomes and design, and support and conduct the Phase 2 studies. The work aimed to contribute to improving the understanding of the virus origins. The overall results and findings would benefit improved global preparedness and response to SARS-CoV-2 and emerging zoonotic diseases of similar origin.
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# Members of the joint international team and methods of work

On 17 August 2020, the WHO Global Outbreak Alert and Response Network (GOARN) issued a call for expressions of interest for experts to participate in the international team to study the origins of SARS-CoV-2 jointly with Chinese experts. In September 2020, the WHO Secretariat evaluated the candidates received as well as candidates proposed by WHO Member States against the expertise needed, including:

- **senior epidemiologists**, with expertise in infectious disease epidemiology and operational research
- **senior data scientists**, with expertise in advanced statistics and infectious disease modelling, particularly in operational contexts
- senior laboratory experts, particularly with experience in SARS-CoV-2 diagnostics and serological studies in human and/or animal populations
- **senior food safety experts**, with experience in persistence of viruses and virus transmission through food and the environment
- **senior veterinary epidemiologists**, with experience in coronaviruses and animals, zoonoses and zoonotic epidemiological investigations
- senior animal health experts, with experience in emerging animal diseases, food animal production and animal disease surveillance.

Among the qualified candidates, additional criteria such as geographical representation and gender were taken into consideration and a list of 10 members was finalised and shared with China officially on 30 September. On 15 October 2020, the Government of China indicated that it had no objection to the list of the international team members.

The joint international team comprised 17 national Chinese, the 10 international experts from Australia, Denmark, Germany, Japan, Netherlands, Russian Federation, Sudan, United Kingdom of Great Britain and Northern Ireland, Viet Nam, and United States of America, plus seven other experts and support staff from the World Organisation for Animal Health (OIE) and WHO. It was headed jointly by Dr Peter K Ben Embarek of WHO and Professor Liang Wannian of the People's Republic of China. The full list of the Chinese members and their affiliations and their international counterparts is available in Annex B. Two staff members from the Food and Agriculture Organization of the United Nations (FAO) participated as observers.

# Declarations of interest

The WHO international team was finalized with the completion of administrative procedures, including a declaration of interest and a confidentiality undertaking. All declared interests were assessed and found not to interfere with the independence and transparency of the work. The declared interests were shared with all team members and were managed by the WHO Secretariat.

# Working procedures

All members of the team served in their personal scientific capacity and not in that of any institution or government with which they were associated. All team members had the same status within the team and all conclusions and decisions were formed jointly, with the same weight being given to the word of each member. Methods of work

The joint study was conducted by the joint team over a 28-day period from 14 January to 10 February 2021 in Wuhan, China. This followed a series of virtual meetings of the WHO international team and the Chinese experts from October to December 2020.

The joint team began working through a series of formal and informal virtual meetings. For the first two weeks, the international team members remained in quarantine and worked exclusively with Chinese experts through video/teleconference calls, exchanging information and presentations through electronic means. For the second 14-day period, Chinese public health regulations required that the international team remained under health monitoring. As a result, all site visits, meetings and interviews were planned and agreed in advance, and conducted with due regard for public health measures including physical distancing.

The joint study began its formal work with a plenary meeting of the international team and the team leading or contributing to the response in China through the National Prevention and Control Task Force. Participants reviewed the initial terms of reference for the work agreed upon for the Phase 1 studies decided on by China and the WHO in July 2020.

A workplan was agreed for the joint study on origins tracing and the development of a joint report with recommendations for Phase 2 studies (Annex A1), as mandated in the July ToRs. It was agreed to establish three focused working groups: (1) epidemiology, (2) molecular epidemiology and bioinformatics, and (3) animal and environment. The schedule of work is available in Annex A2.

Extensive discussions, with full interpretation, site visits and input from a large number of Chinese health professionals, scientists and other experts, culminated in the consideration of an executive summary of the draft final report for presentation at the end of the joint study.

In the July 2020 ToRs, specific studies were agreed by China and WHO. Based on these ToRs, the Chinese team initiated epidemiological, environmental and retrospective studies, the results of which were presented in meetings before and during the visit. The international team reviewed the work done on these agreed Phase 1 studies, some of which were still works in progress. In the course of the discussions the international team gained a deeper understanding of the methods used and discussed additional analyses for some of the data sets provided, reflecting a need for an iterative approach to refine the analyses of such complex studies.

The final report describes the methods and results as presented by the Chinese team's researchers. The findings are based on the information exchanged among the joint team, the extensive work undertaken in China in response to requests from the international team, including re-analysis or additional analysis of collected information, review of national and local governmental reports, discussions on control and prevention measures with national and local experts and response teams, and observations made and insights gained during site visits. The figures have been produced using information and data collected during site visits and with the agreement of the relevant groups. References are available for any information in this report that has already been published in journals. Conclusions and recommendations are based on joint discussions.

In concluding plenary sessions, the joint team consolidated its findings, generated conclusions and proposed further actions.

Presentations

In addition to the exchange of information in working groups, detailed presentations were given on highly relevant topics to help to inform the work of the joint team:

- An overview of the development of the integrated database developed by the China National Center for Bioinformation (Dr Song Shuhui)
- The transmission of SARS-CoV-2 among mink in the Netherlands and steps taken to control outbreaks (Professor Marion Koopmans)
- Pathogen identification of COVID-19 (Professor Shi Zhengli)
- Animal and environmental collection and testing in Huanan Market (Dr William Jun Liu and Dr He Xiaozhou)
- Types and sources of animal products in the Huanan Market (Dr Wu Zhiqiang)
- COVID-19 pandemic traceability and the cold chain virus transmission (Dr Jia Zhiyuan and Prof Jiang Jingkun)
- Progress in tracing and monitoring of SARS-CoV-2 in domestic animals (Drs Ni Jianqiang, Li Dong, Wang Chuanbin and Xin Shengpeng (China Animal CDC)
- The investigation into the outbreak of SARS-CoV-2 in Xinfadi market, Beijing in May-June 2020 (Dr Pang Xinghuo)
- An overview of geographical hotspots for potential emergence of zoonotic viral diseases (in particular coronavirus-related diseases) (Dr Peter Daszak)
- Laboratory detection methods for SARS-CoV-2 detection in animal samples (Dr Ni Jianqiang)
- The activity of the SARS-CoV-2 Laboratory, Hubei Center for Disease Control and Prevention (Dr Huo Xixiang)
- Surveillance of SARS-CoV-2 in wild animals (Dr He Hongxuan)
- The infection risk in cats, dogs and pigs to SARS-CoV-2 from Central China Agriculture University (HZAU) (Dr Jin Maili<sup>1</sup>).
- Presentation of the Wuhan Institute of Virology (Dr Wang Yanyi)
- Presentation of the Wuhan Blood Centre (Dr Wang Ian)

PowerPoint presentations from the plenary sessions are attached in Annex C.

# Site visits

The objective of the site visits was to obtain first-hand information about the places, the environment, the workflows and processes that would be crucial for the study subjects and the origins of the virus, as well as meeting key people. The places were grouped into the following categories:

- 1. sites related to treatment, diagnosis and epidemiological investigation of the first cases, including hospitals, laboratories, the Huanan Market and its neighbourhood, traders and suppliers, the first patients, community leaders and journalists
- 2. centres for human and animal disease control
- 3. key surveillance partners, including municipal and provincial reference laboratories for influenza-like illnesses (ILI) and blood donor centres
- 4. other key partners, including authorities of market regulation, environment and agriculture and researchers.

The schedule of visits is set out in Table 1, and the location of site visits and other relevant points provided in Map 1. During these visits, the team had detailed discussions and consultations; the

<sup>1</sup> In place of a visit to the Huazhong Agricultural University.

annexes listed contain summary reports of the visits. For some of these visits, only part of the team participated while other team members worked in their respective working groups.

29 January, pm	Xinhua Hospital (Hubei Hospital of Integrated	Annex D1
	Traditional Chinese and Western Medicine)	
30 January, am	Jinyintan Hospital for Infectious Diseases	Annex D2
30 January, pm	COVID-19 Exhibition	
21 January am	Deicharthou Wholegale Market	Annov D2
51 January, and	Daisitazitou wholesale Market	Annex D5
31 January, pm	Huanan Seafood Wholesale Market	Annex D4
- 571		
1 February	Hubei Province and Wuhan CDCs	Annex D5
1 Teordary		
2 February	Wuhan Hubei Animal CDC	Anney D6
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3 February	wunan Institute of Virology	Annex D/
4 February	Jianxinyuan Community Centre	Annex D8
, i coruary		

Table 1. Date and location of visits, with annexed summary reports

In addition, experts from the following institutions visited the international team at its hotel to present information and to engage in discussions: Huazhong Agricultural University (4 February), Wuhan Blood Centre (5 February) and Wuhan Central Hospital (6 February).



Map 1. Site visits, Wuhan.

# MAIN FINDINGS

# **EPIDEMIOLOGY**

Before the joint study, the earliest recognized cases of COVID-19 in Wuhan were thought to have occurred in early December 2019.(1) Preliminary information from surveillance of severe pneumonia had suggested no unusual clustering or departure from trends in the weeks and months preceding these first reported cases. As SARS-CoV-2 infection may, however, be asymptomatic or cause only mild illness in many individuals,(2-4) it is likely that others were infected at the time of the recognition of the early cases and that transmission could have been occurring in the community before this point. Investigation into the possible occurrence of earlier cases is therefore important.

Many of the early cases were reported to have a link to the Huanan market, a place where animals and animal products were sold to the public. Some reports have suggested the zoonotic spread of SARS-CoV-2 through this market, although the role of the market, as either the source of the initial transmission of the virus to humans or as an amplifier of the early epidemic, was unclear, as several early cases reported no link to the Huanan market or any other market in Wuhan.(5)

Several Phase 1 studies were agreed following the drafting of the ToRs in July 2020<sup>2</sup>, and work was carried out ahead of the arrival of the international team in January 2021. This work included extensive data collection, data cleaning, review of clinical records, patient interviews and testing, and preparatory analyses. The studies were reviewed in depth by the joint international WHO/Chinese team, and additional analyses were done based on these reviews. The overall focus of the studies was to determine:

- whether there was evidence of transmission of SARS-CoV-2 in Wuhan or Hubei Province in the period preceding the recognized outbreak in Wuhan in December 2019 using routine disease and death surveillance data, review of clinical records and targeted SARS-CoV-2 laboratory testing;
- (2) whether there was evidence of transmission of SARS-CoV-2 in the wider population of Wuhan or Hubei Province at the time the outbreak was recognized in Wuhan in December 2019 using information from the cases reported with onset in that month; and
- (3) whether the epidemiological characteristics of the early cases associated with the Huanan market pointed to a specific time, location or source of the introduction of infection into the market at the beginning of the outbreak.

Surveillance data - morbidity

Epidemiological analysis of influenza-like illness (ILI) and severe acute respiratory infection (SARI) surveillance before January 2020

#### Introduction

This section summarizes work carried out by the Chinese team, together with key findings based on the methods and analyses agreed in the Terms of Reference. A detailed account of this work is attached at Annex E1.

ILI and SARI surveillance, with appropriate laboratory confirmation, is conducted routinely as a measure of the impact of influenza and other respiratory virus infections in the community. (6) The ILI case definition is designed to capture a high proportion of patients with influenza (high sensitivity) but, as the symptoms are also common to other respiratory infections, the case definition is non-specific. To increase the specificity of this surveillance for influenza infection, the ILI and SARI cases are linked with data from laboratory testing for influenza in a subset of cases from which respiratory tract samples are obtained.

China operates a national surveillance system, based on a network of hospitals and Chinese Center for Disease Control and Prevention (CDC) laboratories, to monitor the occurrence of ILI and SARI throughout the year. (7) This system monitors trends in the occurrence of influenza (including new influenza virus types/A subtypes) and provides an early warning of changes in influenza activity. This system also contributes to the surveillance for other respiratory disease syndromes and pathogens. (8)

## Objective

The Phase 1 studies and the subsequent work agreed by the working group set out to:

- review and compare the trends in ILI and SARI surveillance data among the population of Wuhan, Hubei province and neighbouring provinces and municipalities from 2016 to 2019
- (2) seek clusters of illness compatible with COVID-19 in the months preceding the onset of the SARS-CoV-2 outbreak in December 2019.

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

# Methods Population The population of Hubei Province is about 59 million and of Wuhan about 11.1 million.

# Surveillance systems Sentinel surveillance for ILI

The national ILI sentinel surveillance system gathers data for ILI from two hospitals in Wuhan. These data were reviewed in the months preceding the outbreak and compared with previous years. As one general (No. 1 Hospital of Wuhan) and one paediatric hospital (Wuhan's Children's Hospital) in Wuhan contribute data to the national sentinel surveillance system, trends in ILI in children and adults in Wuhan can be examined separately. Elsewhere in China, data are collected from hospitals that include all age groups. In Hubei province, outside Wuhan, ILI surveillance includes 18 sentinel hospitals and 13 associated network laboratories.

The number of cases of ILI and the total number of visits to outpatient and emergency departments are reported weekly by age groups (0-4 years, 5-14 years, 15-24 years, 25-59 years and  $\geq 60$  years).

# Sentinel surveillance for severe acute respiratory illness (SARI)

After the SARS epidemic in 2003, WHO recommended that influenza surveillance systems should also include sentinel surveillance for SARI, which is often defined as ILI plus one additional symptom or sign of severe illness in a hospitalized patient.(9)

In China, the national SARI sentinel system includes a network of sentinel SARI general hospitals located in either a provincial capital cities or other cities with convenient transportation networks. (9) The SARI sentinel hospital for Hubei Province is in Jingzhou; there is no SARI sentinel hospital in Wuhan. In Hubei's neighbouring provinces, there are SARI sentinel hospitals in Luohe (Henan Province), Hefei (Anhui Province) and Changsha (Hunan Province).

The departments responsible for SARI surveillance include respiratory, paediatric internal medicine and infectious diseases, and intensive care units.

Patients who meet the SARI case definition are recorded daily. Cases are counted as hospitalized patients in age groups (0-1, 2-4, 5-14, 15-49, 50-64 and  $\geq$ 65 years).

# Analytical methods

The case information and laboratory results of ILI cases in Hubei, Anhui, Henan, Hunan, Shaanxi, Chongqing and Jiangxi provinces from 2016 to 2019 were reviewed and trends analysed, as were the SARI case information and laboratory results in Hubei, Henan, Anhui and Hunan provinces for the same period. Data, plotted as weekly numbers of cases for the period of January to December 2019, were compared with levels for the same months in previous years to identify deviations from the expected trends.

For ILI, the percentage of all outpatient and emergency department visits to the sentinel hospitals that were categorized as ILI was recorded. The percentage of the subset of ILI cases from which respiratory specimens were examined and reported to be due to influenza virus infection was recorded.

For SARI, the percentage of all outpatient and emergency department visits to the sentinel hospitals that were categorized as SARI was recorded. The percentage of SARI cases from which respiratory specimens were examined and reported to be due to influenza virus infection was recorded.

Results

1. Analysis of ILI surveillance data in Wuhan in 2019, compared with 2016-2018 A similar level of occurrence of ILI cases in the sentinel surveillance systems in Wuhan is seen in 2019 and in the previous three years, until week 48, when a steep increase is seen in 2019, which rapidly exceeds the trend of the previous three years (Fig. 1).



# Fig. 1. Weekly number of ILI cases in the sentinel surveillance in Wuhan in 2019 compared with the average weekly value for the previous three years.

In 2019, most of the ILI cases reported in Wuhan were in children (Figs. 2A and 2B). The number of cases in children increased rapidly from week 49. The number of ILI cases reported in adults was considerably lower than that reported in children. An increase in the number of cases in adults was seen in weeks 4 and 5 of 2019, and smaller peaks in weeks 17, 46 and 52.

Influenza virus infection was prevalent in children with ILI in Wuhan in the early part of 2019 (Fig. 2C) accounting for more than 50% of ILI cases tested in the period from week 3 to 8. Influenza was also seen in adults during this period but accounted for a lower proportion of ILI cases tested. A sharp rise is seen in the proportion of ILI cases due to influenza virus infection in children from week 48 followed, two to three weeks later by a rise in adults. Both influenza B and influenza A (subtype H3N2) were reported by the Chinese team to be circulating in the Wuhan population in December 2019.



Fig. 2A. Weekly number of ILI cases in children in the sentinel surveillance in Wuhan in 2019 (and percentage of outpatient visits categorized as ILI, [ILI %]).



Fig. 2B. Weekly number of ILI cases in adults in the sentinel surveillance in Wuhan in 2019 (and percentage of outpatient visits categorized as ILI, [ILI %]).



# Fig. 2C. Weekly percentage of ILI cases with laboratory-confirmed influenza [FLU %] in the sentinel surveillance in children and adults in Wuhan in 2019.

The weekly percentage of ILI cases in both children and adults in the sentinel surveillance in Wuhan in 2019 laboratory-confirmed to be due to influenza virus infection was compared with the weekly percentages in the previous three years (Annex E1). There was considerable week-to-week variation in the proportion reported positive for influenza virus in both children and adults, with the percentage generally being lower between week 15 and week 40 and higher between week 40 and week 15 of the next year (consistent with the usual seasonal influenza activity). The rise in influenza virus infections, as a proportion of ILI, is apparent in both children and adults at the end of 2019: in children this rise is comparable to rises seen in earlier years; in adults the steep rise in ILI due to influenza virus infection at the end of 2019 is apparent but the percentage positive is little different to that seen at the end of 2016. Only about 20 samples per week were tested.



## 2. Analysis of ILI surveillance data in Hubei province

Fig. 3. Weekly number of ILI cases in all ages in the sentinel surveillance in Wuhan and other cities in Hubei province in 2019.

In 2019, the weekly distribution of ILI cases in all ages in Wuhan was similar to that in other cities in Hubei Province, rising from the week 48 (Fig. 3).

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Also, the ILI% rate in other cities in Hubei Province was similar to that of Wuhan, rising from week 49 (Figs. 4 and 5).

# Fig. 4. Weekly number of ILI cases in children and adults in Hubei Province in 2019 (and percentage of outpatient visits categorized as ILI, [ILI %]).

In 2019, most ILI cases in Hubei Province as in Wuhan city were reported in children (Fig. 4). As in Wuhan (Fig. 1), the weekly number of ILI cases in Hubei Province (and the percentage of all consultations categorized as ILI) rose steeply from week 49 in 2019.

The weekly percentage of ILI cases in Hubei Province in 2019 laboratory-confirmed to be due to influenza virus infection showed less week-to-week variation than the percentage observed for Wuhan alone (likely owing to the larger denominator of ILI cases across the whole province) but exhibited the same general trend of higher rates before and after the end of the year and lower rates in the middle of the year (Annex E1).







Fig. 5B. Percentage of outpatient visits categorized as ILI in Hubei and six neighbouring provinces or municipalities in 2019.



# Fig. 5C. Weekly percentage of ILI cases with laboratory-confirmed influenza in Hubei and six neighbouring provinces or municipalities in 2019.

In 2019, the distribution by week of ILI cases, and the percentage of outpatient visits categorized as ILI [ILI%] in Hubei Province was similar to that observed in the six neighbouring provinces and municipalities (Figs. 5A and 5B). Numbers of cases were high at the beginning of the year, falling by week 10, and rising again steeply from weeks 48 and 49. The rise in the percentage of ILI cases laboratory-confirmed as due to influenza virus infection in Hubei at the end of 2019 was also seen in the six neighbouring provinces or municipalities (Fig. 5C).

#### Conclusions

Based on the sentinel surveillance data for ILI, and the associated laboratory-confirmed influenza activity, in Wuhan as well as Hubei and six surrounding provinces, there was a marked increase in ILI

in both children and adults at the end of 2019 in Wuhan, but no evidence to suggest substantial SARS-CoV-2 transmission in the months preceding the outbreak in December was observed. The increase in ILI is mirrored in the remainder of Hubei Province and in neighbouring provinces and municipalities. While this increase may be explained by a contemporary increase in laboratory-confirmed influenza activity, further time series analyses were recommended and are underway to ensure that no other signals are present.

#### 3. SARI surveillance in Hubei Province

Most cases of SARI reported in the sentinel surveillance in Hubei Province were in children up to the age of 15 years (Fig. 6). The SARI surveillance is based on one hospital only and this is not located in Wuhan. In 2019, the weekly number of SARI cases in Hubei Province, and the percentage SARI cases represented of all outpatient and emergency department visits, varied substantially being generally higher at the beginning and end of the year, and lower in the period from about week 29 to 48. No increase in SARI cases is apparent in adults in the final weeks of 2019 (at the time the outbreak of COVID-19 is now known to have been starting in Wuhan).



Fig. 6. Weekly number of SARI cases in Hubei Province in 2019, by age group (and the percentage of outpatient visits categorized as SARI, [SARI %]).



# Fig. 7. Percentage of outpatient visits categorized as SARI [SARI %] and the percentage of SARI cases laboratory-confirmed to be due to influenza infection [FLU %], Hubei Province, 2019.

The percentage of SARI cases in Hubei Province in 2019 laboratory-confirmed to be due to influenza infection was generally below 0.4%, but rose to 0.6% at the end of 2019, coincident with the rise in influenza activity generally demonstrated by the ILI surveillance (Fig. 7).



# Fig. 8. Percentage of outpatient visits categorized as SARI [SARI %] in the sentinel surveillance in Hubei and neighbouring provinces in 2019.

The percentage of hospital and emergency department visits that were categorized as SARI in the sentinel surveillance in Hubei (Fig. 8) was similar to that seen in other provinces surrounding Hubei, with considerable week-to-week variation. The small increase in this percentage between weeks 46 and 51 of 2019 in the neighbouring provinces, compared with Hubei Province, is unlikely to be significant in the light of the small numbers and week-to-week variation.

## Conclusions

The SARI surveillance data from one single provincial hospital in Hubei Province did not suggest any previously undetected clusters of severe respiratory illness compatible with COVID-19 in the months preceding December 2019. Nor did the SARI surveillance data from Hubei Province provide any clear indication of the onset of the COVID-19 epidemic in Wuhan as was observed in the SARI surveillance data from other provinces. This could either be due to lack of sensitivity or data incompleteness based on the limited information from one hospital only or might reflect that this particular provincial city and area in Hubei Province did not experience any increase in SARI cases in late 2019.

4. SARS-CoV-2 testing of respiratory tract samples from ILI surveillance in late 2019 Respiratory tract samples collected as part of ILI surveillance in Wuhan, elsewhere in Hubei Province and in Shaanxi Province in 2019 were tested retrospectively for SARS-CoV-2 by nucleic acid tests (Table 1). All were negative.

Month	Hubei Province						
	Wuhan				N		Shaanxi
	Sentinel hospital		Other	Sub-total	- Non - Wuhan	Subtotal	Province
	Child	Adult	hospital				
October	80	80	0	160	1610	1770	539
November	80	80	0	160	1782	1942	669
December	100	100	138	338	3068	3406	1196
Total	260	260	138	658	6460	7118	2404

 Table 1. Stored ILI samples tested for SARS-CoV-2 in late 2019.

Retrospective SARS-CoV-2 NAT on ILI surveillance swabs extending the period from 6 October 2019 to 21 January 2020 has been published. (10) This showed that 9 of 120 samples were SARS-CoV-2 NAT positive (tested at the Wuhan CDC) in the first three weeks in January: of the adults sampled 9 of 45 (20%) were SARS-CoV-2 NAT positive. This figure is higher than the proportion for influenza virus detection in the same samples from adults where influenza NAT was positive in 7 of 45 (16%). The nine SARS-CoV-2 NAT positives came from six different districts in Wuhan. There were no co-infections. It should be noted that no samples from adults were available for testing in the last three weeks of December 2019, so conclusions about SARS-CoV-2 causing ILI in adults in December cannot be made. Sample numbers in general are modest in comparison to the risk population size.

5. SARS-CoV-2 testing of respiratory tract samples from SARI surveillance in late 2019 in Hunan and Henan provinces

Respiratory tract samples (n = 274) collected in Hunan (n = 28) and Henan provinces (n = 246) as part of SARI surveillance in late 2019 were tested for SARS-CoV-2 by NAT. In Hunan province, there were 12 paediatric samples and 16 adult samples; in Henan province, there were 218 paediatric samples and 28 adult samples (Fig. 9). All were negative.





# Fig. 9. Distribution and age groups of respiratory tract samples collected in Hunan and Henan provinces as part of SARI surveillance by month in late 2019.

#### Conclusions

Review of retrospective testing of respiratory tract swabs collected within the ILI and SARI surveillance system, and the adult sentinel surveillance data for ILI from one hospital in Wuhan and SARI surveillance data from a provincial hospital in Hubei Province revealed no clear indication of substantial unrecognized circulation of SARS-CoV-2 in Wuhan during the latter part of 2019. Further time series analyses are underway.

#### Recommendations

The joint team recommends further exploration of the weekly ILI trends (especially in adults) in 2019, in comparison to the earlier years, using time series analyses.

Review of purchases of antipyretics, cold remedies and cough medications in retail pharmacies in Wuhan

### Introduction

Community purchase of retail antipyretics, cold and cough medications may provide a general indication of community respiratory tract disease. (11) The joint international team requested information on relevant medications potentially used in community respiratory tract infections.

# Methods

Retail pharmacies in Wuhan provided data of purchases of antipyretics (34 types), cold remedies (47) and cough medications (57) from September to December over four years, 2016-2019.

#### Results

As shown in Fig. 10, purchases of all medications increased in a linear mode over the four-year study period.



# Fig. 10. Purchases of cold medicines, cough medicines and antipyretics in pharmacies in Wuhan in the period September-December for 2016-2019.

#### Conclusions

Analysis of four months of aggregated retail pharmacy purchases for antipyretics, cold and cough medications over a period of four years was unlikely to provide a useful indicator of early SARS-CoV-2 activity in the community.

#### Recommendations

Review pharmacy purchases by week during the period of September to December in 2016, 2017, 2018, and 2019 to look for any signals of increased purchases in the weeks of September to December 2019 compared with the same weeks during the previous years. If any signals are identified, then proceed with analyses for spatial-temporal clusters.

#### Mass gatherings

#### Introduction

Mass gatherings may facilitate transmission of respiratory viruses and there has been speculation that SARS-CoV-2 may already have circulated in the months before December at specific mass gatherings. The joint international team therefore requested information on mass gatherings held in Wuhan in late 2019.

# Results

The Chinese Epidemiology Group provided information on of international gatherings held in Wuhan from September-December 2019 (Table 2). These included the 7<sup>th</sup> World Military Games held from 18 to 27 October 2019 (9308 participants listed as attending), and the 44<sup>th</sup> World Bridge Team Championships in September 2019. In the Military Games, four African participants were diagnosed and treated for malaria, and one U.S. citizen presented with gastroenteritis. The Jinyintan Hospital provided medical support for the games, including on-site clinics (data from these clinics have not yet been evaluated by the joint team). From the Bridge Championships an Italian was admitted with acute gastroenteritis.

Fundamental information	Sep.	Oct.	Nov.	Dec.	Total
Amount of gathering	12	7	11	14	44
Number of participants	3750	9511	34744	21961	69966
The participants number of biggest gathering	1500	9308	34400	21538	9308
Number of foreign participants	1684	9108	301	418	11511
The largest number foreign participants	900	8945	103	71	8945
Number of participating countries	59	136	18	27	146

# Table 2. Statistics on international conferences held in Wuhan, September-December 2019.

## Conclusions

No appreciable signals of clusters of fever or severe respiratory disease requiring hospitalization were identified during review of these events.

## Recommendations

Consideration should be given to further joint review of the data on respiratory illness from the on-site clinics at the Military Games in October 2019.

## Surveillance data – mortality

# Methods

A retrospective study of all-cause mortality from two mortality surveillance systems covering 14 surveillance points (covering all districts) in Wuhan city and 19 mortality surveillance points in Hubei Province outside Wuhan was undertaken to identify and investigate early signals compatible with potential previously undetected COVID-19-associated deaths.

*Death surveillance system.* The first national system was established in 1978 to monitor changes in deaths and disease patterns in the population. In 2004, based on multi-stage stratified cluster random sampling, the National Death Surveillance System expanded its capacity to 161 surveillance points covering 31 provinces, municipalities and autonomous regions nationwide. The death surveillance points system has been proved nationally to be representative and its results reflect changes in deaths and the health status of the entire population. In 2013, it was further integrated and expanded to 605 surveillance points (Fig. 11). The new death surveillance points system became provincially representative and covered more than 300 million people. *(12)* Each surveillance point is a county or a

district, and all deaths occurring in the death surveillance points system are reported. Three of the 22 surveillance points in Hubei Province are in Wuhan city. The mortality data of Wuhan city were obtained from the Wuhan Death Surveillance System, which began in the 1970s and is regarded as one of the earliest surveillance systems authorized by the National Health Commission. By 2009, this system covered all 14 districts in the city, and it receives reports from more than 300 general hospitals and primary medical institutions in Wuhan.

*Population, geography and surveillance system coverage.* The population data for the surveillance point in Hubei Province came from China's National Bureau of Statistics, and those for Wuhan city came from the Wuhan Public Security Bureau. Hubei Province has 103 counties/districts, 14 of which are in Wuhan. Wuhan city was an early participant in the mortality surveillance system. In Hubei Province, 20.3% of the population is covered by the death surveillance points system whereas in Wuhan the total population is covered by the surveillance points.





## Data sources and reporting process

In the case of deaths at medical institutions (including deaths upon arrival at the hospital, deaths in the process of pre-hospital emergency treatment, and deaths in the process of hospital diagnosis and treatment), the admitting doctor makes the diagnosis and completes the Medical Certificate of Cause of Death. For deaths occurring outside hospitals, the local health workers at the township health centre (community health service centre) determine the causes of death according to the medical history, physical signs and/or medical diagnosis provided by the deceased's family or others familiar with the case, and complete the death certificate. All the information in the death certificate is reported online through the cause of death registration and reporting system of China CDC. The underlying causes of death are inferred and coded by a trained coder or the staff of county CDC based on the reported death information. The ICD-10 coding system (International Statistical Classification of Diseases and Related Health Problems (10th revision) as endorsed in May 1990 by the Forty-third World Health Assembly, is applied.

## Classification of causes of death

On 2 February 2020, the Chronic and Non-Communicable Disease Center of China CDC issued guidance on the reporting of COVID-19-related deaths: "For the deaths of confirmed COVID-19 patients due to the deterioration of their condition, the ICD-10 coding of the underlying causes of death shall be U07.9 (novel coronavirus infection, not specific); for highly suspected but unconfirmed COVID-19-related deaths, the ICD-10 coding of the underlying causes shall be J12.8 (other viral pneumonia)". On 18 February 2020, based on the ICD-10 coding system for COVID-19 released by WHO, the Chronic and Non-Communicable Disease Center of China CDC updated the ICD-10 code

to U07.1 (COVID-19, virus identified) for confirmed (including clinically diagnosed) COVID-19 deaths.

The temporal and spatial trends of all causes and pneumonia deaths are analysed in Wuhan and Hubei Province (outside Wuhan), respectively. The ICD-10 codes for the causes of death are shown in Table 3.

Causes	ICD-10 codes	
All-cause	All ICD-10 codes	
Pneumonia	J12-J18.9, J98.4, U07.1	
Confirmed COVID-19	U07.1	
Suspected COVID-19	J12.8*	

Table 3. ICD-10 codes for classification of causes of death

\*J12.8 is the code for deaths of suspected COVID-19 cases only after 2020.

# Statistical analyses

The number of weekly deaths and mortality rates in Wuhan and Hubei Province outside Wuhan from 2016 to early 2020 was calculated, and the weekly all-cause mortality and pneumonia mortality rates in 2019 and early 2020 were compared with the average mortality rate from 2016 to 2018. The age subgroup analysis included all age groups and people over 65 years of age, respectively.

The weekly all-cause deaths and pneumonia deaths from 2016 to 2018 by different districts in Wuhan were calculated. The over-dispersed Poisson regression model accounting for seasonal patterns was established to estimate the weekly baseline deaths (that is, expected deaths) and the 95% confidence interval in different districts in Wuhan in 2019. *(13-15)* Excess deaths are statistically significant when the observed deaths exceed the upper limit of 95% confidence interval.

# Results

# Temporal trends of all-cause mortality

## Wuhan city

*All age groups.* Comparative trends of all-cause mortality for deaths in all age-groups in 2016, 2017 and 2018 allowed for direct comparison with that in 2019 and early 2020 in Wuhan. The trend of average mortality in the months of October to December in 2019 is similar (and slightly lower) to that in previous years until a steep increase beginning from week 3 (15-21 January) of 2020 (Fig. 12). After removal of confirmed and suspected COVID-19 cases, the trend in overall mortality does not change and is still lower than previous years until week 3 of 2020.



Fig. 12. A: Comparison of trends of the all-cause mortality rate in 2019-2020 against average rate for 2016-2018 in Wuhan, for all age groups; B: Comparison of trends of the all-cause mortality excluding confirmed and suspected COVID-19 mortality rates in 2019-2020 against average rate of 2016-2018 in Wuhan, for all age groups.

Age-group:  $\geq 65$  years of age. The trends are similar to overall figure, but the scale is different. The all-cause mortality rate of people 65 years or older in Wuhan during weeks 40-52 of 2019 (from October to December 2019) was lower than the average mortality rate of the same periods of 2016 to 2018. The all-cause mortality rates of people 65 years or older in Wuhan exceeded the average mortality rate in week 4 of 2020 (22-28 January 2020) and increased rapidly (Fig. 13).



Fig. 13. Trends of all-cause mortality. A: Comparison of trends of the all-cause mortality rate in 2019-2020 against average rate of 2016-2018 in Wuhan, for the ≥65-year-old population; B: Comparison of trends of the all-cause excluding confirmed and suspected COVID-19 mortality rates in 2019-2020 against average rate of 2016-2018 in Wuhan, for the ≥65-year-old population.

#### Hubei Province outside Wuhan

*All age groups.* There were no obvious differences between the mortality rate in weeks 40-52 of 2019 (from October to December 2019) and the average mortality rate in the same period from 2016 to 2018 in Hubei Province outside Wuhan. The all-cause mortality rate for 2019 in Hubei Province outside Wuhan was lower than the average level in the same period from week 5 to week 11 of 2020

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(from 29 January to 18 March 2020). After the confirmed and suspected COVID-19 deaths were excluded from all-cause deaths in 2020, the trend was similar to that of all-cause mortality, with the mortality rate from week 5 to week 11 of 2020 lower than the average of the same period. Trends over time show no obvious deviation from average rates from previous years (Fig. 14).



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Fig. 14. A: Comparison of trends of the all-cause mortality rate in 2019-2020 versus the average rate of 2016-2018, Hubei Province outside Wuhan, for all age groups; B: Comparison of trends of the all-cause mortality excluding confirmed and suspected COVID-19 mortality rates in 2019-2020 versus the average rate of 2016-2018 Hubei Province outside Wuhan, for all age groups.

Age-group  $\geq 65$  years of age. The all-cause mortality rate in Hubei Province outside Wuhan from week 5 to week 11 of 2020 (29 January–18 March 2020) was lower than the average level of the same period.

After confirmed and suspected COVID-19-related deaths were excluded from the all-cause mortality among the people over 65 years in 2020, the trend in mortality rate was similar to that of the all-cause mortality rate, and the mortality rate from week 5 to week 11 in 2020 was lower than the average mortality rate of the same period (Fig. 15).



Fig. 15. A: Comparison of trends of the all-cause mortality rate in 2019-2020 against the average rate of 2016-2018, Hubei Province outside Wuhan, for the  $\geq$ 65-year-old population; B: Comparison of trends of the all-cause excluding confirmed and suspected COVID-19 mortality rate in 2019-2020 against the average rate in 2016-2018 for Hubei Province outside Wuhan, for the  $\geq$ 65-year-old population.

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#### Pneumonia mortality

Wuhan city

*All ages.* The mortality rate for pneumonia in Wuhan from week 40 to week 52 of 2019 (from October to December 2019) was not different from the average of the same periods in 2016-2018. From the third week of 2020 (15-21 January 2020), the mortality rate of pneumonia was higher than average value of that in the same period in 2016-2018 and rose rapidly. From October to December 2019, the trends show no obvious deviation from the previous years (Fig. 16).



Fig. 16. Comparison of trends of the pneumonia mortality rate in 2019-2020 against the average rate for 2016-2018, Wuhan, for all age groups.

Age-group  $\geq 65$  years of age. The pneumonia mortality rate among population aged over 65 years in Wuhan during the weeks 40-52 of 2019 (October to December 2019) was not different from the average level of the same periods in 2016-2018. From the third week of 2020 (15-21 January 2020), the mortality rate was higher than the average and rose rapidly. From October to December 2019, the trend shows no obvious deviation from the previous years (Fig. 17).



# Fig. 17. Comparison of trends of the pneumonia mortality rate in 2019-2020 versus the average rate of 2016-2018, Wuhan, for the <u>></u>65-year-old population.

Hubei Province outside Wuhan

*All ages.* From October to December 2019 (weeks 40-52), the pneumonia mortality rate in Hubei Province outside Wuhan was slightly lower than the average level of previous years; no obvious change in the trend of pneumonia mortality rate was found and one minor spike was identified in week 44. The mortality rate for pneumonia in Hubei Province outside Wuhan, from weeks 5-7 of 2020, was higher than the average level of the same period in previous years (Fig. 18).



Fig. 18. Comparison of trends of the pneumonia mortality rate in 2019-2020 against average rate of 2016-2018, Hubei Province outside Wuhan, for all age groups.

Age-group  $\geq 65$  years of age. From October to December 2019 (weeks 40-52), the pneumonia mortality rate among people over 65 years in Hubei Province outside Wuhan was slightly lower than the average value of previous years. There was a minor spike in week 44 and a steep increase and peak in week 6 of 2020 (Fig. 19).



Fig. 19. Comparison of trends of the pneumonia mortality rate in 2019-2020 against average rate of 2016-2018, Hubei outside Wuhan, for the ≥65-year-old population.

# Spatial patterns of mortality in Wuhan

All-cause. Visualization of weekly excess mortality 2019-2020 in maps of the weekly death count by district in Wuhan (Fig. 20) showed increased mortality in week 30 (as seen in trend figures). In week 39 the map indicates an increase in Jiangxia district. This signal was investigated in-depth and revealed a weekly total number of deaths of 77 in this district. The estimated baseline is 59, the upper limit of 95% confidence interval is 76, resulting in only 1 excess death. Stratifying for age groups  $\geq 65$  years of age, provided no change in signal. Only in the third week of January 2020 is excess mortality reported which is fully compatible with COVID-19. The conclusion is that the signal of excess deaths before week 3 of 2020 is considered as unlikely to be compatible with previously undetected COVID-19 deaths.



Fig. 20. Weekly excess mortality of all-cause by districts in Wuhan, 2019-2020.

*Pneumonia deaths.* Weekly excess mortality due to pneumonia in 2019-2020 is visualized in maps of weekly death count by district in Wuhan during 2019-2020 (Fig. 21): increased mortality is seen in week 32 (late summer) and week 40 in Caidian district and week 44 in Jianghan district. These signals were investigated in-depth and revealed a total of three deaths (upper 95% confidence interval: two, thus one excess death) in week 40 and five deaths in week 44 (upper 95% confidence interval: four, thus one excess death). When stratifying for age groups  $\geq 65$  years, there were no changes in signals. The conclusion is that the signals of excess pneumonia deaths are considered unlikely to be compatible with previously undetected COVID-19 deaths.



Fig. 21. Weekly excess mortality of pneumonia by districts in Wuhan, 2019-2020.

# Strengths and limitations

The strengths of this study are that the analysis included large numbers of mortality data from several participating centres at provincial as well as Wuhan city-level, including death surveillance data covering all districts of Wuhan with high quality of cause-specific mortality (<2% ill-defined causes of death).

One limitation of this study is related to the Hubei provincial-level data having a lower representativeness with only 22 surveillance points and a resulting coverage of 20.3% of the total population. Nevertheless, the sample is considered representative of the Hubei provincial population and thus the data are sufficient to indicate overall mortality level and trends of mortality rates in Hubei Province.

# Conclusions

During the period August-December 2019, review of all-cause as well as pneumonia-specific mortality surveillance data provided little evidence of any unexpected fluctuations in mortality that might suggest the occurrence of transmission of SARS-CoV-2 in the population in the period before December 2019. This does not exclude, however, the possibility that some SARS-CoV-2 circulation was occurring in the population at a low level, as changes in mortality at the population level would be unlikely to be sufficiently sensitive to detect this possibility.

Four signals of excess weekly deaths compared to previous years were identified in the period reviewed. In-depth examination of these revealed a total of three excess deaths (one death in week 39 in the all-cause mortality and two deaths in the pneumonia-specific death surveillance data in week 40 and one in week 44, respectively, in two different districts of Wuhan). Based on the few and scattered excess deaths identified, we consider these less likely to be compatible with previously undetected COVID-19 deaths.

Given the time lag from onset of disease to COVID-19-associated death of a median of 17 days (12-22 days) in Wuhan, the documented rapid increase in all-cause mortality in week 3 of 2020 and pneumonia-specific deaths in week 3 of 2020 suggests that virus transmission was widespread among the population of Wuhan by the first week (1-7 January) of 2020. The steep incline in mortality rate occurred with 1-2 weeks' delay among the population in the Hubei Province outside Wuhan, supporting the previously reported *(16)* notion that the epidemic in Wuhan predated the spread in the rest of Hubei Province.

#### Proposals for future studies

The joint team recommends augmenting the mortality review by broadening the approach to include other provinces where phylogenetic analyses (Figure 5, *Molecular Epidemiology* section)have revealed early epidemic clusters, and comparison with other provinces and cities in China.

Clinical review of surveillance data and National Notifiable Disease Reporting System data

Review of reported cases of SARS-CoV-2 in December 2019 in Wuhan

#### Introduction

The outbreak of severe respiratory disease, subsequently determined to be due to infection with SARS-CoV-2, was recognized by Chinese health workers towards the end of December 2019.(17, 18) Searching for additional cases linked to this outbreak began immediately. The cases that were identified with the earliest onset occurred in December 2019 and were reported to the National Notifiable Disease Reporting System (NNDRS) and published. In order to investigate the origin of the outbreak, the clinical and epidemiological features of these early cases were reviewed.

#### Methods

Data sources. The NNDRS was developed and implemented in China in the aftermath of the 2003 severe acute respiratory syndrome (SARS) epidemic. *(19)* The existing paper-based disease-reporting system was transformed into the NNDRS, a web-based system operated by the China CDC to facilitate the complete and timely reporting of infectious diseases. The NNDRS allows for reporting of individual cases from every hospital, township and upper-level primary healthcare clinic directly to the China CDC. Before COVID-19 a total of 39 infections were notifiable as stipulated by the Law on the Prevention and Control of Infectious Diseases of China and included SARS. On 20 January 2020, COVID-19 was officially defined as a Category B infectious disease but to apply measures for it as a Category A infectious disease, namely to be reported to the NNDRS within two hours, albeit that review and confirmation of suspected cases can take longer time at each administrative level of approval (for example, municipal, district, provincial, national). As part of COVID-19 case review, only cases considered sufficiently likely to warrant isolation (whether in hospital or elsewhere) were included in the NNDRS and classified as either clinically diagnosed or laboratory confirmed.

Epidemiological investigation of all cases reported to NNDRS was carried out in the early months following the onset of the outbreak to identify close contacts with, or at risk of, illness, and other relevant exposures. Patients with diagnosed infection with SARS-CoV-2 were asked about close contacts who had been ill in the two weeks prior to onset of illness in the index case.

A detailed description of the methods used to identify cases is provided in Annex E2. Further data and analyses on the cases with links to the Huanan Market are provided in Annex E4. In view of the limited time available during the joint mission in Wuhan in January and February 2021, these data have not yet been analysed in depth by the joint team.

Case-definitions applied during the early phase of the epidemic in Wuhan in December 2019. The case-definitions used have a major impact on the number and characteristics of cases identified. The early case-definitions used are provided at Annex E3.

In the first days of the epidemic in Wuhan, cases were identified on the basis of clinical features, including fever and acute respiratory symptoms, radiology and epidemiological features.

An association with the Huanan market was identified among some of the earliest recognized cases and, for a short period until mid-January 2020, exposure to the Huanan market was included in the case definition. It rapidly became clear, however, that there were cases without a link to the Huanan market, and this element of the definition was dropped a few days after being introduced (Annex E3).

As the wider clinical spectrum of illness associated with infection became apparent, the case definition was modified. When laboratory testing for either SARS-CoV-2 nucleic acid or SARS-CoV-2-specific serological markers became available mid-January 2020, results of such testing were added to the definition, enabling an increasing number of cases to be designated as laboratory-confirmed, including cases with onset before mid-January where specimens were available.

Clinical review of early cases conducted as part of Phase 1 studies

As part of the Phase 1 studies, a review was carried out of all cases reported as potential cases of COVID-19 with onset in December 2019, including all cases that were accepted as formally notified cases in the NNDRS system and other cases that were re-interviewed in December 2020 or January 2021.

# Results

A total of 174 cases of COVID-19 were reported to the NNDRS with onset in December 2019: 100 were retrospectively laboratory-confirmed (by sequencing, NAT or serology) cases and a further 74 were clinically diagnosed cases (see Fig. 22). A detailed description of the cases is provided in Annex E2. Other "cases" were identified as part of the search for other potential cases with onset in December 2019 (including some that were included in early publications). After clinical review by the Chinese team, none of the other cases were considered to be compatible with COVID-19 disease, leaving only the 174 notified cases.

The case with the earliest onset date reported to the NNDRS became ill on 8 December 2019. The clinically diagnosed cases were generally reported in the second half of December with the first



clinically-diagnosed case having onset of illness on 16 December.

# Fig. 22. Notified cases of COVID-19 (laboratory-confirmed and clinically diagnosed) in Wuhan in December 2019 (n = 174).

There were a slightly more males (98) than females (76). The ages ranged from 22 to 92 years, median age 56 years, with most cases in the working age groups up to 60 years. The age and gender profile of the cases, and a comparison with the age and gender structure of the population of Wuhan, is given in the Annex E2. In terms of occupation, 39% were "retired" and 35% were described as being engaged in "business/commerce".

Cases were scattered by place of residence across the city of Wuhan (164) with a further 10 in seven neighbouring cities. There was a concentration of cases, both laboratory-confirmed and clinically diagnosed, in the central districts (which include the Huanan market). The earliest cases were mostly resident in the central districts of Wuhan, but cases began to appear in all districts of Wuhan in mid-to late December 2019 (Fig. 23).



Fig. 23. Notified cases (confirmed and clinically diagnosed) with onset in December 2019 in Wuhan (main figure), with China, Hubei province and areas adjacent to Wuhan shown for context.

For those cases where the information was available, 55.4% had a history of recent exposure to a market:28.0% to the Huanan market only, 22.6% to other markets only, and 4.8% to both. 44.6% had no history of market exposure (see Fig. 24 and Annex E4). Cases with market exposure were more evident among the early cases but exposure to other markets occurred in the earliest cases as much as exposure to the Huanan market. The case reported with the earliest onset date (8 December) had no history of exposure to the Huanan market.



# Fig. 24. Exposure history of 168 of the 174 cases in December 2019 in Wuhan according to association with any market.

Other exposures reported by patients included "dead animals", which included meat and fish (26.4%), live animals (11.8%), cold-chain products (26.4% - with a greater proportion among clinically diagnosed cases), and travel outside Wuhan (8.9%) including one case with international travel (to Thailand).

Seven clusters of cases, accounting for 15 cases in total, were identified among the 174 cases where they reported close contact with others in the cluster at home, in a market or elsewhere. Detailed description of the clusters is provided in Annex E2.

The cases who worked in the Huanan market were plotted in a timeline according to the location of their stalls in the market. Most cases were associated with the western side of the market, but no clear clustering with one specific part of the market was apparent as cases were widely distributed (see Fig. 25). A more detailed description of the association with the Huanan market of those cases who reported links to the market is given in Annex E4. Detailed follow up of all products on the market is described in the section on Animal and environment studies.




# Fig. 25. Spatial distribution of vendor cases associated with the Huanan market by week of onset.

#### Other initially suspected cases in December 2019

Three possible cases with disease onset on 1, 2 and 7 December 2019, respectively, were initially identified as potential cases in the retrospective case search and have been included in some published papers. Clinical review of these three cases by the Chinese expert team led to their exclusion as possible cases on the basis of the clinical features of their illness.

In the case with onset on 1 December, a 62-year-old man with past history of cerebrovascular disease was judged to have had a minor respiratory illness in early December, which responded to antibiotics. He developed a further illness with onset on 26 December 2019, which was later laboratory-confirmed to be COVID-19. This patient had no reported contact to the Huanan market, whereas his wife, who was admitted on 26 December with a COVID-19 compatible illness, reported close contact with the Huanan market. She was also later laboratory-confirmed to have COVID-19. This couple, together with their son, became part of the first recognized family cluster of COVID-19.

In the second case, a 34-year-old woman with onset on 2 December 2019 was assessed to have had venous thromboembolic disease and subsequently pneumonia. She remained negative on SARS-CoV-2 laboratory testing throughout a longer admission period ending in mid-February 2020.

In the third case, a 51-year-old man with onset on 7 December 2019 had symptoms of a cold and fever, and chest X-ray changes ("thickness of texture of both lungs and stripes"). His blood neutrophil count was raised and specific antibodies to *Mycoplasma pneumoniae* were detected. He responded well to antibiotics. Blood collected in April 2020 was reported negative for SARS-CoV-2-specific antibodies.

#### Conclusions and limitations

An explosive outbreak began in Wuhan in early December 2019. Only more severe cases with contact with the healthcare system were recognized. Other milder (and asymptomatic) cases will have been occurring at the same time as the recognized cases but no information is currently available on these milder cases that could add to the epidemiological picture of the early outbreak.

Many of the early cases were associated with the Huanan market, but a similar number of cases were associated with other markets and some were not associated with any markets. Transmission within the wider community in December could account for cases not associated with the Huanan market which, together with the presence of early cases not associated with that market, could suggest that the Huanan market was not the original source of the outbreak. Milder cases that were not identified, however, could provide the link between the Huanan Market and early cases without an apparent link to the market. No firm conclusion therefore about the role of the Huanan Market can be drawn.

#### Recommendations

Limited time was available for a full joint review of the data provided in Annex E4 including analyses of clinical and demographic characteristics, and risk factors, of the 174 notified cases. The joint international team recommends that further work should include a full joint review of these data. Consideration of re-interviewing these cases should be based on the findings of the joint review.

Acknowledging the constant progress in understanding the broad spectrum of COVID-19 disease over time and the insight into mild and/or atypical clinical presentation of the infection, the joint team recommends review of all NNDRS COVID-19 discarded cases (potential or confirmed) registered in Wuhan during the weeks of December 2019 in the search for early cases.

Retrospective search for potential cases of SARS-CoV-2 infection in health institutions in Wuhan from 1 October to 10 December 2019

### Introduction

The full spectrum of the illness caused by SARS-CoV-2 infection has now been recognized to range from asymptomatic infection to severe acute respiratory illness and death. (20)

Severe cases represent the tip of the iceberg and for every severe infection identified, there will have been many milder or asymptomatic infections. It is therefore possible that community transmission had been occurring before the recognition of the explosive outbreak in Wuhan from the middle of December 2019 onwards, but had gone unrecognized owing to the mild and non-specific nature of the illness in many; also, any earlier severe cases may not have been recognized as being potentially linked. Case searching was therefore carried out in Wuhan in the period from 1 October to 10 December 2019 to see if there were any suggestions of previously unrecognized illness due to SARS-CoV-2 infection occurring in the community.

# Methods

An initial case search, for the period 1–31 December 2019, was carried out in January 2021. Altogether 233 health institutions from 15 districts in Wuhan (consisting of all secondary and tertiary hospitals, as well as a selection of community health centres) were contacted through a series of meetings with representatives of the institutions and asked to identify all individuals who had attended those institutions with illness with onset in December 2019 with one of four diagnoses: fever, influenza-like illness (ILI), acute respiratory illness (ARI) and "pneumonia unspecified". In January 2021, it was agreed as part of the joint work plan for the WHO-China study to modify and extend the period for case searching to cases presenting with illness between 1 October and 10 December 2019.

The 233 health institutions inspected their patient records systems to identify patients with the specified four conditions. Each of the patient records identified were reviewed by a team from the health institution. In the two hospitals which described this process in detail during meetings with the joint team in Wuhan, the panel consisted of clinical representatives from respiratory and intensive care medicine, imaging and pathology departments. This process varied, being tailored according to the size, function and expertise of each of the participating institutions. Each institution then determined which of these individual cases might possibly represent cases of SARS-CoV-2 infection. An external multidisciplinary clinical panel then reviewed all the potential cases from these institutions. Those identified were followed up and, where available, blood was obtained and tested for SARS-CoV-2-specific antibodies in January 2021.

# Results

In the period from 1 October to 10 December 2019, 76 253 episodes of fever, ILI, ARI or pneumonia unspecified were presented to Wuhan health institutions by individuals of all ages and were reviewed. Across this period, ARI was the most common diagnosis, followed by fever, ILI and pneumonia unspecified.

A small increase in ILI, ARI and fever was seen in children in early December 2019 consistent with the occurrence of influenza which was observed in the ILI surveillance system to be affecting mainly children (Fig. 26).



# Fig. 26. Distribution of 76 253 episodes of illness identified in the retrospective review, 1 October – 10 December 2019; total by age group; diagnostic category by each age group.

A rise in ARI in early December in the over-60-year age group was observed, together with smaller rises in ILI and fever. Combined ARI, ILI, fever and pneumonia unspecified was higher in some central and western districts of Wuhan throughout the period October to November.

Following review by the health institutions, only 92 cases of the 76 253 episodes were considered to have an illness clinically compatible with SARS-CoV-2infection. These 92 were evenly distributed across the period 1 October to 10 December (Fig. 27). Following further review by the external multidisciplinary clinical team, all these cases were assessed not to be cases of SARS-CoV-2 infection.



# Fig. 27. Distribution of the 92 cases identified as potential cases of COVID-19 following review of the 76 253 episodes of illness presenting from 1 October to 10 December, by date of onset.

The 92 cases were followed up in January 2021 and blood for SARS-CoV-2 serology collected from 67 of them (the remainder either having died, refused or were unobtainable). All 67 sera were reported to be SARS-CoV-2-specific antibody negative.

# Conclusions and limitations

The retrospective search for cases compatible with COVID-19 illness identified 76 253 episodes with one of four indicator conditions. A rise in one of these conditions, ARI (as well as ILI and fever), was seen in this group of individuals in the over-60-year age group in early December. The clinical assessment of the 76 253 individuals revealed 92 cases clinically compatible with COVID-19. It is possible that the application of stringent clinical criteria, resulting in the identification of only 92 clinically compatible cases, may have decreased the possibility of identifying a group or groups of cases with milder illness.

All the 92 cases were rejected as cases of SARS-CoV-2 infection on further clinical review. None of these cases (where blood could be obtained) was positive on SARS-CoV-2 serological testing carried out more than 12 months later. The use of retrospective serological testing so long after the illness cannot be relied on to exclude the possibility of SARS-CoV-2 infection at the time of the presenting illness, given the possible drop in SARS-CoV-2-specific antibody over time and the associated reduced sensitivity of commercial assays. The possibility that earlier transmission of SARS-CoV-2 infection was occurring in this community cannot be excluded on the basis of this evidence.

# Recommendations

The joint international team recommended that further review be made of the methods used to identify and characterise the cases in the retrospective clinical search for patients presenting with relevant conditions to the 233 Wuhan medical institutions, including the 92 cases initially identified as being compatible with a possible diagnosis of COVID-19, as well as others with potentially milder illness, to search for features (such as clustering) that could be suggestive of occurrence of previously unrecognized cases of SARS-CoV-2 infection.

In the light of the increase in ARI in older adults in early December 2019 in the retrospective review of 76 253 records (and the similar increase in ILI in Wuhan in the national sentinel surveillance data described above) further joint review of the ARI data should be performed.

The team also recommends that further testing should be carried out on the 67 specimens obtained in the retrospective clinical review and compared with retesting of a subsample of the 174 confirmed cases from December 2019, and any other groups of specimens of relevance. This should be linked with investigation of new approaches to serological testing using historic samples collected through the blood bank.

## Review of Stored Biological Samples Testing

As part of origins of SARS-CoV-2 study, searches for stored respiratory tract, serum or other samples suitable for SARS-CoV-2 laboratory testing were requested. Sub-set of samples were identified and tested from hospitalized patients related to scientific research projects, including patient samples preserved in the biobank of Tongji Hospital, as well as patient samples preserved by the collaborative research institute jointly developed by Wuhan University and Tongji Hospital of Huazhong University of Science and Technology in late 2019.

# Methods

*Study 1. Tongji Hospital.* Between July and December 2019, 2074 samples were collected; these included 2058 plasma samples, 10 stool samples and six serum samples.

Testing for SARS-CoV-2-specific total antibody (using a Spike protein-based double antigen sandwich assay) was performed on plasma and serum samples. Any sample with SARS-CoV-2-specific total antibody underwent testing for SARS-CoV-2-specific IgG and IgM antibody, followed by confirmation with neutralizing antibody and use of a colloidal gold antibody assay. For stool samples, RNA extraction followed by NAT (Da'an Gene Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit) was performed.

Testing was performed in January 2021.

*Study 2. Tongji and other hospitals*. Some 2334 throat swabs, the majority from children collected between 1 October and 31 December 2019 from four branches of Tongji Hospital (Wuhan Tongji Hospital, the Optics Valley branch, the Sino-French New City branch, and the Children's Hospital) were tested by NAT for SARS-CoV-2 (Da'an Gene Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit).

In addition, 218 throat swab samples collected between October and December 2019 from Wuhan Union Hospital were tested for SARS-CoV-2 nucleic acid (Da'an Gene Novel Coronavirus 2019nCoV Nucleic Acid Detection Kit).

A further 106 samples (20 bronchoalveolar lavage and 11 throat swab samples and 75 sera) collected between October 2019 and January 2020 from three hospitals in Hunan Province (the Second Xiangya Hospital of Central South University, the Third Xiangya Hospital of Central South University, and Hunan Children's Hospital) were tested for SARS-CoV-2 nucleic acid (Sansure Biotech Novel Coronavirus Nucleic Acid Diagnostic Kit). Also, 16 samples (14 bronchoalveolar lavage samples and two sera) collected between October and December 2019 from the First Affiliated Hospital of Zhengzhou University in Henan province were similarly tested for SARS-CoV-2 (BioGerm Shanghai Novel Coronavirus 2019-nCoV PCR Kit) and the two sera were also tested for SARS-CoV-2-specific antibody test (Wondfo Biotech, Guangzhou Novel Coronavirus 2019-nCoV Antibody Colloidal Gold Test Kit).

#### Results

*Study 1*. Plasma samples were collected from 205 patients with renal disease, 1702 patients with gynaecological cancer, 128 from transplant recipients, and 10 from patients with nutritional disorders. Sera was available from six patients with respiratory diseases. The 2051 plasma and sera samples were collected from 192 males and 1858 females; one was of unknown gender. See Table 4 for the distribution of samples.

All plasma and serum samples were negative for SARS-CoV-2-specific total antibody, including 479 patient samples from Wuhan. For thirteen samples too little sample material was available for testing. No further testing was performed.

All 10 stool samples were SARS-CoV-2 NAT negative.

Table 4. Distribution of sources of sera and plasma by age, month of collection and location (Hubei and other provinces).

Age	Wuhan	Hubei outside Wuhan	Other provinces	Total
0-	1	6	1	8
10-	2	18	3	23
20-	43	103	24	170
30-	97	196	41	334
40-	123	340	43	506
50-	138	482	69	689
60-	61	173	32	266
70-	11	38	4	53
80-	3	4	0	7
Unknown	0	3	2	5
Total	479	1363	219	2061

# Distribution of the age

# Distribution of the sampling time

Time	Wuhan	Hubei outside Wuhan	Other provinces	Total
Jul	66	238	34	338
Aug	62	211	45	318
Sep	68	158	18	244
Oct	88	202	33	323
Nov	89	251	29	369
Dec	98	275	59	432
Other	8	28	1	37
Total	479	1363	219	2061

*Study 2*. The distribution of sources of samples by age, month of collection and location (Wuhan and elsewhere in Hubei and other provinces) is listed in Table 5. Samples were mostly from children.

All samples were reported SARS-CoV-2 negative on NAT and/or antibody testing<sup>3</sup>.

<sup>3</sup> SARS-CoV-2 NAT and serological assays used worldwide, especially early in the pandemic, may be accompanied by limited data on assay performance. International Quality Assurance and Harmonization panels are under development.

Table 5. Distribution of sources of samples by age, month of collection and location (Hubei and other provinces).

Age	Henan province	Hubei province	Hunan provinces	Total
0-	0	2130	15	2145
10-	1	165	10	176
20-	1	63	5	69
30-	1	69	6	76
40-	3	25	14	42
50-	3	37	21	61
60-	4	42	17	63
70-	2	15	12	29
80-	1	6	6	13
Total	16	2552	106	2674

# Distribution of the age

# Distribution of the sampling time

Time	Henan province	Hubei province	Hunan provinces	Total
Oct	5	549	36	590
Nov	11	1023	37	1071
Dec	0	979	27	1006
other	0	1	6	7
Total	16	2552	106	2674

# Conclusions and recommendations

The joint international team concluded that no further work is required on the already-investigated clinical samples collection as all laboratory results were negative. If possible, the National Health Commission should continue to identify other biobanks for retrospective laboratory testing, particularly in Wuhan.

# Wuhan Blood Center presentation to the Epidemiology working group

Blood donor serosurveys for SARS-CoV-2 antibodies are used in many countries to understand community prevalence of SARS-CoV-2 and monitor the increasing proportion of the population being infected over time. The testing of convenience samples from research study biobanks did not provide any indications of earlier circulation, but -given the outstanding questions and the potential for limited clusters that would not be detected through the studies done so far, access to systematically collected historic samples would be of great added value for the origins studies. Therefore, the international team invited representatives of the Wuhan Blood Center for discussions. The Wuhan Blood Center has provided a community-based blood donation service for people aged between 18-60 years of age, and operates under national regulations for storage, privacy and re-testing (in the case of disputes).

# Methods

Presentations were given by Professors Wang Yan (Director) and Zhao Lei.

#### Results

In 2020, during the pandemic in Wuhan, and as expected, blood donations dropped. Methods to increase donations through on-line appointments and other systems were introduced. Whole blood donors donate up to every six months and about 15% are regular donors. Donors for other blood products may donate more regularly.

About 200 000 donations are made annually in Wuhan. Blood donor aliquot portions (about 0.5 ml in blood pack tubing) are stored for two years.

SARS-CoV-2 antibody testing is available in the Centre, and the Centre has published its findings on SARS-CoV-2 seropositivity in donations during the pandemic in Wuhan (seroprevalence of 2.2% reported from Wuhan in donations received between January and April 2020) and Hubei and other provinces.(21)

The Blood Centre has also been involved in COVID-19 convalescent plasma collection and trials.

# Further work and recommendations

The Wuhan Blood Centre offers the opportunity to undertake a serosurvey for SARS-CoV-2 in blood donors in the latter part of 2019. The joint international team recommended the investigation of options for performing SARS-CoV-2-specific antibody testing in blood donors (including those who are regular donors) in Wuhan from September to December 2019, within the context of the appropriate local and national regulatory, scientific and ethics approval. This could be expanded to include other blood centres in China and other locations world-wide, focusing on the six months (at least 3-4 months) period before the first cases in each location were identified and ideally using a common laboratory testing approach. Contemporary samples from blood donor populations in other regions of China where COVID-19 cases were not detected before the early months of 2020 could be used as a control group.

#### Summary and recommendations

The joint international team concluded that:

#### Morbidity surveillance, pharmacy purchases and mass gatherings

- 1. Based on the national sentinel surveillance data for ILI, and the associated laboratoryconfirmed influenza activity, in Wuhan as well as Hubei and six surrounding provinces, there is a marked increase in ILI in both children and adults at the end of 2019 in Wuhan. This may be explained by a contemporary increase in laboratory-confirmed influenza activity but whereas the data provided no evidence for substantial SARS-CoV-2 transmission in the months preceding the outbreak in December 2019, sporadic transmission or minor clusters of SARS-CoV-2 cannot be ruled out.
- 2. Analysis of aggregated retail pharmacy purchases for antipyretics, and cough and cold medications did not provide a useful indicator of early SARS-CoV-2 activity in the community.
- 3. No appreciable signals of clusters of fever or severe respiratory disease requiring hospitalization were identified in association with mass gatherings during September to December 2019.

#### Mortality surveillance

- During the period August-December 2019, review of all-cause and pneumonia-specific mortality data provided little evidence of any unexpected fluctuations that might suggest the occurrence of transmission of SARS-CoV-2 in the population in the period before December 2019. This does not exclude, however, the possibility that some circulation of SARS-CoV-2 was occurring in the population at a low level, as changes in mortality at the population level would be unlikely to be sufficiently sensitive to detect this.
- 2. In view of the time lag from onset of disease to COVID-19-associated death, the documented rapid increase in all-cause mortality in week 3 of 2020 and pneumonia-specific deaths in week 4, suggest that virus transmission was widespread among the population of Wuhan by the first week of 2020. The steep increase in mortality occurred 1-2 weeks later among the population in the Hubei Province outside Wuhan, suggesting that the epidemic in Wuhan predated the spread in the rest of Hubei Province.

#### Identification of early cases and role of Huanan Market among early cases

- 3. An explosive outbreak began in Wuhan in early December 2019. Only more severe cases with contact with the healthcare system were recognized. Other milder (and asymptomatic) cases will have been occurring at the same time as the recognized cases but no information is currently available on these milder cases that could add to the epidemiological picture of the early outbreak.
- 4. Many of the early cases were associated with the Huanan market, but a similar number of cases were associated with other markets and some were not associated with any markets. Transmission within the wider community in December could account for cases not associated with the Huanan market which, together with the presence of early cases not associated with that market, could suggest that the Huanan market was not the original source of the outbreak.
- 5. Other milder cases that were not identified, however, could provide the link between the Huanan Market and early cases without an apparent link to the market. No firm conclusion therefore about the role of the Huanan Market can be drawn.

#### **Case-searching**

- 6. The retrospective search for cases compatible with COVID-19 illness identified 76 253 episodes with one of four indicator conditions. A rise in one of these conditions, ARI (as well as ILI and fever), was seen in this group of individuals in the over-60-year age group in early December. The clinical assessment of the 76 253 individuals revealed 92 cases clinically compatible with COVID-19. It is possible that the clinical review, resulting in the identification of only 92 clinically compatible cases, may have decreased the possibility of identifying a group or groups of cases with milder illness.
- 7. All 92 cases identified by the clinical retrospective review of morbidity surveillance episodes were rejected as cases of SARS-CoV-2 infection on further clinical review. None of these cases (where blood could be obtained) was positive on SARS-CoV-2 serological testing performed on samples collected more than 12 months later. The use of retrospective serological testing so long after the illness cannot be relied on to exclude the possibility of SARS-CoV-2 infection at the time of the presenting illness, given the possible drop in SARS-CoV-2-specific antibody over time and the associated reduced sensitivity of commercial assays. The possibility that earlier transmission of SARS-CoV-2 infection was occurring in this community cannot be excluded on the basis of this evidence.

#### Laboratory testing

8. Blood donor screening surveys for SARS-CoV-2 antibodies are used in many countries to understand community prevalence of SARS-CoV-2 and monitor the increasing proportion of the population being infected over time. The Wuhan Blood Centre offers the opportunity to undertake a serosurvey for SARS-CoV-2 in blood donors in the latter part of 2019.

- 9. Testing of convenience samples collected in 2019 from research study biobanks did not provide any indication of earlier SARS-CoV-2 circulation.
- 10. Given the outstanding questions and the potential for limited clusters that would not be detected through the studies done so far, access to systematically collected historic samples, including routinely stored blood bank samples, would be of great added value for the origins studies.

#### Recommendations

The joint international team made the following recommendations:

#### Morbidity surveillance, pharmacy purchase and mass gathering events

- 1. The joint team recommends further exploration of the weekly ILI trends (especially in adults) in 2019, in comparison to the earlier years, using time-series analyses.
- 2. The joint team recommends a review of pharmacy purchases by week during the period of September to December in 2016, 2017, 2018, and 2019 to look for any signals of increased purchases in the weeks of September to December 2019 as compared with the same weeks during the previous years. If any signals are identified then proceed with analyses for spatial-temporal clusters.
- 3. The joint team recommends that consideration be given to further joint review of the data on respiratory illness from the on-site clinics at the Military Games in October 2019.

#### Mortality surveillance

4. The joint team recommends\_augmenting the mortality review by broadening the approach to include other provinces where phylogenetic analyses (Figure 5, *Molecular Epidemiology section*) have revealed early epidemic clusters, and comparison with other provinces and cities in China.

#### Identification of early cases and role of Huanan Market among early cases

- 5. The joint team recommends that further testing of the 67 specimens obtained in the retrospective clinical review of the 92 cases identified by the clinical retrospective review be carried out and compared with retesting of a subsample of the 174 confirmed cases from December 2019, and any other groups of specimens of relevance. This should be linked with investigation of new approaches to serological testing using historic samples collected through the blood bank.
- 6. In view of the limited time available during the visit to Wuhan in January and February 2021, further joint review (including of the data and analyses in Annex E4) should be carried out, including analyses of clinical and demographic characteristics, as well as risk factors, of the 174 notified cases. Consideration of re-interviewing these cases should be based on the findings of the joint review.

#### **Case-searching**

- 7. The joint team recommends further review of the methods used to identify and characterise the cases in the retrospective clinical search for patients presenting with relevant conditions to the 233 Wuhan medical institutions, to search for features (such as clustering) that could be suggestive of occurrence of previously unrecognized cases of SARS-CoV-2 infection.
- 8. This review should include the 92 cases initially identified as being compatible with a possible COVID-19 diagnosis, as well as other cases with potentially milder illness.
- 9. It should also include the increase in ARI in older adults in late 2019, seen in the retrospective search from the 233 Wuhan medical institutions.

Acknowledging the constant progress in understanding the broad spectrum of COVID-19 illness over time and the insight into mild and/or atypical clinical presentation of the infection, the joint team recommends review of all NNDRS COVID-19 discarded cases (potential or confirmed) registered in Wuhan city during the weeks of December 2019 in the search for early cases.

### Laboratory testing

- 10. No further work is required on the convenience clinical sample collection already investigated, as all SARS-CoV-2-specific laboratory results were negative.
- 11. The joint team recommends a collaborative study with the Wuhan Blood Centre for the presence of SARS-CoV-2-specific antibodies in blood samples from adult blood donors in Wuhan collected during the months of September to December 2019, and further back in time until there are two successive months without any evidence of SARS-CoV-2-specific antibodies among the tested samples. This could be expanded to include other blood centres in China and other locations world-wide, focusing on the six months (at least 3-4 months) period before the first cases in each location were identified and ideally using a common laboratory testing approach. Contemporary samples from blood donor populations in other regions of China where COVID-19 cases were not detected before the early months of 2020 could serve as a control group.
- 12. The joint team recommends investigation of new approaches to serological testing to revisit testing performed from cases initially identified in the retrospective clinical review, the early confirmed cases and any other groups of interest. There may be potential for international collaboration on such work.

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#### MOLECULAR EPIDEMIOLOGY

Most emerging viruses originate from animals. Understanding the process that may lead to a crossspecies transmission event, also known as "spillover", and global spread requires a deep understanding of both the virus diversity and evolution in an animal reservoir, the interactions between animals, their environment and humans, and the factors contributing to efficient human to human transmission. A virus causing a global pandemic must be highly adaptive to human environments. Such adaptation may be gained suddenly or may have been evolving through multiple steps with each step driven by natural selection.

The search for the origin of SARS-CoV-2 therefore needs to focus on two phases.(1) The first phase involves viral circulation in animal hosts (such as bat, pangolin, mink or other wild animals) before zoonotic transfer. During this evolutionary process, various animal species may serve as reservoir hosts. Upon circulation, SARS-CoV-2 progenitor strains may have acquired increased ability to infect humans. Finding viral sequences nearly identical to SARS-CoV-2 helps the elucidation of the origin of SARS-CoV-2 from zoonotic transmissions from intermediate host species.

The second phase involves radiative evolution of SARS-CoV-2 during its global spread in human populations following zoonotic transfer. Animal--human contacts permit a progenitor of SARS-CoV-2 to switch its host to humans, and the likelihood of such spillovers increases with the frequency, nature and intensity of contact. (2) Spillovers may have occurred repeatedly, if the genomic features of the virus in the reservoir require further adaptation for efficient onward transmission, and such early spillovers may go undetected. In addition, the evolution or spillover of viruses with pandemic potential may have resulted in substantial clusters in different geographical regions before factors converged and led to the pandemic of COVID-19. Therefore, studies into the origin need to be designed bearing in mind these different potential emergence scenarios.

Evidence from surveys and targeted studies so far have found most highly related viruses in bats and pangolins, suggesting they may be the reservoir of SARS-CoV-2 according to the high sequence similarity between the sampled viruses and SARS-CoV-2. Viruses identified so far from neither bats nor pangolins are sufficiently similar to SARS-CoV-2 to serve as the direct progenitor of SARS-CoV-2.(3) In addition to these findings, the high susceptibility of mink and cats suggests the potential of additional species of animals (belonging to the mustelid or felid family, as well as other species) as potential reservoirs.(4-7) Surveys of virus presence and genetic diversity in potential reservoir species have not been systematic, and potential reservoir hosts are massively under-sampled.

#### Background on molecular epidemiology

The use of pathogen genomic sequencing has become standard in outbreak investigations and pathogen surveillance and has provided deep insights into the evolution of emerging disease outbreaks. (8, 9) The scale of the global sequencing efforts since the start of the COVID-19 pandemic is unprecedented. For instance, very limited full genome sequencing was done during the previous pandemic, caused by 2009 pandemic influenza A virus (H1N1). Mostly targeted sequencing of part of the genome was performed on a Sanger sequencing platform with sequencing of a single DNA fragment at a time. In contrast, implementation of next-generation sequencing platforms during the past decade allowing for sequencing of millions of fragments per run has granted genomic sequencing a pivotal role in SARS-CoV-2 surveillance from the start of the COVID-19 pandemic. (10-13) The first publications used genomic sequencing to characterize the novel virus and provided the first

phylogenetic analysis linking the virus to the genus Betacoronavirus and the lineage Sarbecovirus.<sup>4</sup> Other sarbecoviruses are the viruses that cause SARS and a diverse group of SARS-like coronaviruses identified through surveys of bats mostly conducted following the SARS outbreak. (12, 13) As part of the initial characterization, SARS-CoV-2 was isolated from clinical specimens from the first recognized cases, and the association of this virus with the disease was confirmed through antibody testing (13).

Since the start of the pandemic, viral genome sequences have been collected through GISAID<sup>5</sup> (the global platform that evolved from a global initiative on sharing avian influenza data), which can be accessed by scientists and epidemiologists. With the global dispersal of the virus, the accumulation of mutations has been monitored systematically through bioinformatic analyses. The underlying principle is that virus genomes accumulate mutations during replication. Therefore, with increasing rounds of infection, the accumulated pattern of mutations can be used to track transmission chains.

In addition to the use of genomic sequencing to characterize the new virus and track global dispersal, more granular use of whole genome sequencing has been used throughout the pandemic to track the spread of SARS-CoV-2 and to gain a deeper understanding of suspected clusters identified through epidemiological outbreak investigations. For this, it is essential to combine the genomic data with information from the epidemiological investigation, (6, 14) like time and place of illness onset and case history. (8) Genomic epidemiological analyses have now been widely used to resolve clusters. (14-17)

Phylogenetic and network analyses can provide insights into the spatial and temporal dynamics of virus circulation. Combined with epidemiological and geographical information, phylogeny or haplotype network analysis based on sequence similarity among viral genomic sequences allows the reconstruction of evolutionary history of virus lineages, and can be applied to the analysis of various questions relevant to the studies into virus origin, including: (i) estimation of the number of independent virus founders during the early outbreak of the pandemic; (ii) inference of the population dynamics of virus; (iii) inference of the rates of viral spread; (iv) identification of the existence of infection clusters; and (v) tracing the transmission chains of resurgence (see Fig. 1). *(18)* 

The accumulation of mutations has also been used to estimate time to the most recent common ancestor (tMRCA) of the new coronavirus. (19) There are numerous methods to estimate the tMRCA, but for viral pathogens establishing the timescale of viral evolution relies on determining or using accurately the rate of nucleotide substitution. This rate and known dates of virus isolation from hosts allows for the back calculation of the time when the current viruses or viral clades shared a common ancestor. There are numerous biological and statistical complexities that exist and can be accounted for, and so different methods, from the initial sequencing through to sequence alignment to methods of tMRCA estimation, can give differing results.

<sup>4</sup> SARS-CoV-2 is a *virus* of the severe acute respiratory syndrome-related coronavirus *species*, in the *subgenus* Sarbecovirus and the *genus* Betacoronavirus, **along with three other viruses**. Coronaviruses are positive-sense, single-stranded RNA viruses, in the *family Coronaviridae*. Formally in virology a strain refers to a cell culture isolate. <sup>5</sup> Available at https://www.gisaid.org(accessed 25 March 2021).

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Fig. 1. Examples of molecular epidemiological analyses (modified, based on Martin et al.(18)) (TMRCA: time to the most recent common ancestor)

# 1. Approach

The list of studies was addressed through a combination of plenary and workgroup specific meetings and studies. The working group on molecular epidemiology focused on unlocking the potential information from virus genomic data combined with metadata for the questions related to the origins study. In order to do so, first, an overview was made of the globally available public data and the research support database efforts developed in China to aggregate all SARS-CoV-2 genomic data. During all visits and team discussions the potential availability of additional stored samples was explored in order to identify additional samples accessible for sequencing. Unpublished genomic data were aggregated from ongoing research. For analysis of the earliest phase of the pandemic, sequence providers were contacted to link data to cases in the national registry from China CDC to establish time of illness onset. Raw sequence data were re-analysed to resolve differences between genomic sequences generated by different groups. The data for cases with onset of illness in December 2019 were used for final analysis in combination with data on exposure histories from the questionnaires used as a part of the outbreak investigation.

2. Overview of global databases of SARS-CoV-2

## 2.1 International databases 2.1.1 The GISAID platform

The GISAID initiative is dedicated to providing a rapid data-sharing platform that includes a large proportion of publicly available genomic data on influenza viruses and SARS-CoV-2. GISAID provides data on human-associated viral genome sequences and some related clinical and epidemiological data, as well as data on animal-associated viruses. On 10 January 2020, the first SARS-CoV-2 genomes were made publicly available on GenBank and Virological.org (*10*) and on GISAID. To date (6 February 2021), GISAID has recorded a total of 487 487 SARS-CoV-2 genome sequences from 238 countries and regions, as well as the metadata information corresponding to the sequences.

# 2.1.2 The International Nucleotide Sequence Database Collaboration

The International Nucleotide Sequence Database Collaboration is an initiative between three organizations which since the 1980s has been providing support for molecular biology and genomics research: the NCBI, EMBL-EBI and DDBJ (see below). Through the agreement, the individual regional databases exchange released data on a daily basis. As a consequence, the three data centres share virtually the same data at any given time. The virtually unified database is called the International Nucleotide Sequence Database (INSD). The individual organizations have developed dedicated websites and data repositories specifically for COVID-19.

# National Center for Biotechnology Information (NCBI)

The National Center for Biotechnology Information provides access to a wide range of bioinformatics resources from programmes funded by the United States National Institutes of Health and other public data. It includes the sequence database GenBank and a repository for high-throughput sequencing data. For COVID-19, a dedicated website<sup>6</sup> was developed, providing access to SARS-CoV-2 sequences, raw reads, and publications listed in PubMed.

# The European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI)

The EMBL-EBI is Europe-based support infrastructure for the life sciences. For sequence data, the European Nucleotide Archive was founded in the early 1980s. In April 2020, the European

Commission launched the COVID-19 Data Portal,<sup>7</sup> which includes the repository based in the Archive for raw reads and assembled sequences.

# The DNA Database of Japan (DDBJ)

The DDBJ Center is a Japanese research support database, also providing specific information and resources for COVID-19.<sup>8</sup>

### 2.1.3 Nomenclature

Nomenclature systems have been developed to assign names to the diversifying lineages. (20, https://nextstrain.org<sup>9</sup> and GISAID, reviewed in 20a) The earliest sequences from Wuhan have been designated as lineage A (represented by Wuhan/WH04/2020; sampled 5 January 2020; GISAID accession EPI\_ISL\_406801) and B (represented by Wuhan-Hu-1; sampled 31 December 2019; GenBank accession no. MN908947) respectively, and phylogenetic analysis has been used to track changes. Subsequent lineages were assigned a number, for instance B1, B2 and so on, or letters, depending on the system used. To make tracking of strains accessible for providers of genetic data, GISAID collaborated with bioinformaticians using interactive visualization software that provides rough overviews of the distribution of virus lineages across the world (Fig. 2). Currently, at least 12 Nextstrain clades are recognized globally. There is a clear need for development of a consistent system for nomenclature.



<sup>9</sup> Nextstrain, available at https://nextstrain.org(accessed 25 March 2021).

<sup>&</sup>lt;sup>7</sup> <u>COVID-19 Data Portal - accelerating scientific research through data</u>, available at <u>https://www.covid19portal.org (accessed 25 March 2021)</u>.

<sup>&</sup>lt;sup>8</sup> Available at https://biosciencedbc.jp/blog/20200303-01.html [in Japanese] (accessed 25 March 2021).

Fig. 2. Radial phylogenetic tree showing current grouping of SARS-CoV-2 clades through Nextstrain visualization analysis of data submitted to GISAID. Original viruses from the early pandemic are depicted in blue in the lower left quadrant (Clade 19A and B).<sup>10</sup>

#### 2.2 Databases related to SARS-CoV-2 in China

To better understand the spread of SARS-CoV-2, researchers in China have constructed three important resources (Table 1): (1) the 2019nCoVR *(19, 21,21a)*; <sup>11</sup> (2) the Novel Coronavirus National Science and Technology Resource Service System; <sup>12</sup> and (3) a mirror site of GISAID EpiCoV<sup>TM</sup> Database.<sup>13</sup> The Novel Coronavirus National Science and Technology Resource Service System, developed by National Microbiology Data Centre (NMDC), *(22)* released the first electron microscope photograph of SARS-CoV-2. Also, it provides a part of public sequencing data submitted by Chinese researchers. The mirror site of GISAID EpiCoV<sup>TM</sup> Database (named VirusDIP), maintained by China National Gene Bank, *(23)* provides metadata information on SARS-CoV-2, and the related reports of primary data analysis.

	Database	2019nCoVR	NMDC	VirusDIP
Ho	st/Center	CNCB-NGDC	NMDC	CNGB
1	#Sequences (as of 06/02/2021 14:00)	477,729	58,300	502,772
a)	Quality Assessment	V		
ence	Raw data	٧		V
equ	Public Coronaviruses Sequence	V	٧	V
S	Data Sources: (1 CNCB, 2 NCBI, 3 GISAID, 4 NMDC, 5 CNGB	12345	24	235
	Redundancy Removal	Yes	Yes	No
	Variations	٧	٧	
	Variation Annotation	٧		
uo	Spatiotemporal Dynamics	٧	٧	
iati	Lineage Browse	٧		
Vai	Visualization	V	V	v
	Viral haplotype network	V		
	Phylogenetic tree	٧	V	v
	Genome Assembly	V		
0	Variation Identification	V		
e too	Variation Annotation	V		
aline	Genome Annotation	V	٧	
ō	BLAST	V	V	V
	Phylogeny		V	V
s	Literatures	v	٧	
ther	Clinic Records	V		
0	Protein Structure	V	٧	

#### Table 1. Comparison of content and functionalities of the three database repositories in China.

- <sup>11</sup> Available at https://bigd.big.ac.cn/ncov/ (accessed 18 February 2021).
- <sup>12</sup> Available at http://nmdc.cn/nCov/en (accessed 18 February 2021).
- <sup>13</sup> Available at https://db.cngb.org/gisaid/ (accessed 18 February 2021).

<sup>&</sup>lt;sup>10</sup> Available at <u>https://nextstrain.org/ncov/global?c=GISAID\_clade (accessed 25 March 2021).</u>

The 2019nCoVR database, developed by the National Genomics Data Centre, China National Centre for Bioinformation (CNCB),<sup>14</sup> serves as a database for global data submission and access, and integrates SARS-CoV-2 genome data and metadata accessible from GISAID, National Centre for Biotechnology Information, National Genomics Data Centre and the National Microbiology Data Centre on SARS-CoV-2. It was developed to include quality control of the sequencing data, and provide support for scientists in China and elsewhere through tools for analysis of variations and dynamic trends, haplotype networks, and browsing functionality through GenBrowser.<sup>15</sup> The present version aims to remove redundancy between databases, evaluates data integrity and sequencing quality through manual curation and automated quality assessment. A functionality that allows mapping of genome variation from high-quality genome sequences provides a dynamic landscape of SARS-CoV-2 temporally, it provides the visualization of the dynamic changes in time and space of each mutation and constructs the dynamic evolution map of the virus haplotype network during the outbreak.

As of 4 February 2021, the database has integrated 437 808 non-redundant sequences, of which 2089 are released from China. For the studies related to the origins study, the focus was on early sequences, released in December 2019 and January 2020. There are 768 global early sequences (defined as before 31 January 2020) from 26 countries and 514 Chinese early sequences. For each SARS-CoV-2 sequence, the following five categories of information are established:

- the meta-information of the genome sequence, including sampling time, sampling location, host information, submission time, submission unit, and sample source unit; all meta-information can be downloaded in bulk, and the genome sequence is linked to different database sources and can be downloaded on the link page
- the results of the completeness and quality evaluation of the genome sequence
- when available: raw sequencing data and related information, including sequencing platform, sequencing volume, analysis software and methods
- when available: epidemiological information, including name, age, sex, date of onset of illness, contact with the Huanan market, death, and clinical symptoms
- variation analysis, including the location and type of mutations and functional annotation.

#### 2.2.1 Overview of genomic data on SARS-CoV-2 in China

The 2019nCoVR database has integrated 2089 non-redundant sequences (by 3 February 2021) from 17 provinces and regions of China (see Fig. 3). Of these, 2028 sequences were collected from human cases (Table 2), 28 sequences were collected from the environment (Table 3), and 33 sequences were from possible animal hosts (pangolin and bat), from pets (cats and dogs) or from animal experiments (mouse and hamster). All these sequences are publicly accessible.

<sup>&</sup>lt;sup>14</sup> Available at <u>https://bigstory.big.ac.cn/ncov/</u> (accessed 18 February 2021).

<sup>&</sup>lt;sup>15</sup> Available at <u>https://www.biosino.org/genbrowser/(accessed 22 February 2021).</u>



Fig. 3. Map of the distribution of released genome data in China.

Year	Month	Complete	Partial	Confirmed
2019	12	25	3	27 <sup>b</sup>
2020	1	407	59	11 794
2020	2	401	126	68 147
2020	3	411	43	2663
2020	4	80	52	1754
2020	5	3	5	203
2020	6	11	6	644
2020	7	89	91	2890
2020	8	18	34	2280
2020	9	34	24	659
2020	10	34	16	860
2020	11	12		1656
2020	12	24		3185
2021	1	16		4212
Other		6	27	
		1571	486	
Total		205	57	100 974

Table 2. Summary of genome sequences in China (host is human, as of 3 February 2021).

<sup>a</sup> The numbers are based on the data from National Health Commission of the People's Republic of China (<u>http://www.nhc.gov.cn/xcs/yqtb/list\_gzbd.shtml</u>).

<sup>b</sup> Health Commission of Hubei Province

(http://wjw.hubei.gov.cn/bmdt/dtyw/201912/t20191231\_1822343.shtml).

Based on the number of confirmed cases and early sequences as of 31 January 2020, the cumulative number of confirmed human cases was 11 821, the number of sequenced cases was 494, and the

proportion of confirmed cases from December and January that have been sequenced is about 4.18% (494/11 821).

Accession ID	Data source	Sequence length	Sample collection date	Location	Isolation source
NMDC60013072-01	NMDC	1065	2020-01-01	China / Hubei / Wuhan	NA
NMDC60013070-01	NMDC	28 557	2020-01-01	China / Hubei / Wuhan	NA
NMDC60013071-01	NMDC	25 342	2020-01-01	China/Hubei /Wuhan	NA
NMDC60013073-01	NMDC	29 891	2020-01-01	China/Hubei /Wuhan	NA
NMDC60013074-01	NMDC	29 891	2020-01-01	China/Hubei /Wuhan	NA
EPI_ISL_412425	GISAID	321	2020-01-26	China/ Shandong/ Linyi	NA
EPI_ISL_412426	GISAID	321	2020-01-26	China/ Shandong/ Linyi	NA
EPI_ISL_430743	GISAID	29 782	2020-03-14	China/ Beijing	Environmental swab
EPI_ISL_430744	GISAID	29 778	2020-03-14	China/ Beijing	Environmental swab
EPI_ISL_430745	GISAID	29 732	2020-03-14	China/ Beijing	Environmental swab
EPI_ISL_430746	GISAID	29 782	2020-03-14	China/ Beijing	Environmental swab
EPI_ISL_469256	GISAID	29 903	2020-06-11	China/ Beijing	Environmental swab
GWHANPA01000001	Genome Warehouse	29 858	2020-06-12	China/ Beijing	NA
MT911467	GenBank	1324	2020-08-14	China	Seafood packaging
MT911468	GenBank	1868	2020-08-14	China	Seafood packaging
MT911469	GenBank	1215	2020-08-14	China	Seafood packaging
MT911470	GenBank	1319	2020-08-14	China	packaging
MT911471	GenBank	1612	2020-08-14	China	Seafood packaging
EPI_ISL_591272	GISAID	29 893	2020-09-24	China/ Shandong/ Qingdao	Outer packaging of cold-chain products

Table 3. Summary of genome sequences from environmental samples, collected in China (as at 3February 2021).

EPI_ISL_591273	GISAID	29 873	2020-09-24	China / Shandong / Qingdao	Outer packaging of cold-chain products
EPI_ISL_591274	GISAID	29 869	2020-09-24	China/ Shandong/ Qingdao	Outer packaging of cold-chain products
EPI_ISL_591275	GISAID	29 873	2020-09-24	China/ Shandong/ Qingdao	Outer packaging of cold-chain products
EPI_ISL_591276	GISAID	29 869	2020-09-24	China/ Shandong/ Qingdao	Outer packaging of cold-chain products
EPI_ISL_591277	GISAID	29 873	2020-09-24	China / Shandong / Qingdao	Outer packaging of cold-chain products
EPI_ISL_591278	GISAID	29 876	2020-09-24	China / Shandong / Qingdao	Outer packaging of cold-chain products
EPI_ISL_591279	GISAID	29 888	2020-09-27	China / Shandong / Qingdao	Outer packaging of cold-chain products Outer
EPI_ISL_591280	GISAID	29 888	2020-10-07	China/ Shandong/ Qingdao	packaging of cold-chain products isolated from Vero cells
EPI_ISL_733568	GISAID	29 782	2020-12-10	China / Hong Kong SAR	NA

Among 28 environmental sequences, samples in Wuhan were collected during environmental surveillance of the Huanan market, samples from Qingdao were collected from surveys of cold-chain packaging, samples in Linyi were from seafood packaging, and samples from Beijing were environmental swabs collected from the Xinfadi Market (Table 3).

3. Overview of the sequences of early cases, global overview

To learn more about the initial phase of the pandemic, the 2019nCoVR database was searched for presence of SARS-CoV-2 (or related) genomic data from the first two months in which cases were identified (8 December 2019 - 31 January 2021, by date of sample collection). The joint international team identified a total of 768 sequences globally (Table 4), including 538 from China (Table 4) and 94 of them were from Hubei Province. These data were used as input for haplotype network analyses to visualize the global diversity of sequences in these first two months (section 3.1 and Fig. 4) and for more detailed analysis focusing on the early China data (section 3.2).

3.1 Global analysis of early cases of SARS-CoV-2 genomes

The global haplotype network analysis included 348 early SAR-CoV-2 sequences with high quality and clear sampling location information from China and 142 early high-quality sequences published abroad. Two major sequence clusters were observed (Fig. 4), as has been reported in previous studies.(24, 24a) These clusters have been designated as lineages S/L or A/B, depending on the nomenclature used, and are defined based on a set of two lineage-defining single nucleotide polymorphisms at sites 8782 and 28 144 that have nearly complete linkage. (12, 20, 24-29) When and where these two sublineages diverged remains unclear, and these analyses indicate the origins of SARS-CoV-2 are not yet fully understood. Among the sequences analysed here, the first available sequence for lineage A (also referred to as lineage S) is Wuhan/WH04/2020 (EPI ISL 406801), and these viruses share two nucleotide polymorphisms (positions 8782 in ORF1ab and 28 144 in ORF8) with the closest known bat viruses (RaTG13 and RmYN02). Different nucleotides are present at those sites in viruses assigned to lineage B (also referred to as lineage L), of which Wuhan-Hu-1 (GenBank accession no. MN908947) sampled on 26 December 2019 is an early representative. Evolutionary analyses (20, 30) have suggested that the lineage A sequence might represent the ancestral form and lineage B might be the derived form. Hence, although viruses from lineage B happen to have been sequenced and published first, according to Rambaut et al. (20) it is likely (based on current data) that the most recent common ancestor of the SARS-CoV-2 phylogeny shares the same genome sequence as the early lineage A sequences (for example, Wuhan/WH04/2020). However, the issue of different early lineages has been widely discussed, but there is no consensus on the question of which viruses are older, as evidenced in discussions in writing following the paper published by Foster et al. (30)

		Sample collection date (by year and by week)								
		2019					2020			
Country	49	50	51	52	53	1	2	3	4	5
China				2	26	12	9	25	17 8	286
Italy	1*		3*					1		9
Mexico						3				
Thailand						9	4	6	11	
Spain						1	6	6	7	7
Czech Republic							1			
United States of America							5	30	21	7
Australia									9	11
Cambodia										1
Canada									4	
Finland										2
France									3	5
Germany										7

Table 4. Weekly summary of SARS-CoV-2	genomes of early	cases and enviro	nmental samples
globally for end-2019 and beginning 2020.			

India				4
Japan	1	:	5	5
Luxembourg	1			
Malaysia		(	6	3
Nepal	1			
Philippines			1	5
Singapore		4	4	
Republic of Korea			1	
Sri Lanka				1
Sweden				1
United Arab Emirates				2
United Kingdom of Great Britain and Northern				
Ireland				4
Viet Nam		, -	3	2

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Fig. 4. Haplotype network of 490 complete and high quality early genome sequences globally (A, marked by country/ regions-B, marked by collecting date). The haplotype network was inferred from all identified haplotypes using PopART. SARS-CoV-2 haplotypes were constructed on the basis of short pseudo-sequences that consist of all variants (filtering out variations located in UTR regions). Then, all these pseudo-sequences were clustered into groups, and each group (a haplotype) represents a unique sequence pattern.

**3.2.** Overview of the sequences of early cases (and also other hosts and environments) and their connection with the Huanan market

# 3.2.1. Released early SARS-CoV-2 genomes in China

The publicly available early SARS-CoV-2 genomes in China by week and by province are shown in Table 5.

Table 5. Summary of early SARS-CoV-2 genomes in China (including sequences deposited in GISAID).

		Sample	e collection	date (by	year and	by week)	
_	201	9			2020		
	52	53	1	2	3	4	5
Anhui						1	
Beijing			1		1	5	21
Chongqing					1	2	
Fujian						3	
Guangdong				2	11	23	70
Henan						1	
Hong Kong						10	20
SAR						19	29
Hubei	2	26	11	4	4	20	27
Hunan					2	2	10
Jiangsu						4	1
Jiangxi				2		7	11
Shandong						14	11
Shanghai					1	4	40
Sichuan					1	9	37
Taiwan,						-	
China						3	4
Yunnan					2		
Zhejiang					2	41	15
Other*				1		20	10

\* province could not be specified



Fig. 5. Haplotype network of early sequences of SARS-CoV-2 from China, listed in Table 5. Two viral genomes that carried a T>C variant at site 28 144 (compared to the reference genome) connected the S/A and L/B major lineages, and these two genomes were sampled from Sichuan in late January 2020. One viral genome that carried a C>T variant at site 8782 (compared to the reference genome) connected the S/A and L/B major lineages, and this genome was sampled from Hubei Province in late January.

The haplotype network analysis of the sequence data from China from December 2019 and January 2020 (Fig. 5) reflects the same major lineages (L/B and S/A) as previous publications. This analysis included 348 high-quality genomes. Sequence data from Hubei Province were distributed in both lineages, as were sequences from other parts of China. A cluster of sequences from cases in Zhejiang (black, Fig. 5) was identical to the larger lineage L/B cluster. According to information from the national database and GISAID, this cluster was related to a meeting, with an index case from Wuhan. When analysing the data by week of sampling, the earliest collected samples belonged mostly to lineage L/B.

# 3.2.2. Released early SARS-CoV-2 genomes in Wuhan

There are 85 complete genome sequences of SARS-CoV-2 collected prior to 31 January 2020, of which 81 sequences were from 66 COVID-19 cases, two sequences were from the Huanan market environment and two with unknown sources. In total, all 13 early cases, S01-S13 with onset date before 31 December 2019, were identified (Table 6).

# 3.2.3. Assessment of quality of genomic data from early cases

In line with Chinese national policy, samples from initial patients were sent to more than one laboratory to increase the likelihood of successful sequencing. As a consequence, the database contained genomes from patients generated independently by different institutes (Table 6). The international team performed an in-depth comparison of data from the same patient in order to understand potential effects of platform and quality assessment procedure used by the different institutes on the final genomes.

There were in total 29 sequences for the 13 early cases submitted by different institutes. All of these were generated by de novo sequencing and sequence assembly. The genetic variations of each individual were identified by comparing with the reference sequence (NC\_045512.2). Table 6 summarizes the data generated with different platforms and lists the key parameters that were used to assess quality. Although the overall quality of the genomic sequences submitted by different institutes was high, the team observed some inconsistency among different sequences from the same case. The team therefore collected 26 sets of raw sequencing data for the 12 cases and re-analysed them with uniform single nucleotide variants calling pipelines. The details of the calling procedures include:

- removal of the adaptor sequences of the raw data and the low-quality bases from both 5' and 3' ends
- alignment of the sequence reads to the SARS-CoV-2 reference genome NC\_045512.2 with the Burrows-Wheeler Aligner-maximal exact matches (BWA-MEM) algorithm using the default parameter settings
- identification of single nucleotide variations with the Genome Analysis Toolkit (GATK) HaplotypeCaller (-ploidy 1 -ERC gVCF) and a Genomic Variant Call Format (gVCF) file was generated for each raw data set
- merging all gVCF files to generate a single file in Variant Call Format (VCF) format including all called single nucleotide variants using the GATK Genotype GVCFs default parameters
- filtering the original single nucleotide variant sets obtained above with the GATK VariantFiltration (parameter setting: -filter-expression "MQ < 40.0"--filter-expression "ReadPosRankSum <-8.0"--filter-expression "DP<10" --mask indel.filter.vcf.gz); all single nucleotide variants with coverage below 10 were filtered out to obtain the final set of variations.</li>

There was still some inconsistency among the single nucleotide variants identified from different raw data sets of the same individuals. The team adopted the criteria of high coverage > low coverage and Illumina >Ion Torrent to determine the most likely reliable genome of each individual. The final set of single nucleotide variants identified in the raw genomic sequencing data of the 13 cases is listed in Table 7 and used in the haplotype network and other analyses. Consecutive samples were collected from two patients (S05 and S09), which showed identical genomes. The number of mutations of these 13 early cases ranged from zero to three relative to the reference genome (NC 045512.2).

 Table 6. Details of genomic sequencing of 13 early cases

ID	Onset	Collection date	Virus strain	Mutation position from submitted genome sequences	Mutation position identified by re- analysis	Sequencing platform	Sequencing depth	Indel rate% <sup>16</sup>
S01	2019/12/08	2020/01/01	BetaCoV/Wuhan/IP BCAMS-WH- 05/2020	7866	7866(iSNV)ª	Illumina NextSeq 500	459	0.01
S02	2019/12/13	2019/12/24	BetaCoV/Wuhan/IP BCAMS-WH- 01/2019	3778, 8388, 8987	//b	Illumina NextSeq 500	2278	0.00
S03	2019/12/17	2019/12/26	WH01	6968, 11764	NA	DNBSEQ		
		2019/12/30	BetaCoV/Wuhan/W H19008/2019	24325	24325	NGS	6720	0.01
		2019/12/30	WIV02	21316, 24325	21316, 24325	Illumina MiSeq, MGISEQ 2000	35	0.01
S04 2	2019/12/19	2019/12/30	SARS-CoV- 2/Wuhan_IME- WH02/human/2019/ CHN	//	//	Ion Torrent X5Plus	149	0.56
		2019/12/30	BetaCoV/Wuhan/HB CDC-HB-02/2019	24325	24325	Illumina MiSeq	475	0.01

<sup>16</sup> Rate of insertion and deletion.

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		2019/12/30	hCoV- 19/Wuhan/IVDC- HB-GX02/2019	24325	NA	Sanger dideoxy sequencing		
		2019/12/30	BetaCoV/Wuhan/IP BCAMS-WH- 04/2019	//	376(iSNV) <sup>a</sup>	Illumina NextSeq 500	2491	0.01
S05	2019/12/20	2020/01/01	BetaCoV/Wuhan/W H19004/2020	27493, 28253	//	NGS	2782	0.01
		2020/01/01	BetaCoV/Wuhan/IV DC-HB-04/2020	27493, 28253	NA	missing		
S06	2019/12/20	2019/12/30	Wuhan-Hu-1	//	//	Illumina	530	0.005
S07	2019/12/20	2020/01/02	2019-nCoV WHU01	//	//	Illumina	530	0.01
		2019/12/30	WIV07	8001, 9534	9534(Coverage<10 )	Illumina MiSeq, MGISEQ 2000	11	0.02
S08 2019/12/20	2019/12/30	SARS-CoV- 2/Wuhan_IME- WH04/human/2019/ CHN	//	//	Ion Torrent X5Plus	45	0.51	
500	2019/12/22	2020/01/01	WH03	//	NA	DNBSEQ		
507	2017/12/22	2020/01/02	2019-nCoV WHU02	//	//	Illumina	140	0.01
		2019/12/30	BetaCoV/Wuhan/HB CDC-HB-03/2019	//	//	Illumina MiSeq	3156	0.01
S10 2019/12	2019/12/23	2019/12/30	BetaCoV/Wuhan/IP BCAMS-WH- 02/2019	//	//	Illumina NextSeq 500	7885	0.01

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BetaCoV/Wuhan/W 2019/12/30 // // NGS 45 0.02 H19001/2019 Illumina MiSeq, WIV04 // 0.01 2019/12/30 // 108 Illumina HiSeq 1000 BetaCoV/Wuhan/IV 2019/12/30 // NA missing DC-HB-01/2019 BetaCoV/Wuhan/IP 2019/12/30 BCAMS-WH-6996 Illumina 3371 0.01 // 03/2019 WIV05 2019/12/30 7016, 21137 // MGISEQ 2000 13 0.01 2019/12/23 S11 SARS-CoV-2/Wuhan IME-2019/12/30 Ion Torrent X5Plus // // 37 0.50 WH05/human/2019/ CHN Illumina MiSeq, 2019/12/30 WIV06 // // 0.01 MGISEQ 2000 19 SARS-CoV-2019/12/23 S12 2/Wuhan IME-2019/12/30 24325 24325 Ion Torrent X5Plus 1407 0.55 WH03/human/2019/ CHN SARS-CoV-2/Wuhan IME-4946, 8782, 2019/12/30 ThermoFisher S5Plus 0.53 S13 2019/12/26 4946, 8782, 28144 176 WH01/human/2019/ 28144 CHN

<sup>a</sup> Intra-host single nucleotide variant.

<sup>b</sup>// indicates no mutation.

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#### 3.2.4 Linking with epidemiological data

In order to link the genomic data with the epidemiological data obtained from in-depth interviews of patients, the team acquired the patient information from the submitter of the sequence, and cross-checked this in the epidemiological database (Fig. 6). Eleven early patients had connections with the Huanan market, including seven vendors at the market, three purchasers and one visitor (Table 7, Fig. 6). The other two patients were visitors to other markets. Meanwhile, only one patient with onset date of 17 December had domestic travel history. Concerning animal contact, eight of them had contacts with dead animals and four of them had also mentioned contacts with poultry and aquatic products. Moreover, four patients (S04, S05, S06 and S12) had contact with cold-chain goods with the earliest onset date of 19 December 2019.

Among 11 sequences obtained from samples related to the Huanan market, eight had no mutations, two had the same single mutation and one sequence showed two mutations. Sequences from the two patients not linked with Huanan market had one and three mutations, respectively. Notably, all samples were collected between 24 December 2019 and 2 January 2020, that is 4-24 days after the date of onset of illness; therefore, the genomes obtained may not be necessarily representative of the initial virus at the time of infection. Two sequences were from isolates obtained from environmental samples collected from Huanan market on 1 January 2020; these had zero and two mutations, respectively. As they were collected from either the floor or a wall in the market, the virus is likely to reflect contamination from cases.

# Table 7. The overview of sequences from early patients (with onset date before 31 December 2019)

Sample ID	Sequence ID	Relation to the Huanan market	Stall	Onset date	Collection date	Mutations (gene name) <sup>a</sup>	Lineage
<i>S01</i>	EPI_ISL_40 3928	Visitor to another market		8 Dec	1 Jan 2020	7866 (ORF1a)	L/B
<i>S02</i>	EPI_ISL_40 2123	Vendor	Seafood	13 Dec	24 Dec	0	L/B
S03	EPI_ISL_40 6798	Purchaser		17 Dec	26 Dec	6968 (ORF1a), 11764 (ORF1a)	L/B
S04	NMDC6001 3002-06	Vendor	Frozen goods	19 Dec	30 Dec	24325 (S) <sup>b</sup>	L/B
S05	EPI_ISL_40 3929	Purchaser		20 Dec	30 Dec	0	L/B
	NMDC6001 3002-09	Purchaser		20 Dec	1 Jan	0 <sup>b</sup>	L/B
<i>S06</i>	MN908947 <sup>c</sup>	Purchaser		20 Dec	30 Dec	0	L/B

<i>S07</i>	MN988668	Vendor	Seafood	20 Dec	2 Jan	0	L/B
S08	EPI_ISL_52 9216	Vendor	Seafood	20 Dec	30 Dec	0 <sup>b</sup>	L/B
	MN988669	Visitor		22 Dec	1 Jan	0	L/B
S09	EPI_ISL_40 6800	Visitor		22 Dec	2 Jan	0	L/B
S10	GWHABKG 00000001	Vendor	Vegetab le	23 Dec	30 Dec	0 <i>d</i>	L/B
S11	GWHABKH 00000001	Vendor	Seafood	23 Dec	30 Dec	0 <i>b</i>	L/B
S12	GWHACAU 01000001	Vendor	Dry cargo	23 Dec	30 Dec	24325 (S) <sup>b</sup>	L/B
<i>S13</i>	EPI_ISL_52 9213	Visitor to another market		26 Dec	30 Dec	4946 (ORF1a), 8782 (ORF1a), 28144 (ORF8)	S/A
E1	EPI_ISL_40 8514	Environment			1 Jan	12350 (ORF1a), 29019 (N)	L/B
E2	EPI_ISL_40 8515	Environment			1 Jan	0	L/B

<sup>a</sup> Note that the mutations may arise within a patient within the course of infection. See also Table 6.

<sup>b</sup> Samples had been sequenced multiple times but showed discrepant results, the sequence supported by more submissions or with highest sequence depth being chosen.

<sup>c</sup> NCBI reference genome.

<sup>d</sup> Samples had been sequenced multiple times and showed consistent results.

The sample ID of patients with contact history with dead animals is *italicized*.

The sample ID of patients with contact history with poultry and aquatic products is in **bold** face.





#### 3.2.5 Haplotype analysis of early cases

A haplotype network analysis was performed using the 66 high-quality and non-redundant sequences from December and January (Fig. 7). Note that the timing indicated in the analysis was done by sampling date, as onset times were only available for the 13 cases with illness onset in December. The numbers indicated refer to cases with illness onset in December (Tables 6 and 7). The analysis shows that several of the cases with exposure to the Huanan market had identical virus genomes, suggesting that they were part of a cluster. However, the sequence data also showed that some diversity of viruses was already present in the early phase of the pandemic in Wuhan, suggesting unsampled chains of transmission beyond the Huanan market cluster. There was no obvious clustering by the epidemiological parameters of exposure to animals or aquatic products (Table 7, Fig. 7). Four sequenced cases with cold-chain exposure (in one case cold seafood but unknown in the other three) showed two different genomes; that is, two cases had identical virus strains without mutation and the other two had identical sequences with one mutation. However, another six cases without seafood exposure history also had identical sequences. The current analysis does not provide definitive support for specific exposures explaining the pattern of sequence diversity.



Fig. 7. Haplotype network of early sequences of Wuhan. One viral genome that carried a C>T variant at site 8782 (compared to the reference genome) connected the S/A and L/B major lineages, and this genome was sampled from Wuhan in late January 2020.

#### 3.2.6. Analysis of the time to most recent common ancestor

Different approaches have been used to analyse the SARS-CoV-2 genomes accumulated at different time points as the pandemic developed (Table 8), and the results suggest that the time to most recent common ancestor (tMRCA) inferred by more than 10 groups using different approaches is similar: between mid-November and mid-December 2019.(19, 31-42)

The tMRCA and mutation rate were estimated with the genomic sequences of 66 early cases (from Wuhan, before 31 January 2020). The inferred date of the tMRCA was 11 December 2019, with the 95% confidence interval ranging from 13 November 2019 to 23 December 2019, and the mutation rate was estimated to be  $6.54 \times 10^{-4}$  per site per year, with the confidence interval ( $3.32 \times 10^{-4} - 9.54 \times 10^{-4}$ ) (Table 9). The team also inferred the tMRCA with fixed mutation rate values (from previous studies), listed in Table 9. Overall, all these values are consistent with existing results, indicating a recent common ancestor of these viral genomic sequences.

Table 8. Time to the most common ancestor	(tMRCA	) inferred in	different studies.
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Reference	Sample size	Country	Inferred tMRCA <sup>17</sup>	Method
$\mathbf{D} \stackrel{\cdot}{\cdot} \left( 1 \right) $	622	China	2019, late September	Strict clock model
Bai et al. $(31)$			(95% CI	(BEAST v2.6.2)

<sup>&</sup>lt;sup>17</sup> Note that the 95% confidence intervals cited include highest posterior density, Bayesian credible intervals and frequentist confidence intervals; see individual publications for details.
			2019.8.28 -	
			2019.10.15	
			(95% CI	Rate-informed strict
Li et al. (41)	32	China	2010 5 2	clock model
			2019.5.2 - 2020.1.17)	(BEAST v1.8.4)
			2019.12.6	Pata astimated releved
Li et al. <i>(41)</i>	32	China	(95%BCI	clock model
			2019.11.16 -	(BEAST v1.8.4)
			2019.12.21)	
~			2019.11.25	Relaxed clock model
Giovanetti et al. $(34)$	54	Italy	(95%Cl	
			2019.9.28 - 201912.21)	(BEAS1 v1.10.4)
			2019.12.3	
Hill & Rambaut	116	UK	(95%CI	Unreported clock model
(36)			2019.11.16 - 2019.12.17)	(BEAST v1.7.0)
			2019.12.1	
Lu et al <i>(40</i> )	53	China UK	(95%HPD	Strict clock model
		china, ore	2019.11.15 -	(BEAST v1.10.4)
			2019.12.13)	
			2019.11.19	
Duchene et al. (33)	47	Australia	(95%HPD	Strict clock model
			2019.10.21 -	(BEAST v1.10)
			2019.12.11)	
			2019.11.12	Dalama dala ala sha sa adal
Duchene et al. (33)	47	Australia	(95%HPD	Relaxed clock model
			2019.9.26 - 2019.12.11)	(BEAST v1.10)
			2019.12.8	
Volz et al (42)	53	UK	(95%CI	Strict clock model
			2019.11.21 -	(BEAST v2.6.0)
			2019.12.20)	

Volz et al. (42)	53	UK	2019.12.5 (95%CI 2019.11.6 - 2019.12.13) 2019.11.18	Maximum Likelihood regression (treedater R package v0.5.0) A Bayesian framework
Lai et al. <i>(37)</i>	52	Italy	(95%CI 2019.9.28 - 2019.12.13)	using a Markov chain Monte Carlo (MCMC) method (BEAST v.1.8.4)
Nie et al. <i>(39)</i>	124	China	2019.11.12 (95%CI 2019.10.11 - 2019.12.9)	A Bayesian framework using a Markov chain Monte Carlo (MCMC) method (BEAST v.1.8.4)
Chaw et al. <i>(32)</i>	137	Taiwan, China	2019.12.11 (95%CI 2019.11.13 - 2019.12.23)	A Bayesian framework using a Markov chain Monte Carlo (MCMC) method (BEAST v1.10.4 )
Gómez-Carballa et al. <i>(35)</i>	4721	Spain	2019.11.7 (95%CI 2019.8.18 - 2019.12.2)	Strict clock model (BEAST v2.6.2)
Gómez-Carballa et al. <i>(35)</i>	4721	Spain	2019.11.12 (95%CI 2019.8.7 - 2019.12.8)	Relaxed clock model (BEAST v2.6.2)
Liu et al. (19)	12 909	China	2019.11.28 (95%CI 2019.10.20 - 2019.12.9)	Maximum likelihood method

# Table 9. The inference of tMRCA using the genomic sequences of the 66 early cases with different mutation rates.

Mutation rate (per site per year)	Date of the MRCA
6.54×10 <sup>-4</sup> (3.32×10 <sup>-4</sup> – 9.54×10 <sup>-4</sup> ) <sup>a</sup>	11 December 2019 (13 November 2019 – 23 December 2019)

8.69×10 <sup>-4</sup> (8.61×10 <sup>-4</sup> - 8.77×10 <sup>-4</sup> ) <sup>b</sup>	19 December 2019 (14 December 2019 – 23 December 2019)
5.42×10 <sup>-4</sup> (4.29×10 <sup>-4</sup> - 8.02×10 <sup>-4</sup> ) °	5 December 2019 (16 November 2019 – 21 December 2019)
6.05×10 <sup>-4</sup> (4.46×10 <sup>-4</sup> – 8.22×10 <sup>-4</sup> ) <sup>d</sup>	9 December 2019 (16 November 2019 – 22 December 2019)

<sup>a</sup>: estimating both mutation rate and tMRCA by virusMuT.(19)

<sup>b</sup>: using mutation rate of reference.(19)

c: using mutation rate of reference, (35) uncorrelated relaxed-clock method.

d: using mutation rate of reference, (35) strict-clock model.

In summary, the tMRCA analysis based on molecular sequence data suggested that the pandemic onset occurred before the end of December 2019. The tMRCA analyses can be considered a statistical inference but do not provide definitive proof of time of origins. The point estimates for the time to most recent ancestor ranged from late September to early December, but most estimates were between mid-November and early December.

**3.3. Evidence for the early occurrence of SARS-CoV-2 from other studies** It remains to be determined where SARS-CoV-2 originated. Although the virus was first identified as the cause of a cluster of cases of severe pneumonia in Wuhan, to date it is uncertain from where the first cases originated. A few studies suggest that cases may have occurred before December 2019, the time when circulation of SARS-CoV-2 was thought to have started in Hubei Province. In a retrospective survey, sewage samples collected on 12 March 2019 in Barcelona, Spain, were positive for SARS-CoV-2 RNA, but other samples collected between January 2018 and December 2019 were all negative. The PCR signals has not been confirmed by sequencing and could be false-positive signals. *(43)* 

In Italy, the first known COVID-19 case was reported in the town of Codogno in the Lombardy region on 21 February 2020. Since then, a few studies have suggested evidence for earlier circulation. La Rosa and others (44) found the first positive sewage sample in northern Italy mid-December 2019, using a sewage testing protocol with nested PCR. In the same region, SARS-CoV-2 was detected by PCR in a throat swab from a child with suspected measles early in December. (45) Gianotti et al. (46) reported reactivity by in situ hybridization with a range of probes for SARS-CoV-2 in skin biopsies from a 25-year-old woman sampled in November 2019. She tested negative by PCR but in June 2020 was serologically positive. A serological survey among participants in a lung cancer screening programme described finding a few persons with neutralizing antibodies as early as October 2019.(46a)

In France, an oropharyngeal sample from a haemoptysis patient who was admitted to hospital on 27 December 2019 was identified positive by RT-PCR for SARS-CoV-2 RNA.(47) A separate, serological study found evidence for a significant increase in prevalence of neutralizing antibodies in mid-December, suggesting considerable earlier circulation of the virus.(47a) In Brazil, testing of sewage by RT-PCR yielded SARS-CoV-2-positive results in samples collected on 27 November 2019, much earlier than the first reported case in the Americas.(48, 49)

In the United States of America, a serological survey of 7389 archived donated blood samples collected between 13 December 2019 and 17 January 2020 from nine states identified 106 positive samples, suggesting that SARS-CoV-2 might have been introduced into United States of America before the first identified case in the country.(50)

Collectively, these studies from different countries suggest that SARS-CoV-2 circulation preceded the initial detection of cases by several weeks. Some of the suspected positive samples were detected even earlier than the first case in Wuhan, suggesting that circulation of the virus in other regions had been missed. So far, however, the study findings were not confirmed, methods used were not standardized, and serological assays may suffer from non-specific signals. Nonetheless, it is important to investigate these potential early events.

#### 4. Zoonotic origins of SARS-CoV-2

SARS-CoV-2 is thought to have had a zoonotic origin. (51) Genome analysis reveals that bats may be the source of SARS-CoV-2 (Fig.8). (13, 41, 52, 53) However, the specific route of transmission from natural reservoirs to humans remains unclear. Initial analysis revealed that the SARS-CoV-2 genome (WH-Human 1) was closely related to SARS-like coronaviruses previously found in bats, (10) and the whole-genome sequence identity of the novel virus has 96.2% similarity to a bat SARS-related coronavirus (SARSr-CoV; RaTG13). (13) In contrast, the SARS-CoV-2 genome is less similar to the genomes of SARS-CoV (about 79%) or MERS-CoV (about 50%). (12, 53, 54) Notably, a novel bat-derived coronavirus, denoted RmYN02, shares 93.3% nucleotide identity with SARS-CoV-2 at the genomic scale. (11)

In addition, SARS-CoV-2 has a unique insertion of four amino acids between the S1 and S2 domains of the spike (S) protein, which creates a cleavage site for the furin enzyme. This furin-cleavage site is not present in most other betacoronaviruses (for instance, SARS-CoV), and it may increase the efficiency of virus infection of cells.(38) As with SARS-CoV-2, RmYN02 was also characterized by the insertion of multiple amino acids at the junction site of the S1 and S2 subunits of the spike protein, providing evidence that such insertion events occur naturally in animals.

Besides RaTG13 and RmYN02, very recently SARS-CoV-2-related coronaviruses were isolated from two *Rhinolophus shameli* bats (RshSTT200 and RshSTT182). These animals were sampled in Cambodia in 2010, and samples were processed for sequencing recently. (55) The whole genome comparisons indicated that these viruses overall shared the nucleotide identity of 92.6% with SARS-CoV-2. The results suggest that the geographical distribution of SARS-CoV-2 related viruses is much wider than previously expected. (55) Another study found related viruses in Thailand, in *Rhinolophus acuminatus* bats, where near identical viruses were found in five animals from a single colony, suggesting a colony-specific sequence signature. (55a) The above-mentioned bat viruses differ in their ability to bind to the human ACE2 receptor from RmYN02, but both RmYN02 and RshSTT200/182 share part of the furin-cleavage site unique to SARS-CoV-2. There is evidence of recombination in the evolutionary history of these Thailand bat coronaviruses. These findings do show that the ongoing search for the origins of SARS-CoV-2 should consider wider geographical ranges, multiple potentially susceptible species, and a sampling design that includes knowledge on number and densities of colonies.

Current studies have demonstrated that Malayan pangolins (*Manis javanica*) hosted two sub-lineages of SARS-CoV-2-related coronaviruses (see Fig.8). In the first study, animals (including four Chinese pangolins (*M. pentadactyla*) and 25 Malayan pangolins (*M. javanica*)) had been obtained during antismuggling operations by the Guangdong customs in March and August 2019.(56) The viruses from the animals (termed pangolin-CoV-GDC) shared a genomic similarity of 90.1% to SARS-CoV-2. The pangolin-CoV-GDC has 100%, 98.6%, 97.8% and 90.7% amino acid identity with SARS-CoV-2 in the E, M, N and S proteins, respectively.(56) Both SARS-CoV and SARS-CoV-2 bind to angiotensin-converting enzyme 2 (ACE2) receptors through the receptor-binding domain of the S protein to enter human cells.(13, 54, 57-61) Five of the six critical amino acid residues in the receptor-binding domain differ between SARS-CoV-2 and SARS-CoV, and structural analysis revealed that the spike of SARS-CoV-2 has a higher binding affinity to ACE2 than SARS-CoV.(61) Although SARS-CoV-2 is closely related to RaTG13, only one out of the six critical amino acid sites is identical between the two viruses. However, these six critical amino acid sites are identical between SARS-CoV-2 and pangolin-CoV-GDC. (56, 62, 63) Although some researchers thought these observations served as evidence that SARS-CoV-2 may have originated in the recombination of a virus similar to pangolin-CoV with one similar to RaTG13, (56, 63) others argued that the identical functional sites in SARS-CoV-2 and pangolin-CoV-GDC may actually result from coincidental convergent evolution. (24, 62) Interestingly, upon farm-to-farm passage of SARS-CoV-2 in mink in the Netherlands, a mutation was observed in a receptor-binding residue that is common to bat and pangolin and rarely found in the human SARS-CoV-2 database, suggesting adaptation (Oude Munnink et al, unpublished).

The second sublineage of pangolin-CoV (termed pangolin-CoV-GXC) was isolated from 18 Malayan pangolins obtained during anti-smuggling operations performed by Guangxi customs officers between August 2017 and January 2018.(62) This study obtained six complete or near complete genome sequences, which were highly similarly to each other (>99%) and had a sequence similarity of 85% to SARS-CoV-2 at the genomic scale.(62) A small-scale serological survey found neutralising antibodies to a bat SARSr-CoV in pangolins seized in Thailand.(55a) Based on recombination analysis of currently known SARSr-CoV viruses, pangolins have been proposed as the original reservoir, but the inclusion of mosaic sections of the genome complicates the use of phylogenetic analyses.(55b) When removing recombinant sections of the genomes, Boni et al. (3) concluded that the binding to the human ACE2 receptor is a trait shared with bat viruses, and that the lineage giving rise to SARS-CoV-2 has been circulating unnoticed in bats for decades

Although inconclusive, these studies (3, 64), collectively demonstrate that pangolins should be included in the search for possible natural hosts or intermediate hosts of the novel coronaviruses.

Comparative genomic analyses have revealed that extensive recombination events occurred during the divergence between SARS-CoV-2 and other SARS-CoV-2-related coronaviruses. (12, 37, 51, 65) Although the overall genomes differ by about 3.8% (nucleotides) between SARS-CoV-2 and RaTG13, the divergence at neutral sites (dS, number of synonymous changes in the synonymous sites of the protein-coding regions) was 17% between these two viruses. In contrast, the proportion on non-synonymous changes (dN, number of non-synonymous changes in the non-synonymous sites of the protein-coding regions) was only 0.8%, reflecting strong negative selection pressure. Calculating sequence differences without separating these two classes of sites may underestimate the extent of molecular divergence by several fold. Overall, these results suggest that, during the divergence between SARS-CoV-2 and RaTG13, more than 95% of the amino-acid-changing mutations have been removed by purifying selection. (24)



# Fig. 8. The phylogenetic tree of SARS-CoV-2 and other coronaviruses in bats and pangolins (based on the concatenated protein sequences of all the genes).

An initial search for bat betacoronaviruses provided 1501 results<sup>18</sup> and for sarbecovirus sequences from all non-human hosts through GenBank<sup>19</sup> 467 results. These include some SARS-CoV-2 sequences related to the current pandemic (for example, nine from tigers) or sequences from animal infection experiments (for example, murine 62). Most were bat viruses (310) but again this number included repeats of viruses or gene fragments. Seventy-one reliable genomes were obtained from 13 species, comprising 11 bat species, humans (SARS-CoV and SARS-CoV-2) and Malayan pangolins (*Manis javanica*); these are presented in Table 10. The genomes include bat sarbecoviruses from Japan (*66*) and Cambodia (*55*). The vast majority of data was collected in China, reflecting more comprehensive research efforts in China compared to other parts of the world. Also, metadata associated with globally shared genome data typically are incomplete. For instance, the location of sequences reflects where samples were taken, but not the geographical origin of the species sampled. For instance, pangolin virus genomes were listed as having been sampled in Guangdong and Guanxi provinces, whereas they were from imported animals. Further work is needed to develop integrated genomic and epidemiological data collections on animals to support the origin-tracing studies.

5. Genomic sequencing data of SARS-CoV-2 viruses in naturally infected animals Since the emergence of SARS-CoV-2 in humans, the virus has been detected in domestic and farmed animals exposed to infected humans. The first evidence of this was from reported cases of SARS-CoV-2 infection in dogs in Hong Kong SAR and cats in Belgium and Hong Kong SAR, respectively. Subsequently, infection was diagnosed in a Siberian tiger in a zoo in the Bronx (New York, United States of America). In all cases, infection was diagnosed by detection of viral RNA in respiratory samples, and in some animals further supported by detection of specific antibodies. *(67)* Experimental infections have confirmed species' susceptibility, with cats and ferrets considered to be highly infectious as evidenced by transmission experiments. *(5, 9)* In line with the susceptibility of ferrets, natural infections have been observed in farmed mink, animals also belonging to the family of mustelids. *(6, 7)* By now, mink farm infections have been reported from Canada, Denmark, France, Greece, Lithuania, The Netherlands, Poland, Spain, Sweden and the USA. Animals may display

<sup>18</sup> Database of bat-associated viruses, available at <u>http://www.mgc.ac.cn/cgi-bin/DBatVir/main.cgi</u> (accessed 25 March 2021)

<sup>19</sup> National Center for Biotechnology Information available at <u>https://www.ncbi.nlm.nih.gov/nucleotide/</u> (accessed 25 March 2021).

symptoms of respiratory disease and increased mortality, but not all farms are equally affected and circulation of the virus may go unnoticed. (7, 68) Sequencing has shown that SARS-CoV-2 may evolve during circulation on mink farms, with selection of variants with mutations in the contact residues of the ACE2 receptor-binding domain of the spike protein. (6, 69) The governments of Denmark and The Netherlands have ordered the culling of all mink in order to reduce the potential for adaptation to circulation in high density mink farms. The high susceptibility and transmissibility of SARS-CoV-2 in mink was confirmed by experimental infections (70).

Virus name	Species	Sample location	Accession no.	Year	Month	Day
RshSTT182	R_shameli	Steung Treng, Cambodia	EPI_ISL_852604	2010	12	NA
RshSTT200	R_shameli	Steung Treng, Cambodia	EPI_ISL_852605	2010	12	NA
Rc-0319	R_cornutus	Iwate, Japan	LC556375	2013		
RpShaanxi2011	R_pusillus	Shaanxi	JX993987	2011	9	NA
HuB2013	R_sinicus	Hubei	KJ473814	2013	4	NA
279_2005	R_macrotis	Hubei	DQ648857	2004	11	NA
Rm1	R_macrotis	Hubei	DQ412043	2004	11	NA
JL2012	R_ferrumequinum	Jilin	KJ473811	2012	10	NA
JTMC15	R_ferrumequinum	Jilin	KU182964	2013	10	NA
HeB2013	R_ferrumequinum	Hebei	KJ473812	2013	4	NA
SX2013	R_ferrumequinum	Shanxi	KJ473813	2013	11	NA
Jiyuan-84	R_ferrumequinum	Henan-Jiyuan	KY770860	2012	NA	NA
Rf1	R_ferrumequinum	Hubei-Yichang	DQ412042	2004	11	NA
GX2013	R_sinicus	Guangxi	KJ473815	2012	11	NA
Rp3	R_pearsoni	Guangxi-Nanning	DQ071615	2004	12	NA
Rf4092	R_ferrumequinum	Yunnan-Kunming	KY417145	2012	9	18
Rs4231	R_sinicus	Yunnan-Kunming	KY417146	2013	4	17
WIV16	R_sinicus	Yunnan-Kunming	KT444582	2013	7	21
Rs4874	R_sinicus	Yunnan-Kunming	KY417150	2013	7	21
YN2018B	R_affinis	Yunnan	MK211376	2016	9	NA
Rs7327	R_sinicus	YunnanKunming	KY417151	2014	10	24
Rs9401	R_sinicus	Yunnan-Kunming	KY417152	2015	10	16
Rs4084	R_sinicus	Yunnan-Kunming	KY417144	2012	9	18
RsSHC014	R_sinicus	Yunnan-Kunming	KC881005	2011	4	17
Rs3367	R_sinicus	Yunnan-Kunming	KC881006	2012	3	19

Table 10. Sarbecovirus genomes (Extracted from 55, 66 Boni et al, 2020)

WIV1	R_sinicus	Yunnan-Kunming	KF367457	2012	9	NA
YN2018C	R_affinis	Yunnan-Kunming	MK211377	2016	9	NA
As6526	Aselliscus_stoliczkanus	Yunnan-Kunming	KY417142	2014	5	12
YN2018D	R_affinis	Yunnan	MK211378	2016	9	NA
Rs4081	R_sinicus	Yunnan-Kunming	KY417143	2012	9	18
Rs4255	R_sinicus	Yunnan-Kunming	KY417149	2013	4	17
Rs4237	R_sinicus	Yunnan-Kunming	KY417147	2013	4	17
Rs4247	R_sinicus	Yunnan-Kunming	KY417148	2013	4	17
Rs672	R_sinicus	Guizhou	FJ588686	2006	9	NA
YN2018A	R_affinis	Yunnan	MK211375	2016	9	NA
YN2013	R_sinicus	Yunnan	KJ473816	2010	12	NA
Anlong-103	R_sinicus	Guizhou-Anlong	KY770858	2013	NA	NA
Anlong-112	R_sinicus	Guizhou-Anlong	KY770859	2013	NA	NA
HSZ-Cc						
(SARS COV 1)	Homo sapiens	Guangzhou	AY394995	2002	NA	NA
YNLF_31C	R_Ferrumequinum	Yunnan-Lufeng	KP886808	2013	5	23
YNLF_34C	R_Ferrumequinum	Yunnan-Lufeng	KP886809	2013	5	23
F46	R_pusillus	Yunnan	KU973692	2012	NA	NA
SC2018	R_spp	Sichuan	MK211374	2016	10	NA
LYRa11	R_affinis	Yunnan-Baoshan	KF569996	2011	NA	NA
Yunnan2011	Chaerephon_plicata	Yunnan	JX993988	2011	11	NA
Longquan_140	R_monoceros	China	KF294457	2012	NA	NA
HKU3-1	R_sinicus	Hong_Kong SAR	DQ022305	2005	2	17
НКИЗ-3	R_sinicus	Hong_Kong SAR	DQ084200	2005	3	17
НКU3-2	R_sinicus	Hong_Kong SAR	DQ084199	2005	2	24
НКU3-4	R_sinicus	Hong_Kong SAR	GQ153539	2005	7	20
HKU3-5	R_sinicus	Hong_Kong SAR	GQ153540	2005	9	20
НКU3-6	R_sinicus	Hong_Kong SAR	GQ153541	2005	12	16
HKU3-10	R_sinicus	Hong_Kong SAR	GQ153545	2006	10	28
НКИЗ-9	R_sinicus	Hong_Kong SAR	GQ153544	2006	10	28
HKU3-11	R_sinicus	Hong_Kong SAR	GQ153546	2007	3	7
HKU3-13	R_sinicus	Hong_Kong SAR	GQ153548	2007	11	15
HKU3-12	R_sinicus	Hong_Kong SAR	GQ153547	2007	5	15
HKU3-7	R_sinicus	Guangdong	GQ153542	2006	2	15

HKU3-8	R_sinicus	Guangdong	GQ153543	2006	2	15
CoVZC45	R_sinicus	Zhoushan-Dinghai	MG772933	2017	2	NA
CoVZXC21	R_sinicus	Zhoushan-Dinghai	MG772934	2015	7	NA
Wuhan-Hu-1 (SARS-CoV-2)	Homo sapiens	Wuhan	MN908947	2019	12	NA
BtKY72	R_spp	Kenya	KY352407	2007	10	NA
BM48-31	R_blasii	Bulgaria	NC_014470	2008	4	NA
RaTG13	R_affinis	Yunnan	EPI_ISL_402131	2013	7	24
P4L	pangolin	Guangxi	EPI_ISL_410538	2017	NA	NA
P5L	pangolin	Guangxi	EPI_ISL_410540	2017	NA	NA
P5E	pangolin	Guangxi	EPI_ISL_410541	2017	NA	NA
P1E	pangolin	Guangxi	EPI_ISL_410539	2017	NA	NA
P2V	pangolin	Guangxi	EPI_ISL_410542	2017	NA	NA
Pangolin-CoV	pangolin	Guangdong	EPI_ISL_410721	2019		NA

 $R_{\rm is}$  Rhinolophus bat genus. Pangolin is Manis javanica.

# 6. Summaries and perspectives

## 6.1. Summaries

The joint international team concluded that:

- 1. Linking genomic data with epidemiological data is essential for molecular analysis in support of origin-tracing studies.
- 2. Quality control of genome sequencing is important to provide reliable results.
- 3. Viruses from some Huanan market cases were identical, suggesting a spreading event.
- 4. Analysis of early case genomes also showed some diversity, suggesting additional sources and unrecognized circulation.
- 5. Estimates of the time to most recent common ancestor (from literature and re-analysis) suggest that virus transmission or circulation date might be recent, in late 2019.
- 6. Up to now, the most closely related genomic sequences have been found in bats.
- 7. Reports of detection of SARS-CoV-2 in cases and environmental samples before January 2020 in different parts of the world require follow-up.

# 6.2. Recommendations

The joint international team made the following recommendations:

- 1. Conduct further retrospective and systematic research around earlier cases and possible hosts for SARS-CoV-2 around the world.
- 2. In view of the team's re-analysis of the data quality of early cases in Wuhan, China, early cases or samples collected in future SARS-CoV-2-global tracing studies need to be sequenced using multi-platforms and high-depth sequencing (more than 40-fold coverage) in order to obtain reliable high-quality data.
- 3. Continue to develop an integrated database that includes global SARS-CoV-2 genome and raw sequences with epidemiological and clinical data, and linked analysis results.

4. Develop a comprehensive information database to combine molecular data, global distribution data and other metadata of potential animal hosts.

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### ANIMAL AND ENVIRONMENT STUDIES

#### Introduction

Nearly three quarters of emerging human infectious diseases have animal reservoirs, including wildlife (for instance, bats, primates, rodents and birds) and domesticated animals (such as poultry, pigs and camels). (1, 2) For example, in recent years, A/H5N1, A/H5N6, A/H7N9 and other avian influenza viruses have infected humans after cross-species transmission from live birds; and publications suggest that henipaviruses have emerged in people after being transmitted from bat reservoir hosts via domesticated intermediate hosts (horses and pigs). (3, 4) These and other zoonotic viruses have been responsible for some of the most significant emerging disease threats to human health and economic development.

Research on wildlife reservoirs of some of these zoonoses have revealed a high diversity of related viruses distributed globally (for example, within the coronaviruses of the Sarbecovirus subgenus or Merbecovirus subgenus carried by bats, or the hantaviruses carried by rodents). (5-10) In appropriate conditions, these viruses break through the interspecies barrier, infect humans and cause epidemics or pandemics. Analyses show that these spillover events are driven by factors that include large-scale environmental and socioeconomic changes, including land use change, deforestation, agricultural expansion and intensification, trade in wildlife, and expansion of human settlements. (11, 12)

The coronaviruses now endemic in humans that emerged in our recent past (such as HCoV-HKU1, HCoV-NL63, HCoV-OC43 and HCoV-229E) are thought to have originated in cattle, rodents, bats or birds, but the exact circumstances of their spillover are not known. (13-15) SARS-CoV-2 is also thought to have its ecological niche in an animal reservoir. (16) It is a member of a clade of betacoronaviruses (SARS-related CoVs) that is almost exclusively found in bats (5), and the viruses most closely related to it were identified in *Rhinolophus* spp. (horseshoe) bats sampled in Yunnan Province in China (RaTG13 and RmYN02), (16, 17) in Japan (Rc-0139), (18) in Cambodia (RshSTT182 and RshSTT200), (19) and in Thailand (RacCS203). (20) Two other closely-related viruses with 85.5% to 92.4% sequence similarity to SARS-CoV-2 were sequenced from custom-seized trafficked Malayan pangolins that were housed in rehabilitation facilities in Guangxi and Guangdong provinces, China. (21)

Two other  $\beta$ -coronaviruses (MERS-CoV and SARS-CoV) have caused largescale epidemics in people, but their exact origins remain elusive. However, CoVs with high sequence similarities with SARS-CoV or MERS-CoV have been identified in bats.(22, 23) Evidence suggests that dromedary camels are the intermediate host of MERS-CoV, and data suggest that civets or related species may be the intermediate host of SARS-CoV.(24, 25) Although no intermediate hosts have so far been implicated in the origin of COVID-19, a range of species can be infected by SARS-CoV-2 experimentally (for example, raccoon dogs, ferrets, rabbits, cats, golden Syrian hamsters, bats, macaques, marmosets and white-tailed deer) or by presumed or demonstrated exposure to humans with COVID-19 (for example, mink, gorillas, captive large felids, domesticated cats and dogs).(26) Cattle, pigs and poultry are not thought to be receptive to infection with SARS-CoV-2 (see Annex F, Tables 1 and 2).

Although the exact route of exposure of people to the putative wildlife reservoir or potential intermediate hosts of SARS-CoV-2 is unknown, circumstantial evidence supports a range of potential spillover pathways. Direct spillover from bats to humans may have occurred, or as with MERS-CoV and likely SARS-CoV, transmission to humans may have involved an intermediate host. Candidate intermediate host species may include mink, pangolins, rabbits, raccoon dogs and domesticated cats that can be infected by SARS-CoV-2,(26) or species such as civets and ferret badgers and related mustelids that were shown to be infected by SARS-CoV during the outbreak in Guangdong Province, China. (25) Spillover of viruses from animals to humans can occur through direct contact with infected animals, indirectly through animal products or excreta, or via intermediate hosts. (25) Therefore, the investigations so far conducted focused on the Huanan market and included a comprehensive sampling plan bearing such transmission routes in mind. The study in the Huanan Market was designed on the basis of these scientific principles. Here, the focus on animals and animal products is described. Other potential routes for the emergence of SARS-CoV-2 in people associated with the Huanan market in late 2019 include exposure to contaminated animal meat or food products that are refrigerated or frozen, or the introduction of the virus by people infected elsewhere.

Three recent COVID-19 outbreaks in China have been linked to exposure to imported refrigerated or frozen seafood products.(27-30) An outbreak in Beijing linked to the Xinfadi market was first identified on 11 June 2020 after 56 days without a single known community case of COVID-19 in Beijing. Full genome sequencing and phylogenetic analysis of publicly available genomes suggests that the virus was from the L lineage European branch 1 with specific mutations characteristic to the market outbreak. However, it is not possible to fully infer the source of contamination from this work yet (31). In October 2020, an outbreak occurred in Qingdao. (32) The index cases for the cluster were two dock workers from the city's port with no history of travel or recognized contact with anyone with confirmed COVID-19; the only epidemiological link which could be established between the cases was exposure to SARS-CoV-2 on the surface of cold-chain packaging. In addition, SARS-CoV-2 viruses were isolated from swabs of the outside surfaces of imported cold-chain packages in Qingdao(33). Based on these observations, China has launched a programme for systematic screening of packaged frozen imported food. Although re-introduction of a pandemic virus to epidemic-free areas can occur via various transmission routes including imported goods during a pandemic, the similarities between the outbreaks in the Beijing Xinfadi market and Qingdao, leading to the consideration of potential introduction of the virus through frozen products into the Huanan market in late 2019.(34) For research focusing on the origin of SARS-CoV-2, this will need to be aligned with sources of those products.

In this report, published and unpublished surveillance studies and surveys conducted in China were reviewed according to clearly defined objectives, differentiating studies that investigated the origin of SARS-CoV-2 from those that aim to identify potential infection of animals by COVID-19-infected people. These surveys included environmental, products and animal sampling as part of the initial outbreak investigation and a detailed review of the supply chain of the Huanan market. Retrospective testing of samples from wildlife and livestock animals in China was also conducted and the results included.

#### Methods

#### 1. Sample collection

(1) Environmental samples: Using full personal protective equipment, investigators applied sampling swabs to the floors, walls or surfaces of objects and then preserved them in virus

preservation solution. Swabs and virus preservation solution were commercial products (Disposable Virus Sampling Tube, V5-S-25, Shen Zhen Zi Jian Biotechnology Co., Ltd., Shenzhen, China).

(2) Animal samples: Depending on the type of animal and whether it was alive or frozen, pharyngeal, anal, body surface and body cavity swabs or tissue samples were collected for nucleic acid testing (NAT), and blood samples from domesticated animals were collected for serum antibody tests.

(3) Sewage (silt) samples: Collected by the use of virus sampling swabs to probe into the silt at the bottom of drainage channels in the market, sewage and silt samples were preserved in virus preservation solution (Disposable Virus Sampling Tube, V5-S-25, Shen Zhen Zi Jian Biotechnology Co., Ltd., Shenzhen, China); for the sewage well, a container was used to take a silt-water mixture from a location near the bottom of the well, and an appropriate amount of sample was collected by using virus sampling swabs and then preserved in virus preservation solution (Disposable Virus Sampling Tube, V5-S-25, Shen Zhen Zi Jian Biotechnology Co., Ltd., Shenzhen, China); for the sewage well, a container was used to take a silt-water mixture from a location near the bottom of the well, and an appropriate amount of sample was collected by using virus sampling swabs and then preserved in virus preservation solution (Disposable Virus Sampling Tube, V5-S-25, Shen Zhen Zi Jian Biotechnology Co., Ltd., Shenzhen, China).

#### 2. Nucleic acid extraction

A virus nucleic acid extraction kit (Xi'an Tianlong) was used to extract viral nucleic acid from samples using an automated nucleic acid extraction instrument according to the manufacturer's instructions.

#### 3. SARS-CoV-2 real-time PCR assay

Real-time (RT) PCR was performed on extracted nucleic acid samples with a SARS-CoV-2 nucleic acid assay kit. The reagent brands include BioGerm (40/38, cycle number/cut-off value, the same as below), DAAN (45/40), and BGI (40/38).

#### 4、Animal coronavirus test

An RT-PCR method was used to complete surveys for animal coronaviruses. The primers were designed and synthesized by China Animal Health and Epidemiology Center (CAHEC), and the relative papers and patents are being prepared and will be submitted soon.

# 5. Metagenomic sequencing of positive samples

Metagenomic sequencing was conducted at Wuhan BGI. Nucleic acid was extracted using Qiagen's viral RNA microextraction kit and human nucleic acid was removed using an enrichment kit to improve the sensitivity of viral RNA detection. Extracted RNA was reverse transcribed into cDNA and segmented into 150-200 bp by enzyme digestion. After repair, fitting, purification, PCR amplification and purification, sample concentration was assayed and SE50+10 sequencing performed by DNBSEQ-T7, and an average output of more than 200 million reads was obtained. Sequencing data were compared with those in a SARS-CoV-2 database to determine whether the samples contained coronavirus sequences.

# 6. Serological testing

(1) SARS-CoV-2-specific antibody screening

Initial screening for serum SARS-CoV-2-specific antibodies was done using a double-antigen sandwich ELISA. This kit has been used in animal infection models in relevant laboratories in China and has been shown effective for both animal and human samples. (35)

(2) SARS-CoV-2-specific antibody confirmation

Samples with positive ELISA results were confirmed using a neutralization assay.

# Results

Environmental sampling and description of vendors at the Huanan market

Environmental samples in the Huanan market were collected to represent exhaustively as possible, from a wide diversity of surfaces, animals and products (Table 1). Some environmental samples tested positive for SARS-CoV-2 nucleic acid, and the virus was isolated from some of these samples. The distribution of positive environmental samples was assessed relative to sites where people with early cases had worked and the types of products sold.

Huanan market was officially closed on 1 January 2020 and on early morning of that same day China CDC began collecting environmental and animal samples. Staff from China CDC entered the market about 30 times before the market's final clean-up on 2 March 2020. The environmental and animal samples in and around the market were collected according to different sampling principles.

The range of in-market sampling covered: (1) environmental samples from stalls related to early cases; (2) environmental samples from doors and floors of all stalls in the blocks where the early cases were located; (3) environmental samples in the east wing of the market were collected according to blocks; (4) transport carts, trash cans and similar objects; (5) environmental samples from stalls that sold livestock, poultry, farmed wildlife (also called "domesticated wildlife" or "domesticated wildlife products" in this report); (6) samples of sewage and silt from drainage channels and sewerage wells; (7) stray cats, mice and other potential vector animals in the market; (8) animal products and other commodity samples kept in the cold storages and refrigerators in the market; (9) the market's ventilation and air-conditioning system; and (10) public toilets, public activity rooms and other places where people gathered in the market.

At the same time, environmental or animal samples were collected from other sites, mainly including: (1) other markets around the Huanan market; (2) sewerage wells in the neighbouring communities of the Huanan market; (3) animal products and other commodities stored in warehouses and cold-storage facilities related to the Huanan market and the environment; and (4) stray cats from around the Huanan market.

Between 1 January 2020 and 2 March 2020, 923 environmental samples were collected and tested, among which 73 samples were SARS-CoV-2 NAT positive. Among the positive samples, 69 were environmental samples from or related to the Huanan market, of which 61 were collected from or related to the west area of the market. The other four samples were collected from other markets or community sewerage wells in Wuhan. The PCR cycle threshold (Ct) values of most samples ranged from 23.9 to 41.7, and SARS-CoV-2 strains were successfully isolated from three samples with Ct values below 30 (Table 1).

Table 1. Overview of environment sample sampling and testi	ng in the Huanan market

	Number of samples	Number positive by RT-PCR	Number virus isolated from
Huanan market	718	40	3
Warehouses related to the Huanan market	14	5	
Other markets in Wuhan*	30	1	
Drainage system in the Huanan market	110	24	
Sewerage wells in surrounding areas	51	3	

Total	923	73	3	

\*The other markets were Dongxihu Market and Huanggang Center Market.

The nature of merchants' activities was assessed against the NAT results of the environmental samples. The sampling covered 19.8% (134/678) of vendors in the market (95% confidence interval (CI): 16.8-23.0%). Of the positive samples, 60% (44/73) were distributed among 21 vendors in the market (95% CI: 48.1-71.5%), 19 of whom were located in the west area of Huanan market and the remaining two located in the east area (Table 2). Some vendors sold more than one product type, leading to differences in the denominators: 16/87 (18.4%) of vendors selling cold-chain products were positive (95% CI: 10.9-28.1%) while five did not; 13/73 (17.8%) of the vendors selling aquatic products were positive (95% CI: 9.8-28.5); six of the vendors selling seafood products were positive (11%, 6/56: 95% CI: 4-21.9%), eight of the vendors selling poultry were positive (22%, 8/37: 95% CI: 9.8-38.2%), five of the vendors selling livestock were positive (14%, 5/36: 95% CI: 4.7-29.5%), one vendor selling wildlife products was positive (11%, 1/9: 95% CI: 0.3-48.2%) and two vendors who sold vegetables were positive (25%, 2/8: 95% CI: 3.2-65%) (See Figure 1). While these results provide some indication of association of cases with different products, further analyses are required to identify their significance. Of the 110 samples collected from sewers or sewerage wells in the market, 24 samples were positive for SARS-CoV-2 nucleic acid, suggesting that either contaminated sewage may have played a role in the cluster of cases in the market or that infected people in the market contaminated the sewage.

		Product types						
Vendors No.	Location	Cold- chain products	Aquatic products	Seafood products	Pou ltry	Live stock	Wildlife products	Vege tables
1	West	-	-	-	+	-	-	-
2	West	+	+	+	-	-	-	-
3	West	+	+	-	+	+	+	-
4	East	+	-	-	+	+	-	-
5	West	-	-	-	-	-	-	-
6	West	-	+	-	+	+	-	-
7	West	+	-	-	+	-	-	-
8	West	+	+	+	+	-	-	-
9	West	+	+	+	-	-	-	-
10	West	+	+	+	+	+	-	-
11	West	+	+	-	-	-	-	-
12	West	+	+	+	-	-	-	-
13	West	+	+	-	-	-	-	-
14	West	+	+	-	-	-	-	-
15	West	+	+	-	-	-	-	-
16	West	+	+	-	-	-	-	-
17	West	-	-	-	-	-	-	-
18	West	+	-	-	+	+	-	-
19	West	-	-	-	-	-	-	+

Table 2.	<b>Twenty-one</b>	vendors	of NAT	test	positive	in	Huanan	market	
	•								

20	West	+	-	-	-	-	-	+
21	East	+	+	+	-	-	-	-
Sum of NAT positive vendors		16	13	6	8	5	1	2
Vendors sampled in the study selling such products		87	73	56	37	36	9	8



Figure 1: Positive environmental samples associated with different products in the Huanan Market. Dots represent the percentage of positive environmental samples associated with each product. Bars represent 95% confidence intervals for the binomials in the text above. Note that the CI for some products (e.g. vegetables, farmed wildlife) have broad error bars that are likely due to the low number of vendors for these categories in the market. Nine of the 10 vendors selling farmed wildlife have been sampled.

The typical coronavirus morphology was observed by transmission electron microscopy in the strains isolated from three environmental samples (see Annex F, Figs. 1 and 2), two of which were from the stalls with confirmed patients. Genome sequences of the three isolated strains were obtained by applying high-throughput sequencing technology (sequences uploaded to GISAID). Through comparison with the SARS-CoV-2 reference strains from the cases, the consistency is more than 99.9%, suggesting that the three strains may have originated from the contamination by infected persons' expelled virus. (Sequencing data of the three strains were analysed and presented in the molecular epidemiology working group's report.)

# Animals, supply chains and professional customers in the Huanan market

The profile of the animal businesses, supply chains, and downstream sales in the Huanan market and other markets were reviewed and no significant changes were reported in the period leading up to the epidemic and the closure of the market. Extensive collection and testing of animal samples in the market and animals in upstream supply farms took place; the SARS-CoV-2 PCR test results were all negative.

# (1) Animal selling and supply chain in the market

Discussions with the authority of market regulation and supervision, and review of records obtained identified 10 animal-selling stalls in the Huanan market, accounting for 1.5% of the total. They were located in the south-western corner of the west area and the north-western corner of the east area (see



Figure 2). The authority of market regulation and supervision verified that there was no substantial change in the type of animal business in these 10 stalls in the 12 months before the outbreak.

# Figure 2: Map of the Huanan Market, showing locations of stalls where domesticated wildlife products were sold in relation to environmental testing results, and confirmed human cases of COVID-19.

According to sales records, in late December 2019, 10 animal stalls sold animals or products from **n**, snakes, avian species (chickens, ducks, gooses, pheasants and doves), Sika deer, badgers, rabbits, bamboo rats, porcupines, hedgehogs, salamanders, giant salamanders, bay crocodiles and Siamese crocodiles, among which snakes, salamanders and crocodiles were traded as live animals (Annex F, Table 3). Other products sold were frozen goods or *bai tiao* (remaining parts of poultry or livestock after removal of hair and viscera). Snakes and salamanders were slaughtered before being sold, but crocodiles were alive when sold.

The sources of farmed wildlife within Hubei Province included other local markets in Wuhan or farms in Tianmen, Xiaogan, Jingmen, Suizhou, Jianli, Xiangyang, Huangshi, Wuxue and Jingshan. The sources outside Hubei Province included farms in the following provinces: Heilongjiang, Jilin, Shanxi, Henan, Hunan, Jiangxi, Guangdong, Guangxi and Yunnan. No living or dead animals of foreign origin were identified from the sales records in late December 2019.

Market authorities have confirmed that all reported live and frozen animals sold in the Huanan market were from farms that were legally licensed for breeding and quarantine, and that no illegal trade in wildlife has been found. Although there is photographic evidence in a published paper that live mammals were sold at the Huanan market in the past (2014) *(36)* (date confirmed by author in statement in Annex F) and unverified media reports in 2020, no verified reports of live mammals being sold around 2019 were found.

On-site visits and telephone interviews by the market supervision authority with the owners and vendors of the 10 animal stalls in the Huanan market suggest that all the downstream customers of animal sales were retail customers. Further information on the Huanan market characteristics are given in the description of the site visit by the WHO-China joint team (see Annex D5).

# (2) Animal sample testing in the market

A total of 457 animal-related samples from 188 individuals of 18 species were collected and tested between 1st January and 2nd March. The sources of the samples include unsold goods kept in refrigerators and freezers in the Huanan market, goods kept in warehouses and refrigerators related to the Huanan market, vector animals such as stray cats and dogs (including animal faeces) in the market, and animal products sold in other markets in Wuhan. The animal species include rabbit, snake, badger, cat, bamboo rat, rat, chicken, and salamander, etc. All samples were SARS-CoV-2 NAT negative (Tables 3 and 4). The badgers were carcasses found in freezers and were identified visually. DNA barcoding has not yet been conducted on them to verify their identity.

At the same time, samples from animals raised by some Huanan market suppliers in Hubei were also sampled and tested between February and March 2020 (Table 5.1). Meanwhile, SARS-CoV-2 surveillance within wild animals were also done in some other provinces (Table 5.2). Altogether 2480 samples were collected and tested, and the results were all NAT negative (Table 5).

Table 3. Resu	lts of animal	samples testing	within and	outside Huanan	Market
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Collection sites	Sample number	<b>RT-PCR</b> positive number
Huanan market	327	0
Warehouses related to the Huanan market	32	0
Cats, rats and other vectors and their droppings	92	0
Wuhan and other surrounding markets	6	0
Total	457	0

Species	Sample	Animal number	RT-PCR positive	Remarks
Rabbit/Hares	104	52	0	
Stray cat	80 <sup>a</sup>	27	0	Including faeces
Snake	80	40	0	
Hedgehog	67	16	0	
Muntjac	18	6	0	
Dog	17	7	0	Including one stray dog
Badger	16	6	0	5 0
Bamboo rat	15	6	0	
Mouse	12	10	0	Captured around the market
Pig	6 <sup><i>b</i></sup>	NA <sup>c</sup>	0	
Chicken	5	5	0	
Chinese giant salamander	5	3	0	
Crocodile	4	2	0	
Wild boar	4	2	0	
Soft-shelled turtle	3	2	0	

#### Table 4. Details of animal samples within and outside Huanan Market

Weasel	2	1	0	Captured around the market
Fish	2	2	0	
Sheep	1	1	0	
Others	16	NA <sup>c</sup>	0	
Total	457	188	0	

<sup>*a*</sup> Six of the cats were from the Huanan market.

<sup>b</sup> Other markets.

<sup>c</sup> Not applicable.

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Nucleic Acid Testing (NAT)					
	Hubei				
Number of species	10				
Specific types of	Bamboo Rat, Porcupine, Duck, Snake, Rabbit/Hare, Chicken,				
animals	Ostrich/Turkey, Wild Boar				
Total sample size	616				
Test results	Negative				

Table 5.2. Survey of wild animals from	Yunnan, Guangdong an	d Guangxi for the	e SARS-CoV-2 NAT
- 5	, , ,	0	

Nucleic Acid Testing (NAT)								
	Yunnan	Guangdong	Guangxi					
Number of species	27	1	1					
Specific types of animals	Chinese pangolin, Malay pangolin, Civet cat, Rhinolophus affinis bat, Miniopterus schreibersi bat, Bamboo rat, Macaque, Bear monkey, Porcupine, Fox, etc.	Pangolin	Pangolin					
Total sample size	1287	92	485					
Test results	Negative	Negative	Negative					

# National domestic animal testing

In order to conduct a widespread scan of potential indicators of exposure to SARS-CoV-2 in animals, or evidence of potential animal sources of infection, samples from a range of animal species across the country were tested. The SARS-CoV-2-specific antibody and NAT results show no positive results in livestock and poultry tested before and after the COVID-19 epidemic. The survey did not find evidence for enzootic presence of SARS-CoV-2 in the main food animals (pigs, cattle, sheep, chicken).

# (1) Results of SARS-CoV-2 specific antibody testing

In 2019, as part of routine animal surveillance aimed at investigating the epidemic situation of major animal diseases in China, a total of 5638 livestock and poultry serum samples were collected from 31 provinces across China, including 946 pig, 1002 bovine, 962 sheep, 2479 chicken, 215 duck, and 34 goose sera. Samples came from 222 farms, including 130 small and medium-sized farms, 67 scattered

households in towns and villages, and 25 slaughterhouses. A retrospective study was performed to test whether these samples contained antibodies against SARS-CoV-2. In 2020, a total of 6070 livestock and poultry serum samples were collected from 31 provinces across the country, including 1045 pig, 767 bovine, 1058 sheep, 3,030 chicken, 169 duck and one goose sera. Sera came from 240 farms, including 135 small and medium-sized farms, 78 scattered households in towns and villages, and 27 slaughterhouses. All of the results of the SARS-CoV-2-specific antibody tests performed during 2020 were all negative (Table 6).

Table 6. Location, species	and number of livestock and poultry indivi	duals tested for SARS-
CoV-2-specific antibodies.	Samples were collected in 2019 and 2020 a	nd tested in 2020

Location	Goose	Duck	Chicken	Sheep	Cattle	Pig	In total
Beijing	0	0	180	94	15	70	359
Tianjin	0	0	208	60	80	50	398
Hebei	0	0	200	15	95	70	380
Shanxi	0	0	197	90	19	70	376
Inner Mongolia	0	0	191	80	70	30	371
Liaoning	0	0	177	66	44	70	357
Ji Lin	0	0	177	35	95	50	357
Heilongjiang	0	0	184	0	110	69	363
Shanghai	0	11	185	95	15	70	376
Jiangsu	0	30	162	71	39	70	372
Zhejiang	0	0	191	55	40	70	356
Anhui	0	0	198	80	30	70	378
Fujian	0	94	96	46	64	70	370
Jiangxi	0	0	185	40	55	85	365
Shandong	1	35	157	55	55	50	353
Henan	0	0	196	33	76	70	375
Hubei	0	20	165	15	75	99	374
Hunan	0	0	198	75	35	70	378
Guangdong	0	60	140	75	35	70	380
Guangxi	0	95	95	50	60	70	370
Hainan	34	39	127	90	20	70	380
Chongqing	0	0	200	70	40	70	380
Sichuan	0	0	192	97	13	70	372
Guizhou	0	0	191	70	40	69	370
Yunnan	0	0	200	20	90	69	379
Tibet	0	0	100	80	95	15	290
Shaanxi	0	0	199	39	71	70	379
Qinghai	0	0	193	70	80	30	373
Gansu	0	0	100	120	78	15	313
Ningxia	0	0	183	94	35	50	362
Xinjiang	0	0	168	100	30	50	348
Xinjiang Production and Construction Corp.	s <sup>0</sup>	0	174	40	70	70	354
Total	35	384	5509	2020	1769	1991	11708

# (2) Retrospective testing of livestock and poultry using SARS-CoV-2 NAT

A total of 12 092 animal tissue and swab samples, collected in 2018-2019 from 26 provinces and autonomous regions, including Heilongjiang, Liaoning, Tianjin, Hebei, Fujian, Anhui, Shandong, Henan, Hunan, Guangxi, Guangdong, Yunnan, Sichuan, Shaanxi, Xinjiang, Jiangsu, Jiangxi, Ningxia, Tibet, Jilin, Shanghai, Hubei, Zhejiang, Qinghai, Inner Mongolia and Guizhou, were tested for SARS-CoV-2 nucleic acid, including: 5000 pig, 131 cattle, 368 sheep, and 6593 poultry samples. The sample information is shown in Table 7. They have been tested retrospectively for SARS-CoV-2 nucleic acid, and the results are all negative.

	Ca	ttle	She	eep	Pig		Р	oultry
Location	Sample number	Sample type	Sample number	Sample type	Sample number	Sample type	Sample number	Sample type
Heilongjiang	40	Tissue			235	Tissue/Swab	102	Swab
Liaoning					213	Tissue/Swab	87	Swab
Tianjin	20	Tissue			215	Tissue/Swab	403	Swab
Hebei					354	Tissue/Swab	645	Swab
Fujian					258	Tissue/Swab	105	Swab
Anhui	14	Tissue			292	Tissue/Swab	340	Swab
Shandong					821	Tissue/Swab	601	Swab
Henan	46	Tissue			811	Tissue/Swab	413	Swab
Hunan			127	Swab	290	Tissue/Swab	86	Swab
Guangxi					497	Tissue/Swab	390	Swab
Guangdong					384	Tissue/Swab	366	Swab
Yunnan					203	Tissue/Swab	326	Swab
Sichuan					280	Tissue/Swab	691	Swab
Shaanxi	11	Tissue			12	Tissue/Swab	79	Swab
Xinjiang					135	Tissue/Swab	65	Swab
Guizhou			122	Swab				
Jilin			119	Swab			379	Swab/Feces
Jiangsu							130	Swab
Inner Mongolia								Swab
Shanghai							160	Swab
Zhejiang								Swab
Hubei							326	Swab
Jiangxi							305	Swab/Feces
Ningxia							267	Swab
Qinghai							105	Swab
Tibet							222	Swab
Total	131		368		5000		6593	

Table 7. Location, species and number of livestock and poultry individuals tested using SARS
CoV-2-NAT. Samples were collected in 2018 and 2019 and tested in 2020

# (3) Animal coronavirus test results

A subset of 26 807 samples of different animals stored in 2019-2020 from 24 provinces and autonomous regions, including Heilongjiang, Shanghai, Liaoning, Tianjin, Hebei, Fujian, Anhui, Shandong, Henan, Hunan, Hubei, Guangxi, Guangdong, Yunnan, Sichuan, Shaanxi, Xinjiang,

Jiangsu, Jiangxi, Ningxia, Tibet, Zhejiang, Inner Mongolia and Shanxi, were tested using NAT with pan-coronavirus and SARS-CoV-2 primer sets. Primers were designed and synthesized by China Animal Health and Epidemiology Center (CAHEC), and the relative papers and patents are being prepared and will be submitted soon.

The results of SARS-CoV-2 NAT were all negative, and 1711 samples tested for pan-coronavirus NAT were positive. Animal coronaviruses detected include: 1095 samples with avian infectious bronchitis virus, 167 samples with duck coronavirus, 50 samples with pigeon coronavirus, 25 samples with avian deltacoronavirus, 151 samples with porcine epidemic diarrhoea virus, and 36 samples with porcine transmissible gastroenteritis virus, six samples with porcine hemagglutinating encephalomyelitis virus, one sample with porcine del coronavirus, 74 samples with bovine coronavirus, 14 samples with mink coronavirus, 74 samples with feline coronavirus and 18 samples with canine coronavirus, as shown in Fig. 1. The genetic evolution analysis showed that the genetic distance between these viruses and SARS-CoV-2 was far (homology  $\leq$ 54.2%), and there was no evidence of SARS-CoV-2 in domestic animals, poultry and pets.



# Fig. 2. Animal coronaviruses detected in livestock and farmed animals. Samples were collected in 2019 and 2020 and tested in 2020

Further testing of livestock and captive wildlife for SARS-CoV-2

The results of SARS-CoV-2-specific NAT and serology of wild animal samples collected and stored from 2015 to 2020 were all negative, and no anomaly was found in the national surveillance system for wild animal disease in China.

# (1) Results of SARS-CoV-2 specific antibody testing

In total, 1914 serum samples were collected from 35 different species between November 2019 and March 2020. No SARS-CoV-2-specific antibodies were detected (Table 8).

Species	Number	Result
Dia	187	Nagativa
Fig	107	Negative
Cow	107	Negative
Sheep	133	Negative
Horse	18	Negative
Chicken	153	Negative
Duck	153	Negative
Goose	25	Negative
Mice	81	Negative
Rat	67	Negative
Guinea pig	30	Negative
Rabbit	34	Negative
Monkey	39	Negative
Dog	487	Negative
Cat	87	Negative
Camel	31	Negative
Fox	89	Negative
Mink	91	Negative
Alpaca	10	Negative
Ferret	2	Negative
Bamboo rat	8	Negative
Peacock	4	Negative
Eagle	1	Negative
Tiger	8	Negative
Rhinoceros	4	Negative
Pangolin	17	Negative
Leopard cat	3	Negative
Jackal	1	Negative
Giant panda	14	Negative
Masked	10	Negative
civet	10	1.08001.0
Porcupine	2	Negative
Bear	9	Negative
Yellow-	4	Negative
throated		1 ve Gattive
marten		
Weasel	1	Negative
Red nandas	3	Negative
Wild boar	5	Negative
with boat	1	negative

Table 8. Testing (by ELISA) of livestock, domesticated animals and captive wildlife during the epidemic period (Wuhan and surrounding areas, November 2019 – March 2020). *(35)* 

# (2) Results of SARS-CoV-2 NAT

In total, 648 samples (tissue, swab, blood and faeces) from 90 captive animals (nine species), including red pandas, white foxes, badgers, civets, bamboo rats, porcupines, guinea pigs and macaques, were collected between 8 February and 11 March 2020 in Wuhan, Dazhi, Yangxin, Jingmen, Jiangling and several provinces other than Hubei, and the SARS-CoV-2 NAT results were all negative.

After 8 April 2020, 2995 samples of 37 species of captive or farmed wildlife, including bamboo rats, porcupines, guineapigs and macaques, were collected in 14 cities in Hubei Province. The results of SARS-CoV-2 NAT were all negative.

Between May and September 2020, 27 000 samples of wild animals were collected in China, including primates, lagomorphs, artiodactyls, chiropterans, rodents and many kinds of wild birds (including Galliformes, Passeriformes and storks). All SARS-CoV-2 NAT were negative (Table 9).

	Nucleic Acid Testing (NAT)		
	Hubei Province	Nationwide	
Number of	74	208	
species			
Specific types of	Yunnan horse, Pony,	Green guenons, Green iguanas, Green monkeys,	
animals	Kangaroo, Arctic fox,	Bactrian camels, Horned owls, Dwarf musk deer,	
	Dezhou donkey, leopard,	Hyenas, Falcons, Cheetahs, Cinnamon bittern,	
	Ocelot, Tibetan macaque,	Northwest wolves, Blue macaws, Cockatoos,	
	Red-necked kangaroo,	Snub-nosed monkey, Leopards, Festival-tail	
	Skunk, Sichuan horse,	monkeys, Wildebeest, Muntjacs, Grey parrots,	
	Elephant, Giant panda,	Grey rock rats, Grey owls, Grey wolves, Grey	
	Siberian tiger, Sheep,	kangaroos, Grey monkeys, Reeves's muntjac,	
	Auricular fox, African lion,	Yellow monkeys, Ringtail raccoons, Ring-tailed	
	Baboon, Dog, Civet, Nutria,	lemur, Ring-necked pheasants, Rat snakes, South	
	Porcupine, River muntjac,	Chinatigers, Masked foxes, Tiger frogs, Red	
	Golden monkey, Black	foxes, Red-beaked blue magpies, Red-faced	
	bear, Red fox, Fruit bat,	monkey, Orangutan, Red-cheeked bamboo rat,	
	Pangolin, Tiglon, South	Black bear, Chimpanzee, Black swan, domestic	
	China tiger, Ring-tailed	chicken, Beauty rat snake, spider monkey, Black	
	lemur, Raccoon, Yellow	eyebrow monkey, Black monkey, Black panther,	
	muntjac, Grey kangaroo,	Black spotted frog, Black and white colobus	
	Muntjacs, Snub-nosed	monkey, Black and whitetegu, Brown winged	
	monkey, Grey wolf, Dwarf	crow cuckoo, Hippopotamus, River muntjac,	
	musk deer, Bactrian camel,	Porcupine, nutria, Gecko, Civet, badger, Gansu	
	Mongolian horse, Red deer,	zokor, Crested eagle, Yellow baboon, Scarlet	
	Yak, Sika deer, Stump-	parrot, African elephant, Auricle fox, Crocodile	
	tailed macaque, Squirrel,	lizard, Sheep, East African baboon, Siberian	
	Argalı, Grey goat, Muskrat,	tiger, Panda, Asian elephant, King snake, Giant	
	Black goat, Capybara, Red	anteater, Great ewe, Great egret, Pangolin, River	
	squirrels, Squirrel monkey,	horse, Skunk, Red kangaroo, Red lemur, Red-	
	Prairie dog, Guinea pig,	bellied lemur, Pond heron, Toad, Striped Water	
	Pig-footed bandicoot,	Snake, Tibetan macaque, De Brazza's monkey,	
	Northwest wolf, Tibetan	Fruit bat, Leopard cat, Leopard, Zebra, White	
	wild ass, Meerkat, Xiang	rhino, White-headed langur, White fallow deer,	
	Pig, Panda, Alpaca, Chinese	Lion, Hoolock gibbon, White eyebrow monkey,	
	nare, wild boar, Bamboo	White manage of North are sublide the sheet of 1	
	rat, Brown bear, etc.	white peacock, Northern White-cheeked gibbon,	
		Liger, while lox, while bellied langur,	
		Rangaroo, while nose monkey, Yunnan norse,	
Total comple size	2642	27 000	
Total sample size	JU4J Nagatiya	27 000 Nagatiya	
rest results	negative	inegative	

Table 9. Survey of wildlife (captive) in China for SARS-CoV-2 NAT, post-epidemic in Wuhan (after March 2020).

## (3) Retrospective test results of animal coronaviruses

Retrospective SARS-CoV-2 NAT was performed on 6811 animal samples collected from Beijing, Shanghai, Jiangxi and Xinjiang from 2015 to 2019, involving species of primates, Carnivora, Artiodactyla, Anciformes and Marabiformes. The results were all negative.

As part of national active surveillance plan of important animal diseases, animal samples were collected every year and these stored samples were retrospectively tested for SARS-CoV-2 after the outbreak of SARS-CoV-2. In December 2019, 2328 samples of 69 animal species, including macaque monkeys, forest musk deer, tigers, camels, bamboo rats, porcupines, goats and guinea pigs, were collected from tourist areas, zoos and artificial breeding sites in Hubei Province. All were SARS-CoV-2 NAT negative (Table 10).

	Nucleic acid testing	
	Hubei Province	Nationwide
Number of species	69	14
Specific types of animals	South China tiger, Raccoon, Siberian tiger, African lion, Stump-tailed macaque, Civet, Red fox, Meerkat, Porpoise, Skunk, Brown bear, Red kangaroo, Red squirrel, Marmot, Porcupine, Fennec fox, Nutria, China rabbit, squirrel, Guinea pig, Bamboo rat, Muskrat, Sika deer, Bactrian camel, Grey wolf, Hare, Mule, Chinese water deer, Lynx, Racoon dog, Asian elephant, Black bear, Leopard, Ring-tailed lemur, Tibetan macaque, African baboon, Panda, Snub-nosed monkey, DeZhou donkey, lion, Pallas's cat, kangaroo, Elk, Giraffe, African elephant, Hippo, White rhinoceros, Zebra, Red panda, Francois's leaf monkey, etc.	Angora ferret, Snub-nosed monkey, Sika deer, Wild boar, Elk, Mallard, Bar-headed goose, Heron, Night heron, Chicken, Duck, Pigeon, Fruit bat, Pangolin, etc.
Total sample size	2328	6811
Test results	negative	negative

## Table 10. Survey of SARS-CoV-2 in wildlife before the epidemic

(4) Other information on SARSr-CoVs from unpublished studies reported during meetings of the international joint team in Wuhan

• Tests on samples of more than 1000 bats from Hubei Province showed that none was positive for viruses related to SARS-CoV-2 (see Annex F, Table 4).

Study on cold-chain products<sup>20</sup>

# (1) Description of frozen food vendor operations in the Huanan market

There were 390/678 cold-chain related vendors in the Huanan Market. From September to December 2019, no substantial changes were reported in the type or quantity of import and sales of cold-chain products in the market. Information of upstream wholesalers of cold-chain products from 256 stores in the market was collected and analysed, including 10 vendors of domestic frozen farmed wild animals and 26 wholesalers of imported cold-chain products. Through tracking and inquiry of these 26 wholesalers, partial information was obtained about 17 upstream wholesalers from nine provinces and cities in China who imported cold-chain products into the Huanan market. Further trace-back showed that in addition to China, there were altogether 20 imported cold-chain product source countries and regions, and 29 kinds of imported cold-chain products. Information, including product name, import custom, source province (domestic) or country (international) and product quantity, was collected. Information about all imported cold-chain products in Wuhan from September to December 2019 was also collected and reviewed, involving a total of 440 kinds of cold-chain products from 37 import source countries or regions (Table 11). Information about the farms supplying the 10 vendors of farmed wild animal products were also collected (Annex F, Table 3).

Group	Wholesaler site	Source country or region	Number of different types of goods
Upstream wholesalers in the Huanar market	Fuzhou, Fujian; Foshan, Fujian; Guangzhou, Guangdong; Shenzhen, Guangdong; Zhanjiang, Guangdong; Fangchenggang, Guangxi; Hebei; Dalian, Liaoning; Shanghai	Argentina, Australia, Brazil, Canada, Chile, Denmark, France, Iceland, Japan, New Zealand, Norway, Russian Federation, Spain, Thailand, United Kingdom of Great Britain and Northern Ireland, United States of America, Uruguay, Viet Nam	29
Imported cold-chain products in Wuhan	NA	Argentina, Australia, Brazil, Canada, Chile, Hong Kong SAR, Denmark, Ecuador, Estonia, Faroe Islands, Finland, France, Germany, India, Indonesia, Ireland, Japan, Kazakhstan, Malaysia, Mauritius, Mongolia, Mexico, the Netherlands, New Zealand, Norway, Poland, Russian Federation, Saudi Arabia, Singapore, South Africa, Spain, Switzerland, Thailand, United Kingdom of Great Britain, Northern Ireland, United States of America, Uruguay and Viet Nam	About 440

Table 11. Country of origin for cold-chain products imported into the Huanan market and Wuhan from September to December 2019.

 $^{20}$  In this report, cold-chain products are defined as those supplied frozen or chilled to market. They do not include live animals.

Total	9	20+37	About 29+440
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# (2) Correlation between confirmed cases and cold-chain in Huanan market

The proportion of cases in stalls with cold-chain goods (5.6%) is significantly higher than those without cold-chain goods (1.7%), and the relative risk of cases in stalls with cold-chain goods is 3.3 times higher than those without cold-chain goods (relative risk = 3.3, 95% CI:1.2-8.6), and the morbidity rate of vendors of cold-chain products is higher than others (3.3%) compared with 1.4%), but there is no statistically significant difference. Epidemiological analysis showed that the first three cases in Huanan market all had a history of exposure to cold chain. (Annex E4, Table 6 and Fig 8).

# (3) Type of goods dealt by environmental positive stalls

Analyses show that 60% (44/73) of the positive samples are related to 21 stalls, 19 of which were located in the western part of the Huanan market, and the remaining two stalls were located in the eastern part. 16 stalls were dealing with cold-chain product.

# (4) Retrospective study on the cold chain in 2019

An inventory was made of imported cold-chain products in large and medium-sized cold warehouses in Wuhan from September to December 2019. It has been confirmed that cold-chain products were still in stock during the above period. From 4-6 February 2021, samples were collected and SARS-CoV-2 NAT were performed on a total of 1055 samples of imported cold-chain food products (no domestic-origin cold chain products could be located at that time) including 330 pieces with outer packages, 244 pieces with inner packages and 481 food samples. The results of SARS-CoV-2 NAT were all negative.

# (5) The persistence of live SARS-CoV-2 in environments related to the cold-chain It was noted that in one study, the infectivity of SARS-CoV-2 on cold-chain products did not decline after 21 days at 4 °C (refrigerated food) or at -20 °C (frozen food). Even at 21-23 °C, SARS-CoV-2 on cardboard surface remained infective up to 24 hours. (37, 38)

# (6) Examples of introduction of COVID-19 into China through imported cold chain products

After China successfully controlled the COVID-19 epidemic in Wuhan in April 2020, a series of clustered epidemics occurred in various places. According to the experience of prevention and control of these epidemics, especially the successful traceability results of Xinfadi in June, Dalian in July and Qingdao in October 2020, it is confirmed that SARS-CoV-2 can survive and maintain infection activity in cold chain products and packaging for a long time, which provides a scientific basis for the possibility of introduction of SARS-CoV-2 through cold chain products.

# Conclusions

- 1. CoVs that are phylogenetically related to SARS-CoV-2 were identified in different animals from different countries, including bats (*Rhinolophus* spp) and customs-seized trafficked Malayan pangolins. Sampling and testing of >1,100 bats in Hubei Province, however, has been conducted but none were positive for viruses close to SARS-CoV-2. Sampling of wildlife across China has been conducted but no samples were positive for SARS-CoV-2.
- 2. The Huanan market had evidence of extensive sale of frozen products, fresh sea and aquatic animals and products, livestock meat, and limited farmed wildlife products. All the product samples retrieved during the outbreak investigation tested negative for the SARS-CoV-2 nucleic acid.

- 3. SARS-CoV-2 can persist in conditions found in frozen food, packaging and cold-chain products. Index cases in recent outbreaks in China have been linked to the imported cold chain. These indicates a possibility of transmission of SARS-CoV-2 through frozen products. The supply chains to the markets in Wuhan included cold-chain products (including the seafood, aquatic products, vegetables, animal products and farmed wildlife products) from several provinces in China and 20 other countries. Suppliers included countries and regions where SARS-CoV-2 (NAT and serum) tested positive before the outbreak of SARS-CoV-2, countries where cold chain imported products were sourced, provinces where domestic wildlife farms were sourced, and where the relatives of SARS-CoV-2 are found in bats and pangolins. There is evidence that some domesticated wildlife species sold in the Huanan market are susceptible to SARS-CoV-2 or SARS-CoV, but none of the animal products sampled in the market tested positive. Apart from frozen farmed wildlife products, cold-chain products in Huanan market were not tested specifically in early 2020. These findings do, however, raise the possibility for different potential pathways of introduction, stressing the need for careful trace-back of these supply chains and sample testing.
- 4. Preliminary sampling and testing at other markets in Wuhan and upstream suppliers to the Huanan market taken during 2020 did not reveal evidence of SARS-CoV-2 circulating in animals. Evidence was not found of presence of SARS-CoV-2 among animal products in the Huanan market and upstream suppliers.
- 5. Environmental sampling in the Huanan market demonstrated widespread contamination of surfaces with SARS-CoV-2, compatible with the virus shedding from infected people in the market at the end of December 2019. However, through extensive testing of animal products in the market, no evidence of animal infections was found. One environmental sample collected on Jan 22, 2020 on a second market tested positive, implying an environmental contamination from the patients in the communities.
- 6. Of 923 environmental samples in Huanan market 73 were positive; Forty-four of those positive were from the stalls of 21 vendors dealing in the following products: aquatic animals and products (n = 13), cold-chain products (n = 16), poultry meat (n = 6), seafood products (n = 6), livestock meat (n = 5), vegetable products (n = 2) and farmed wildlife meat (n = 1). Sampling and testing of 38 515 livestock and poultry samples and 41 696 wild animal samples from 31 provinces in China during 2018 to 2020 resulted in no positive SARS-CoV-2 antibody or nucleic acid tests. No evidence was found of circulation of SARS-CoV-2 among domestic livestock, poultry and wild animals before and after the SARS-CoV-2 outbreak in China.

#### Recommendations

The joint international team made the following recommendations:

#### Recommendations for work related to the pathway of emergence from wildlife to people

#### Global-level recommendations

Although a large SARS-CoV-2 survey has been conducted in the animals in China, no positive samples were found so far. Therefore, tracing the origin of the SARS-CoV-2 worldwide in relevant wildlife species predicted to harbour diverse CoVs through international cooperation mechanisms should be conducted for viral discovery of diverse beta-coronaviruses in emerging disease hotspots.

#### Specific recommendations

• Despite large surveys of wildlife in China for CoVs, there are limits to the power of detection for wildlife populations over large geographic areas. Therefore, further surveys to identify coronaviruses related to SARS-CoV-2 is needed in bats and pangolins in China as well as in Southeast Asia (which is undersampled), and in *Rhinlophus* spp. bats in other countries where

this bat genus is found. This should focus in particular on regions where insufficient prior sampling has been done and where analyses show spillover to people is most likely.

• Surveys of other wild animals known to be infected by SARSr-CoVs should be conducted where they occur (e.g. civets, mustelids such as mink and ferrets, raccoon dogs).

# Recommendations for work related to the pathway of emergence involving intermediate hosts

#### Specific recommendations

- Further trace-back at the wildlife farms that previously supplied Huanan market and other Wuhan markets linked to positive cases, including interviews and serological testing of farmers and their workers, vendors, delivery staff, cold-chain suppliers and other relevant people and their close contacts.
- The surveys of livestock and farmed wildlife described in this report are large, but due to often large geographic area and animal populations, there are limits to the power to detect positive individuals. Therefore, surveys for SARSr-CoVs in farmed wildlife or livestock that have potential to be infected, including species bred for food such as ferret-badgers and civets, and those bred for fur such as mink and raccoon dogs in farms in China, in South-East Asia, and in other regions.
- DNA barcoding of the meat product samples from Huanan market to identify more precisely species involved and potential intermediate hosts or wildlife reservoirs of CoVs that might have been involved in the food chain.

#### Recommendations for work related to the cold chain

#### High-level, global recommendation

• Conduct retrospective testing for SARS-CoV-2 from products manufactured in 2019 supplied to the Huanan market and still available.

#### Specific recommendations

- Analyse virus persistence and viability at different temperatures to simulate the freeze-thaw cycle that would happen naturally as products are shipped from one port to another, then through the supply chain.
- Analyse the different role of the cold chain in the possible introduction of the virus in a market and the possible spread within a market following the introduction of the virus in a market by an infected human.

#### General high-level recommendations

• Establish a global expert group to support joint traceability research on the suspected origin of the epidemic. For example, conduct related traceability research on countries and regions with reported positive results in sewage, serum, human or animal tissues/swab and other SARS-CoV-2 test by the end of 2019.

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# POSSIBLE PATHWAYS OF EMERGENCE

The joint international team examined and discussed four main scenarios for introduction (see Fig. 1 and below):

- direct zoonotic transmission (also termed: spillover)
- · introduction through an intermediate host followed by zoonotic transmission
- introduction through the cold/ food chain
- and introduction through a laboratory incident.



Fig. 1. Overall schema for possible pathways of emergence, providing a conceptual framework for possible routes for SARS-CoV-2 emergence. The icons are meant to be interpreted in a generic manner and the location and timing is not stated. The animals depicted reflect animal species that have been discussed in relation to potential infection but can be replaced by other species as well. Arrows indicate directions of possible transmission. The symbols indicating "evolution" are meant to reflect any mutations, recombination, variant selection leading to enhanced ability to infect other species and/or transmit.

For each of these possible pathways of emergence, the joint team conducted a qualitative risk assessment considering the available scientific evidence and findings. The team assessed the relative likelihood of these pathways using an arbitrary Likert opinion scale of "extremely unlikely", through "unlikely", "possible", "likely" to "very likely"(*l*) and suggested further international and national phase 2 scientific studies as described in the recommendations. The diagrams are meant to be used as

a dynamic risk assessment framework and can be reviewed periodically when new information or studies become available.

In summary, the joint team considered the following ranking of potential introduction pathways, from very likely to extremely unlikely: (1) through an intermediate host; (2) direct zoonotic introduction; (3) introduction through cold/ food chain; and (4) introduction resulting from a laboratory incident. Building from the evidence for the studies conducted so far, follow-up research studies were proposed for the first three options. The arguments considered and underpinning these choices are summarized for each scenario in the section below.

# Direct zoonotic transmission

# Explanation of hypothesis

In this case, there is transmission of SARS-CoV-2 (or very closely-related progenitor virus) from an animal reservoir host to human, followed by direct person-to-person transmission with (top row of human icons) or without (bottom row) the need for adaptation of the virus to humans (Fig. 2). The speed of dissemination will depend on chance events such as superspreading events (indicated by the icon for the market, and for groups).



# Fig. 2. Schema for direct zoonotic transmission. Arrows relevant for this scenario are indicated in red.

# Arguments in favour

The majority of emerging diseases originate from animal reservoirs and there is strong evidence that most of the current human coronaviruses have originated from animals. Regarding plausible zoonotic reservoir hosts: surveys of the bat virome conducted following the SARS epidemic in 2003 have found SARSr-CoV in various bats, particularly *Rhinolophus* bats, and viruses with the high genetic similarity to SARS-CoV-2 have been found in *Rhinolophus* bats sampled in China in 2013, Japan in 2013, Thailand in 2020 and Cambodia in 2010. Recently, two distinct types of SARSr-CoV were

detected in Malayan pangolin (*M. Javanica* sampled in rescue centres in China for smuggled imported wildlife). The RaTG13 and pangolin coronaviruses do bind to hACE2, although the fit is not optimal. Seeding of SARS-CoV-2 in mink populations has shown that these animals are highly susceptible as well and the current evidence available cannot rule out the possibility for minks as the primary source of SARS-CoV-2. Antibodies to bat coronavirus proteins have been found in humans with close contact to bats. Bats are a known reservoir for many zoonotic viruses (with high virus diversity globally); they have the highest proportion of projected zoonotic viruses of any mammalian order. (2) In addition, bat ecology favours virus circulation (large populations, birthing waves, and closely spaced communities).

# Arguments against

Although the closest genetic relationship with SARS-CoV-2 was a bat virus, more detailed analysis found evidence for several decades of evolutionary space between the viruses. Although many betacoronavirus sequences have been found in a range of bats, isolation of viruses from them is rare, and only a few of the identified full genomes have human ACE2 binding properties. Because several contact residues between the bat and pangolin viruses and the hACE2 receptor are distinct from those in SARS-CoV-2, the affinity is low, and the viruses are genetically still quite distinct from SARS – CoV-2. In addition, the link with and focus on bats may be spurious as far less sampling has been done of other animal species. Confirmation of this potential bias is the identification of SARSr-CoVs from pangolin and from bats in Cambodia, Japan and Thailand, in studies that were completed since the start of the pandemic. The findings of high susceptibility of mink also raise the potential for certain mustelids as reservoir hosts. Also, contacts between humans and bats or pangolins are not likely to be as common as contact between humans and livestock or farmed wildlife, and virus presence in host animal is likely variable and seasonal, further decreasing the likelihood of an infectious contact. Despite consumption of bat and other wild animal meat in some countries, there is no evidence for transmission of coronaviruses from such encounters, and the trace-back investigation found no evidence for presence of bats or pangolins (or their products) in the market. The range of known mammals permissive to SARS-CoV-2 is expanding, suggesting alternative reservoir hosts are possible.

## Assessment of likelihood

Based on the arguments listed, the zoonotic introduction scenario was listed as possible to likely.

# What would be needed to increase knowledge?

To further investigate possible direct zoonotic introduction, detailed trace-back studies of the supply chain of the Huanan market (and other markets in Wuhan) have provided some credible leads to be followed. These leads can be followed to develop further surveys of potential reservoir hosts, including genomic surveys and serosurveys of high-risk potential reservoir hosts and their human contacts. Given the geographic range of the animal species in which closest relatives of SARS-CoV-2 have been found, such surveys should be expanded to include other countries, guided by knowledge on ecology and smuggling routes.

Introduction through intermediate host followed by zoonotic transmission

Explanation of hypothesis

SARS-CoV-2 is transmitted from an animal reservoir to an animal host, followed by subsequent spread within that intermediate host (spillover host), and then transmission to humans. The passage through an intermediate host can be without (group of animals, top) or with (group of animals, bottom row) virus adaptation (Fig. 3).



Fig. 3. Schema for introduction of SARS-CoV-2 through an intermediate host followed by transmission. Arrows relevant for this scenario are indicated in red.

# Arguments in favour

Although the closest related viruses have been found in bats, the evolutionary distance between these bat viruses and SARS-CoV-2 is estimated to be several decades, suggesting a missing link (either a missing progenitor virus, or evolution of a progenitor virus in an intermediate host). Highly similar viruses have also been found in pangolins, suggesting cross-species transmission from bats at least once, but again with considerable genetic distance. Both these putative hosts are infrequently in contact with humans, and an intermediary step involving an amplifying host has been observed for several other emerging viruses (Henipaviruses, influenza viruses, SARS-CoV and MERS-CoV). SARS-CoV-2 infection and intraspecies spread (including further transmission to humans) has been documented in an increasing number of animal species, particularly mustelids and felids. SARS-CoV-2 adapts relatively rapidly in susceptible animals (such as mink). The increasing number of animals shown to be susceptible to SARS-CoV-2 includes animals that are farmed in sufficient densities to allow potential for enzootic circulation. High-density farming is common in many places across the world and includes many livestock species as well as farmed wildlife. There was a large network of domesticated wild animal farms, supplying farmed wildlife. In high-density farms, there often are connections between farms (for instance, through the workforce and food supply), leading to complex transmission pathways that may be difficult to unravel, as was observed in other zoonotic outbreaks
involving farmed animals. Optimized conditions for sustained virus transmission chains in large-scale animal farms may also impact on virus seasonality in favour of a year-round endemic transmission pattern, and thereby increasing the zoonotic risk in winter months.

### Arguments against

SARS-CoV-2 has been identified in an increasing number of animal species, but genetic and epidemiological studies have suggested that these were infections introduced from humans, rather than enzootic virus circulation. In addition, since the containment of SARS-CoV-2 in China, new outbreaks have occurred for which genomic sequence data was generated. Based on epidemiological analysis and genetic sequencing of viruses from new cases throughout 2020, there is no evidence of repeated introduction of early SARS-CoV-2 strains of potential animal origins into humans in China. There was no genetic or serological evidence for SARS-CoV-2 in a wide range of domestic and wild animals tested to date. The screening of the major livestock species was done across the country and provided no evidence for circulation of a related virus. The scale of testing in these species was such that widespread circulation is extremely unlikely. Screening of farmed wildlife was limited but did not provide conclusive evidence for the existence of circulation.

### Assessment of likelihood

Based on the above arguments, the scenario including introduction through an intermediary host was considered to be likely to very likely.

### What would be needed to increase knowledge?

Given the literature on the role of farmed animals as intermediary hosts for emerging diseases, further surveys including further geographic range are needed. Studies of the supply chain of the Huanan market (and other markets in Wuhan) have not found any evidence for presence of infected animals, but the analysis of supply chains has provided potential information that will inform a targeted design of follow up studies. For instance, there was evidence for supply chains leading to wild-life farms from provinces where the higher prevalence of SARSr-CoVs have been detected in bat surveys. While this does not prove a link, it does provide a meaningful next step for surveys, as model for similar studies in neighbouring regions. Meanwhile animal products from areas outside southeast Asia where more distantly related SARSr-CoVs circulate should not be disregarded. Surveys should be designed using a One health approach in larger areas and more countries, including genomic surveys and structured serosurveys of high-risk potential reservoir hosts and their human contacts.

### Introduction through the cold/food chain

### Explanation of hypothesis

### SARS-CoV-2 is introduced and/or amplified through the cold/food chain

Food-chain transmission can reflect direct zoonotic transmission, or spillover through an intermediate host. Meanwhile cold chain products may be a vehicle of transmission between humans. This would also refer to food-contamination events in addition to introductions. The focus of this paragraph is on cold/food chain products and their containers as potential route of introduction of SARS-CoV-2. Here, it is important to distinguish between contamination of cold chain products leading to secondary outbreaks in 2020 and the potential for cold chain acting as the entry pathway for the origin of the pandemic in 2019.



# Fig. 4. Schema for introduction of SARS-CoV-2 through the cold/food chain. Arrows relevant for this scenario are indicated in red.

### Arguments in favour

The arguments are similar as those listed for zoonotic introduction, but with an emphasis on the potential for initial introduction through food animals or cold/ food chain products, or through contamination of food and food containers (for instance by animal waste). This includes frozen food items that are commonly sold and their packages in markets, including the Huanan market. Since the near-elimination of SARS-CoV-2 in China, the country has experienced some outbreaks related to imported frozen products in 2020. Screening programmes have found some limited evidence for the presence of SARS-CoV-2 by nucleotide acid tests in different batches of unopened packages and containers in different cities. In the epidemiological investigation of Qingdao outbreak, the live virus was isolated from the outer package of imported frozen products. SARS-CoV-2 and related CoVs have been found to persist in conditions (time/temperature/humidity) found during trade of frozen products suggesting the virus could persist on contaminated frozen products.

Foodborne outbreaks with enteric viruses are common, and - when entering the food supply - may lead to geographically dispersed outbreaks that can be difficult to detect. Seafood is known as a source of foodborne outbreaks, and food as a vehicle of zoonotic infections, but most evidence is for contamination of food with human viruses that are dispersed in growing areas through sewage or contaminated water for irrigation. Sewage treatment typically does not remove all infectious viruses prior to release of wastewater in the environment. These processes have been investigated widely for non-enveloped viruses but far less for enveloped viruses in the food chain, but there is widespread evidence for SARS-CoV-2 nucleic acid in sewage. There is some literature suggesting SARS-CoV-2 may have been circulating earlier as indicated by sewage testing in Spain and Italy. Although typical foodborne infections are thought to be restricted to enteric pathogens, there is some evidence that the oral route could lead to infection for SARS-CoV-2 from hamster infection experiments, and the virus replicates in gut organoids. Many animal CoVs have dual respiratory and enteric tropism. For SARS, food animal handlers had increased prevalence of SARS-CoV-specific antibodies. Humans infected with SARS-CoV-2 shed virus through faeces and can have gastrointestinal symptoms, suggesting involvement of the gastrointestinal tract. Humans can also be exposed to contaminated fomites, as suggested from the studies on markets in China in 2020.

### Arguments against

There is no conclusive evidence for foodborne transmission of SARS-CoV-2 and the probability of a cold-chain contamination with the virus from a reservoir is very low. While there is some evidence for possible reintroduction of SARS-CoV-2 through handling of imported contaminated frozen products in China since the initial pandemic wave, this would be extraordinary in 2019 where the virus was not widely circulating. Industrial food production has high levels of hygiene criteria and is regularly audited. Most viruses have been found in 2020 in low concentrations and are not amplified on cold-chain products. It is not clear what the infection route would be (possibly oral, touch, or aerosol). There is no evidence of infection in any of the animals tested following the Wuhan outbreak. Risk-assessments have concluded that the risk of foodborne transmission of SARS-CoV-2 through these known transmission pathways is very low in comparison with respiratory transmission.

### Assessment of likelihood

The consensus was that given the level of evidence, the potential for SARS-CoV-2 introduction via cold/ food chain products is considered possible.

### What would be needed to increase knowledge?

In order to further study the potential for (frozen) food as a source of infection or the cold chain as an introduction pathway of SARS-CoV-2, case-control studies of outbreaks in which the cold chain product and food supply is positive would be useful to provide support for cold chain products and food as a transmission route. There are some preliminary reports of SARS-CoV-2 positive testing in other parts of the world before the end of 2019. There is also evidence of more distantly related SARSr-CoV in bats outside Asia. Some producers located in these countries were supplying products to the markets. If there are credible links to products from other countries or regions with evidence for circulation of SARS-CoV-2 before the end of 2019, such pathways would also need to be followed up. Screening of leftover frozen cold chain products sold in Huanan market from December 2019 if still available is needed, particularly frozen animal products from farmed wildlife or linked to areas with evidence for early circulation of SARS-CoV-2 from molecular data or other analyses.

### Introduction through a laboratory incident

### Explanation of hypothesis

SARS-CoV-2 is introduced through a laboratory incident, reflecting an accidental infection of staff from laboratory activities involving the relevant viruses. We did not consider the hypothesis of deliberate release or deliberate bioengineering of SARS-CoV-2 for release, the latter has been ruled out by other scientists following analyses of the genome (3).



# Fig. 5. Schema for introduction of SARS-CoV-2 through a laboratory incident. Arrows relevant for this scenario are indicated in red.

### Arguments in favour

Although rare, laboratory accidents do happen, and different laboratories around the world are working with bat CoVs. When working in particular with virus cultures, but also with animal inoculations or clinical samples, humans could become infected in laboratories with limited biosafety, poor laboratory management practice, or following negligence. The closest known CoV RaTG13 strain (96.2%) to SARS-CoV-2 detected in bat anal swabs have been sequenced at the Wuhan Institute of Virology. The Wuhan CDC laboratory moved on 2<sup>nd</sup> December 2019 to a new location near the Huanan market. Such moves can be disruptive for the operations of any laboratory.

### Arguments against

The closest relatives of SARS-CoV-2 from bats and pangolin are evolutionarily distant from SARS-CoV-2. There has been speculation regarding the presence of human ACE2 receptor binding and a furin-cleavage site in SARS-CoV-2, but both have been found in animal viruses as well, and elements of the furin-cleavage site are present in RmYN02 and the new Thailand bat SARSr-CoV. There is no record of viruses closely related to SARS-CoV-2 in any laboratory before December 2019, or genomes that in combination could provide a SARS-CoV-2 genome. Regarding accidental culture, prior to December 2019, there is no evidence of circulation of SARS-CoV-2 among people globally and the surveillance programme in place was limited regarding the number of samples processed and therefore the risk of accidental culturing SARS-CoV-2 in the laboratory is extremely low. The three laboratories in Wuhan working with either CoVs diagnostics and/or CoVs isolation and vaccine development all had high quality biosafety level (BSL3 or 4) facilities that were well-managed, with a staff health monitoring programme with no reporting of COVID-19 compatible respiratory illness during the weeks/months prior to December 2019, and no serological evidence of infection in workers through SARS-CoV-2-specific serology-screening. The Wuhan CDC lab which moved on 2<sup>nd</sup>

December 2019 reported no disruptions or incidents caused by the move. They also reported no storage nor laboratory activities on CoVs or other bat viruses preceding the outbreak.

### Assessment of likelihood

In view of the above, a laboratory origin of the pandemic was considered to be extremely unlikely.

What would be needed to increase knowledge?

Regular administrative and internal review of high-level biosafety laboratories worldwide. Follow-up of new evidence supplied around possible laboratory leaks.

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### CONCLUDING REMARKS

The international team recognized the impact of the epidemic on Wuhan, from affected individuals and communities to government officials, scientists and health workers. The team commended the engagement of all the professionals who had spent long hours analysing very large quantities of data to support its work. In conclusion, the team called for a continued scientific and collaborative approach to be taken towards tracing the origins of COVID-19.

# WHO-convened Global Study of Origins of SARS-CoV-2: China Part

Joint WHO-China Study 14 January-10 February 2021

Joint Report - ANNEXES

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# ANNEXA: Background documents

# Annex A1 - Joint WHO-China study on origins of SARS-CoV-2: China Part - Workplan for the working groups (relating to the ToRs)

# Section I: Workplan specific outlines A: Cross-cutting workplan

### A1: General overview

#### A1.1: Outline of work process

The proposed work will be done by a combination of meetings and discussions in working groups, and plenary meetings. The composition of the working groups may change depending on the need for specific input.

The three working groups are:

Working group 1: Epidemiology

Working group 2: Animal and environmental

Working group 3: Molecular epidemiology and bioinformatics

The starting point across working groups will be an in-depth review of the initial cluster of cases in Wuhan, and all associated studies, sampling, data analysis, as specified below. Working group-specific discussions will focus on methodological aspects of the initial cluster analysis, and on the list of defined retrospective studies for which the China team has provided updates. All work will be regularly reviewed in plenary meetings to discuss findings, provide input for conclusions, and identify potential synergies. All outputs will be reviewed and endorsed by the entire team for integration in the joint study report.

### A1.2: Planned output for the first study

The working groups will compile a synthesis of all data and analyses as input for the joint evidence synthesis report describing the current knowledge on the early stages of the COVID-19 pandemic, starting from the initial observations of the Wuhan cluster that lead to the identification of SARS-CoV-2. This will include a discussion of methodological aspects specific to epidemiological approaches, molecular epidemiological analyses, and analyses of the role of animals in the emergence of the virus and the environmental and animal surveillance systems. The workplans will include an inventory of all available sources of information, a scientific quality assessment and systematic review of the existing data, a discussion of methodological aspects specific to the epidemiological analyses, a review of key hypotheses for the early chain of events, and identification of knowledge gaps and proposed future studies for these.

#### A1.3: Detailed workplan for the Phase 1 studies

A1.3.1: Prepare for systematic review of the data for this work-package, by customizing the generic template for assessment of data quality based on PRISMA or related criteria

Output: template details for review for each workplan

Timeline: Wednesday 20-1-2021

Overall aim: to provide a systematic way to scientifically assess all available evidence from published and unpublished reports and studies.

# *A1.3.2:* Detailed reconstruction of current knowledge starting with the initial cluster of cases, starting at the earliest recognized cases, and including the study related to the Huanan market cluster

Output: input for the joint study report

Timeline: first draft by Feb 1

Overall Aim: To collect and analyse all possible sources of information as well as data from observational and analytical studies that can be used to create a detailed map of the events involving the first reported COVID-19 cases in Wuhan (identified before and in December 2019), and anywhere else if necessary, in order to:

- Understand the epidemiology of the earliest human cases and associated potential risk factors for infection
- Understand the potential role of the Huanan market/spillover event at the market
- Explore possible sources and transmission modes that may have led to the introduction and amplification of SARS-CoV-2 in humans.
- To provide demographic, epidemiological, trade and ecological background data that may help to inform the choice of follow-up studies.
- Overall questions for first study:
  - What is the current state of knowledge on the initial notified outbreak based on published literature (including reports, pre-prints and Chinese language articles)?
  - What information is available on the earliest human cases (including interviews in December 2019? (e.g. list of all variables available).
  - What unpublished data are available? (e.g. data from completed/preliminary data from ongoing studies).
- Review of the above in light of possible hypotheses for disease emergence.
- Description of follow-up plans and timelines.

### Methods

- 1. Prepare a detailed timeline (including information on epidemiological and laboratory data available) of the initial cases and events until 31 December 2019.
- 2. Specify case definitions, laboratory methods etc. and indicate changes therein in the course of the initial studies.

- 3. Overview of currently published and unpublished reports. This includes clinical, epidemiological, virological, immunological, and imaging data, and data from additional surveys including animals and environmental (including sewage) swabs.
- 4. Review of raw interview data from early cases associated with Huanan market, and those with no relation to market.
- 5. Review of the Analytical Epidemiology Study agreed during the previous China-WHO study1.
- 6. Detailed discussion in the individual working groups on specific parts of the work, as described in the specific workplans below.

Output:

- Background literature reviews for the joint study report.
- Detailed timeline (including information on epi and lab data available) of the early cases and events.
- Summary of phase 1 studies, description of data and results of interim analyses.
- Evaluation of the above in light of possible hypotheses for disease emergence.
- Suggestions for next phase.

*A1.3.3:* Detailed review of retrospective studies, and design of additional studies based on in depth inventory of potential sources of information, related to the different hypotheses generated throughout the work

Output: input for the joint study report

Timeline: first draft by 1 February 2021

Overall aim: *to* review and develop retrospective studies capturing available data on human illness, animal, food products, cold chain and the environment among others, from December 2018 to December 2019 and, where relevant, control periods from previous years in order to investigate all possible sources of the origin of SARS-CoV-2.

Topics to address (details in the individual workplans per working group)

- 1. Review of primary data from the (ongoing) outbreak studies
- 2. Mapping the supply chains for all relevant animals and products
- 3. Retrospective studies
  - a. Review of hospital data
  - b. Review of respiratory disease surveillance/animal health surveillance
  - c. Review of trends in mortality
  - d. Serological studies
  - e. Surveillance studies in animals and food products
  - f. Surveillance studies involving environmental sampling (e.g. avian influenza program)
  - g. Other studies and approaches, as appropriate

<sup>1</sup> https://www.who.int/publications/m/item/who-convened-global-study-of-the-origins-of-sars-cov-2

Initial assessment of background information needs:

The workplan specific background is given in the working group specific work plans however, a number of general and cross cutting information of interest to the work of the three working groups should be collected. These include:

- How is the healthcare and public health system organised in the region? What types of data and statistics are available that could provide information about disease trends in the region and its administrative entities (e.g. districts/communes?) and communities (e.g. urban and rural)? Which selected hospitals would cover severe illness observed in those populations?
- What genomes are available publicly? What data are available in the various national or regional databases? What is the list of metadata available from the collected genomes? Who is doing molecular epidemiology studies? Is sequencing capacity available in the China CDC system? The animal health system? If in academia: how is data governance arranged?
- What are the data storage and sequencing agreements? What is the metadata standard? (methods used, software used, metadata collected and stored). Is there an agreement with the agricultural side?
- For the priority studies identified in the epidemiology and animal and environmental working groups: what types of data and sample collections are available (for instance from other surveillance programs) that could be included for sequencing?
- What is the role of the agricultural and environmental sectors in surveillance of diseases linked to wildlife; what do we know about wildlife and farmed wildlife value chains?
- What other relevant studies in the world are ongoing that may provide data to inform this part of the work?

### A2: Specific locations to visit and individuals to interview

During the first week of joint work, the detailed plans on locations to visit and individuals to interview will be finalised.

### A3: Final outputs

The joint study report will include the following points:

- 1. Background (literature reviews)
- 2. Study methods (process, components, objectives)
- 3. Study results (major findings, major conclusions, analyses of available data etc.)
- 4. Generate and discuss virus emergence pathways
- 5. Suggestions for next steps

## Section II: Workplan specific outlines

## B: Epidemiology working group

Output: input for the joint study report Timeline: first draft by Feb 1.

Colour-code for in yellow has been added indicating that the task is not considered urgent.

B1: Detailed reconstruction of current knowledge starting with the initial cluster of cases, starting at the earliest cases (prior to 31 December 2019), and including the study related to the Huanan market cluster

Discuss, analyse and review working group specific aspects of the cross-working group study of the initial cluster.

### B2: Review of primary data from the (ongoing) joint outbreak studies:

In-depth interviews and reviews of currently and jointly agreed identified early cases, suspected cases and potential earlier cases (prior to December 2019) identified through the above-described studies regarding to their exposure history. All potential exposure factors will be considered, including place of residence, travel history, occupational (including healthcare workers) exposure, market exposure, animal contact, consumption of raw/exotic animal meat/products (milk)/medicine with raw ingredients, intake and/or handling of wild/exotic animal meat at restaurants or at home, characteristics of their social contacts at the time and others.

### **B3:** Retrospective studies

Thorough review of existing data from surveillance systems, records of healthcare facilities, community clinics, sickness absenteeism data registries etc. looking for cases compatible with even mild COVID-19 from 1 October 2019 to 10 December 2019.

B3.1: Hospital focus based on the above selection: In-depth reviews of hospital records for cases compatible with COVID-19 before December 2019. This will focus on Wuhan and anywhere else if necessary, but potentially needs to be expanded further, based on the preparatory analysis.

Review of data from all type and location of hospitals (private and public) in Wuhan and suburban districts (n=233 healthcare facilities). This work is ongoing as of Jan 21, 2021

The descriptive epidemiology of the early cases needs to include information about how they were ascertained, and what definitions were used to identify further cases (applies to all sections where relevant).

Surveillance systems and hospital records:

Review of databases of hospitalised and outpatient clinic patients with the purpose to define the cohort of potential earlier COVID-19 cases.

Kindly provide the method applied for the systematic review of 233 Wuhan-based healthcare facilities using the fever, ILI, ARI and unspecified pneumonia data that resulted in the identification of potential COVID-19 cases. Please provide a description of the resulting dataset from the surveillance system and the final dataset of potential COVID-19 cases including all variables available for analysis (e.g. transfer to ICU, mechanical ventilation, laboratory diagnostic outcomes etc. that are potentially compatible with COVID-19, availability of stored samples for potential future testing).

Output will be:

- 1. Weekly numbers of patients >15 years of age with fever, ILI, ARI, and/or unspecified pneumonia from 1 October to 10 December 2019 overall (only expanding if relevant results), and by home address (e.g. district), outcomes by cluster analysis, etc.
- 2. A line-list of all the potential COVID-19 cases which can be populated in accordance with suggestions in Annex 1 (descriptive data already available). From a joint review of this this list, we can decide on the way forward for assessing the exposure history of the potential COVID-19 cases using relevant variables, including laboratory and radiology outcomes. This list will also be used to plan for face-to-face meetings post-quarantine with relevant experts (clinicians, hospital and provincial laboratory microbiologists, epidemiologists). Please find suggestions of exposure variables in Annex 2.

B3.2. Focus on high-risk groups: searching for previously unidentified cases compatible with COVID-19 among high-risk groups such as healthcare workers and laboratory staff. Other groups of consideration include: veterinarians, traditional medicine providers, farmers/farm workers, food-value chain workers (market vendors, port workers, storage house works etc). In-depth reviews of routinely collected employee sickness absence data. Where such data are available.

Routinely collected data e.g. sickness absenteeism, antibody (serology) or NAT screening, other?

Clusters of illness (especially among high-risk groups) might be of high relevance for the detection of early cases (this work has high priority but can be done when time permits).

Is information from months prior to outbreak (e.g. September – December 2019) on any relevant observations available from review of routinely collected data, e.g. sickness absenteeism of frontline hospital workers (e.g. infectious disease/ pulmonary disease/ICU departments) and of laboratory personnel at the major Wuhan research, public health and diagnostic laboratories.

If this information is not currently available, are there other ways to obtain it?

- a. Are there any data about sickness absenteeism, or occurrence of respiratory disease, or deaths in health care workers (HCWs) and/or laboratory workers during 2019 to January 2020?
- b. If any signal detected, kindly provide detailed information such as clustering.
- c. Is NAT/antibody results available from screening/testing of any of the above listed high-risk groups from 2019?

B3.3: Focus on mild respiratory disease surveillance and community cases: Review of surveillance trends for disease in the months preceding the outbreak to compare to baseline levels of similar months in previous years to identify any departure from expected trend through appropriate statistical analyses. For example, identify departure from trends of pneumonia of unknown origin, or review of syndromic surveillance trends for ILI and SARI, by comparing trends in the second half of 2019 to that of similar periods in previous years.

- 1. Are there any surveillance systems available for respiratory disease (including pneumonia) at community level?
  - a. Weekly numbers of patients >15 years of age with community-level records of ILI, SARI, and/or PUE (pneumonia of unknown etiology) from September to December 2016-2019 overall (only expanding if relevant results), and by home address (e.g. district), outcomes by cluster analysis, etc.
- 2. What type of respiratory surveillance data reviews have been conducted in and around Wuhan and in other provinces? (it would be interesting to look at the trends elsewhere in China outside Hubei Province).
- 3. Overview of geographic clusters identified in the surveillance data analysed so far.
- 4. Overview of the viral pathogen testing and results (e.g. influenza virus, respiratory syncytial virus etc.) in the surveillance data that could explain any changes seen in syndromic surveillance during the relevant periods?

# B3.4: Review of trends of all-cause mortality at provincial level, and review death registers for specific causes of death compatible with COVID-19.

- 1. Numbers of all-cause mortality for 2016, 2017 and 2018 in individuals >15 years of age by week to allow direct comparison with 2019 (specifically for Wuhan, Hubei)?
- 2. Numbers for specific causes of death compatible with COVID-19 from the second half of 2016, 2017 and 2018 to allow direct comparison with the second half of 2019? (Our understanding is that identification of cases is based on ICD-10 codes: J.12-18 and J.98.4).
- 3. Type and method used for reviews conducted in and around Wuhan and in other provinces?
- 4. Identification of temporo-spatial clusters where possible.

B4: Serological studies based on specific studies and/or stored blood/serum samples collected in weeks and months before December 2019. Consideration should be given to high-risk groups/workers as defined in section B3.2. Additionally, the work may include targeted serological testing on stored serum samples of suspect COVID-19 patients and contacts, cases of unexplained pneumonia, and suspect deaths identified through retrospective reviews.

- 1. Overview of serological study on stored blood (e.g. serum, dried blood spot samples etc., including from pregnant women and neonatal screening) to check for the presence of SARS-CoV-2-specific antibodies in the period leading up to December 2019, including samples from early 2019 and the previous two years to exclude previous circulation of SARS-CoV-2?
- 2. Are specimens from neighbouring parts of China available for serological testing?
- 3. Are serological studies possible on identified possible early COVID-19 cases (B3.1, B3.2, B3.3).

- 4. Overview of testing carried out on specimens from blood donors performed from 2019 and 2020.
- 5. What laboratory methods have been used for serological studies?
- 6. What health monitoring is carried out on food handlers, animal handlers, healthcare workers and laboratory personnel.?

#### B5. Other studies and approaches, as appropriate

To be discussed during the coming weeks, if the search for early cases can be narrowed down to specific locations.

Some examples of information of interest to increase potential case finding in districts indicated in B3.1:

#### Laboratory-specific questions:

The WHO Int team has some questions specifically related to the virology laboratory activities, as these listed below.

- 1. What is the scope and capacity of virus detection work (NAT, serology, virus isolation, sequencing), including sample storage, for those cases identified in SARI and/or unexplained pneumonia surveillance, in the diagnostic hospital laboratories?
- 2. What is the process for referral of samples for specialised virus testing within Wuhan, Hubei and other provinces? How does the WHO Influenza Centre interact with local hospital and public health laboratories? What virology research (sequencing, serology, isolation etc.) is carried out in the hospital laboratories? How are the clinical infectious diseases services linked to the diagnostic or regional laboratories?

#### Other questions:

 Can you provide a listing of mass-gatherings (involving humans and humans/animals and food festivals) in Wuhan and other places if needed (type, number of participants, duration of the event, geographical location, known health issues among participants, increased influenza-like illness (ILI)/ severe acute respiratory infections (SARI)/ pneumonia of unknown etiology admission-rates to hospitals following a period of 2-3 weeks after the gathering) from September 2019 until December 2019.

2. What kind of statistical information is available and can be provided on sales of pharmaceutical products indicative of respiratory syndromes (cough medicine, influenza medicines etc.) and other potential information sources? Have any of these data-sources already been reviewed?

### C. Animal and environmental working group

Output: input for the joint study report

Timeline: first draft by Feb 1

C1: Detailed reconstruction of current knowledge starting with the initial cluster of cases, starting at the earliest recognized cases, and including the study related to the Huanan market cluster

Discuss, analyse and review working group specific aspects of the cross-working group study of the initial cluster.

### C2: Review of primary data from the (ongoing) outbreak studies:

To be covered in EPI group, but of relevance to Anim/Env. Group: In-depth interviews and reviews of currently identified early cases, suspected cases and potentially earlier cases identified through the above-described studies regarding to their exposure history. All potential exposure factors will be considered, including travel history, occupational (including healthcare workers) exposure, market exposure, animal contact, consumption of raw meat/products (milk, cheese, organs, blood products) from farmed (including for food, fur and other products) and wild-caught animals, characteristics of their social contacts at the time and others. The process will likely be iterative and should include interviews of a few non-cases in order to easily identify susceptible patterns.

### C3: Review of studies focussing on animals, products and environmental contamination

C3.1: A mapping of activities and items traded at the Huanan (and potentially other relevant) market(s) in late November and December 2019, including types of animals (captured wild, farmed wild and livestock and domestic animals) and stalls (for all types of goods) present at the Huanan market.

To do this work with sufficient rigor, it will be necessary to review detailed datasets, and meet or interview members from key agencies, laboratories, and organizations. This will include:

Data on the species and countries of origin for imported animals being sold at the market both alive and dead for the months of November and December 2019? If any seasonal animals or produce were present in period before this are these known also?

- A full list of traders or stall holders, including the goods they are selling, available for the same period?
- Information on wild animal and other mammal suppliers, traders and stall holders over the 12 months prior to the outbreak, to see if there were any changes over the period prior to the outbreak?
- To understand what animals were sold in markets in Wuhan that might not be publicly disclosed (e.g. illegal species such as frozen or live pangolins), we need to speak with Market Regulation

Authorities to ask if there were any seizures of illegal wildlife at the Huanan market (and other markets in the study) in the months prior to the outbreak (December 2018 to January 2020). If so, what species were found in the seizures? What triggered the seizures?

- To understand the epidemiology of early spread, we need to review a list of local markets and professional customers (restaurants, large-scale customers, etc) to which the Huanan wholesale market was supplying, and, where relevant, similar information for nearby animal/food markets (work will be completed in later phase).
- Data on the presence of pets, stray, wild feral and pest animals (cats, dogs, rodents, bats, etc) found in and around the Huanan market.
- The map of the Huanan market will need to be populated with data on where animal and environmental samples were taken (with information on sample type, sampling time and test result), information on location of animal stalls (indicating species or type of animal where possible) and other product stalls, the drainage system (with direction of flow), exit/entry points, vehicle unloading point, organisation of the ventilation systems etc.
- For positive environmental samples, we will need to review details (what type, from what surface, in what area/stall of the market, sequencing and phylogenetic analysis) and whether virus been cultured (and sequenced) from environmental samples.
- Data on serological testing of meat juices from heart and diaphragm tissue (or other similar samples) of animal meats stocked in China before and around December 2019
- We would like to meet with the leaders of the animal study of the Huanan market that took place in December 2019/January 2020. We would like to discuss the rationale for the selection of the different animals from the market that were tested for SARS-CoV-2 and how was the testing done (sample collection, assay, etc.). There was an update of the PCRs in the course of January as initial assays were thought to be less sensitive. We need to look at details on the validation of the assays used.
- Data on environmental samples taken at the market or other, similar markets in Wuhan prior to Dec 2019 and if these have been retrospectively tested? It was noted that there were no samples taken, so a retrospective study is not possible.

Lower priority work to be considered for a later phase:

• From discussion with Market authorities, we require information on what animal sanitation and food safety measures were in place. We need information on whether there were live animals traded on the market, and if yes, how long were they kept at the market (broken down by species) and if health checks performed, if there is a day when the market is emptied and thoroughly cleaned, and what pest control measures are in place, as well as other important details.

C3.2: Mapping the supply chains for all relevant animals and products, including food products, sold at the Huanan market and other markets in Wuhan as informed by the results of the epidemiological studies. Supply chains could be local, national or international.

Based on current knowledge about animal susceptibility, the team will develop a list of high-risk animals traded at the market, and their supply chains, to develop an animal sampling strategy. This mapping exercise may provide additional clues about possible geographic areas suitable for future animal and

human serological surveys. Data and interviews with the key agencies, authorities and laboratories will be critical for a rigorous study, particularly on the following:

- A breakdown of the percentage of animals and products (frozen and otherwise) traded at the market during late November and December 2019 by source, i.e., local, national and international.
- Comprehensive information on cold-chain product supply chains during September to December 2019.
- Recent images of the market in operation during September to December 2019 around the location where the cases occurred.
- The proportion of live animal to dead animals sold in the market, and details on which species are usually killed at the market prior to sale, and which stalls would normally kill and butcher animals.
- Vendors involved in trading of non-aquatic animals, especially mammals, in the Huanan market will be assessed to see if their suppliers or delivery workers have links to domesticated wildlife farms or farmers that are in contact with different farmed species, farming of animals for fur (e.g. mink, raccoon dogs fox etc.) or are from farms located close to fur farms (mink, raccoon dogs, fox etc.). The first step will involve vendors dealing with domesticated wild animals. Additional studies will be guided by risk assessment and our knowledge on the susceptibility of different species.
- Learn about the source of cold-chain products on key stalls; sampling and test of cold-stored and cold-cain products linked to Huanan Market and Wuhan from September to December 2019.
- Evaluate the possibility of the introduction of the virus via cold chain or animal supply chain and search for clues of cold-chain transmission or animal supply chain in Huanan market;
- Description of an overview of production systems, statistics, and results of surveys of animals with known high susceptibility to SARS-CoV-2. I.e. mink, raccoon dogs, cats, etc.

Work that will be completed in a later phase if possible:

• Data on the movements of traders, workers and transporters and what other markets locally or regionally that they may have attended.

### C4: Retrospective studies

### C4.1: Surveillance studies in animals and food products and samples

- Animal surveillance will be guided by the results of the human epidemiological work. We will need to conduct the following work:
- Identification of livestock, domestic and wild animals of most interest.
- Can we compile a list of organizations that have conducted animal surveillance, e.g. academia/researchers; local government; central government; wildlife groups?
- Identification of what sampling and testing of the cold chain food products has been done and what more can be done.
- What baseline information is there for animal surveillance (routine, disease study; research; import/export).

- Information on sampling in mixed animal/domesticated wildlife markets and along the wild animal supply chain.
- Are there samples from surveillance programs above and could the China team have access to them for testing if necessary?
- Specific information on SARS-CoV-2 studies in animals to date (date of sampling species, number of samples, location, tests, results)
- Surveillance results (from before December 2019 and separately after December 2019) from testing in mink, raccoon dog and other farmed wildlife and susceptible animal populations in China prior to and since the emergence of SARS-CoV-2
- A list of samples collected from mammals (including *Rhinolophus* and other relevant bat genera) and other relevant animals from Hubei Province, and from other provinces in Southern China, and what testing has been done for coronaviruses, as well as further samples that are not yet tested, if available.

The following work is considered more of a longer-term effort. We will use published papers as a source of information in this phase of the work.

- A list of laboratory groups, or research teams that have conducted sampling of bats in Yunnan or Hubei province since January 2020
- Data on all universities (including agricultural, ecological and environmental), research labs, other government agency and public health groups approved to conduct wildlife sampling across China in last 10 years
- A list of data on all universities (including agricultural), research labs, other government agency and public health groups approved to conduct sampling of wildlife farms in last 10 years
- Data on isolation of viruses, uncharacterised cytopathic effects (CPE) from cell cultures, sequencing from each of the labs/public health agencies working on wildlife and animal sampling

### C4.2: Surveillance studies involving environmental sampling (avian influenza program)

This will assist in identifying presence of SARS-CoV-2 in other markets or sites at different times prior to and during the early outbreak

- Information on baseline environmental surveillance (routine, disease study; research)
- Surveillance results from environmental sampling campaigns at markets and along the supply chains of interest; both domestic and wild (from before December 2019 and separately after December 2019)
- Data on sites of environmental sampling for avian influenza, laboratories engaged in this work, and samples collected since January 2017.

### C4.3: Other studies and approaches, as appropriate.

• Access to databases of virus holdings, genetic sequences and epidemiological information on provenance of specimens for the SARS-like coronavirus research program in China (Those existing are publicly available); including confirmation of the provenance and analytical processes undertaken in the key horseshoe bat and pangolin virus samples that are currently central to the origins of SARS-CoV-2.

## D. Molecular Epidemiology and bioinformatics working group

### Output: input for the joint study report Timeline: first draft by Feb 1.

D1: Detailed reconstruction of current knowledge starting with the initial cluster of cases, starting at the early cases, and including the study related to the Huanan market cluster

Discuss, analyse and review working group specific aspects of the cross-working group study of the initial cluster.

D2: Assessment of the potential utility of other surveillance and research programmes in humans and animals for testing and genomic sequencing, including an overview of genomic surveillance programmes on respiratory and enteric diseases in humans and animals and review their biobanking and sequencing practices. In case of viral (meta) genomic sequencing up to 31 January 2020, map the availability of raw sequence data for re-analysis, relevant to gaining a full understanding of early cases

Specifically address the following:

For this work, it is important to develop an integrated database including genomic, epidemiological and clinical information. This includes cases and findings from the early study in Wuhan, supplemented with data from cases and studies outside of Wuhan, and outside of China.

- Various institutions have undertaken viral genomics in China. Can you provide an overview of how the data from these initiatives have been integrated to give an overall national approach to presenting sequencing results (from humans or animals, clinical-, public health-, research institutes), especially for sequences generated from early cases in Wuhan or elsewhere if necessary, or from environmental (e.g. cold-chain samples, sewage) or animal samples? Has the sequencing been standardized (e.g. platforms, workflows)?
- Current data have used fully curated quality controlled whole genome data, but partial genomic information may be relevant as well. Can you inventory the availability of any unpublished (whole and partial) genome sequences available from PCR-positive SARS-CoV-2 samples, especially early in the pandemic e.g. December 2019?
- In order to allow deeper analysis, it is critical to ensure a complete metadata set stratified by time of sampling, geography, interpersonal relationships, risk behaviour, occupation, relation to markets.
- This work includes integration of international genomic sequences with available epidemiological and clinical information

### D2.1: Develop a database of all available genomic data and metadata

Develop a combined list of metadata and samples linked to the early confirmed and suspected cases, and from potential cases identified through the above-described studies. Add all available genomes for all of

these and integrate additional pathogen genome information for all available biobanked samples (human-, animal-, environmental-, food samples, cell culture isolates) using standardized methods for data comparability. All potential exposure factors will be considered for the metadata set, including travel history, occupational (including healthcare workers) exposure, market exposure, animal contact, consumption of raw/exotic animal meat/products (milk, cheese), characteristics of their social contacts at the time and others.

### D2.2: Review of genomic data linked to the retrospective studies focussing on epidemiology in humans

For the list of potential suspected cases identified in working group 1 from different studies, develop a list of samples that may still be accessible for sequencing, as well as possible available non-published genomic data. For the sequencing of samples from humans, the relevant studies are:

- Hospital-based review of suspected cases
- Samples from historic ILI, SARI and pneumonia of unknown aetiology surveillance

The analysis can include sequencing of newly identified suspected cases from the retrospective studies.

# D2.3: Review of genomic data linked to the studies focussing on animals, products and environmental contamination

For the list of animals and samples produced by working group 2, review potential sources of samples and sequence data.

Retrospective studies

- Surveillance studies in animals and food products
- Surveillance studies involving environmental sampling (e.g. avian influenza programme)
- Other studies and approaches as appropriate

D2.4 retrieve international genomic data with available epidemiological and clinical information under coordination of WHO, if possible

- Collection of international genomic sequences with available epidemiological and clinical information under coordination of WHO
- Combination of all international available genomic sequences with available epidemiological

#### D3. Methodological questions to address

D3.1. Provide a re-analysis of all available initial sequences (complete or partial genomes, published or unpublished) from the early cases in Wuhan and elsewhere. Assess comparability of data. Assess the potential impact of technical issues on the result of clustering analyses.

- How comparable are the data generated in the different institutes? (e.g. were they produced on the same sequencing platforms, was the consensus calling standardized and comparable)
- What was the process for quality control of the sequence data?
- How reliable is the base calling for the initial genomic sequence data?
- Was the diversity observed in the initial genomic sequence data random or were specific mutations observed in residues that may have functional implications?
- What was the prevalence and location of minor variants in early cases?

D3.2 Provide an analysis of the resolution of genomic sequencing for source tracking, taking into account the coverage of international genomic sequencing as part of focus of the joint study. Assess possible effects of coverage gaps on conclusions regarding sources and potential solutions to improve the reliability of source tracking.

- What proportion of cases was sequenced from the initial outbreak?
- What proportion of cases in currently being sequenced from ongoing clusters? (if possible)
- What is the protocol for background sampling and is there a national reference dataset reflecting regional differences in strains from imported cases and secondary cases resulting from local transmission?

D3.3. If available, provide a review of sewage SARS-CoV-2 detection and sequencing studies (metagenomic or target-specific), and map potentially available frozen sewage samples that could be accessed to test for evidence of SARS-CoV-2 circulation prior to December 2019. This list needs to be guided by the outcomes of the back tracing of cases and animals in working groups 1 and 2. (WHO to provide relevant information)

- Has the testing of frozen sewage samples from before December 2019 been carried out? Please provide a list of locations, sample collections, and time covered.
- What protocol was used and how was that validated?
- Given the well-known issues of presence of low levels of viruses in food microbiology, what is the quality control process around frozen food testing?
- Has sewage testing been done in areas and premises where contamination of frozen food samples was detected?
- What has been the geographic focus of these studies to date? Only surrounding Huanan market, only Wuhan, or other provinces as well? How does the selection of samples relate to the market chain analysis?

### D4.5.Other studies and approaches, as appropriate.

To be defined by working group.

## E. Specific locations to visit and individuals to interview Timeline: prepare by Jan 24

# Annex A2 - Schedule of work

Global study of the origins of SARS-CoV-2 China Part 14 January – 10 February 2021

Timelines for tasks and milestones (Agenda)

14/01/2021 International team arrived in Wuhan

15/01/2021 First virtual meeting of international team and Chinese team

Timeframe	Deliverables/objectives	Tasks and activities	Outputs
15-27 January	<ol> <li>Overview of information on background, work conducted, and ongoing</li> <li>Develop workplan of the phase 1 study</li> <li>Develop the outline of the the joint study report</li> </ol>	<ul> <li>Detailed workplan for the Phase 1 studies</li> <li>Develop draft outline of the joint study report workplan for the Phase 1 studies</li> <li>1. Overview of study conducted / ongoing by three Chinese teams</li> <li>Epidemiology <ul> <li>Animal-human/environment Interface</li> <li>Bioinformatics /Molecular epidemiology</li> </ul> </li> <li>2. Experience sharing on relevant studies and work <ul> <li>The mink outbreak studies in Europe</li> <li>How to identify worldwide hotspots for emerging zoonotic diseases</li> <li>Wuhan Institute of Virology work on bats and coronaviruses</li> <li>Study of the June 2020 outbreak in the Xinfadi market</li> </ul> </li> </ul>	<ul> <li>PPTs</li> <li>Workplan document</li> </ul>

		Develop the outline of the joint study report	• Outline of the the joint study report
27 January - 7 February	Prepare input for and develop the draft joint study report	Prepare for systematic review of the data for this work-package, by customizing the generic template for assessment of data quality based on PRISMA or related criteria	• Summary of published literature
		<ul> <li>Detailed reconstruction of current knowledge starting with the initial cluster of cases, and the earliest cases; and including the srudy related to the Huanan market cluster</li> <li>1. Introduction of relevant China systems (market regulation and management, laboratory testing, agriculture, animal and wild animal surveillance and management, health system structure and operations)</li> <li>2. Overview of currently published and unpublished reports. This includes clinical, epidemiological, virological, immunological, and imaging data, and data from additional surveys including animals and environmental (including sewage) swabs.</li> <li>3. Review of raw interview data from early cases associated with Huanan market, and those with no relation to market.</li> <li>Prepare a detailed timeline (including information on available epidemiological and laboratory data) of the initial cases and events until 31 December 2019.</li> <li>Specify case definitions, laboratory methods etc. and indicate changes therein during the course of the initial studies</li> </ul>	<ul> <li>PPTs</li> <li>An inventory of all available sources of information</li> <li>Detailed timeline (including information on the available epidemiological and laboratory data) of the early cases and events</li> <li>Summary of Phase 1 studies, description of data and results of interim analyses.</li> </ul>

<ul> <li>Interviews including list of all</li> </ul>	
variables	
4. Review of the Analytical Epidemiology Study agreed during the previous China-WHO study	
Detailed review of retrospective studies,	Evaluation of
and design of additional studies based on in depth inventory of potential sources of information, related to the different hypotheses generated throughout the work	the above in light of possible pathways for disease emergence. • Workplan
<ol> <li>Review of primary data from the (ongoing) outbreak investigation(s)</li> </ol>	Suggestions for next phase.
2. Mapping the supply chains for all relevant animals and products to Huanan market	
3. Retrospective studies	
<ul> <li>Review of hospital data</li> <li>Review of respiratory disease surveillance/animal health surveillance</li> <li>Review of trends in mortality</li> <li>Serological studies</li> <li>Surveillance studies in animals and food products</li> <li>Surveillance studies involving environmental sampling (e.g. avian influenza programme)</li> <li>Other studies and approaches, as appropriate</li> </ul>	
Detailed reconstruction of current knowledge starting with the initial cluster of cases, starting at the earliest cases (before 31 December 2019), and including the study related to the Huanan market cluster	• Weekly numbers of patients > 15 years of age with fever, ILI, ARI, and/or unspecified pneumonia from 1 October to 10 December 2019

Review of primary data from the (ongoing) joint outbreak study(s):	expanding if relevant results).
Retrospective studies	and by home address (e.g.
	district),
Hospital focus based on the above	cluster analysis
records for assas compatible with	etc.
COVID-19 before December 2019	
This will focus on Wuhan include	• A line-list of all
Hubei and anywhere else if necessary.	the potential
but potentially needs to be expanded	COVID-19 cases
further, based on the preparatory	(descriptive data
analysis. Review of data from all type	already
and location of hospitals (private and	available).
public) in Wuhan and suburban	
districts (n=233 healthcare facilities).	
This work is ongoing as of 21 January	
2021.	
Focus on high-risk groups: searching	
for previously unidentified cases	
compatible with COVID-19 among	
high-risk groups such as healthcare	
workers and laboratory staff. Other	
groups of consideration include:	
veterinarians, traditional medicine	
providers, farmers/farm workers, food-	
value chain workers (market vendors,	
port workers, storage house works etc).	
In-depth reviews of routinely collected	
such data are available	
• sickness absenteeism	
<ul> <li>antibody (serology) or NAT</li> </ul>	
screening	
• other	
Focus on mild respiratory disease	
Surveillance and community cases:	
disease in the months preceding the	
outbreak to compare to baseline levels	
of similar months in previous years to	
identify any departure from expected	
trend through appropriate statistical	
analyses.	
For example, identify departure from	
trends of pneumonia of unknown	
origin, or review of syndromic	

surveillance trends for ILI and SARI, by comparing trends in the second half of 2019 to that of similar periods in previous years	
Review of trends of all-cause mortality at provincial level, and review death registers for specific causes of death compatible with COVID-19	
Serological studies based on specific studies and/or stored blood/serum samples collected in weeks and months before December 2019. Consideration should be given to high-risk groups/workers as defined in section B3.2. Additionally, the work may include targeted serological testing on stored serum samples of suspect COVID-19 patients and contacts, cases of unexplained pneumonia, and suspect deaths identified through retrospective reviews	
Specific locations to visit and individuals to interview: See annex Field visits	
Review of studies focusing on animals, products and environmental contamination	
A mapping of activities and items traded at the Huanan (and potentially other relevant) market(s) in late November and December 2019, including types of animals (captured wild, farmed wild and livestock and domestic animals) and stalls (for all types of goods) present at the Huanan market.	
Mapping the supply chains for all relevant animals and products, including food products, sold at the Huanan market and other markets in Wuhan as informed by the results of the epidemiological studies. Supply chains could be local, national or international.	

Retrospective studies	
Renospective studies	
Surveillance studies in animals and food	
products and samples	
· ·	
Surveillance studies involving	
environmental sampling (avian	
influenza programme)	
Other studies and approaches, as	
appropriate	
Assessment of the potential utility of	
other surveillance and research	
programmes in humans and animals for	
testing and genomic sequencing,	
including an overview of genomic	
surveillance programmes on respiratory	
and enteric diseases in humans and	
animals and review their blobanking and	
(meta) genomic sequencing up to	
January 31, 2020, man the availability	
of raw sequence data for re-analysis.	
relevant to gaining a full understanding	
of early cases	
Develop a database of all available	
genomic data and metadata	
- Davidor a combined list of materiate	
• Develop a combined list of metadata	
confirmed and suspected cases and	
from potential cases identified	
through the above-described studies.	
Review of genomic data linked to the	
retrospective studies focusing on	
epidemiology in humans	
Review of genomic data linked to the	
studies focusing on animals, products	
Retrieve international genomic data with	
available epidemiological and clinical	
information under coordination of	
WHO, if possible	

	Methodological questions to address Provide a re-analysis of all available initial sequences (complete or partial genomes, published or unpublished) from early cases in Wuhan and elsewhere. Assess comparability of data. Assess the potential impact of technical issues on the result of clustering analyses. Provide an analysis of the resolution of genomic sequencing for source tracking, taking into account the coverage of international genomic sequencing as part of focus of the joint study. Assess possible effects of coverage gaps on conclusions regarding sources and potential solutions to improve the reliability of source tracking.	
	If available, provide a review of sewage SARS-CoV-2 detection and sequencing studies (metagenomic or target- specific), and map potentially available frozen sewage samples that could be accessed to test for evidence of SARS- CoV-2 circulation prior to December 2019. This list needs to be guided by the outcomes of the backtracing of cases and animals in working groups 1 and 2. (WHO provides relevant information)	

8-10 February	Develop the joint study report	1.	Develop the first draft of joint study report	•	Joint study report
		2.	Review the draft report		
		3.	Finalize the joint study report		

# ANNEXB: Team members

### COVID-19 Origins Study

# Chinese team members

Team leader	
Liang Wannian	Executive Vice-President, Vanke School of Public Health, Tsinghua University
Epidemiology	
Feng Zijian*	Deputy Director-General of China CDC
Shi Guoqing	Deputy Director of Public Health Emergency Center of China CDC
Zhou Lei	Professor, Public Health Emergency Center of China CDC
Zhang Xianfeng	Director-General of Hubei Provincial CDC
Tong Yeqing	Deputy Director of Institute of Infectious Diseases of Hubei Provincial CDC
Chen Banghua	Deputy Director of Office of Health Emergency of Wuhan CDC

## Molecular epidemiology

Yang Yungui*	Deputy Director, China National Center for Bioinformation
Song Shuhui	Associate Professor, China National Center for Bioinformation
Wang Qihui	Professor, Institute of Microbiology, Chinese Academy of Sciences
Huo Xixiang	Deputy Director of Institute of Health Inspection and Testing of Huber Provincial CDC
Peng Mingwei	Technologist-in-charge, Institute of Pathogenic Biology of Wuhanv

### Animal and environment

Tong Yigang*	Director of Life Science and Technology College, Beijing University of
	Chemical Technology

William Jun Liu	Professor, Deputy director, Chinese National Influenza Center, National Institute for Viral Disease Control and Prevention, China CDC
He Hongxuan	Professor, Institute of Zoology, Chinese Academy of Sciences
Yang Guoxiang	Senior Engineer, Hubei Wildlife Epidemic Source and Disease Surveillance Center
Gao Yanhong	Deputy Director of Wuhan Zoo

(\*Subgroup lead)

# International experts, observers and WHO team members

### **Team leader**

Peter Ben Embarek	Scientist, Monitoring Nutritional Status & Food Safety Events, Nutrition and Food Safety, World Health Organization, Geneva, Switzerland
Epidemiology	
Thea K Fischer*	Director of Clinical Research, Nordsjællands University Hospital, Hillerød, Denmark
Dominic Dwyer	Director, NSWHP-Public Health Pathology State-wide Service and Director, New South Wales Health Pathology - Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, Australia
Farag Elmoubasher	Acting Head, Communicable Disease Control Programmes, Public Health Department, Ministry of Public Health, Qatar
John Watson	Adviser, Public Health England, London, United Kingdom and Northern Ireland
Marion Koopmans	Head, Department of Viroscience, Erasmus University Medical Centre, Rotterdam, The Netherlands

## Molecular epidemiology

Marion Koopmans*	Head, Department of Viroscience, Erasmus University Medical Centre, Rotterdam, The Netherlands
Fabian Leendertz	Robert Koch-Institute, Berlin, Germany
David Hayman	Co-Director, Molecular Epidemiology and Public Health Laboratory, Massey University, Palmerston North, Manawatu, New Zealand (OIE Collaborating Centre)

## Animal and environment

Peter Daszak*	President and Chief Scientist, EcoHealth Alliance, New York, United States of America
Vladimir Dedkov	Deputy Director-General for Research, Head of Epidemiology Department, Institute Pasteur, St Petersburg, Russian Federation
Ken Maeda	Director, Department of Veterinary Science, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Japan
Hung Nguyen-Viet	Co-Leader, Animal and Human Health Programme, International Livestock Research Institute (ILRI), Nairobi, Kenya

Keith Hamilton	Scientific and Technical Department, World Organisation for Animal Health (Office International des Epizooties, OIE), Paris, France
Observer	
Sophie von Dobschuetz	Animal Production and Health Division, Food and Agriculture Organization, Rome, Italy
Junxia Song	Animal Health Services, Food and Agriculture Organization, Rome, Italy
WHO Team	
Pat Drury	Deputy Incident Manager for COVID-19/Unit Head, Global Outbreak Alert and Response Network (GOARN) and Global Health Emergency Workforce, Emergency Response, World Health Organization, Geneva, Switzerland
Li Jian	Technical Officer, Emergency Operations Centre, Strategic Health Operations, Emergency Response, World Health Organization, Geneva, Switzerland
Lisa Scheuermann	Technical Officer, Human Animal Interface for IHR, Health Security Preparedness, Emergency Preparedness, World Health Organization, Geneva, Switzerland
David FitzSimons	Consultant (Rapporteur), Prévessin, France

(\*Subgroup lead)

# ANNEX C: Presentations to the joint international team in January 2021

Disclaimer: Presentations were given as part of the process of informing the joint study team on on-going and recent studies. Data presented may change as the studies progress. No permission was granted to publicize some of the presentations in the report.

Annex C1 - A Global Knowledge Hub for SARS-CoV-2: 2019nCoVR (Dr Song Shuhui)

# A Global Knowledge Hub for SARS-CoV-2: 2019nCoVR

2021-01-23

Shuhui Song


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## **Data submission**



## **Data sharing with INSDC**

#### Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/Gilgit1/human/2020/PAK, complete genome

GenBank: MT240479.1

FASTA Graphics

#### Go to: 🗹

LOCUS	MT240479 29836 bp RNA linear VRL 25-MAR-2020
DEFINITION	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/Gilgit1/human/2020/PAK, complete genome.
ACCESSION	MT240479 GWHACDD01000001
VERSION	MT240479.1
KEYWORDS	
SOURCE	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Severe acute respiratory syndrome coronavirus 2
	Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae; Orthocoronavirinae; Betacoronavirus; Sarbecovirus.
REFERENCE	1 (bases 1 to 29836)
AUTHORS	Javed,A., Niazi,S.K., Ghani,E., Saqib,M., Janjua,H.A., Corman,V.M. and Zohaib,A.
TITLE	Direct Submission
JOURNAL	Submitted (25-MAR-2020) Department of Healthcare Biotechnology, National University of Sciences and Technology (NUST), Islamabad, Islamabad 46000, Pakistan
COMMENT	This record was submitted to GenBank on behalf of the original submitter through Genome Warehouse (GWH,
	https://bigd.big.ac.cn/gwh/) of the China National Center for
	Bioinformation (CNCB)/National Genomics Data Center (NGDC.
	https://bigd.big.ac.cn).

# **Coronavirus sequences**

Nucleotide Protein 5	79,427						
Filters    Download	all available nucleotide sequences	Go to dai	ily updates				
Select all Deselect all	Download selected inetadata	Dovenload	selected sequences	Select columns			
Showing 1 to 10 of 87,708 entries	87708					Search:	
Accession Specie	5	×.	Genus	Collection Date -	Country / Region	Host =	Isolation Sour
MW485917 (NCBI) Severe	acute respiratory syndrome-related cor	onavirus	Betacoronavirus	2021-01-12	USA: California	Homo sapiens	
MW490591 (NCBI) Severe	acute respiratory syndrome-related cor	ronavirus	Betacoronavirus	2021-01-11	USA: MD	Homo sapiens	oronasopharyn
MW490588 (NCBI) Severe	acute respiratory syndrome-related cor	ronavirus	Betacoronavirus	2021-01-10	USA: MD	Homo sapiens	oronasopharyn
MW490589 (NCBI) Severe	acute respiratory syndrome-related cor	ronavirus	Betacoronavirus	2021-01-10	USA: MD	Homo sapiens	oronasopharyn
MW493699 (NCBI) Severe	acute respiratory syndrome-related cor	ronavirus	Betacoronavirus	2021-01-09	USA: New Mexico	Homo sapiens	oronasopharyn
	acute respiratory syndrome-related cor	conquira in	Retacoronauirus	2021.01.00	LICA: Mary Maying	Lieme coniene	oronoconhonin

**SARS-CoV-2** sequences



6

	1		
laccri	ntion	ot cod	liancae

	Virus Strain Name	Accession ID	Gender	Age Dat	a Source Related	Lineage	Nuc.Completeness	Sequence Length		
	Submitting Lab	Create Date	Country/Region	Province	Province Last Update Time					
Ge	nome Annotation	BLAST Download	Selected Sequences	Download All	Sequences Download	Table Download (	Current Table Select Colum	nn • Show 10 • entr		
0	Virus Strain Name	0	4	Lineage 🕢	Nuc.Completenes	s Sequence Len	gth Quality Assessmen	nt 🕜 Sample Collection I		
	SARS-CoV-2/huma	an/ITA/SARS-CoV-2_	Milan_Dec2019/2019	NA	Partial	409		2019-12-05		
0	BetaCoV/Wuhan/IF	BCAMS-WH-01/201	9	В	Complete	29899		2019-12-24		
	WH01			в	Complete	29866		2019-12-26		
	BetaCoV/Wuhan/H	BCDC-HB-01/2019		В	Complete	29848		2019-12-30		
	BetaCoV/Wuhan/IV	/DC-HB-01/2019		В	Complete	29891		2019-12-30		
0	BetaCoV/Wuhan/IV	/DC-HB-05/2019		в	Complete	29891		2019-12-30		
0	BetaCoV/Wuhan/IF	BCAMS-WH-02/201	9	в	Complete	29889		2019-12-30		
0	BetaCoV/Wuhan/IF	BCAMS-WH-03/201	9	в	Complete	29899		2019-12-30		
0	BetaCoV/Wuhan/IF	BCAMS-WH-04/201	9	В	Complete	29890		2019-12-30		
0	WIV02			в	Complete	29825		2019-12-30		
F	Raw data	Genome Information	Sample	on	Epidemiologica	l Clin	ical Information	Genome Variation		

# Sequenced raw data

Raw data	Geno Informa	me ation Ir	San	mple mation	Epidemiolo Informat	ogical ion	Clinical Info	rmation	Ge Va	enome ariation
Home / Run										
aw data of SA	RS-CoV-2									
Accession \$	Accession \$	GSA	¢	Sample Accession	BioProject \$	Platform \$	Data Source \$	Library source	e ¢	Release date
CRR152713	CRX126678	CRA003003		SAMC201979	PRJCA003106	Illumina HiSeq X Ten		TRANSCRIPTO	NIC	2021-01-01
CRR152712	CRX126677	CRA003003		SAMC201978	PRJCA003106	Illumina HiSeq X Ten		TRANSCRIPTO	NIC	2021-01-01
CRR152711	CRX126676	CRA003003		SAMC201977	PRJCA003106	Illumina HiSeq X Ten		TRANSCRIPTO	NIC	2021-01-01
CRR152710	CRX126675	CRA003003		SAMC201976	PRJCA003106	Illumina HiSeq X Ten		TRANSCRIPTO	NIC	2021-01-01
CRR152709	CRX126674	CRA003003		SAMC201975	PRJCA003106	Illumina HiSeq X Ten	-	TRANSCRIPTO	VIIC	2021-01-01
CRR152708	CRX126673	CRA003003		SAMC201974	PRJCA003106	Illumina HiSeq X Ten	-	TRANSCRIPTO	NIC	2021-01-01
CRR152707	CRX126672	CRA003003		SAMC201973	PRJCA003106	Illumina HiSeq X Ten		TRANSCRIPTO	NIC	2021-01-01
CRR152706	CRX126671	CRA003003		SAMC201972	PRJCA003106	Illumina HiSeq X Ten		TRANSCRIPTO	NIC	2021-01-01
CRR147451	CRX121346	CRA002833		SAMC195330	PRJCA002866	Illumina Nextseq		METATRANSCRIP	TOMIC	2020-06-23

## **Genome basic information**

Raw data	Genome Information	Sample Information	Epidemiological Information	Clinical Information	Genome Variation
Genome Informatio	on				
Virus Strain Name	WIV02				
Accession ID	GWHA	BKK00000001			
Host	Homo	sapiens			
Data Source	Genom	ne Warehouse			
Sample Collection Da	te 2019-1	2-30			
Location	China /	Hubei / Wuhan			
Related ID	EPI_IS	L_402127,MN996527			
Nuc Completeness	Comple	ete			
Quality Assessment	0/0/0/2	/NO			
Originating Lab	CAS K Acader	ey Laboratory of Special F ny of Sciences	Pathogens and Biosafety and Center	for Emerging Infectious Diseases, Wuhan I	nstitute of Virology, Chinese
Submitting Lab	CAS K Acader	ey Laboratory of Special F ny of Sciences	Pathogens and Biosafety and Center	for Emerging Infectious Diseases, Wuhan I	institute of Virology, Chinese
Create Date	2020-0	1-30 23:38:41			
Sequence	Down	load Genare Amolaik	BLAST		10

## **Epidemiological & Clinical information**

w data	Genome Information	Sample Information	Epidemiological Information	Clinical Information	Genome Variation						
Age		32									
Gender		M									
Occupation	1	Fresh seafood market peddlers or deliverymen									
Onset Date		2019-12-19									
Admission	date	2019-12-29									
Travel histo	ory/ Contact history	A frequent visitor to the seafood	wholesale market								
Severity of	illness	Serious									
Patient Sta	tus	Fever and intermittent cough (2020/1/13)									
Clinical syr	mptoms and latent days	Fever, cough and dyspnea									
Clinical tes	ts	Blood IgM tests,legionella pneur influenza A virus, influenza B vir	nophilia, mycoplasma pneumoniae, chla us, parainfluenza virus	imydia pneumoniae, respiratory syncytial virus, a	adenovirus, rickettsia,						
Chronic Me	edical Illness	NA									
Co-infectio pathogens	n with other respiratory	NA									
Comorbid o	conditions	NA									
Clinical me	dication and treatment	NA									
Physiologic indicators	cal and biochemical	BALF, oral swab, blood(Ab), ana	al swab, blood(PCR)								
Discharge	date	NA									
Related pu	blication	A pneumonia outbreak associate	ed with a new coronavirus of probable ba	at origin. [PMID: 32015507]							

## **Genome variation information**

Raw data	Genome Information	Sample Information	Epidemiological Information	Clinical Information	Genome Variation				
Genome	Variation Statist	ics							
Reference		2019-nC	CoV (MN908947.3)						
Virus Strai	n Name	WIV02 (	GWHABKK00000001)						
SNP No.		2	2						
Insertion N	lo.	0							
Deletion N	0.	2							
Indel No.		0							
Similarity		99.99							
Quality As	sessment Grade	А							
Annotation	Statistics	4(synon	ymous_variant:1; interg	enic_variant:2; missense_vari	iant:1)				
File Downl	oad (GFF3)	*							

## **Evaluation of completeness and quality**



Virus Strain Name 😧	Accession ID	Lineage 🕜	Nuc.Completeness	Sequence Length	Sequence Quality 🚱	Quality Assessment
hCoV-19/Denmark/DCGC-28632/2021	EPI_ISL_844885	B.1.258.11	Complete	29776	High	
hCoV-19/Denmark/DCGC-28571/2021	EPI_ISL_845141	B.1.258.11	Complete	29782	High	
hCoV-19/Denmark/DCGC-28357/2021	EPI_ISL_845397	B 1 177 12	Complete	29782	High	
hCoV-19/Colombia/BOG-INS-VG-288/2021	EPI_ISL_845653	B.1.5	Partial	29323	Low	

## Sequence search and download

Search by Metadata	Search by Genome Position Variation	Beta
Search by Metadata	Sedicit by Genotite Position variation	Contraction of the local division of the loc

Country/Region		Provin	ice			City		
	•				~			~
Host	Database			с	ollect Date (Start)		Collect Date (End)	
Homo Sapiens X			•		2019-12-01		2020-01-31	
Nuc.Completeness		Range	of Sequence Length			Lineag	e 0	
All	~	1		-	34791			•
Quality Assessment		Unknown Base(s)				Degen	erate Base(s)	
All	~	<=	Linknown taise(s): n	um	bai	<=	Dependente base(s) number	

۵	Virus Strain Name 🕢	Lineage 🔞	Nuc.Completeness	Sequence Length	Quality Assessment 🕜	Sample Collection Da
۵	SARS-CoV-2/human/ITA/SARS-CoV-2_Milan_Dec2019/2019	NA	Partial	409		2019-12-05
	BetaCoV/Wuhan/IPBCAMS-WH-01/2019	в	Complete	29899		2019-12-24
D	WH01	в	Complete	29866		2019-12-26
	BetaCoV/Wuhan/HBCDC-HB-01/2019	В	Complete	29848		2019-12-30
0	BetaCoV/Wuhan/IVDC-HB-01/2019	в	Complete	29891		2019-12-30

## Overview of global early sequences (before 31Jan 2020)



# Meta and raw sequencing data of international samples are absent !

## Variation identification

Reference: MN908947.3 Variants detection method: Genome sequences alignment using muscle

Reference: TCAGACTTACCTACGTACCTATTGCACCCAATTACTACGTACTA--GACGA | | | | | \* | | | | | | | | | | | | \*\* | | | | | \*\* | | | | \*\* | | | | |||\*||| Sequence: TCAAACTTACCTACTTACCTATTGCACCCAATT--TACGTACTACTGACGA

A new sequenced virus genome

		$\sim 40$ seconds							
Sample Collection Date	Virus Strain Name	SNP* No.	Insertion No.	Deletion No.	Indel No.	Similarity	Quality Assessment Grade 😰	Annotation Statistics 😧	Amino Acids No.
2020-08-04	hCoV- 19/Switzerland/230051_752_D05/2020 (EPI_ISL_516589)	31	0	2	O	99.89	C	33(synonymous_variant:4; intergenic_variant:2; missense_variant:9; upstream_gene_variant:1; coding_sequence_variant:17)	9
2020-01-22	SARS-CoV- 2/human/CHN/WHUHnCoV004/2020 (MT079846)	30	2	1	2	99.88	с	35(synonymous_variant:2; intergenic_variant:31; missense_variant:2)	2

## Early warning of abnormal number of variation

病毒株名 🚱	序列号	谱系 🕜	序列完整度	序列长度	质量评估 🕤 👘	宿主	采样日期 -	采样地点
hCoV-19/England/204941557/2020	EPI_ISL_754302	B.1.1.7	Complete	29826		Homo Sapiens	2020-12-28	United Kingdom / England
hCoV-19/England/205261303/2020	EPI_ISL_754255	B.1.1.7	Complete	29858		Homo Sapiens	2020-12-23	United Kingdom / England
hCoV-19/England/205260873/2020	EPI_ISL_754330	B.1.36.17	Complete	29819		Homo Sapiens	2020-12-22	United Kingdom / England
hCoV-19/England/205261305/2020	EPI_ISL_754285	B.1.1.7	Complete	29803		Homo Sapiens	2020-12-22	United Kingdom / England
hCoV-19/England/205261295/2020	EPI_ISL_754279	B.1.1.7	Complete	29849		Homo Sapiens	2020-12-22	United Kingdom / England
hCoV-19/England/205260869/2020	EPI_ISL_754246	B.1.1.7	Complete	29819		Homo Sapiens	2020-12-22	United Kingdom / England
hCoV-19/England/NOTT-121798/2020	EPI_ISL_741657	B.1.177	Partial	29611		Homo Sapiens	2020-12-21	United Kingdom / England
hCoV-19/England/205260452/2020	EPI_ISL_754380	B.1.177	Complete	Det	aatad marie	unter 41	020-12-21	United Kingdom / England
hCoV-19/England/205261290/2020	EPI_ISL_754376	B.1.177	Complete	Evn	ected varia	$\frac{1115:41}{25}$	020-12-21	United Kingdom / England
hCoV-19/England/205260453/2020	EPI_ISL_754364	B.1.177	Complete	Слр	varia	unts. 25	020-12-21	United Kingdom / England

Expected/theoretical value of variation quantity = 29904 X (0.8X10<sup>-3</sup>) X (days/365)

## Statistic and distribution of all variation



## **Comprehensive list of all variants**

ene nam	ne/Regions		Annotation Type		Mutation Type			Impact			
				•							
/irus nun	nber with variation		Genome position								
1	- 669	7	1 🔄 - 299	903 🖨							
Search	Reset										
w 5 *	entries	Download	Table						Search	c	
enome osition	Gene name/Regions	Virus number with variation	Annotation Type	Mutation Type	Base change:Virus number	Evidence Level 🔞	Protein.P	osition.Amir	no acids cl	hange	
2550	S	5	missense_variant	SNP	C->T:5	ш	QHD434	16.1:p.330P>	s		
1658	S	5	synonymous_variant; coding_sequence_variant	SNP	C->T:4; C-> Y:1	ш	QHD434	16.1:p.32F; C	HD43416	1;p.32-	
2530	S	2	missense_variant	SNP	C->T:2	ш	QHD434	16.1:p.323T>	4		
2525	S	1	synonymous_variant	SNP	A->G:1	ш	QHD434	16.1:p.321Q			
	S	1	missense_variant	SNP	A->T:1	ш	QHD434	16.1:p.321Q>	۰L		
2524											
2524											

20

## Spatiotemporal dynamics of genomic variants



https://bigd.big.ac.cn/ncov/variation/heatmap

## Spatiotemporal dynamics of genomic variants



## Spatiotemporal dynamics of genomic variants



## Haplotype network construction

## Example:

病毒1:	TCAGACTTACCTACGTACCTATTGCACCCAATTTACGTACTAGACGA
病毒2:	TCAAACTTACCTACTTACCTATTGCACCCAATTTACGTACTAGACGA
病毒3:	TCAAACTTACCTACTTACCTATTGCACCCATTTTACGTACTAGACGA
病毒4:	TCAAACTTACCTACTTACCTATTGCACCCATTTTACGTAGTAGACGA
病毒5:	TCAAACTTACCTACTTACCTATTGCACCCAATTTACCTACTAGACGA



## Dynamic haplotype network



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## Lineage browse



C	Online too	ols				
	CONTROL CON	Genome annotation	Alternative (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	1-50 00-50 10 Blast Hills or 1000 1000 1000	5 86 - 266 109 subject s 22000 2	2 23 equet Cr 77500
	11.00 21.000 21.000 M	SARS.Cov.2	Reference	Start	End	Ba
Intelligent diagnosis system			SARS-CoV-2	Position 1	1 Position	1
interingent diagnosis system	al day, "was	input Genome	SARS-CoV-2	10	10	0
of novel coronavirus		17858	SAR5-CoV-2	1	1	1
pneumonia based on			SARB-GAV.2	1	Ť	
Artificial Intelligence		0	philip philip	- 100	1.4	
B		REF: A 👄 G			m	- 41
	Variants identify	Variants identify	Variant	s ann	otate	

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## Users across the world



Unique users: >600,000; Countries/regions: 175 Data downloads: >250 million

## **Referred by other resources**



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# Ongoing improvement or suggestions

30

# What we need

- ✓ Open data sharing with GISAID
- ✓ Assistance from WHO to collect meta- and raw sequencing data of international samples

#### Annex C2 - SARS-CoV-2 in Dutch mink farms (Prof Marion Koopmans)



#### Mink farming in the Netherlands ((until 2000)

- 125 locations with on average 5000 female mink
- 4 million mink / pelts produced 2019
- "Closed" farms
- Fed on offall (poultry, fish)
- 1200 fulltime and 400 parttime employees
- Sales through auctions (DK, Fin)
- Prohibited since 2013, with final closing planned 2024

handing of mink	Period
Paring males and females	March
Gestation Whelping and nursing	March - April Half April – Half May
Vaccination kits	June
6 Suckling Whelping and nursing. Dams and kits	8 Growth









Figure 1. Schematic representation of the time-line of events in the first month of a SARS-CoV-2 outbreak on two mink farms, the Netherlands, April 2020

Figure 2. Lung from a necropsied mink, SARS-CoV-2 outbreak on two mink farms, the Netherlands, April 2020



Oreshkeva et al, 2020

#### **Current situation**

- · All farms pelted: mink farming has ended in the Netherlands
- · 69 farms diagnosed & culled since end of April
- · Most farms in South-East of the Netherlands
- 1 farm in Putten (Province Gelderland: no mink-specific cluster found







## The importance of combining data from different sources

Database human genomes SARS CoV 2, The Netherlands (Approx 4700)

> Potential local cluster



Oude Munnink et al, unpublished

### Mink farm clusters, genomic epidemiology

· 5 separate introductions from vicinity of farms BD humans to mink High proportion of people on farms infected Е · No related cases based on genome sequences from same postal code area (no community spread Human cases in vicinity of farms

Human cases in Human cases in vicinity of farms С

0.00-5

A

## Combined with animal samples

> large zoonotic cluster



Oude Munnink et al, unpublished

## Detailed investigation to track modes of transmission



- Not always same cluster for linked farms with same owners or shared personnel
- No clear links related to feed company, veterinarian, etc.
- Farms with related sequences are further investigated in tracking & tracing documents to find epidemiological links

## Observations relevant for early detection



Infection may be inapparent Throat sampling is more sensitive than stool sampling

Velkers et al, unpublished information

1 Occupational and environmental exposure to SARS-CoV-2 in and around

#### z infected mink farms

3 Authors

4

Myrna M.T. de Rooij<sup>a</sup>', Renate W. Hakae-Yan der Honing<sup>1</sup>, Marcel M. Hulst<sup>1</sup>, Frank Harders<sup>1</sup>, Marc

5 Engelsma<sup>1</sup>, Wouter van de Hoef<sup>1</sup>, Rees Meliefste<sup>1</sup>, Signid Nieuwenweg<sup>1</sup>, Bas B, Dude Munnink<sup>1</sup>,

6 Isabelia van Schothorst<sup>1</sup>, Reina S. Sikkema<sup>1</sup>, Arco N. van der Spek<sup>4</sup>, Marcel Spierenburg<sup>4</sup>, Jack

- Spithoven<sup>1</sup>, Ruth Bouwstra<sup>1</sup>, Robert-Jan Molenaar<sup>1</sup>, Marion Koopmans<sup>1</sup>, Arjan Stegeman<sup>1</sup>, Wini H.M
- 8 van der Poel<sup>2</sup>, Lidwien A.M. Smit<sup>1</sup>

High viral loads in dust (dust collection devices)



https://www.medrxiv.org/content/10.1101/2021.01.06.20248760v1.full.pdf

		Inhalable		PM 10		Total suspended particles		
Sampling- date	Measurement	Negative sample(s)	Positive sample(s) - virus concentration (copies/m <sup>3</sup> )	Negative sample(s)	Positive sample(s) - virus concentration (copies/m <sup>3</sup> )	Negative sample(s)	Positive sample(s) - virus concentration (copies/m <sup>3</sup> )	
13/05/2020	Multiple-day outdoor: spot A only	none	A: 7.1 * 10 <sup>2</sup>	A	none	Not collected	Not collected	
16/05/2020	Multiple-day outdoor: spot A only	none	A: 3.6 * 10 <sup>3</sup>	A	none	Not collected	Not collected	
19/05/2020	6-hr indoor stationary (sampling spots I, II, III, IV, V, VI)	I; II; V	III: 6.6 * 10 <sup>3</sup> IV: 9.8 * 10 <sup>3</sup> VI: 1.9 * 10 <sup>4</sup>	I; II; III; V	IV: 5.5 * 10 <sup>3</sup> VI: 2.5 * 10 <sup>3</sup>	Not collected	Not collected	
	8-hr indoor personal (person X and person Y)	none	X: 4.5 * 10 <sup>4</sup> Y: 4.3 * 10 <sup>4</sup>	None	X: 2.0 * 10 <sup>4</sup> Y: 3.0 * 10 <sup>3</sup>	Not collected	Not collected	
21/05/2020	Multiple-day outdoor: expanded (spot A + B,C,D)	none	A: 2.6 * 10 <sup>3</sup>	none	A: 1.1 * 10 <sup>2</sup>	A; C; D	B: 6.9 * 10 <sup>2</sup>	
25/05/2020	Multiple-day outdoor: expanded (spot A + B,C,D)	A; B; C; D	none	A	none	B; D	A: 4.5 * 10 <sup>2</sup> C: 3.1 * 10 <sup>2</sup>	
28/05/2020	Multiple-day outdoor: expanded (spot A + B,C,D)	A; D	none	none	A: 1.2 * 10 <sup>3</sup>	D	A: 4.8 * 10 <sup>3</sup> B: 1.7 * 10 <sup>3</sup> C: 2.7 * 10 <sup>2</sup>	

#### Table 2. Overview of SARS-CoV-2 RNA in PM<sub>10</sub> and inhalable dust samples collected at a mink farm (NB4) in a more acute phase of SARS-CoV-2 outbreak

Note. I, II, III, IV, V, VI: stationary indoor air sampling spots, see Supplemental Figure S1 for a map of the lay-out of the farm and measurement spots A, B, C, D: stationary outdoor air sampling spots, see Supplemental Figure S1 for a map of the lay-out of the farm and measurement spots X: personal air sampling of fieldworker X Y: personal air sampling of fieldworker Y

## Ongoing: assessment of the potential risk or role for Wildlife

Route	Risk to species likely to be susceptible for SARS-CoV2	-
Wild carnivores / escaped mink	<ul> <li>Fenced farmyards and catching cages on premises</li> <li>In progress: Risk assessment of possible role of wild carnivores in the spread of SARS-CoV-2 between mink farms</li> <li>Observations by zoologists on/around infected and non-infected farms for accesibility and presence of wild carnivores</li> <li>Results of first 10 visits: part of farms are accessible for marters, foxes and mink</li> <li>Collection and testing of dead Mustelids (including minks) in risk areas + feces of marters, foxes and badgers</li> <li><b>2 escaped minks PCR positive</b>, 10 wild mustelids PCR negative</li> </ul>	
Wildlife as mechanical spreaders: birds, bugs and rodents	Rodents: only small numbers of mice and rats caught after culling: • 1 Rattus rattus tested 24-8 (PCR negative). In progress: • Risk assessment of possible role of birds by omithologists (SOVON)	
Bats	In progress: Risk assessment of possible role of wild carnivores in the spread of SARS- CoV-2 between mink farms - Observations by bat experts to assess suitablility and likely presence of different bat species - Collection and testing of feces (validates for coronavirus surveillance in bats) and dead bats in risk area - Bat tracking data	



LEX 5003



# Spike 501, 453 and 452 mutation present in early clusters









Lu Lu et al.



Lu Lu et al.

# F486L in only 9 other sequences wordwide (checked on 12-11-2020, GISAID)

Sampling Date GISAID id



Seq ID



Scotland/CVR4501/2020	9/21/2020	EPI_ISL_585364	Original (Human)
USA/MI-MDHHS-SC22125/2020	10/6/2020	EPI_ISL_614176	Original (Human)
USA/MI-MDHHS-SC22140/2020	10/6/2020	EPI_ISL_614191	Original (Human)
bat/Yunnan/RaTG13/2013	7/24/2013	EPI_ISL_402131	Original
pangoun/Guange/P1E/2017	2017	EP1_IS1_410539	Original
pangolin/Guangki/P2V/2017	2017	EPI_ISL_410542	Vero E6
pangolin/Guangki/P4L/2017	2017	EPI_ISL_410538	Original
pangolin/Guangxi/P5E/2017	2017	EPI_ISL_410541	Original
pangolin/Guangxi/P5L/2017	2017	EPI_ISL_410540	Original

Animal adaptation?

Type sample

# Conclusions & ongoing research

- SARS COV 2 can spread very efficiently among mink
- Virus circulation among animals may lead to adaptive changes in the viruses, that may affect receptorbinding en recognition by antibodies
- Unravelling modes of transmission is very complex
- Combining data from multiple sources is essential to provide insight in spread



Photograph. Pasily Fertosenko/Reuse



# Annex C3 – Analysis of geographic hotspots of viral disease emergence (Dr Peter Daszak)





Allen et al. (2017) Nature Communications; Pike et al. (2014) PNAS



**Emerging Infectious Disease Events 1960-**

https://eidr.ecohealthalliance.org

## Global Emerging Disease 'Hotspots'



### Global trends in emerging infectious diseases

Kate E. Jones<sup>1</sup>, Nikkita G. Patel<sup>2</sup>, Marc A. Levy<sup>3</sup>, Adam Storeygard<sup>3</sup>†, Deborah Balk<sup>3</sup>†, John L. Gittleman<sup>4</sup> Nature 2008 & Peter Daszak<sup>2</sup>



## Global Environmental Change Drives Disease Emergence



#### Analysis of Viral Data for All Mammals

#### 2805 unique mammal-virus associations

#### 754 mammal species

374 genera, 80 families, 15 orders

#### 586 ICTV unique viruses found in mammals

- 28 viral families
- 382 RNA; 205 DNA viruses
- 263 detected in humans (44%); 75 exclusively human.
- 188 (71.5%) of human viruses are 'zoonotic'

Olivation at 2007.7-Matter-

#### **Observed Viral Richness in Mammals**



Ohval et al. 201 |, Nature



(Ilival et al. 7017, Nature

Where Are the Unknown or 'Missing' Zoonoses?



#### Distribution of SARSr-CoVs likely much wider than reported



Distribution of bats harboring SARSr-CoVs



Distribution of *Rhinolophus affinis* (host of RaTG13)



#### Risk of a bat CoV emergence is highest in SE Asia

Novel coronaviruses have emerged repeatedly in last 1,000 yrs

HCoV-NL63	HCoV-229E	HCoV-OC43	TGEV"	PHEV'	PEDV*	PRCoV*	PRRSV*	BCoV*	SARS-CoV	PDCoV*	MERS-CoV	SADS-CoV	SARS-CoV-2	17
APA .	Alph		1	1	AN	1	1	1	MAN	1		APA	Aqua	1
A	-A.	-A_	<u>_</u> _	-0		_	0	0	A	0	A		1	0
			<b>W</b>	- 🐷	- 🔘 -	- 🕑 -		-0	-U.			F 🔘 🗆	- V-	U
T	7 11	Ter	T	T	T	T	T	T	M	190	ATAN	T	1	
-1218-151	9 -1718-1818	~1898	-19405	-19506	-19705, 2010	-1993	-1993	-1999	-2003	-2009	-2012	-2017-2019	-2019-	2

### Early SARS cases, Guangdong China

Table 2. SARS cases (%	) by month of onset and occupation	onal status, Guango	dong,
Occupational status <sup>b</sup>	Jan 2003 or before no. (%)	Feb 2003 (%)	M
Retired	2 (9)	44 (10)	
Worker	2 (9)	40 (9)	
Student	0 (0)	29 (7)	
Civil servant	3 (13)	43 (10)	
Housewife	0 (0)	20 (5)	
Food industry worker	9 (39)	20 (5)	
Farmer	1 (4)	10 (2)	
Teacher	1 (4)	7 (2)	
Child	0(0)	9 (2)	
Other	2 (9)	49 (11)	
Unknown	3 (13)	157 (37)	
Total	23 (100)	428 (100)	

\*SARS, severe acute respiratory syndrome. \*Excluding healthcare workers or case-patients with known exposure.

Xu et al. EID 2004

#### DFAT - Released under FOI Act 1982



### LETTER

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

Xing, 'H Ge<sup>1a</sup>, Ho - Lu L<sup>1a</sup>, Xing, Low Yang<sup>1</sup>a, Akisse A, Chunan<sup>2</sup>, Guangian Zhu<sup>2</sup>, Jonathan H, Epoten<sup>2</sup>, Iorna K, Maset<sup>2</sup>, Ben Ha<sup>1</sup>, Wei Zhang<sup>1</sup>, Chong Peng<sup>2</sup>, Yu-B Zhang<sup>2</sup>, Chu, Ming Luo<sup>1</sup>, Hing Tan<sup>4</sup>, Ming Wang<sup>2</sup>, Yan Zhu<sup>2</sup>, Gary Cranterl<sup>4</sup>, Shu-Yi Zhang<sup>2</sup>, Lin-Fai Wang<sup>20</sup>, Peter Dosal & Elling, Li Shi<sup>1</sup>



94 Human SARS CoV Tor2



Ge et al. (2013) Nature

doi:10.1018/www.srs.12711



#### SADS-CoV: A novel bat-origin CoV causing lethal swine disease

Zhou et al. Nature 2018

Bat Species	Individuals tested	# positive	SARSr-CoV mean prev.	SARSr-CoV prev. range
Rhinolophus sinicus	1,328	113	8.5%	7.1 - 10.1%
R. macrotis	70	3	4.3%	0.9 - 12%
R. ferrumequinum	406	12	3.0%	1.5 - 5.1%
R. spp.	331	10	3.0%	1.5 - 5.5%
R. affinis	792	7	0.9%	0.4-1.8%
R. pusillus	1,023	8	0.8%	0.3 - 1.5%
Aselliscus stoliczkanus	269	2	0.7%	0.1-2.7%
Hipposideros pratti	323	2	0.6%	0.1-2.2%
H. armiger	1,188	1	0.1%	0.0-0.5%



Evolutionary origins of α- & β-CoVs from bats in China (RdRp)

## 781 sequences of novel bat-CoVs discovered in China

Size of circle proportional to the number of samples with identical viral sequences.

405  $\alpha$  – CoVs (SADSr), 361 from China






## PLOS PATHOGENS

RESEARCH ARTICLE

Discovery of a rich gene pool of bat SARSrelated coronaviruses provides new insights into the origin of SARS coronavirus

Ben Hu<sup>1+</sup>, Lei-Ping Zeng<sup>1+</sup>, Xing-Lou Yang<sup>1+</sup>, Xing-Yi Ge<sup>1</sup>, Wei Zhang<sup>1</sup>, Bei Li<sup>1</sup>, Jia-Zheng Xie<sup>1</sup>, Xu-Rui Shen<sup>1</sup>, Yun-Zhi Zhang<sup>23</sup>, Ning Wang<sup>1</sup>, Dong-Sheng Luo<sup>1</sup>, Xiao-Shuang Zheng<sup>1</sup>, Mei-Niang Wang<sup>1</sup>, Peter Deszak<sup>1</sup>, Lin-Fa Wang<sup>6</sup>, Jie Cui<sup>1+</sup>, Zheng-Li Shi<sup>1+</sup>

Hu et al. (2017) PLoS Pathogens

#### Concurrent bat-human surveillance, China



Populations highly exposed to bats in Yunnan Province, China



#### Predictors of self-reported ILI/SARI



People	seropositive	for bat-	CoVs, S.	China	2017-18

Site	# tested	Bat CoV + (%)	SARSr-CoV Rp3 + (%)	HKU10 + (%)	HKU9 + (%)	MERS-CoV+ (%)
Jinning, Yunnan	209	6 (2.87%)	6 (2.87%)	-	÷.,	+
Mengla, Yunnan	168	1 (0.6%)	1 (0.6%)		-	1
Jinghong, Yunnan	212		-		-	
Lufeng, Yunnan	144	-	2	4	1	-
Guangdong	420	8	*		-	
Guangxi	412	2 (0.48%)		2 (0.48%)		

Wang et al. 2018 Virol. Sinica

Risk factors for bat CoV seropositivity in people (n=9/1585)

	Exposu	ıre/n, (%)			
Variable	Bat CoV	Bat CoV	Odds ratio (95%	P value	Phi
	+	-	CI)		value
Livelihood in construction	2/9	39/1555	11.106 (2.235-	0.021	0.093
industry	(22.2)	(2.5)	55.191)		
Animal exposures					
Raising carnivores	1/4	12/1132	31.111 (3.016-	0.045	0.133
	(25.0)	(1.1)	320.898)		
Presence of dogs in house	5/7	255/1192	9.186 (1.772-	0.007	0.093
	(71.4)	(21.4)	47.625)		
Presence of cats in house	3/4	278/1128	9.173 (.950-	0.049	0.069
	(75.0)	(24.6)	88.539)		
Purchased live animals from	7/9	495/1562	7.544 (1.562-	0.006	0.075
market	(77.8)	(31.7)	36.448)		

High probability of hidden 'spillover' events & undiagnosed outbreaks across SE Asia prior to SARS or COVID-19



Site in SW Yunnan where up to 3% of people had antibodies to bat-coronaviruses

#### Across Southeast Asia:

>1 million people exposed to bat-coronaviruses every year



#### Collaborators

- 100+ partners in 24 countries
- · Hongying Li, Guangjian Zhu, Aleksei Chmura (EHA)
- Wuhan Inst. Virology (Zhengli Shi, Peng Zhou)
- CAS Inst. Microbiol., China CDC (G. Fu Gao)
- Yunnan CDC (Yun-Zhi Zhang)
- S. China Agric. Univ. (Jing-Yun Ma)
- Beijing Inst. Microbiol/Epidemiol. (Yi-Gang Tong)
- Duke-NUS Singapore (Linfa Wang)
- PREDICT Consortium
- Columbia Univ. (Ian Lipkin; Simon Anthony)
- UNC (Ralph Baric)

Annex C4 - Progress in tracing and monitoring of SARS-CoV-2 in domestic animals (Drs. Ni Jianqiang, Li Dong, Wang Chuanbin& Xin Shengpeng)



Progress in tracing and monitoring of SARS-Cov-2 in domestic animals

Ni Jianqiang, Li Dong,

#### Wang Chuanbin & Xin Shengpeng

China Animal Disease Control Center

## Outline



> Laboratory detection methods for SARS-CoV-2

#### > Surveillance of SARS-CoV-2 in animals

- > livestock and poultry samples
- Companion animal
- > Fur animal
- recommended detection techniques

National Surveillance program of animal disease

## Laboratory detection methods for SARS-CoV-2 detection in animal samples

**X** Serum samples from animal were used for detection SARS-CoV-2 specific antibodies using double antigen sandwich ELISA.

*Verified by standard positive sera prepared by Harbin Veterinary Research Institute* 

The micro-neutralization test was used as the confirmatory method.

The method was used in some countries, e.g. Italy, USA, etc

**X RT-PCR was used to detect the pathogen** 

Surveillance of SARS-CoV-2 in samples from livestock and poultry:

A brief review

#### Livestock and poultry Surveillance of SARS-CoV-2 nucleic acid in livestock and poultry samples, 2018-2019



From: HLJ, LN, TJ, HeB, FJ, AH, SD, HeN, HuN, GX, GD, YN, SC, SN, XJ, GZ, JL, JS, NM, SH, ZJ, HuB, JX, NX, QH and XZ

## **Monitoring of common coronaviruses** in animals (2019-2020)

RT-PCR was used to monitor 25655 animal samples and non Avian infectious bronchitis (1095) SARS-CoV-2 positive samples were detected. duck coronavirus (167)



- Pigeon coronavirus (501)
- Avain delta coronavirus (25)
- Porcine Epidemic Diarrhea (151)
- Transmissible gastroenteritis of swine
- Porcine hemagglutinating encephalomyelitis (6)
- Porcine delta coronavirus (1)
- Bovine coronavirus (74)
- mink coronavirus (14)
- feline coronavirus (74)
- canine coronavirus (18)

1711 positive samples for common coronaviruses were detected.

## Monitoring of common coronaviruses in animals (2019-2020)

- > The homology between common coronaviruses and SARS-Cov-2 was less than 54.2%.
- The possibility of source of SARS-CoV-2 from domestic animals (e.g. pig, chicken, cattle, goat) and pets (e.g. cat & dog) was ruled out.

Animal	Num. of samples	Num. of Positives	Num. Provinces	Province	Num. of different coronviruses
Avian	17912	1337	19	HLJ, SH, LN, TJ, HeB, FJ, AH, SD, HeN, HuN, <b>HuB</b> , GX, GD, YN, SC, SN, XJ, JS, JX, NX, XZ	IBV:1095; Duck coronavirus:167; Pigeon coronavirus:50; Avian delta coronavirus 25.
Swine	5664	194	22	HLJ, LN, TJ, SH, ZJ, HeB, FJ, AH, SD, HeN, HuN, GX, GD, YN, SC, SN, XJ, JS, JX, NX, XZ, NM	PEDV: 151; TGEV:36; Porcine hemagglutinating encephalomyelitis:6; Porcine Delta coronavirus 1
Bovine and Goat	1517	74	10	NX, SX, HeN, HeB, NM, JX, HLJ, GZ, HuN, JL	Bovine coronavirus 74;
Mink	67	14	1	SD	Mink coronavirus 14
Cat and Dog	1647	92	4	SD, JS, HeB, ZJ	Feline coronavirus 74; Canine coronavirus 18.

## Serological survey of SARS-CoV-2 antibodies in livestock and poultry samples, 2019-2020





# Surveillance of SARS-CoV-2 in Companion animal:

A brief review

## Detection of SARS-CoV-2 in dogs and cats (2019-2020)

#### Sampling before the outbreak:

**246** samples collected from dogs and cats in Shandong and Jiangsu Provinces were negative for SARS-CoV-2 (performed by CAHAEC)

Sampling after the outbreak:

**40** oropharyngeal swabs collected from cats and dogs in Beijing were negative for SARS-CoV-2.

## Serological survey of SARS-CoV-2 in 35 different animals species

**1914** serum samples collected from **35** animal species were used for detection of SARS-CoV-2 specific antibody using double antigen sandwich ELISA, and the result indicated that no sample was detected to be positive for SARS-CoV-2 specific antibody.

- Collected during the period of November 2019 to March 2020
- 487 serum sample from dogs, including 90 beagle dogs, 147 pet dogs and 250 street dogs. Among which, serum samples from 15 pet dogs and 99 street dogs were collected from Wuhan City.
  - One sample collected from pet dog came from confirmed SARS-CoV-2 infected patient, and other two samples from dogs that had close contact with this dog
- 87 sample from cats, including 66 pet cats and 21 street cats.

#### No SARS-CoV-2 specific antibodies were detected.

Junhua Deng,Yipeng Jin,Yuxiu Liu,Jie Sun,LiyingHao,JingjingBai,TianHuang,D egui Lin, Yaping Jin,KegongTian. Serological survey of SARS-CoV-2 for experimental, domestic, companion and wild animals excludes intermediate hosts of 35 different species of animals. *Transboundaryand Emerging Diseases*, 2020, 67(4):1745-1749.

序号	animal	物种	检测数量	检测结果(ELISA 读值范围)	判定
1		pig	187	0.005-0.134	negative
2		cow	107	0.002-0.18	negative
3	1	sheep	133	0.002-0.169	negative
4	Livestock	horse	18	0.002-0.189	negative
5	and poultry	chicken	153	0.005-0.134	negative
6		duck	153	0.004-0.189	negative
7		goose	25	0.005-0.121	negative
8	Companion	dog	487	0.004-0.198	negative
9	animals	cat	87	0.005-0.045	negative
10		mice	81	0.004-0.19	negative
11		rat	67	0.004-0.095	negative
12	7	rhesus	30	0.005-0.031	negative
13		rabbit	34	0.005-0.029	negative
14		monkey	39	0.001-0.141	negative
15		camel	31	0.005-0.178	negative
16		fox	89	0.005-0.197	negative
17		mink	91	0.001-0.195	negative
18		alpaca	10	0.004-0.02	negative
19		ferret	2	0.036-0.038	negative
20		bamboo rat	8	0.005-0.008	negative
21	-	peacock	4	0.006-0.009	negative
22		eagle	1	0.006-0.006	negative
23	Other	tiger	8	0.004-0.077	negative
24	animals	rhinoceros	4	0.005-0.006	negative
25		pangolin	17	0.004-0.156	negative
26		Leopard cat	3	0.005-0.007	negative
27		jackal	1	0.01-0.01	negative
28		Giant panda	14	0.005-0.05	negative
29		Masked civet	10	0.004-0.014	negative
30		bear	9	0.005-0.006	negative
31		Yellow-throated marten	2	0.005-0.095	negative
32		weasel	4	0.006-0.006	negative
33	1	Red pandas	3	0.005-0.005	negative
34	1	Wild boar	1	0.005-0.005	negative
35		porcupine	2	0.007-0.007	negative

## Surveillance of SARS-CoV-2 in Fur animals:

## A brief review

## Surveillance of SARS-CoV-2 in Fur Animals

The samples, collected from minks in the main fur animal producing areas of China during the period of November 2019 to July 2020, were detected, and the results indicated that all samples were negative for SARS-CoV-2, demonstrating that no Chinese mink was infected with the virus before and after the COVID-19 epidemic.

species of animals	Types of samples	Collected sites	Total	methods	Results	Completed by
minks	swabs	Shandong	67	real-time RT-PCR	negative	China animal health and epidemiology center
minks	visceral tissue, throat swabs	Shandong,Jili n , Liaoning , Heilongjiang	328	real-time RT-PCR	negative	Institute of Special Animal and Plant Sciences , Chinese Academy of Agricultural Sciences
	serum		35	ELISA	negative	
minks	serum	unknown	91	ELISA	negative	College of veterinary medicine, Northwest A&F University,



#### Surveillance of SARS-CoV-2 in fox after the COVID-19 outbreak

### Sampling and real-time RT-PCR detection after

#### the outbreak

ALL samples below were negative for SARS-CoV-2:

- The SARS-CoV-2 nucleic acid of 2 white fox tissue samples in Wuhan city were negative.
- The SARS-Cov-2 nucleic acid of 7 fox tissue samples in Wuhan city were negative.

## The National Animal Disease Surveillance

## System

A brief review

#### Animal Disease Surveillance and Epidemiology Survey Plan

The Animal Husbandry and Veterinary Bureau, MARA, China, is in charge of national animal disease surveillance and epidemiology survey and is responsible for making the surveillance plan, as well as releasing the results of surveillance.

- CADC, IVDC and CAHEC are national institutions working on animal disease surveillance.
- National Ref. Labs, professional labs and animal disease diagnosis related institutions are main supporting units.
  - > HVRI, SHVRI....
- Provincial husbandry and veterinary agencies make local animal disease surveillance and survey plan, based on their local situations, including breeding, natural environment, animal disease epidemiology.
- Surveillance data submitted to animal disease control and animal health supervision platform



#### Annual surveillance plan

MARA makes surveillance and epidemiology survey plan annually for major animal diseases. In addition, CADC organizes annual surveillance on main animal diseases in Spring and Autumn.

- Diseases: AHS, FMD, HPAI, Brucellosis, PPR, Glanders, EIA, PRRS, CSF, ND, Schistosomiasis, Echinococcosis, BTB, Rabies
- Species: pig, cattle, goat/sheep, chicken, duck, goose, water fowl, etc
- Animal disease control and prevention strategies are made annually based on these above

## Reporting and investigation of emergent animal disease outbreak



#### Animal Disease Situation of Hubei Province, 2017-2020

During the year of 2017 to 2020, about 50 kinds of animal disease were reported in domestic animals, including pig, cattle, sheep and poultries, etc. The total incidence rate ranged from 2.47% to 12.45% and the total mortality rate ranged from 0.75% to 4.30%. Overall, the epidemic situation was steady and no zoonotic prevalence occurred.

Time	Type of Disease	Num of country	Sampling time	Total infected premise	Total num of incidence	Total morbidity	Total dead animals	Total mortality
2017	58	78	1375	1373	273023	2.47	82869	0.75
2018	55	83	1433	1432	238365	9.05	74658	2.83
2019	50	79	951	950	175240	12.45	60560	4.30
2020	57	90	1180	1180	155225	7.80	45651	2.28

The main diseases occurred include porcine epidemic diarrhea (PED), infectious gastroenteritis disease, swine streptococcal disease, Eperythrogoonosis, pullorum disease (PD), avian coccidiosis, fowl cholera, E.coli infection.

#### **National Surveillance of Avian Influenza Virus**

The National AIV Surveillance Project is Conducted by the OIE/National Ref. Lab for Avian Influenza, cooperating with provincial labs. From 2017 to 2020, samples were collected from LBMs, farms and slaughter houses in 20 provinces/cities, and a total of 8 times of surveillance had been performed.

Year	Market	Farm	Slaughter house	Sample total	Province	Num of virus isolate
2017	208 (3)	19	3 (3)	1889	GX, YN, SC, GD, CQ, JS, HuN, HuB, HeN, AH, FJ, JX, SD, NX, ShaX, GZ, HuB, XJ	AIV: 202 NDV: 90
2018	54 (4)	2 (1)	9 (2)	815	JS, FJ, ZJ, GZ, GX, SD, HeN, HuN, HuB, HeB, QH, SC, XJ	AIV: 61 NDV: 21
2019	16	0	1	287	GX, HuN, GZ< HeB, JS, CQ	AIV: 29 NDV: 12
2020	20 (4)	3	2	301	HuB, XJ, ZJ, QH, GX, JS, HuN	AIV: 12 NDV: 3

Note: 1. the numbers in bracket indicates the total numbers in Hubei (no sampling HuB in 2019 and no sampling in Wuhan)

2. Samples were collected in HuB in April of 2020 include those from Dafangping Market and Minda LBM of Yichang, Market in Huangshantou County, Boyuan Wholesale Market in Jingzhou.

## Surveillance of Avain Influenza Hubei Province, 2017-2020

A total of 31,000 sites and 1365000 samples were detected. Samples were collected from breeding farms, large-scale chicken farms, households, trading market, slaughter houses, etc.

Subtype of AIV	Sample sites	Num. of samples	Num. of Positive	Market	farms	individual positive rate	group positive rate
H5	3438	8.6万	95	14	1	0.1%	0.4%
Н7	7272	10.7	40	10	12	0.04%	0.35%

**%**All samples detected before 2020 have been treated harmless.

## Surveillance of Avain Influenza Wuhan City, 2017-2020

Breeding process: A total of 3776 sites including 116836 serum samples and 11408 pathogen samples were detected.

No positive samples have been detected since 2017.

Trading process: A total of 343 sites including 630 serum samples and 3069 pathogen samples were detected.

No positive samples have been detected since 2017.

H5 and H7 : The qualified rate of immune antibody was over 95%.



Annex C5 – Basic Information on the Hubei Provincial CDC laboratory (Dr Huo Xixiang)

## Basic Information of Lab of Hubei Provincial CDC

## Outlines

Main activities

□ COVID-19 testing

## **Main activities**

- To provide etiology evidences for infectious disease prevention and control;
- To provide evidences for health hazard risk monitoring and evaluation;
- To provide etiology evidences for public health emergency responses.

## **Main activities**

- Microbiological tests: bacteria, viruses
- Physical and chemical tests public places, drinking water, workplaces, food



## Lab management

## □ Quality control

Certified by China Lab Accreditation Committee;

Passed National Metrology Certification.



## Lab management

- □ BSL-1, BSL-2: management by record
- □ BSL-3: Mandatory management



## Lab management-BSL-3

- equipment functions: meeting the requirements
- protective equipment: sufficient and of reliable quality
- □ staff: qualified
- health monitoring: access registration, health monitoring, vaccinated, serum samples reserved, antibody tested

## Microorganism testingbacteria

- bacterial strain collection
- verification & identification
- □ serotyping
- D PFGE
- drug sensitivity test





## **Microorganism testing-viruses**

- antigen and antibody tests
- viral isolation and culture
- variation of prevalent strains
- genetic variation analysis



## Flu surveillance in Hubei Province

- □ Sentinel hospitals (23) –ILI registry, sampling
- □ Network labs (17) PCR, viral isolation
- Provincial reference lab virus verification antigenicity analysis genetic sequencing



## **COVID-19 testing - requirements**

- Hazard classification: Class 2 pathogen management
- Active lab classification: viral culture-BSL-3 lab

operation involving infective materials: BSL-2 lab +PPE

1.1		清潔之行-	2.47-		实现	全活动所得	產業物度企	主要检查的	及例。"	运输包	築分乗り	1.0
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	Secon option originalizations	87498 9 <b>8</b>	<b>机组织是</b> 和 ·	8-S	855.)	ABALS	016-11-	pairs.	816.0	A.	wan	9.9 MA
e l	Attaction	11.用社会年		#*#	BSL+	ABIL	83L01	PHO:	810.1	.81	(IDAH)	17

## **COVID-19 testing - requirements**

Criteria for confirmed cases (diagnosis protocol)

□ Jan 2020: nuclear acid test

sequencing

viral culture

□ Mar 2020: IgM+IgG: positive

IgG titer:  $\geq$  4 times higher/positive



## **COVID-19 testing**





#### **COVID-19 testing**

> Field sampling: surfaces, air, etc



### **COVID-19 testing**

#### Nuclear acid test (NAT)

screenings of suspect cases, close contacter monitoring of environmental contaminat

#### Viral isolation and identification

isolation and culture of NAT positive samples

fund witch

## **COVID-19 testing**

#### Genetic sequencing

nuclear acid + samples, viral strains

To understand viral mutation and share sequencing results

Antibody test: colloidal gold

method





## Thank you!



Annex C6 - Surveillance of SARS-CoV-2 in wild animals (Dr He Hongxuan)

## Surveillance of SARS-CoV-2 in Wild Animals

HongXuan He

The development history of the national terrestrial wildlife epidemic disease surveillance system



#### System construction



#### **Monitoring content**

#### **Order of State Forestry Administration**

#### No. 31

«Administrative Measures for Monitoring and Control of Epidemics and Diseases of Terrestrial Wild Animals», which were considered and adopted at the executive meeting of the State Forestry Administration on December 25, 2012, are hereby promulgated and shall come into force as of April 1, 2013.

#### 2013.1.22

There are 28 articles in the Measures, which make specific provisions on strengthening the monitoring, prevention and control of terrestrial wild animal epidemic diseases and preventing the spread and spread of terrestrial wild animal diseases.

#### National active surveillance plan

Important Wildlife-borne Diseases in China

Important diseases	Attribute	Key epidemic source species	Key area 🖬	
HPAI	Zoonoscs	More than 100 kinds of migratory birds and resident birds such as the spotted goose, swan, wild goose and duck	Nationwide, lakes, rivers, wetlands, beaches and other wild bird domestication and breeding sites	
West Nile fever	Latent foreign disease	More than 200 resident and migratory birds mainly of the family Corvidae Culex pipiens as vector	Eastern and western migration routes, focusing on Xinjiang, China's border areas with Pakistan, Tajikistan, Kyrgyzstan and Kazakhstan	
Rabies	Zoonoses	Carnivorous animals such as wolves, jackals, foxes, and ferrets and bat of the order Chiroptera	Nationwide, with a focus on the northwest, southwest and south china	
Brucellosis	Zoonoses	Domesticating and breeding cloven-hoofed animals	The domestication and breeding area (field) of the focal species	
Tuberculosis	Zoonoses	Domesticating and breeding cloven-hoofed and primates		
African swine fever	Latent foreign disease	Eurasian wild boar	Border areas of Xinjiang, Inner Mongolia, and Heilongjiang	
Marmot Plague	Zoonoses	Mainly Himalayan marmot, wild animals including grey marmot, long-tailed marmot, Siberian marmot and otter beasts	Marmot distribution areas in Xinjiang, Qinghai, Gansu, Tibet, Sichuan and Inner Mongolia	
Sheep infectious pleuropneumonia	Endangered species suffering from disease	Tibetan antelope, Tibetan antelope, rock sheep and other small cloven ungulates	Epidemic areas in Tibet, Qinghai, Xinjiang, Sichuan, etc.	



12th Asia-Pacific Conference on Wildlife-Borne Diseases

> Developing surveillance system and joint consortium to promote and address emerging disease issues in wildlife populations

> Identifying risks and developing management approaches to mitigate disease impacts, promote global awareness of threats posed by emerging wildlife diseases

Developing activities to enhance regional countries capacity to respond to emerging wildlife diseases that threaten agriculture or have zoonotic implications.



#### Motivating MOU about wildlife diseases between CAS and USDA (May,2010)



#### **Asia-Pacific Wildlife Diseases Network**



亚太野生动物疫病合作网络 (Asia-Pacific Wildlife Diseases Network , APWDN)

The network basically includes leading units such as the Bureau of Life Sciences and Biotechnology of the Chinese Academy of Sciences, the Bureau of International Cooperation of the Chinese Academy of Sciences, the National Wildlife Disease Monitoring Station of China, and the Chinese Academy of Inspection and Quarantine Sciences, as well as from the International Association of Wildlife Diseases, the International Zoological Society, and the United States. International organizations such as the National Wildlife Disease Research Center and countries or international organizations in the Asia-Pacific region such as the United States, Russia, Canada, Mongolia, Japan, and South Korea.

 A total of 161 scientists from more than 30 countries and representatives of 5 international organizations have signed network cooperation agreements.



## **Overview of Wild Animals in Hubei Province**

There are **893 species** of wild vertebrates in Hubei Province, including 639 species of terrestrial species (121 species of mammals, 456 species of birds, and 62 species of reptiles), 48 species of amphibians, and 206 species of aquatic species, accounting for approximately 19% of the national total.

species	animal species	distribution
Primates	rhesus monkeys, Tibetan chief monkeys, and golden monkeys	Shennongjia, Enshi, Shiyan, Yichang, Xiangyang, Huanggang and other places. Tibetan chief monkeys and golden monkeys are mainly distributed in Shennongjia and Enshi;
Scales	Chinese pangolin	historically distributed in Xianning, Huangshi, Huanggang, Enshi and other places, but it has not been found in field surveys in recent years;
Carnivore	jackal, black bear, yellow-throated mink, otter, big civet, little civet, golden cat, clouded leopard, leopard, etc.,	Shennongjia, Shiyan, Enshi and other places;
Artiodactyls	Anhui musk deer, forest musk deer, roe, sika deer, elk, Chinese goral, Chinese hyena, etc.	Huanggang, Enshi, Shennongjia, Shiyan;
Pterodactyls	bat	most areas, but because they are not protected animals, no relevant investigations have been organized.



#### Statistical table of artificial breeding wild animals

### Surveillance of SARS-CoV-2 in wild animals in Hubei Province before the outbreak

- On December 7, 2019, coordinated and cooperated with the scientific research team, we collected 69 kinds of animal samples including macaques, forest musk deer, tigers, camels, bamboo rats, porcupines, muntjac, and guinea pigs in tourist areas, zoos and artificial breeding places in Hubei Province.
- A total of 2328 samples of blood, feces and blood.
- · The results showed they were all negative for SARS-CoV-2 .

### Surveillance of SARS-CoV-2 in wild animals in Hubei Province after the epidemic

- From February 8 to March 11, 2020, coordinated and cooperated with the scientific research team, we collected red pandas, white foxes, badgers, civet cats, bamboo rats, porcupines, guinea pigs, macaques, etc. in Wuhan, Dazhi, Yangxin, Jingmen, Jiangling and other places There are 648 samples of tissues, swabs, blood, and feces in nine kinds of 90 animals. The SARS-CoV-2 test results all showed negative.
- After April 8, 2020, we sampled 37 species of wild animals in close contact with humans, including civets, bamboo rats, porcupines, guinea pigs, and macaques, from 31 wild animal farms in 14 cities and prefectures. A total of 2,995 samples were collected and tested., The SARS-CoV-2 test results were all negative.

#### National wild animal SARS-CoV-2 retrospective detection

- Detection method: Refer to the "New Coronavirus Pneumonia Laboratory Testing Technical Guidelines" issued by the National Health Commission, the new coronavirus is detected by RT-qPCR, and the coronavirus universal primers are used for RT-PCR detection.
- Sample source: 6811 animal samples were tested for the new coronavirus, including 4,025 wild animal samples and 2,786 poultry samples. These samples were collected from my country's Beijing, Shanghai, Jiangxi and Xinjiang regions in 2015-2019, involving species such as Primates, Carnivora, Artiodactyla, Anseriformes, and Catoroides.
- Test result: No SARS-CoV-2 positive was found, only a small amount of infectious bronchitis-like virus nucleic acid positive and ferret coronavirus nucleic acid positive were detected.

## Detection after the SARS-CoV-2 epidemic in wild animals nationwide

- Pathogenic testing: 27,000 wild animal samples were tested for the new coronavirus. These samples were collected from Beijing, Shanghai, Jiangxi, Yunnan, Guangxi, Xinjiang, etc. after the epidemic, involving primates, lagomorphs, scaly, carnivora, etc. Swabs and lung tissue samples of odd-hoofed, artiodactyl, pterodactyl, rodents, etc. Using qPCR and metagenomic inspection. The test results were all negative.
- Serological testing: collected camels, civets, porcupines, alpacas, ferrets, bamboo rats, peacocks, eagles, tigers, rhinos, pangolins, ocelots, jackals, giant pandas, foxes, bears, weasels, wild boars, red pandas, A total of 1914 serum samples from 35 animals including yellow-throated mink were tested by ELISA, and the test results were all negative.

#### **Research on wild bats in Hubei Province**

- Species and distribution: Mainly include chrysanthemum head bat, hoof bat, myotis bat, long-wing bat, low-wing bat, etc. Among them, Fuyi is a house-dwelling bat, widely distributed in towns and villages. Chrysanthemum-head bats, hoofed bats, and myotis bats are cave-dwelling bats, which are distributed in caves in Xianning, Shiyan, Yichang, Enshi, Shennongjia and other places.
- Seasonal dynamics: Hubei Province belongs to a temperate zone. The size of the same cave bat population varies with the seasons. It gradually increases from April to July and August, then gradually decreases, and enters hibernation at the end of October
- Species characteristics: The bats distributed in Hubei Province are small insectivorous bats, lacking the long-distance migration ability of fruit-eating bats in tropical areas, and the general migration range of the population is within 30-50km.
- Monitoring results: SARS-like coronavirus, hoofed bat β coronavirus ZJ2013, myotis coronavirus HKU6, bat coronavirus HKU5 and other coronaviruses.

## Abnormal situation of wild animals in Hubei Province



#### **Reasons:**

- SARS-CoV-2 test negative;
- · African swine fever;
- The impact of the epidemic has caused food shortages.

月份年度	1月	2月	3月	4月	5月	6月	7月	8月	9月	10月	11月	12月
2020	9	46	70	8	2	2	0	0	1	0	11	3
2019	0	1	1	1	1	0	2	1	1	0	1	0
2018	1	1	0	0	0	0	0	0	0	3	0	3
2017	1	1	2	0	0	0	0	1	0	0	0	1
2016	0	0	2	3	1	0	1	0	0	0	0	1

#### **Research on wild bats in Hubei Province**

- The bat SARS-like coronaviruses and new coronaviruses found in Hubei Province are very different and belong to different evolutionary branches; several viruses (Rf1, Rm1, etc.) that have been sequenced are less than 80% similar to the new coronavirus genome sequence; in Hubei No coronavirus related to the new coronavirus has been found in bats in the province.
- The bat SARS-like coronavirus S protein found in Hubei Province has two deletions and cannot use the ACE2 receptor, and the risk of cross-species infection is low.

## 2016-2020 Abnormal situation of wild animals in Hubei Province (specific)

Time	Wild animal abnormalities (specific)					
2020	152 cases of wild animal abnormalities occurred, 3 cases were caused by epidemics, and 9 wild animal abnormalities, a total of 1337 wild animals of 35 species died					
	bird	mammalia	reptiles			
	201 of 18 species	726 of 16 species	140 of 1 species			
2019	9 cases of wild animal abnormalities, a total of 27 wild animals of 5 species died					
	bird	mammalia				
	11 of 2 species	16 c	of 3 species			
2018	8 cases of wild animal abnormalities, a total of 1,000 wild animals of 3 species died					
	bird	m	ammalia			
	996 of 2 species	4 o	f 1 species			
2017	6 cases of wild animal abnormalities, 2 cases caused by diseases, and a total of 155 wild animals of 4 species died					
2016	8 cases of wild animal abnormalities, 5 caused by epidemics, and 150 wild animals of 14 species died.					

## 2016-2020 abnormal wildlife situation in six provinces surrounding Hubei

Time	Wild animal abnormalities (specific)					
2020	55 cases of wild animal abnormalities occurred, a total of 1331 wild animals of 31 species died					
	bird	mammalia				
	1260 of 17 species	70 of 0species				
2019	7 wild animal abnormalities, 1 outbreaks, a total of 21 wild animals of 5 species died					
	bird	mammalia				
	10 of 3 species	11 of 2 species				
2018	13 cases of wild animal abnormalities, 2 outbreaks, a total of 1720 birds of 4 species died					
2017	14 cases of wild animal abnormalities, 1 outbreaks, a total of 405 birds of 6 species died					
2016	5 cases of wild animal abnormalities, 1 outbreaks, 831 wild animals of 7 species died. Among them, 830 of 6 species of birds and 1 of mammals died.					


#### Species affected Member Date of first report Hong Kong Dog and cat 21/03/2020 Cat Feline (tiger, lion, cat), Belgium 28/03/2020 06/04/2020 USA dog, mink 26/04/2020 Netherlands Mink France Cat 02/05/2020 Mink 25/11/2020 Spain Cat 11/05/2020 Mink 16/07/2020 13/05/2020 Germany Cat and dog 26/05/2020 Russia Cat Mink Denmark 17/06/2020 United Kingdom Cat 28/07/2020 Japan Dog 07/08/2020 06/11/2020 Cat South Africa 11/08/2020 Puma 30/10/2020 Italy Mink Ca 09/12/2020 Sweden Mink 29/10/2020 Chile 22/10/2020 Cat 28/10/2020 Canada Dog Mink 09/12/2020 Brazil Cat 29/10/2020 Mink 16/11/2020 Greece Argentina Cat and dog 18/11/2020 Lithuania Mink 30/11/2020 Switzerland USA 03/12/2020 11/01/2021 Cat gorilla

SOURCE:www.oie.int

### Laboratory infection test

### Susceptible animal:

1. **Domestic animals**: Ferrets, minks, rabbits and raccoon dogs are susceptible, but pigs, cattle and poultry are not.

2.Companion animals: cats and dogs are susceptible;

3. Wildlife: Deer mice and fruit bats are susceptible.

		species	Susceptibility	symptoms	Transmission capacity
1	the second	ferret	high	a few	Horizontal propagation
	domestic animals	rabbit	high	no	no
		raccoon dog	high	no	Horizontal propagation
		cattle	low	no	no
		pig	low	no	no
		Poultry (chicken, duck, Turkey)	no	no	no
	companion animal	cat	hĩgh	a few	Horizontal propagation
		dog	low	no	no
	wildlife animal	Pangolin (Malay)	high	no	Horizontal propagation
		Fruit bats	high	no	Horizontal propagation
		deer mice	high	no	Horizontal propagation
	Other animal	golden hamsters	high	vary in severity	Horizontal propagation
		Monkeys	high	vary in severity	Horizontal propagation
		Marmoset	high	no	Horizontal propagation
		treeshrew	high	no	unknown

#### SARS-CoV-2 infection animal case report around the world

## **Preliminary Conclusions**

- Wild animal shops in the Huanan Market account for a small portion (10/678);
- The commodities in the Huanan Market are all from artificially bred wild animals and are legal;
- There are no cases of infection in personnel related to wildlife product shops;
- The wild animals test results were all negative;
- National wildlife disease surveillance system has not found abnormalities.

The current results don't indicate that the SARS-CoV-2 comes from any of the above mentioned wild animals.

### Wildlife and SARS-CoV-2

#### To trace the source

To restore its process, to identify exactly where the virus came from, where it went, and what kind of transmission and development trajectory it has gone through, so as to prevent the recurrence of similar outbreaks;

#### Continued monitoring of wildlife

The mobility of wild animals is relatively large, and continuous monitoring should be strengthened;

#### Predict scientifically

Scientific prediction is to prevent SARS-COV-2 from being introduced into wild animals and becoming a natural infectious disease.

Annex C7 - The infection risk in cats, dogs and pigs to SARS-CoV-2 (Dr Jin Meilin)



### -、华中农业大学基本情况

学校前身是张之洞1898年创办的湖北农务学堂 1952年,成立华中农学院 1985年,更名为华中农业大学







5	生物学、园艺学、畜牧学、兽医学、农林经济管理5个学科入选世界 一流建设学科,入选学科数并列全国第21位,在一流学科建设高校 中列第2位
7	全国第四轮学科评估(2017年)4个门类7个学科获评A类,A类 学科综合比较位列全国高校第19位
9	植物学与动物学、农业科学、化学、生物学与生物化学、分子生物学与遗传学、微生物学、环境科学与生态学、工程学、药理学与毒理学等9个学科领域进入ESI全球前1%,分布于农学、生命科学、理学、工学、医学等5个门类,实现农学、生命科学2个门类的全覆盖,全国并列27
2	植物学与动物学、农业科学等2个学科领域进入ESI全球前1‰, 实现农学门类的全覆盖,全国并列15

### 二、猫、犬、猪等动物感染风险调查

- (一) 猫流行病学监测
- (二) 犬血清学动态监测
- (三) 猪场疫情影响的排查
- (四) 驯养野生动物排查





建立相关监测方法

### 建立适用于猫、犬等动物的新型冠状病毒ELISA抗体检测技术



✓利用真核表达纯化的RBD蛋白作为包被抗原;分别建立适用于猪、猫和犬的 ELISA抗体检测方法。



✓ 动物样本复杂,且动物冠状病毒具多样性,基于生物信息学分析,建立了更适用于 动物的荧光PCR检测技术,提高检测敏感性,检测体系引入内参基因,排除假阴性。

### 建立了适于哺乳动物的荧光测流免疫层析抗体检测技术



▶开发了基于量子点纳米球的荧光测流免疫层析检测技术,用于检测人与哺乳动物 新冠病毒抗体,检测灵敏度相对胶体金试纸条提高了100倍,适合现场快速检测。



### 1. 新冠病毒在猫体的血清学调查

▶疫情流行1-3月间,收集猫血清102份,采用RBD-ELISA抗体检测技术检测出15 只猫血清呈阳性(14.7%);

>检测疫情前收集的2019年武汉39份猫血清样本,进行回顾性调查,均为阴性。



#### 口中和试验和Western blot试验,进一步确定其特异性

Cat NO.	ELISA (OD450)	Neutralization titre	Background of cats		
#1	0.353	1/40	from pet hospital		
#2	0.334	1/80	abandoned cat		
#3	0.348	1/40	abandoned cat		
#4	0.687	1/360	COVID-19 patient owne		
#5	0.394	1/40	from pel hospital		
#6	0.401		from pet hospital		
#7	0.379		from pet hospital		
#8	0.345	1/20	from pet hospital		
#9	0.351	1/40	from pet hospital		
#10	0.624	1/20	abandoned cat		
#11	0.342	1/40	abandoned cat		
#12	0.852	-	abandoned cat		
#13	0.437	-	abandoned cat		
#14	1.432	1/360	COVID-19 patient owne		
#15	1.095	1/1090	COVID-19 patient owne		

>对15份ELISA阳性猫开展空斑减少中和试验(PRNT),结果
 显示15份血清中,11份具有针对新冠病毒中和抗体;
 >中和活性最强的4、14和15号血清均源自患者家庭的猫,中和
 滴度分别为1/360、1/360、1/1080;





### 2. 两只新冠病毒抗体阳性猫的持续性监测

>RBD-ELISA抗体和中和抗体可持续近4个月,高抗体猫与新冠患者亲密接触有关。







▶2020年4月26日, 武汉市COVID-19病例清零; 随后, 猫阳性病例也很快消失, 表明血清阳性猫与武汉地区COVID-19流行趋势密切相关。

(二) 犬血清学动态监测

实时跟踪监测: 2020年1-12月, 共监测犬血清样本1274份, 16份RBD-ELISA阳 性, 阳性率1.25%



— 2020.1月-5月阳性率分别是0% (0/10), 7.14% (1/14), 7.89% (3/38), 7.37% (9/122)和 3.52% (3/85); 2020.6月-12月1005份则均为阴性。

来源:

◆疫情前: 36份阴性
◆疫情发生1-12月: 1274份
来自动物收容所、动物医院、新冠 患者家庭

8	ELISA	Neutralization		14	-		- 554	in a la				
0.	(00459)	Titer	Background of dogs		20	60	-	180	540	-	1620	-
1.22	0.554	1 180	COVID-19 patient owner	1 3	1#				16		100	
2.44	0.38	1/80	COVID-19 patient owner							1		1
311	0,44	None	Stray dog		20	40	20	80	160	_	480	1
414	0.384	1 (20	From pet hospital		2#	-			AV/C		16-	
511	0.395	1.10	From pet Inspital		- C.							1
68	0.424	1/20	From pet hospital		20	40		80	160		480	
74	0.454	1/20	COVID-19 patient owner		54	14	- 1	4	$\mathcal{M}$		16	1
\$77	4.445	1/20	I rom pet hospital									1
9.52	0,491	1.20	From pet hospital							-		-
04	0.402	1/20	From per los-piul	Inc	out C	- 16		1	14		110	
1#	0.300	None	From per hospital									
24	0.369	None	From pet hospital	D						1		-
3#	0.415	1/20	From pet hospital	D	kDa M	14	2#	54	D-N	H-P	H-N	
40	0.576	None	From pet hospital		55	-	-	-		-		<-N
5.11	0.382	None	Trom per bospital		10	-	-	-				
077	0.394	None	From pet hospital		33	-		1000				

16份ELISA阳性犬血清中,10份具有新冠病毒中和抗体,其效价范围为1/20至1/180;
 送而采用Western blot试验进一步证实1、2、5号犬血清具有较强的新冠病毒特异性;
 中和活性最强的1号和2号血清(中和滴度为1/80和1/180)均来自患者家庭的犬。

### (三) 猪场疫情影响的排查 (2月份)

#### 武汉周边县、市

2020年2月下旬,监测麻城、鄂州、孝感、 东西湖、蔡甸、汉南、黄陂、汉川等猪场血 清样本178份,结果均为阴性。

> 监测鼻拭子、肛拭子及环境样本共399份, 荧光PCR(N、E基因靶标)结果均为阴性。

#### 2019年各地血样

▶ 回顾性调查:对2019年采集的1970份猪血清 样本进行新冠病毒抗体检测,RBD-ELISA结果 均为阴性。



### (四) 驯养野生动物排查 (2月份)

◆ 武汉周边黄石(阳新)、荆州、荆门采集果子狸(30只)、竹鼠(19只)
 和 豪猪(15只)、豚鼠(10只)样本。

— 采集咽、鼻、肛拭子共295份,进行实时荧光PCR检测,结果均为阴性。 — 采集的血样本73份,进行抗体检测,结果均为阴性。



小结

1. 在疫情流行的早期,2020年1-3月血清学调查发现猫可感染新冠病毒, 抗体阳性猫与新冠肺炎患者密切接触相关,随着新冠肺炎患者清零, 传染源逐渐消除,抗体阳性猫也逐渐清零;

2. 自然状态下犬可被动感染新冠病毒,感染率及抗体滴度低于猫,猫 较犬易感;

 3. 采集武汉周边猪的样本,抗体及核酸检测均为阴性,表明猪不易感;
 4. 武汉周边驯养的果子狸、竹鼠、豪猪、豚鼠等野生动物抗体及核酸 检测均为阴性。

# Thanks for your attention !



### ANNEX D: reports on site visits

Disclaimer: All records of site visits are based on notes taken by expert team members and went through language interpretation. Recording bias may exist. Personal statement and response by interviewees to questions from the expert teams are based on memories of events as long as 12 month prior, and it is possible that some biases in these memories affect their accuracy.

### Annex D1 - Xinhua Hospital

#### 29 January 2021, pm

**Participants from the Xinhua Hospital** (also known as the Hubei Hospital of Traditional Chinese and Western Medicine):

- Vice President
- Zhang Jixian, Chief of Respiratory and Critical Care Medicine Department
- Chief of Cross Infection Control Office
- Health professionals from radiology department and clinical laboratory
- Members of the joint study team

The earliest cases and the hospital's response to COVID-19

- After an introduction to the hospital, Dr Zhang Jixian described the timeline and actions of the hospital's response after the identification of the first case of pneumonia of unknown etiology on 27 December 2019.
- On 27 December, after receiving three patients from one family with similar symptoms and test results, Dr Zhang reported to the hospital authorities. The hospital had a small group joint consultation, and then reported to the district level CDC, who sent staff for sampling and epidemiological surveillance in the afternoon.
- At 2 pm on 29 December, a hospital-wide joint consultation meeting was held and it was decided to report the disease cluster to a higher-level authorities (Wuhan CDC, Hubei Provincial CDC, Hubei Provincial Health Commission).
- On 30 December, Wuhan CDC came for sampling and epidemiological surveillance. All patients were sent to Jinyintan Hospital for treatment. No samples from the early cases remain. Because of national legislation for laboratory management, all samples have to be disposed after testing. No storage is allowed.
- A retrospective study among patients from 1 September to 31 December 2019 revealed:
  - the earliest case identified was the one reported on 26 December with onset date of 12 December
- The number of outpatient visits at fever clinics in Xinhua Hospital was similar in January-November in 2018 and 2019, but the number increased by 40% in December 2019 in comparison with December 2018.

• Since SARS, China has established a PUE surveillance system for pneumonia of unknown origin and each hospital has to report to its local CDC on every 5<sup>th</sup> and 20<sup>th</sup> of the month if there is any case pneumonia of unknown origin. No case had been reported for many years.

#### Repurposing the hospital

- 25 January 2020, Xinhua hospital was repurposed as one of the hospitals for COVID-19 patients only. All health professionals were involved in the COVID-19 response, and additional staff were seconded from Guangdong and Beijing on 27 January. A group of high-level experts on severe disease and respiratory diseases was formed to support the hospital.
- 24 inpatient wards were established with 950 quarantine beds. For treatment, patients were categorized as: normal, severe, critically severe and in rehabilitation. The hospital treated 1133 patients and another 1011 patients from the shelter hospital that Xinhua hospital was responsible for. Both traditional and western medicine were used for treatment in this hospital.
- A follow-up survey was done by Xinhua hospital among those who recovered from COVID-19. Among the findings 40% of respondents reported fatigue and 25% post-traumatic stress disorder. A further follow-up will be conducted in March 2021.

Interview with patient recovered from COVID-19

- The patient in the COVID-19 reporting system with the earliest date of onset date (8 December 2019) agreed to a face-to-face interview with the joint international study team.
- The WHO team asked some questions about the patient's history and family and were told that the person was an accountant by profession and worked for his family company.
- While details of the interview are not disclosed here to protect the person's privacy, the interview found no evidence for high-risk exposures (wild animals, mass gatherings, contacts with healthcare settings, contact with symptomatic individual, travel, etc.). The person mentioned one relative working in a healthcare setting, and one relative visiting a local market, but there were no illness reports related to these locations at that time. The person commuted to work by public transport, and had not travelled outside Wuhan.
- The WHO team was told that earlier potential cases were given the opportunity to be interviewed, but were unwilling or unable to attend.

#### Annex D2 - Jinyintan Hospital for Infectious Diseases

#### 30 January 2021

- **Participants** from the Jinyintan Hospital include Dr Zhang Dingyu, the former President of Jinyintan Hospital who was in charge during COVID-19 (now promoted as the DDG of Hubei Provincial Health Commission), President of the hospital, and health professionals from departments including respiratory disease, ICU, radiology, and laboratory testing.
- The WHO team expressed their great appreciation for the cooperation of so many staff from the world-renowned hospital.
- The president of the hospital introduced staff concerned with COVID-19 and the hospital and its work dealing with public health-related diseases. Its work included:
  - routine drills and rehearsals for public health emergencies
  - training for responses to outbreaks of infectious diseases, including emerging diseases
  - emergency response and training (such as for the military games in Wuhan in 2019)
  - development of treatment protocols (as for example for MERS and Ebola virus disease)
  - specialization in infectious diseases, most patients are referrals from other hospitals.
- On 29 December 2019, the hospital staff learned of COVID-19 from Wuhan Health Commission and the Wuhan CDC. The first batch of patients with pneumonia of unknown etiology was transferred to Jinyintan Hospital for treatment. The Hubei Health Commission and the Nation al Health Commission told the hospital to consult with Xinhua Hospital on cases of pneumonia of unknown etiology. A task force was organized and the infectious disease workforce and wards were reorganized. Multiple laboratory tests eliminated potential pathogens. The response was described as that of clinicians facing cases of viral pneumonia.
- On 31 December 2019, the National Health Commission sent an expert team to help with treatment, guidance and training and internal coordination was increased.
- By around 4 January 2020, the hospital had received about 40 referrals from several hospitals in Wuhan (the exact number is not sure). The diagnosis was based on clinical and imaging evidence, not pathogen identification. ICD-10 codes were used. PCR testing was introduced when commercial kits became available (around 20 January 2020).
- It seems that no measures were in place by the hospital for discussion with or sampling from families of early cases.
- The WHO team asked if there had been any medical histories towards the end of 2019 that had been unusual. None had been, but that was not unexpected as the hospital was not the first point of entry into the health system and it dealt mainly with referred severe cases. In October-November cases of influenza had been seen, but 99% were in children.
- Retrospective analysis found no adult case of viral pneumonia that could be confused with COVID-19 before 29 December.
- During the Military Games in October 2019 (held in Wuhan), emergency response plans had been prepared and temporary hospitals established with triage. Although five imported cases of malaria and dengue fever were detected (malaria and dengue fever are not endemic in Hubei Province) nothing resembling COVID-19 had been seen.
- The only other recent mass international gathering in Wuhan had been a marathon in April 2019.
- In the outbreak period (December 2019 April 2020), 2800 COVID-19 patients were seen; the age range was 16-94 years and more than half were classified as severe or critical. (Jinyintan

Hospital is a designated hospital for the treatment of severe cases, and severe cases from other hospitals would be transferred to Jinyintan Hospital)

- With regard to samples:
  - none was stored for longer than was laid down in statutory provisions and were destroyed after a week
  - in early days, none was taken for pathology or at post-mortem (not a culturally accepted practice so was difficult to get agreement from the families)
  - the laboratory of the hospital was not yet qualified to preserve samples (biobanking) and it was not known which hospitals in Wuhan might be qualified for this purpose
  - further tests on samples cannot be conducted without informed consent
  - the hospital does not engage in research projects that might be the source of clinical specimens, such as those on non-polio enteroviruses for which it is mandatory to collect samples and send them to reference laboratories to eliminate poliovirus
  - system exists collating ethical approvals given to the use of patients' samples.
- The hospital acknowledged several useful suggestions made by the WHO team, such as collecting samples and biobanking; establishing a network of specialized, infectious disease hospitals; and increasing participation in research projects and would be followed up.
- Asked about its ideas about the origins, staff referred to early cases in other countries, imported food product and contaminate packaging and possibility in cold chains.

#### Annex D3 - Baishazhou Market

31 January 2021, am

**Participants:** All members of the Chinese and international teams. Market officer, representative of Hubei CDC, Market control officer of Hongshan district.

#### The market

- Baishazhou Market is the largest wholesale market in Wuhan, with 350 staff and 1200 merchants, provides 70% of frozen food for Wuhan, receiving frozen foodstuffs from abroad through points of entry and from domestic suppliers across the country. It is a regional distribution hub. Currently, trade sees the arrival of more than 10 000 customers and more than 7000 vehicles a day, but in high season this figure rises to 12 000-14 000 vehicles a day, including private vehicles.
- For imported materials, strict controls are in place at points of entry, including sterilization and heath quality controls, all of which are certificated. Imports, ranging from cattle and sheep to seafood come from places as far apart as Argentina, Brazil, Ecuador and Oceania.
- No live animals are sold; only frozen food, ingredients and kitchenware. No frozen products from domesticated wild animals are sold; only frozen packaged food like meat and animal organs.
- Imported and domestic products are separated on arrival at the complex, which comprises nine warehouses and a capacity of 150 000 tons of storage. It is divided into several dedicated sections. At the international loading bay and warehouse (where material is stored at -18°C) pallets (which are not opened) are sampled, tested, sterilized and their traceability ensured. The cold-chain is ensured by liquid ammonia refrigeration; the machinery is housed in a separate building.
- Other dedicated areas include frozen goods shops where samples are displayed in freezers and areas where spices and kitchen utensils are sold. The showrooms are no longer importing products and are exhausting their existing stocks of imported materials.
- The entire market is closed on Sunday afternoons in order to allow complete disinfection. Merchants also must disinfect their products; the cost of that has been systematically lowered.
- Personnel management is done on site and the market regulatory authorities have their offices in the market.

#### Comparison with the Huanan market

- Baishazhou market was a competitor to Huanan market when it was open in terms of wholesale frozen food products, but it was much bigger, and includes individuals and restaurants as customers.
- Huanan market sold mainly frozen and fresh seafood.

#### COVID-19 response

- Testing for SARS-CoV-2 was introduced on 4 November 2020, since when no positive result has been found. Testing is done by a third-party certified commercial company to ensure openness and transparency. The local Government of Hongshan district subsidizes the cost of testing.
  - Employees are tested for SARS-CoV-2 nucleic acid every seven days, free of charge. Samples for the surface of some 200 000 packages have been tested.

- Only the in-stock imported frozen products were tested.
- Domestic products are not tested but only disinfected.
- Since the outbreak, four positive cases have been detected among the employees but no package surface sample has been found to be positive. Most of the cases were detected in January 2020 after the Chinese New Year.
- Nucleic acid testing is performed by a third-party commercial laboratory with testing qualifications. The Inspection Center is responsible for the quality audit and control of the third-party commercial laboratory. CDC carries out parallel sampling tests on market environment, goods and personnel. No information was provided on the quality control protocol and positive CT values for nucleic acid tests. Statistical data of nucleic acid testing shall be collected and archived by market management personnel and reported to CDC and the Market Supervision and Administration. Since November 2020 the market no longer imports frozen products, based on a policy issued by the district government.

#### Annex D4 - The Huanan Seafood Wholesale Market

31 January 2021, pm

Participants

The Huanan Seafood Wholesale Market manager, two vendors, two suppliers of frozen products, market regulators and two neighbourhood residents.

Members of the Chinese and WHO teams.

The market – layout and activities

- Although the market was closed on 1 January 2020 and subsequently disinfected, it remains shuttered and closed to the public. The team visited the area of the west part which had most links with cases of COVID-19, with various stalls, the remnants of the ventilation system (which has been shut off since the prohibition of live poultry markets in China).
- *Layout and condition.* Apart from the central street, the alleys of the market are very narrow, dark and poorly ventilated, with a roof covering the whole wing. The ventilation system had been closed since the live poultry trade had been stopped following the outbreak of avian influenza. The "sewerage" was an elaborate semi-open drainage system in poor condition running through the entire market. Storage areas above the stalls seemed to have partly served as (temporary) living quarters, but mostly as storage areas.
- There was a mixed smell of animals and disinfection in some areas of the market, even a year since its closure. It was later clarified by the manager in a subsequent meeting that those were the smell of rotten meat since all stalls were closed suddenly without removal of their products. Those products were disposed of a few weeks later, so it could also be the smell of sewage.
- *Products sold.* Most shops sold frozen food products including seafood and meat. Some shops sold live seafood or aquatic animals (the WHO international team was told live fish, amphibians e.g. salamanders, and turtles were sold. In Dr Liu's presentation on the environmental sampling of the Huanan market he described sampling from live snakes). According to the manager, 10 shops had been found to be selling frozen domesticated wild animals like bamboo rats and snakes, and no live animals had been seen before the market was closed; only storage and fridges. It was further stated that no live animals were sold and no animals were butchered on the premises.
- There was evidence of the sale of live aquatic animals in the presence of fish tanks and shallow tanks that would have been used for turtles or amphibians.
- No evidence of the sale of live mammals was found in our visit e.g. cages of the type used to house mammals like raccoon dogs, as seen in some of the photographs taken by Dr E.C. Holmes about seven years ago (Zhang YZ, Holmes EC. A genomic perspective on the origin and emergence of SARS-CoV-2. Cell, 2020.) or other unverified photographs and videos in media reports.

Meeting with people related to the Huanan market

• The joint study team had a face-to-face meeting with a large group of participants, and talked with two vendors of the Huanan market who sold frozen beef, and frozen seafood, two suppliers of frozen product, market regulators and the market manager, as well as two neighbourhood residents.

- The manager informed the teams the market was cleaned twice a day, morning and evening. Pests and rats are sought out and killed; holes were closed. Rigorous cleaning was done once or twice a week. Even though there are rooms above some stalls, vendors were not allowed to live in the market. The rooms were only for storage.
- The WHO team was told by the China team and the vendor that the market was not a purely wholesale market and that many ordinary people bought their food there.
- The WHO team was told by Hubei CDC representative that around 10 000 people per day visited the market.
- Employees ate in the canteen of the market. Vendors usually brought food from home or ate in neighbouring canteens, and most lived close to the market.
- *Health and other regulatory issues*. Market regulations demand that vendors have a health certificate for work and a health check every year. If they fell ill, most vendors and employees would consult Wuhan No.11 Hospital nearby. If symptoms were mild, traditional Chinese medicine or western medicine practitioners of choice were consulted.
  - National regulation mandates double-random checking, and in October and December 2019 cross-sectoral joint inspections had taken place. All necessary certificates had to be displayed in the stall.

Response during the outbreak

- More than 20 cases occurred at the beginning of the outbreak, some but not all were in vendors.
- The market was closed (1 January 2020) after being notified by public health department and the market regulators on 31 December 2019. Sterilization and disinfection measures were undertaken.
- All the tools, machines and products were left in the market and could not be recovered by their owners.
- *Interviewees* reported hearing no rumours of disease at the time and no "unusual" events came to mind. Vendors (mostly of beef and frozen seafood products (imported and domestic) and suppliers (frozen food products) confirmed these accounts. After the closure of the market, some vendors went to other markets and some others switched to other lines of work.
- Imported foods were mostly from Australia, Ecuador, India, New Zealand, Thailand and Viet Nam.
- *Neighbourhood residents*. Two citizens living close by had responded to a community invitation to participate in this meeting. The citizens have been shopping regularly in the market for 20 and 30 years. They provided very similar details: nothing out of the ordinary noticeable, all vendors had certificates and inspection certificates displayed in their stalls, they had never witnessed any live animals being sold, the market was kept clean and tidy and they had not noticed any stray cats or dogs, and there had been no confirmed cases in their residential block.

#### Annex D5 - Hubei CDC and Wuhan CDC

1 February 2021, am and pm respectively

**Participants**: from the provincial and municipal CDCs include directors-general of the CDC, head of infectious disease prevention, head of inspection and testing and head of emergency response. The Deputy Director-General of China CDC and the Jianghan District CDC attended the meeting with Wuhan CDC.

Members of the Chinese and WHO teams.

#### Overview

• The team visited Hubei CDC and Wuhan CDC and their laboratories, where colleagues from China CDC and Jianghan District CDC were also present. It was the first meeting with CDCs from all four levels together since the beginning of the pandemic. The meetings provided the WHO team with a full picture of the CDC system in China and the role of each during the early outbreak, contributing to a clearer timeline of the early outbreak and better understanding of the epidemiology surveillance done.

The role of CDCs and laboratories at different levels during the early outbreak

- <u>Jianghan District CDC</u> (one of 13 District CDCs in Wuhan): it received a report from Xinhua Hospital (also known as the Hubei Provincial Hospital of Integrated Traditional Chinese and Western Medicine Hospital), collected a sample for influenza testing, transferred it to the Municipal CDC, implemented epidemiology surveillance, searched for cases related to Huanan Market and reported to the Municipal CDC.
- <u>Wuhan Municipal CDC</u> (with a BSL-2 laboratory): it collected a sample for respiratory pathogen testing, organized city-wide case screening and environment sample collection, PCR testing (from 24 January 2020), transferred the sample to the Provincial CDC, and retrospectively tested serologically samples from HIV/AIDS patients.
- <u>Hubei Provincial CDC</u> (with several BSL-2 and one BSL-3 laboratory): it conducts antigen and antibody tests, virus isolation and culture, genome sequencing (from January 2020), PCR testing (from 16 January 2020), transfer samples to China CDC, collect environmental samples, provide training and provincial guidelines.
- <u>China CDC</u>: the CDC conducted back-to-back sequencing for the earliest cases, together with the Chinese Academy of Medical Sciences and the Wuhan Institute of Virology, published results internationally, provided technical consultation, training and development of national guidelines
- In total, there are around 90 CDCs in Hubei Province, including the Provincial CDC, several Municipal CDCs and District/County level CDCs. CDCs include Epidemiology departments and laboratories.

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Tim	e	lıne	

Date	What happened			
27 Dec 2019	<ul> <li>Xinhua Hospital reported three viral pneumonia cases (a family: father, mother and child) to Jianghan District CDC.</li> <li>Jianghan CDC together with Wuhan CDC went to the hospital for epi surveillance and sample collection.</li> <li>Jianghan CDC did influenza tests, all negative</li> </ul>			
28 Dec	<ul> <li>The samples were transferred to Wuhan CDC for testing for 23 respiratory pathogens, all negative.</li> <li>From 28 to 30 December, human and environment samples also went through FilmArray multi-respiratory pathogens testing.</li> </ul>			
29 Dec	<ul> <li>Xinhua Hospital reported another four viral pneumonia cases, all of which were workers at the Huanan Market.</li> <li>Wuhan CDC and Jianghan CDC went to the hospital for throat swab sample collection.</li> <li>Experts of Wuhan CDC, Jianghan CDC and two other doctors did joint consultation.</li> <li>The samples were transferred directly to Wuhan CDC laboratory.</li> <li>All seven cases were transferred to Jinyintan Hospital.</li> <li>In the afternoon at 5 pm, the Hubei Health Commission received a call about the seven viral pneumonia cases.</li> <li>Hubei CDC visited Jinyintan Hospital in the evening at 7 pm together with Wuhan CDC.</li> </ul>			
30 Dec	<ul> <li>A city-wide case screening was conducted targeting people with pneumonia of unknown origin, abnormal blood routine test (normal WBC, lymphocytopenia), and exposure history with Huanan market. More cases with similar symptoms were identified, traced and quarantined.</li> <li>Wuhan CDC and Jianghan CDC went to Huanan market for environment sample collection.</li> <li>Hubei CDC verified the pathogen testing for all samples; all negative.</li> <li>Hubei CDC reported to China CDC.</li> <li>A test report showing SARS-like testing results was circulating on social media in the afternoon.</li> </ul>			
31 Dec	<ul> <li>Continued epidemiology surveillance at several hospitals (close to Huanan market), Huanan market and the neighbourhood of Huanan market.</li> <li>China CDC experts arrived to launch a three-level joint taskforce. It was agreed that the identified cases were pneumonia of unknown etiology.</li> <li>Jianghan CDC together with a third-party organization disinfected Huanan market around midnight.</li> </ul>			
1 Jan 2020	- Huanan market was closed at 1 am.			

Epidemiological surveillance and its analysis for the early cases

- Details were presented for first three reported cases (a family cluster) and the four cases reported on 29 December 2019 (all in workers at the Huanan market).
- The findings of a retrospective study included the fact that almost one third of early cases had no exposure history to Huanan market or even any market, including before 10 December, some sporadic cases. The onset date of those with no exposure history to Huanan market is earlier than those had exposure history to the market (onset dates all after 10 December 2019). Among cases with onset date in December 2019, all cases from suburban areas of Wuhan had a history of exposure at the Huanan market. Starting from 20 December, the number of cases related to Huanan market increased sharply and spread widely. When the Hubei Provincial CDC was notified about the cases, there was already transmission in clusters.
- Epidemiological information for sporadic cases has been stored in a database. Contact tracing were done for all cases identified, but the focus was hospitalized pneumonia cases, not cases with mild symptom or asymptomatic cases because of the limited understanding of the disease at the time.
- The Head of the Infectious Disease Prevention Department of Hubei CDC concluded that it is likely the virus was introduced into Huanan market via an individual or animal. It is more likely from an individual given the sporadic cases prior to 10 December had no exposure to Huanan market.
- There was no significant variation in the virus between January and May 2020.

Findings from retrospective ILI surveillance

- Hubei CDC tried to search ILI cases from October to December 2019 through surveillance system for SARS-CoV-2 testing. All results were negative. Testing was done by the sentinel hospitals, under supervision of Hubei CDC. A guideline was developed by China CDC. Every week, the sentinel hospitals submit around 20 samples to Hubei CDC.
- SARS-CoV-2-positive cases were first identified by the ILI surveillance system in the first week of January 2020, demonstrating community transmission by that date.

Environment samples collected from the Huanan market

• The positive samples were mostly at stalls where the positive cases had worked – on the floor, walls, chopping boards, and cleaning tools. A working assumption was that the virus was likely being transmitted from people to surfaces.

Other samples available for retrospective study

- *Issue*. Although the WHO team highlighted the importance of serum samples from October to December 2019 for identifying earlier cases and origin tracing, the District CDC cannot store samples.
- SARS-CoV-2 testing by Wuhan CDC on 381 HIV-positive serum samples collected in October-December 2019 and by China CDC on 2000 serum samples collected in the same period from Wuhan Tongji Hospital all tested negative.
- *Polio*. Hubei CDC performs virus culture of stool samples for acute flaccid paralysis surveillance. If positive a sample will be sent to China CDC. If negative, the sample will normally be preserved for one year and then destroyed as it is too smelly. The cultures will be saved. It will review all the preserved early samples and check if any are available for retesting for SARS-CoV-2.

Laboratories' research and staff

- Hubei CDC. All its laboratory staff have been tested for SARS-CoV-2-specific antibodies: all had negative IgM and IgG results.
- Wuhan CDC. one of its staff was confirmed SARS-CoV-2 seropositive after infection due to family cluster transmission. All other staff have tested negative. A health check is mandatory for all BSL-2 laboratory workers, but no serum is preserved.
- Jianghan CDC. All PCR tests for SARS-CoV-2 of all laboratory workers in June 2020 were negative.

#### Sequencing

- China CDC sequenced early samples, submitting the results to the China National Center for Bioinformation.
- A sequencing kit was available on the market in the second-half of January 2020. Since then, Hubei CDC has sequenced material from imported cases, environment positive samples (mainly packaging of the imported cold-chain products, not from Huanan market).
- Later samples were kept, but early samples were all sent to China CDC, Chinese Academy of Medical Sciences and Wuhan Institute of Virology for sequencing.

#### Interview with media journalists

• The journalists informed the teams of their activities. They went to Huanan market on 31 December before it was closed and had footage of the market, which could be provided to the WHO team. They did not hear anything about the disease or virus on social media prior to the report by the Government.

#### Annex D6 - Hubei Animal CDC

#### 2 February 2021, am

#### Participants

- Government Official of Hubei Province Agricultural and Rural Bureau.
- Director-General of Hubei Animal CDC and the chief of laboratory, general office, pathogenic laboratory, and serological laboratory.
- Director-General of Hubei Province Wild Animal Surveillance Centre.
- Director of Hubei CDC.
- Chinese team and WHO team (except the Epidemiology working group).

Introduction of Hubei Animal CDC

- The Provincial Animal CDC is in charge of diagnostics for animal diseases, serology and pathogenic surveillance and epidemiology surveillance, laboratory management, guideline development and providing training to city, county and village level animal CDCs.
- In total there are 27 staff members, of whom 18 are veterinarians.

The prevention and control system for animal-related diseases

- Agricultural authorities are in charge of disease prevention and control for bred animals.
- The Grassland and Forestry authorities are in charge of disease prevention and control for wild animals.
- A joint prevention and control committee for animal-related diseases was established between the health authorities, agricultural authorities and the forestry and environmental protection authorities. The joint committee meets regularly to update the surveillance results. If anything abnormal occurs, one authority will report to the other two. They have experienced SARS, influenza A/H7N9 and African swine fever outbreaks.
- There is a bottom-up reporting system within animal CDCs, from village to the State Council, depending on the seriousness of the disease (major disease: no more than two hours for reporting at each level).
- A retrospective study was done among pig, cows, sheepand poultry. All tested negative.
- The Hubei Wild Animal Surveillance Centre conducts regular surveillance among migratory birds, wild boar and fur animals.
- During COVID-19, the Hubei Wild Animal Surveillance Centre sampled wild animals and fur animals in 14 cities of Hubei Province for SARS-CoV-2 testing. All the results were negative.

Legislation about animal trade and its enforcement

- With effect from May 2020, Hubei Province issued legislation and banned consumption of all terrestrial wildlife (including domesticated wild animals), aquatic wild animals and other wild animals that are protected by law.
- China has an office in charge of protecting endangered wildlife animals.
- Wild animals were grouped into three level based on numbers of animals in China. The highest risk level (the smallest number) is level 1, including panda and elk. Any person found guilty of capturing or selling these animals will be sentenced for 10 years; levels 2 and 3 both carry jail sentences.

• There is no regulatory authority in charge of animal trade activities conducted by small-scale breeders or individuals.

Challenge in capacity-building for animal CDCs and possible solutions

- The Provincial Animal CDC is short-staffed.
- A BSL-3 laboratory was built in the compound of Hubei Animal CDC, but has not been used owing to lack of staff.
- The WHO team suggested:
  - more international collaboration with experts and academics from South-East Asia to build capacity and experience in laboratory research in order to apply for more funding and support from the Government
  - starting to build capability for public health in veterinarian students and professionals as well as the veterinary medicine capability of public health students and professionals, in order to have capable professionals for sustainable animal disease prevention and control work in China
  - connecting public health professionals, veterinary experts and socio-economic experts for joint studies and projects.
- The Chinese team appreciated all above suggestions and welcomed more from WHO team regarding the human CDCs.

#### Annex D7 - Wuhan Institute of Virology

#### 3 February 2021

**Participants**: Professors Wang Yanyi, Yuan Zhiming, Xiao Gengfu, Shi Zhengli, Deng Fei, Zhou Peng, Chen Quanjiao, and Chen Jianjun.

Members of the Chinese and international teams.

- The Laboratory Director, Professor Yuan Zhiming, gave the joint team a tour of the BSL-4 facility and Professor Wang Yanyi introduced the staff and the work of the Institute. She concluded with a plea for endorsement of the application by the Wuhan Institute of Virology (WIV) to become a WHO Collaborating Centre and Reference Laboratory.
- The Laboratory Director stated that WIV BSL4 laboratory was constructed in collaboration with France between 2014 and 2017, accredited in 2017, and became operational in 2018 (this information was featured on a poster in the laboratory and mentioned in the Director's talk).
- The animal room in the P4 facility can handle a variety of species, including primate work with SARS-CoV-2.
- WIV has engaged in international collaborations with Insert, Merieux, Pasteur on Nipah virus; CAS Sino-Africa collaboration with Kenya; in the USA its collaborations are with EcoHealth Alliance and are funded by NIH and USAID.
- WIV has been a member of the World Federation for Culture Collections since 1989.
- The WIV laboratory director is a member of the "Group of High-Containment Laboratory Directors".

The role of WIV during COVID-19 response

- Rapid response: WIV received 7 samples from Jinyintan Hospital on 30 December 2019 and did sequencing and virus culture immediately. The results were conveyed to WHO, and the 5 genome sequences were published on 12 January 2020. Testing of various samples from Huanan market, wildlife markets, urban stray cats, domestic cats etc., revealed positive results in cats (not from Huanan market).
- WIV worked with commercial companies to develop a nucleic acid testing kit for SARS-CoV-2. It also worked on animal models, drug and vaccine development, and providing inactivated virus; it also shared the virus strain.
- WIV was heavily targeted by conspiracy theories. Staff talked to media and scientific journalists to dispel the myths.

Bat coronaviruses

- Professor Shi Zhengli gave an extensive scientific report on her team's work on bat coronaviruses. She covered issues including the following:
  - the team has collaborated internationally since 2004
  - about 19 000 samples had been collected, coronaviruses were detected in about 13% (2481 positive for CoV) of the tested samples by RdRp sequencing and triaged according to phylogeny. Clade 4 SARSr-CoVs only found in Yunnan
  - all fieldwork is done with full PPE

- One virus strain with high homology with SARS-CoV-2, was renamed as RaTG13, and the information published in *Nature*
- the low likelihood that RaTG13 was the precursor of SARS-CoV-2
- the limited amount of live virus available for research
- her laboratory used recombinant viruses to test whether bat CoVs could use ACE2 to bind but used bat spike protein on a bat-CoV backbone, not human SARS. It is important to use this approach because it is difficult to isolate these viruses and envelope protein is most important part to understand function. For example, other researchers engineered SHC014 spike so did not need isolates for mouse experiments. WIV began recombinant work in 2015 with WIV-1. It received ACE2 mice in 2016 and started recombinant experiments with WIV +SHC014 in 2018 but did not finish them owing to the COVID outbreak.
- She noted that viruses can be shared internationally but in compliance with Chinese laws and regulations. Her team collaborates with external partners, recently in Kenya, Thailand and Uganda. All samples are stored, but not all have been examined yet. Other collaborators in Huangzhou, Huazhong Agricultural University China, have worked on frozen samples and with stray cats. With regard to possible reservoirs of SARS-CoV-2, other species of animals than bats are sensitive hosts.

#### Conspiracy theories

- The WIV Director raised the issue of conspiracy theories, reiterating that the Institute had worked with the media to stress the need to respect science in the fight against COVID-19 and to rebut the theories. The international team's visit could help to defuse some of the theories that were circulating.
- Staff had to report any symptoms every day after the outbreak of COVID began. Serum samples were preserved annually for laboratory staff. There was extra testing during COVID outbreak according to the Yuang Zhiming (laboratory director). The Institute did not respond to conspiracy theories but understood why the WHO team needed to ask. There had been no reports of unusual diseases, none diagnosed, and all staff tested negative for SARS-CoV-2 antibodies.
- Asked about positive influenza cases in October-November 2019, he replied that WIV performed retrospective research in collaboration with Wuhan Xiehe (Union) Hospital, testing ILI samples from that hospital. In total 1 001 samples were collected from patients in the hospital (the samples were not from staff of WIV). No SARS-CoV-2 NAT positive samples were found in the samples from December 2019 and four coinfections with influenza and SARS-CoV-2 were found in the 700 samples from January 2020. With regard to whether a laboratory audit had been done in response to conspiracy accusations, it was stated that annual external audits were conducted routinely. No problems had been identified. The four coinfection cases are not WIV staff.
- With regard to the matter of morbidity and mortality in miners in a mine in Mojiang, Yunnan Province, where bats were present, Professor Shi said that the events had been clarified in an addendum to her *Nature* article. Doctors sent her the samples for testing after something like three months of illness. Miners had been to the cave 2-3 times and it was 1 meter thick with bat feces. Professor Shi's team went there in 2012-15 about seven times to look for novel viruses. They found no viruses close to SARS-CoV but there was a rat henipa-like virus (Mojiang paramyxovirus), as reported by another group in China. Samples taken during subsequent visits to the cave were found to contain no viral sequence related to SARS-CoV-2 (like RaTG13). However, none of them has higher similarity to SARS-CoV-2 than the RaTG13 has. Therefore, none of them are the progenitor virus of SARS-CoV-2 (this would usually entail >99% in genome). None could be isolated. The reported illnesses associated with the miners, according to the WIV experts, were more likely explained by fungal infections acquired when removing a thick layer of guano. The WHO team suggested

surveillance of the local population. More generally, global surveillance of bats, other animals and humans with close exposure was recommended.

- With regards to questions about laboratory workers, all underwent a strict training regime that includes three levels with strict rules on number of hours training and in-laboratory experience prior to being allowed on own in lab, or to supervise others. P4 staff also undergo psychological evaluation before being allowed to work in the laboratory. Physical and mental health was monitored; no unusual respiratory infections had been noted in the previous year. Good compliance with mask use and hand hygiene was observed. Surveillance during the outbreak had been stringent; no suspected or confirmed case of COVID-19 was seen by PCR and antibody testing of all staff was negative. (If any worker had been infected, it would have been likely that close contacts would have shown signs of infection.) Sera were tested twice a year, and all had been negative. There had been no turnover of staff in the coronavirus team.
- Some reports identified one former laboratory worker as "missing". This person according the WIV staff was an alumnus who graduated in 2015 and was now working in a different province and did not accept to talk with media. The person had been contacted and tested and ascertained to be healthy.
- The rumour about missing data was discussed. This related to an Excel spreadsheet that had been on the website for 10 years as part of a national databank of samples. It had been used for internal analyses and metadata. It had been planned to make this an interactive system with visualized data to fit in with the national system. They received attacks from hackers more than 3000 cyber-attacks, so was kept offline.
- The rumours of a leak from the laboratory were refuted categorically by the laboratory director for the following reasons:
  - among the three SARS-like viruses cultured in the laboratory, none are closely related to SARS-CoV-2. The only SARS-CoV-2-like virus found by this group is RaTG13, which is neither a live (cultured) virus nor the progenitor of SARS-CoV-2
  - a paper by leading virologists in *Nature* rebutted the idea of a bioengineered source
  - WIV has a strong biosafety management system; the biosafety laboratory comes under different authorities, with independent assessments for the National Health Commission, for instance
  - The reserved sera in April 2019 and March 2020 from all the workers and students in research group led by Professor Shi Zhengli were seronegative for SARS-CoV-2 antibodies.
  - The laboratory director, responding to laboratory-leak theories, commented that from 2010, including the P3 laboratory, WIV has conducted experiments with more than 10,000 entries, and the P4 laboratory has conducted experiments with more than 3,000 entries in the last 3 years. No infection was ever reported. Close contacts would have been infected if there had been a laboratory leak. But serum samples from Professor Shi's team were all negative.

#### Origins of virus

- Asked about this and the possible route into humans, WIV staff responded by noting:
  - A natural origin close relatives of SARS-CoV-2 were found in bats and pangolins, and diverse genera of bats were distributed across the world and some bats could migrate.
  - Intermediate hosts may have an important role.
  - In the Wuhan outbreak the pathogen might not have been related to animals and transmission could have been from human to human.
  - Transmission from bats or pangolins directly to human is possible but of low probability; a more likely scenario is animal to farm animal to human to human.

- The joint team's study was the China part of the origins tracing work; attention should focus on other animals besides bats.
- The recent confirmation of a closely related coronavirus in Cambodia supported the call for a large international, multidisciplinary collaborative project on all possible hosts and reservoirs and host sensitivities to these viruses. WIV would be willing to participate. The WHO team suggested that the location of the highest density farms of susceptible animals such as mink could be determined.

#### Cold-chain

- A link with frozen products had been established in the outbreak in the Xinfadi market in Beijing, but in the Hunan market frozen samples of wild animal products and other products collected in early January 2020 were all negative. Contamination of the environment in the Huanan market by infected humans could not be eliminated, and the positive human cases could not be conclusively linked with specific products.
- More work on the cold chain and different types of products for possible contamination was needed.

#### Annex D8 - Jianxinyuan Community Centre

4 February 2021

**Participants**: Director of the Jianxinyuan Community Centre, a member of the community whose spouse had died of COVID-19, survivors of COVID-19 and other members of the community.

Members of the Chinese and WHO teams, including Professor Liang Wannian and Dr Peter Ben Embarek.

- The joint team was welcomed at the gated community centre by its leader, who explained the functional organization of community centres in the country. The Jianxinyuan Community Centre serves a community of about 23 000 people in about 7200 families. Many were old; 480 lived alone and 90 were handicapped. The center provides services including those for senior citizens, health services, activities for children, employment and entrepreneurship services, sports and cultural activities and more. It had received an award for its anti-COVID-19 work.
- News about the new coronavirus pneumonia filtered through the community in mid-January, causing some attention. Building and floor leaders went from door to door conveying epidemic prevention knowledge and emphasizing the need for public health measures to be taken wearing masks, regular washing of hands, good ventilation, no crowding or use of lifts, and to stay indoors. Compliance was said to be "relatively high".
- During the outbreak and lockdown in early 2020, which started around 25-26 January, 19 cases of COVID-19 were recorded in the community. No obvious risk or exposure factor stood out.

Functions and services during lockdown

- The volunteers and block managers were divided into 13 teams that
  - supplied food, vegetables and other necessary provisions, all free of charge; the longest interval between provision was four days
  - undertook health promotion, healthcare and other services, including care for senior citizens, minors and single people, and people with chronic illnesses; they also contacted people who had left before the Chinese New Year, telling them that they should not return
  - provision of medical supplies and other medical needs
  - group buying through ecommerce was conducted by 13 teams
  - provisions were delivered, announced with a knock on the door before the teams left, in order to eliminate face-to-face contact.
- A public-access control team restricted movement into and out of the blocks.
- Volunteers worked with families to reduce mental stress, encouraging calm acquiescence and taking activities such as practicing cooking, show talent, and building a healthy body. One public health message that succeeded was "As long as I stay indoors and respect prevention and control measures, that is my contribution".
- Like healthcare workers, volunteers and community workers were tested monthly by PCR free of charge. The community leader herself had been vaccinated (2 doses) already.

The selected participants' thoughts and views.

- A married couple in their 70s, who both contracted COVID-19, recounted their fears and experiences. They expressed thanks to the Government for covering all their expenses and to the volunteers for smoothing their return to their apartment with practical and moral support. Of the joint team's work on origin tracing, the husband welcomed experts to Wuhan and expressed that origin tracing should be extended to cover the whole world.
- Another representative of the community who is also building director described their activities as a volunteer, urging everybody to stay indoors, delivering food and medicines, and frequently telephoning the old and lonely, concluding that quarantine brought people together as one big family.
- The final witness lost their spouse due to COVID-19 and described how they were unable to see them during the final two weeks of their life due to their isolation. They acknowledged the continuing support of colleagues and was grateful to the State for psychological support and counselling. Their suffering was still very evident.

Both teams acknowledged the poignant and powerful testimonies, and the strength and organization of the community.

### ANNEX E: EPIDEMIOLOGY WORKING GROUP

#### Annex E1 - ILI surveillance supplementary data

To identify unusual changes during the months preceding the early epidemic weeks of December 2019, the weekly percentage of laboratory-confirmed influenza rates of ILI were plotted and compared to the same period of the previous three years 2016-2018:

#### Trend comparisons of 2019 influenza-rates to previous influenza seasons 2016-2018

Laboratory-confirmed influenza rate **in children** in Wuhan in 2019 was lower between weeks 25 and 46 but increased from week 47 in 2019 and exceeded that of the previous three years from week 50 and onwards (Fig. 1). The graphs were quite irregular reflecting a limited number of cases in the surveillance system.



Fig. 1. Weekly distribution of laboratory-confirmed influenza rates in paediatric cases of ILI in Wuhan from 2016 to 2019.

Compared with the same period in the previous three years, the laboratory-confirmed influenza rate in **adults** in Wuhan in 2019 varied, with low levels from weeks 27 to 46, increasing from week 47 and exceeding the levels in the previous three years from week 51 (Fig. 2).



Fig. 2. Weekly percentage of laboratory-confirmed influenza rates in adult cases of ILI in Wuhan from 2016 to 2019.



Fig. 3. Weekly distribution of the number of adult ILI cases in 2019 compared with the mean of the previous three years in Wuhan.

In 2019, the trends of ILI% rates and laboratory-confirmed influenza rates in Wuhan were slightly different, with the ILI% keeping relatively steady compared with the laboratory-confirmed influenza rates. The laboratory-confirmed influenza rates were high in early 2019 but increased from week 47; with a time lag of two weeks the ILI% rate increased significantly from week 49 (Fig. 3).



Fig. 4. Distribution of ILI% and laboratory-confirmed influenza rates in 2019 in Wuhan.

In 2019, the overall ILI rate in Wuhan was at a lower level from weeks 1 to 48, increased from the 49<sup>th</sup> week. The laboratory-confirmed influenza rate fluctuated before the 27<sup>th</sup> week, and was at a low level from 27<sup>th</sup> week to 47<sup>th</sup> week, then increased from the 47<sup>th</sup> week (Fig. 4).

In 2019, the ILI% rate for all ages in Wuhan was similar to or slightly lower than the mean of the previous three years from weeks 1 to 49, but higher than the mean of the previous three years from week 49 (Fig. 5(A)).



Fig. 5A. Weekly distribution of previous three years' mean value of ILI% compared to 2019 in Wuhan.

In 2019, the ILI% rate in children in Wuhan was similar to or slightly lower than the mean of the previous three years from weeks 1 to 49, but higher than the mean of the previous three years from week 49 (Fig. 5B).


Fig. 5B. Weekly distribution of the previous three years' mean value of ILI% in children compared with the rate in 2019 in Wuhan.

In 2019, the ILI% rate in adults in Wuhan fluctuated, but was similar to or slightly lower than the mean of the previous three years over the whole year (Fig. 5C).



Fig. 5C. Weekly distribution of the previous three years' mean value of the ILI% rate in adults compared with that in 2019 in Wuhan.

2. Analysis of ILI surveillance data in Hubei Province

In 2019, most ILI cases in Hubei Province were in children (Fig. 6).



Fig. 6. Population distribution of ILI cases in Hubei Province in 2019.

In 2019, the ILI% rate remained relatively stable from week 33 to week 48 and began to increase from week 49. The laboratory-confirmed influenza rate fluctuated throughout the year, being relatively high from week 2 to week 6, gradually decreasing from week 7, and increasing again from week 47 (but the peak was lower than that in week 4) (Fig. 7).



Fig. 7. Distribution of the ILI% rate and laboratory-confirmed influenza rate in Hubei Province in 2019.

The trend of the weekly number of ILI cases in 2019 was similar to the mean of the previous three years and higher than the mean from week 48. The trend of the ILI% rate in 2019 was relatively stable, lower

than that in the same period of the previous three years, and higher than that in the same period of the previous three years from week 49 (Fig. 8).



Fig. 8. Distribution of the mean number of ILI cases in Hubei Province in 2019 compared to the previous three years.



Distribution of the average ILI% rate in Hubei Province in 2019 and the previous three years.

In 2019, the weekly distribution trend of ILI cases and the ILI% rate in Hubei Province was similar to that in the six neighbouring provinces and municipalities, and the number of cases began to increase from week 48. From week 50, the ILI% rate in Hubei Province was higher than that in the six neighbouring provinces. The laboratory-confirmed influenza rate in Hubei and the six neighbouring provinces or municipalities began to rise from week 45, but in Hubei Province the rise was slightly higher than that in the other provinces from week 47 (Figs. 8 and 9).

# Annex E2 - A report on early cases

#### 1. COVID-19 Prevention and control

1.1 Incorporating COVID-19 into Class B statutory infectious diseases in a timely manner.

Since the COVID-19 outbreak began in the country, China has taken prevention and control measures in responding to the epidemic. After confirming that the SARS-CoV-2 has human-to-human transmissibility, on 20 January 2020, a decision was taken to classify COVID-19 as a Class B infectious disease in compliance with the Law of the People's Republic of China on Prevention and Treatment of Infectious Diseases, but to apply to it the preventive and control measures for a Class A infectious disease. At the same time, China has brought COVID-19 under quarantinable infectious disease management in accordance with the Frontier Health and Quarantine Law of the People's Republic of China. These policies mandate all detected COVID-19 cases shall be reported to the National Notifiable Infectious Diseases, all COVID-19 cases found in Wuhan were reported to the infectious disease reporting system as unspecified pneumonia, which have been later revised as COVID-19 according to the requirements of the prevention and control protocols.

## 1.2 Updating the prevention, control and treatment protocols continuously.

With the deepening understanding of the clinical and epidemic characteristics of SARS-CoV-2, China has revised the relevant prevention and treatment protocols, updating the diagnosis and treatment protocols of COVID-19 in an average of four days, and updating the prevention and control protocols in an average of six days. On January 15, 2020, China unveiled the first version of Diagnosis and Treatment Protocol for COVID-19, and Protocol on Prevention and Control of COVID-19. Three days later on 18 January 2020, the General Office of National Health Commission (NHC) issued a Notice on the Issuance of Diagnosis and Treatment Protocol for COVID-19 (Second Trial Edition) and the Confirmation Procedure for the First COVID-19 Case in All Provinces (Districts and Municipalities) in China, which has clarified the reporting procedures for the first suspected and confirmed COVID-19 case in each province. On January 20, China included COVID-19 into the Protocol on Prevention and Control of COVID-19 (Second Edition) released on the same day, and two days later the Diagnosis and Treatment Protocols for COVID-19 (Third Edition) was released, as shown in Fig. 1.



Fig. 1. Timeline of the release of COVID-19 Prevention, Control, Diagnosis and Treatment Protocols in China in 2020.

Note: PPT is the protocol of diagnosis and treatment, and PPC is the protocol of prevention and control.

The case definition, case reporting and management and other measures in each version of the prevention, control, diagnosis and treatment protocols were constantly revised based on findings regarding the transmissibility of SARS-CoV-2, domestic testing capacity and medical resources by then in China. Starting from the second version of prevention and control protocol, direct online reporting of cases within two hours and epidemiological study of cases within 24 hours were required. In addition, in view of the epidemiological characteristics and the actual situation of Hubei Province (when the nucleic acid testing capacity was relatively low at the early stage), the case definition for Hubei Province was formulated in the fourth version of the Protocol on Prevention and Control of COVID-19. Later, the case definition was revised as the testing capacity improved. With the deepening understanding of the COVID-19 diagnosis and treatment, immunity, vaccine research and development, virus mutation and relevant prevention and control measures, China is still revising the protocols to adapt to the current stage of prevention and control efforts by far.

1.3 Adjusting the diagnostic criteria when necessary.

On February 4, 2020, NHC released the Diagnosis and Treatment Protocol for COVID-19 (Trial Version 5), which stated that Hubei and other provinces should adopt different diagnostic criteria for COVID-19 cases, i.e., in provinces other than Hubei, the relevant cases were classified into "suspected cases" and "confirmed cases", while in Hubei one more group of "clinical diagnostic cases" were added to the above two groups. This change was due to the limited nucleic acid testing capacity in Wuhan at that time, which meant that not all cases could be tested within a short period of time. To put all potential COVID-19 patients under the prevention and control process and proper treatment, "clinical diagnostic cases" were added so that all patients who should be treated could receive timely medical care, which would be conducive to lower the fatality rate.

On February 18, 2020, NHC released the Diagnosis and Treatment Protocol for COVID-19 (Version 6), writing off the difference between Hubei Province and other provinces in terms of COVID-19 diagnostic criteria, and unifying them into "suspected cases" and "confirmed cases". The main reason for this change

was that the testing capacity in Hubei greatly increased. All suspected cases could be tested quickly, so the "clinical diagnostic cases" were removed from the criteria.

#### 2. Methodology

## 2.1 Data Sources

2.1.1 China's National Notifiable Disease Reporting System (NNDRS) has been put into service nationwide since 2004. In accordance with the Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases, Center for Disease Control and Prevention (CDCs), medical institutions, blood collection and supply institutions and their staff shall report the cases of infectious diseases that they detect through the specialized network within the required time limit.

2.1.2 Outpatient records and inpatient records of all medical institutions (including community health service centers and various clinics) in Wuhan from 1 October to 10 December 2019.

#### 2.2 Data collection

2.2.1 Wuhan Center for Disease Control and Prevention (Wuhan CDC) searched the NNDRS for COVID-19 cases with the onset date in 2019, and clinical experts were organized to review and discuss the medical records of all cases. Moreover, Wuhan CDC conducted an in-depth epidemiological study of all the cases, mainly focusing on the travel history, occupational exposure, market exposure, animal contact and social contact and other exposure history. Taking into consideration the epidemiological study results and the opinions of clinical experts, 174 cases of COVID-19 were identified with onset date in 2019, including 100 laboratory-confirmed cases and 74 clinically diagnosed cases.

2.2.2 The medical institutions searched and registered all the four categories of patients with fever, influenza-like illness, acute respiratory infection and unspecified pneumonia. Clinical physicians, laboratories and imaging experts investigated and verified the four categories of patients one by one, and to determine whether each case met the COVID-19 suspected case standard based on all available clinical manifestations, laboratory tests, imaging examinations and other information of the patients. For the patients identified as suspected cases, Wuhan Municipal Health Commission conducted epidemiological study and serum detection of coronavirus antibody.

Suspected case evaluation criteria

We should combine epidemiological history and clinical manifestations for a comprehensive analysis.

Epidemiology history

- A history of travel or residence in the community where COVID-19 was reported within 14 days prior to onset;
- ➤ A history of exposure to SARS-CoV-2 infection or asymptomatic COVID-19 infection within 14 days prior to onset.
- A person with fever or respiratory symptoms who had come into contact with the community where the case was reported within 14 days prior to onset of illness
- Cluster disease (2 or more cases of fever and/or respiratory symptoms within 2 weeks in a small area such as home, office, school, class, etc.)

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# Clinical manifestations

- Covid-19 related clinical manifestations such as fever and/or respiratory symptoms.
- COVID-19 imaging characteristics such as Subpleural lesions and/or patchy consolidation and ground-glass opacity.
- ➢ In the early stage of the disease, the total number of white blood cells is normal or decreased, and the lymphocyte count is normal or decreased.
- $\geq$

# Evaluation criteria

Any 1 epidemiological history + any 2 clinical manifestations = suspected

0 epidemiological history + 3 clinical manifestations = suspected

0 epidemiological history + 2 clinical manifestations + SARS-COV-2-IgM positive = suspected

## Evaluation procedure

An expert group was formed including 2 radiological experts, 3 respiratory clinical experts, and 1 infectious disease clinical expert to evaluate each suspected case. The cases were selected by local clinical doctors from 233 hospitals in Wuhan. Each case was evaluated by the 6 experts from the perspective of clinical characteristics, disease process, CT image, laboratory tests, and treatment response. When there were different views on each case, a consensus would be decided. Finally, all the cases selected by the local health committee of Wuhan were regarded as not supportive for the diagnosis of COVID-19.

## 2.3 Analytical Methods

2.3.1 Through analyzing the temporal and spatial distribution of the incidence and the population characteristics, the study will address the trend of early COVID-19 epidemic, and preliminarily determine the outbreak process and transmission path.

2.3.2 Through analyzing different exposure histories of confirmed cases, market, animal, and other relevant products, the study will figure out whether there was clusters of cases with different exposure histories, which could provide the direction for origin tracing.

2.3.3 Through searching suspected cases from four types of patients and conducting epidemiological study on suspected cases, the study will identify possible early cases of COVID-19, which could provide the direction for origin tracing.

2.3.4 Among the early cases, cluster cases were defined as those with two or more cases found within 14 days in a small area, such as the workplace or home.

3. Findings

3.1 Overview

A total of 174 COVID-19 cases with onset date in 2019 in Wuhan were found in the NNDRS, including 100 laboratory-confirmed cases and 74 clinically diagnosed cases.

A search was conducted on all the fever patients, influenza-like illness patients, acute respiratory tract infection patients and unspecified pneumonia patients in all medical institutions in Wuhan. No suspected COVID-19 cases were found.

3.2 Epidemiological Characteristics

#### 3.2.1 Temporal distribution

Among the 174 cases, the earliest onset was on December 8, followed by a gradual increase, clinically diagnosed cases appeared on December 16, and reached the peak on December 30 (a total of 30 cases), as shown in Fig. 2.



Fig. 2. Epidemiological curve of the 174 COVID-19 cases.

#### 3.2.2 Spatial distribution

All the 174 COVID-19 cases live in Hubei Province, including 164 in Wuhan City. 51 of the 174 cases were related to the Huanan Market while the other 113 were not. Besides, it was not clear for six cases whether they were related to the market. See Figs. 3 and 4 for the spatial distribution according to whether they were linked to the market.



Fig. 3. Spatial distribution of the 174 cases by home address.



Fig. 4. Spatial distribution of the 164 cases living in Wuhan by home address.

# 3.2.3 Population distribution

Among the 174 cases, 98 were male and 76 were female. The male-to-female ratio was 1:0.78, the median age was 56 years old, the oldest was 92 years old, the youngest was 22 years old, and the interquartile range was from 46 to 67 years old, as shown in Fig. 5.

Compared with the entire population in Wuhan by age and gender (Fig. 6), the "40-", "50-" and "60-" age groups accounted for a higher proportion among the 174 cases (70.7% vs 42.3%).



Fig. 5. 174 COVID-19 cases - by age and gender.



Fig. 6. Entire population in Wuhan - by age and gender in 2020 (ten thousand).

Among the 174 cases, retirees and commercial services accounted for the largest proportion, 38.5% (67 cases) and 35.1% (61 cases), respectively. Among them, there were two cases of health care workers, as shown in Fig. 7. Fig. 8 shows the onset curve of the top four occupations.



Fig. 7. Occupational distribution of 174 COVID-19 cases.



Fig. 8. Onset curves of various occupations: A: retirees; B: commercial services; C: housework; D: office clerk.

#### (III) Exposure history

Among the 174 COVID-19 cases, those with exposure history to the Huanan Market accounted for 32.7% (55 cases), the highest proportion of all exposure history. The proportion of cases with exposure history to dead animals was 26.4% (39 cases), the proportion of cases with exposure history to live animals was 11.8% (18 cases), the proportion of cases with exposure history to cold-chain products was 26.4% (29 cases), and the proportion of cases with a travel history was 8.9% (15 cases), as shown in Table 1.

Type of exposure	Yes		No	Total*	
Type of exposure	n	%	n	%	1000
Market	93	55.4	75	44.6	168
Huanan Market only	47	28.0	121	72.0	168
Others only	38	22.6	130	77.4	168
Huanan Market and others	8	4.8	160	95.2	168
Dead animals	39	26.4	109	73.6	148
Live animals	18	11.8	134	88.2	152
Cold-chain products	29	26.4	81	73.6	110
Travel history	15	8.9	144	93.5	154

Table 1. Exposure history of 174 COVID-19 cases

\* Excluding cases with unknown exposure history.

#### 1. Market exposure history

Among the 168 cases, 93 cases had been to markets, 47 cases had only been to the Huanan Market, and 38 cases had only been to other markets. Among the 38 cases, 3 clusters (2 persons in each group) had been to the same market, the remaining 32 cases had been to different markets. Other 8 cases had been to both the Huanan Market and other markets. According to the onset date, the peak incidence of cases with market exposure history was earlier than cases without market exposure history, as shown in Figure 9.



Fig. 9. onset curve of 168 cases breakdown by with or without market exposure history.

According to an analysis of 168 COVID-19 cases with or without a history of exposure to the Huanan Market, the first case had no history of exposure to the Huanan Market, and the incidence of cases with and without history of exposure to the Huanan Market basically increased simultaneously. However, the cases with exposure history decreased after reaching the peak on 25 December, and the cases without exposure history reached the peak on 30 December, as shown in Fig. 10.



Fig. 10. Morbidity curve of 168 COVID-19 cases with or without a history of exposure to Huanan Market.

#### 2. History of dead animals exposure

Among 148 COVID-19 cases, 39 cases had a history of dead animals exposure. Both of the first and the second COVID-19 cases had a history of dead animals exposure, and the incidence rate of cases without a history of dead animals exposure was higher than that of cases with exposure history, as shown in Figure 11.



Fig. 11. onset curve of 148 cases with or without a history of dead animals exposure.

#### 3. History of live animals exposure

Among 152 COVID-19 cases, there were 18 cases with a history of live animal exposure, and the onset date was uniformly distributed, as shown in Fig. 12.



Fig. 12. Onset curve of 152 cases with or without a history of live animals exposure.

#### 4. History of cold-chain exposure

Among 110 COVID-19 cases, 29 cases had a history of cold-chain exposure. The onset of the first case was on 15 December. It showed an increasing trend before 25 December, and then gradually decreased, as shown in Fig. 13.



Fig. 13. Onset curve of 110 cases with or without a history of cold-chain exposure.

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#### 5. Travel history



Among 154 COVID-19 cases, 15 cases had travel history, and the onset date of the first case was 15 December, with an irregular distribution, as shown in Fig. 14.

Fig. 14. Incidence curve of 110 cases with or without travel or residence history.

(IV) Analysis of cluster cases

Among the 174 cases, as to whether there were two or more cases found within 14 days of onset in the same area, a total of seven cluster outbreaks were identified and 15 cases, including 13 laboratory-confirmed cases and two clinically diagnosed cases were involved.

## 1. Temporal distribution

Among the 15 cluster cases, the earliest onset date was 15 December 2019, and the last onset date was 31 December 2019, as shown in Fig. 15.



Fig. 15. Cluster outbreaks among the 174 cases.

Among the seven clusters of cases (Fig. 16), there were two clusters with high possibility of market infection, four clusters with high possibility of family infection, and one cluster happened at home due to the introduction from market infection.



Fig. 16. Seven cluster outbreaks.

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# Spatial distribution

The first stage of onset: 8-11 December 2019, cases were sporadic.

The second stage of onset: 8-16 December 2019, the number of cases increased, and clusters of cases which were family-related emerged.

The third stage of onset: 8-21 December 2019, more patients were infected. And clusters of cases were on the rise, among which the market-related clusters increased significantly.

The fourth stage of onset: 8-26 December 2019, the number of cases continued to rise, and clusters of cases included market-related clusters and family-related ones.

The fifth stage of onset: 8-31 December 2019, more and more patients were infected, clusters increased, and the cases spread to even larger areas, as shown in Fig. 17.



Fig.17. Spatiotemporal distribution of cluster and sporadic cases.

#### 3. Population distribution

Among the 15 cluster cases, 10 were male and 5 were female. The ratio of male to female was 2:1. The ages ranged from 32 to 70 years old, with a median age of 58 years.

In terms of the occupations of cases, there were 6 cases of commercial service, 6 cases of retirees, 2 cases of housework, and 1 case of office clerk. Among the 15 cases, there were 6 cases related to the Huanan Market. Among them, there were 5 vendor cases at fixed stalls in the Huanan Market and 1 case of long-term purchasers in the Huanan Market.

#### 4. Analysis of the source of infection in cluster cases

Personal details were available to the joint team, but are excluded here for protecting the privacy of the individuals.

**Cluster 1:** Including two confirmed cases, living together as husband and wife. Both of them denied case contact history, as well as history of exposure to Huanan Market. Spouse one, 62 years old, fell ill on 15 December 2019, spouse two, 62 years old, fell ill on 26 December 2019.



Source of infection: Spouse one had a travel history to Thailand in November 2019, so imported infection cannot be ruled out. The married couple had bought shrimps from a Supermarket at Yangchahu, so cold chain food contact history cannot be ruled out either. The couple denied history of exposure to Huanan Market, however they had purchased and contacted chickens slaughtered in a market at Yangchahu, they might have been exposed to infection in other markets. The onset interval between the two was 11 days, because the couple lived together, it cannot rule out the possibility that spouse one was infected by spouse two.

**Cluster 2:** there were 3 confirmed cases, all of whom were traders of the same stall in Huanan Market. Stall employee one, 40 years old, fell ill on 17 December 2019; stall employee two, 32 years old, fell ill on 19 December 2019; stall employee three, 57 years old, fell ill on 25 December 2019. It was a fixed stall in Huanan Market, dealing in frozen products such as pastry and soy products. Employee two was purchasing goods from the Baishazhou market and Huanan Market back and forth. Employee three was delivering goods in Huanan Market.



Possible sources of infection: employee one might be infected from environmental exposure in Huanan Market, employee two and employee three might be infected from market environmental exposure or human to human transmission.

**Cluster 3:** there were two confirmed cases, living together as husband and wife, and both of them denied animal contact history and history of travel. Spouse one, 61 years old, fell ill on 20 December 2019; Spouse two, 57 years old, fell ill on 25 December 2019. Spouse one had been engaged in restaurant distribution for a long time, and often stocked up in Huanan Market. Spouse two denied a history of exposure to Huanan Market or other markets.



Possible source of infection: Spouse one might be infected from environmental exposure in Huanan Market, and spouse two was likely to be infected from spouse one.

**Cluster 4:** There were two confirmed cases, both of whom were employees of the same stall in Huanan Market, and both of them denied contact history of poultry and animals, as well as contact history of travel. Employee one, 56 years old, fell ill on 20 December 2019; employee two, 45 years old, fell ill on 26 December 2019. It was a fixed stall in the Huanan Market, dealing in aquatic products such as catfish and perch.



Possible source of infection: Employee one might be infected from environmental exposure in Huanan Market, and employee two might be infected from environmental exposure in Huanan Market or human-to-human transmission.

**Cluster 5:** there were two confirmed cases, living together as husband and wife. Both of them denied exposure to Huanan Market, confirmed exposure to Jinyinhu Farmers Market, denied contact history of poultry and animals, and denied history of travel. Spouse one, 57 years old, fell ill on 22 December 2019; spouse two, 58 years old, fell ill on 24 December 2019.



Possible source of infection: Spouse one be infected from Jinyinhu market environmental exposure or community exposure; They might be infected from human-to-human transmission or Jinyinhu market environmental exposure or community exposure.

**Cluster 6:** There were two clinically diagnosed cases, living together as husband and wife. Both of them denied exposure to the Huanan Market, confirmed exposure to Yangchahu market and the Gusaoshu market, denied animal contact history, and denied exposure history of travel. Spouse one, 70 years old, fell ill on 26 December 2019; Spouse two, 70 years old, fell ill on 28 December 2019.



Possible source of infection: Spouse one might be infected from market environmental exposure or community exposure, and Spouse two might be infected from market environmental exposure or human-to-human transmission or community exposure.

**Cluster 7:** There were two confirmed cases, living together as husband and wife. None of them had links with Huanan Market. Both of them denied animal contact history and travel history. Spouse one, 62 years old, fell ill on 30 December 2019; Min X, male, 70 years old, fell ill on 31 December 2019. Shen had visited a small supermarket near Huanan Market, and Min had visited a market at Changgang Road.

Case 1	Visited a small supermarket near HN market, within 2 weeks before onset	12/30 Onset	1/1 Visit a doctor Admission		2/2 Diagnosis	2/7 Dead
Case 2	Visited a farmer's market, and living with Case 1	12 Or Visit a Adm	/31 iset doctor ission	1/1 Diagr	L9 nosis	
Case 2						

Possible source of infection: might be infected from supermarket environmental exposure or community exposure, and spouse two might be infected from market environmental exposure or human-to-human transmission or community exposure.

# Annex E3 - Case definitions: evolution over time

Version	Issue Date	Epidemiological history	Clinical manifestations	Suspected case	Clinically diagnosed case	Confirmed case	mild case	common case	severe case	critical case
VI	2020-01-15	(1) Travel history in Wuhan within 14 days before the onset of illness; (2) Direct and indirect exposure to related markets in Wuhan especially farmers market in Wuhan within 14 days before the onset of illness.	<ul> <li>(1) fever;</li> <li>(2) With the imaging characteristics of pneumonia mentioned above;</li> <li>(Multiple small patchy shadows and interstitial changes appeared early, and the extrapulmonary bands were obvious. Furthermore, it develops multiple ground glass infiltration and infiltrates in both lungs. In severe cases, pulmonary consolidation may occur, but pleural effusion are rare.)</li> <li>(3) The total number of white blood cells is normal or decreases, or</li> </ul>	Observed case: Have any one of the epidemiol ogical history and have the clinical manifestati ons.	NA	Observed cases with whole genome sequence of virus isolated from respiratory specimens such as sputum and throat swab is highly homologo us to known 2019- nCoV.	NA	NA	NA	Cases meeting any of the following criteria: (1) Respirato ry failure ; (2) Septic Shock; (3) With other concomit ant organ failure that requires ICU monitorin g and treatment

the lymphocyte count decreases in the early stage of disease. (4) After three days of standard antibiotic treatment, the disease had no significant improvement but had progressive aggravation.

V2	2020- 01-18	(1) Travel history or residence history in Wuhan within 14 days before the onset of illness; (2) Contact history with patient who had fever and concomitant respiratory symptom from Wuhan within 14 days before the onset of illness; (3) Cluster.	<ol> <li>Fever;</li> <li>With the imaging characteristics of pneumonia mentioned above;</li> <li>(Multiple small patchy shadows and interstitial changes appeared early, and the extrapulmonary bands were obvious.</li> <li>Furthermore, it develops multiple ground glass infiltration and infiltrates in both lungs. In severe cases, pulmonary consolidation may occur, but pleural effusion are rare.)</li> <li>(3) The total number of white blood cells is normal or decreases, or the lymphocyte count decreases in the early stage of disease</li> </ol>	Suspected cases (cases originally placed under observatio n): Have any one of the epidemiol ogical history and have the clinical manifestati ons.	NA	Suspected cases with one of the following pathogeni c evidence: 1. 2019- nCoV nucleic acid is tested positive in respiratory specimens such as sputum, throat swab, and lower respiratory secretion by real- time fluorescen t RT-PCR; 2. Gene sequence of virus is highly homologo us to known 2019- nCoV.	NA	NA	Cases meeting any of the following criteria: (1) Increased respiratory rate ( $\geq$ 30 breaths/ min), difficulty in breathing, and cyanosis of lips; or When inhaling air, oxygen saturation is $\leq$ 95%; or Arterial partial pressure of oxygen (PaO2)/ fraction of inspired oxygen (FiO2) $\leq$ 300 mmHg (1 mmHg=O. 133kPa); (2) Pulmonary imaging shows multilobular lesions or lesion progression exceeding	Same with V1 Cases meeting any of the following criteria: (1) Respirato ry failure ; (2) Septic Shock; (3) With other concomit ant organ failure that requires ICU monitorin g and treatment
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NA

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50% within 48 hours; (3) qSOFA score  $\geq 2$ : (4) CURB-65 score ≧ 1: (5) With concomitant aerothorax; (6) Other clinical conditions that require hospitalizati on.

01-22 (1) Travel history or residence history in Wuhan within 14 days before the onset of illness; (2) Contact history with patient who had fever and concomitant respiratory symptom from Wuhan within 14 days before

the onset of

Same with V2

Same with V2 Same with (1) Fever; V2 (2) With the Suspected cases characteristics (cases ofpneumonia originally mentioned placed under (Multiple small observatio patchy shadows n): and interstitial Have any one of the appeared early, epidemiol ogical extrapulmonary history and bands were have the clinical Furthermore, it manifestati ons.

imaging

above:

changes

and the

obvious.

develops

V2 Suspected cases with one of the following pathogeni c evidence: 1.2019nCoV nucleic acid is tested positive in respiratory specimens such as sputum.

Same with NA

NA

Cases Cases meeting any meeting ofthe any of following the criteria: following criteria: (1) Increased (1) respiratory rate ( $\geq 30$ Respirato breaths/ ry failure min), and requiring difficulty in mechanic breathing. and cyanosis al ventilatio of lips; (2) When n; inhaling air, (2) Shock; oxygen (3) With saturation is ≦93% other

2020-V3

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illness; multiple ground (3) Cluster.

glass infiltration and infiltrates in both lungs. In severe cases, pulmonary consolidation may occur, but pleural effusion are rare.) (3) The total number of white blood cells is normal or decreases, or the lymphocyte count decreases in the early stage of disease.

throat swab, and lower respiratory secretion by realtime fluorescen t RT-PCR: 2. Gene sequence of virus is highly homologo us to known 2019nCoV.

(3) Arterial concomit partial ant organ failure pressure of oxygen that (PaO2)/ requires fraction of ICU inspired monitorin g and oxygen (FiO2)≦300 treatment mmHg(1 mmHg=O. 133kPa); (4) Pulmonary imaging shows multilobular lesions or lesion progression exceeding 50% within 48 hours; (5) With other concomitant clinical conditions that require hospitalizati on.

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V4	2020- 01-28	(1) Travel history or residence history in Wuhan area or other areas with sustained local transmission within 14 days before the onset of illness; (2) Contact history with patient who had fever or respiratory symptom from Wuhan City or other areas with sustained local transmission within 14 days before the onset of illness; (3) Cluster or has epidemiological link to NCP infection person.	Same with V2 (1) Fever; (2) With the imaging characteristics of pneumonia mentioned above; (Multiple small patchy shadows and interstitial changes appeared early, and the extrapulmonary bands were obvious. Furthermore, it develops multiple ground glass infiltration and infiltrates in both lungs. In severe cases, pulmonary consolidation may occur, but pleural effusion are rare.) (3) The total number of white blood cells is normal or decreases, or the lymphocyte count decreases in the early stage of disease.	Have any one of the epidemiol ogical history and have any two of the clinical manifestati ons.	NA	Suspected cases with one of the following pathogeni c evidence: 1. 2019- nCoV nucleic acid is tested positive in respiratory specimens or blood specimens by real- time fluorescen t RT-PCR; 2. Gene sequence of virus isolated from respiratory specimens or blood specimens is highly homologo us to known 2019- nCoV.	NA	Cases who have fever or respirator y symptom s, and imaging shows pneumon ia	Cases meeting any of the following criteria: (1) Respiratory distress (RR ≥30 breaths/ min); (2) At rest, oxygen saturation is ≤ 93% (3) Arterial partial pressure of oxygen (PaO2)/ fraction of inspired oxygen (FiO2)≤300 mmHg (1 mmHg=O. 133kPa).	Same with V3 Cases meeting any of the following criteria: (1) Respirato ry failure and requiring mechanic al ventilatio n; (2) Shock; (3) With other concomit ant organ failure that requires ICU monitorin g and treatment
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V5	2020- 02-04	<ul> <li>(1) Travel history or residence history in Wuhan and surrounding areas or other community with reported NCP patient within 14 days before the onset of illness;</li> <li>(2) Contact history with NCP infection person (2019- nCoV nucleic acid positive) within 14 days before the onset of illness;</li> <li>(3) Contact history with patient who had fever or respiratory symptom from Wuhan and surrounding areas or other community with reported NCP patient within 14 days before the onset of illness;</li> <li>(4) Cluster.</li> </ul>	Provinces other than Hubei Province: (1) Fever and/or respiratory symptom; (2) With the imaging characteristics of pneumonia mentioned above; (Multiple small patchy shadows and interstitial changes appeared early, and the extrapulmonary bands were obvious. Furthermore, it develops multiple ground glass infiltration and infiltrates in both lungs. In severe cases, pulmonary consolidation may occur, but pleural effusion are rare.) (3) The total number of white blood cells is normal or decreases, or	Provinces other than Hubei Province: Have any one of the epidemiol ogical history and have any two of the clinical manifestati ons. Or without obvious epidemiol ogical history, but have three of the clinical manifestati ons. Province: With any one of the epidemiol ogical history or without epidemiol ogical history or without epidemiol ogical history or without epidemiol ogical history or without epidemiol ogical history, but have two of the epidemiol ogical history or without epidemiol ogical history, but have two of the clinical	Hubei Province: Suspeted case with imaging characteristics of pneumonia mentioned above ;	Same with V4 Suspected cases with one of the following pathogeni c evidence: 1. 2019- nCoV nucleic acid is tested positive in respiratory specimens or blood specimens by real- time fluorescen t RT-PCR; 2. Gene sequence of virus isolated from respiratory specimens or blood specimens isolated from respiratory specimens or blood specimens isolated from respiratory specimens is highly homologo us to known 2019- nCoV.	The clinical symptom s were mild, and there was no sign of pneumon ia on imaging	Same with V4 Cases who have fever or respirator y symptom s, and imaging shows pneumon ia	Same with V4 Cases meeting any of the following criteria: (1) Respiratory distress (RR ≥30 breaths/ min); (2) At rest, oxygen saturation is ≤ 93% (3) Arterial partial pressure of oxygen (PaO2)/ fraction of inspired oxygen (FiO2)≤300 mmHg (1 mmHg=O. 133kPa).	Same with V3 Cases meeting any of the following criteria: (1) Respirato ry failure and requiring mechanic al ventilatio n; (2) Shock; (3) With other concomit ant organ failure that requires ICU monitorin g and treatment
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			the lymphocyte count decreases in the early stage of disease.	manifestati ons.						
			Hubei Province: (1) Fever and/or respiratory symptom; (2) The total number of white blood cells is normal or decreases, or the lymphocyte count decreases in the early stage of disease.							
V5 revisio n	2020- 02-08	Same with V5	Same with V5	Same with V5	Same with V5	Same with V5	Same with V5	Same with V5	Same with V5	Same with V5

V6 2020- 02-18	Same with V5 (1) Travel history or residence history in Wuhan and surrounding areas or other community with reported NCP patient within 14 days before the onset of illness; (2) Contact history with NCP infection person (2019- nCoV nucleic acid positive) within 14 days before the onset of illness; (3) Contact history with patient who had fever or respiratory symptom from Wuhan and surrounding areas or other community with reported NCP patient within 14 days before the onset of illness; (4) Cluster.	Same with the part of provinces other than Hubei Province in V5: (1) Fever and/or respiratory symptom; (2) With the imaging characteristics of pneumonia mentioned above; (Multiple small patchy shadows and interstitial changes appeared early, and the extrapulmonary bands were obvious. Furthermore, it develops multiple ground glass infiltration and infiltrates in both lungs. In severe cases, pulmonary consolidation may occur, but pleural effusion are rare.) (3) The total number of white blood	Same with the part of provinces other than Hubei Province in V5: Have any one of the epidemiol ogical history and have any two of the clinical manifestati ons. Or without obvious epidemiol ogical history, but have three of the clinical manifestati ons.	ΝΑ	Suspected cases with one of the following pathogeni c evidence: 1. 2019- nCoV nucleic acid is tested positive by real- time fluorescen t RT-PCR; 2. Gene sequence of virus is highly homologo us to known 2019- nCoV.	Same with V5 The clinical symptom s were mild, and there was no sign of pneumon ia on imaging	Same with V4 Cases who have fever or respirator y symptom s, and imaging shows pneumon ia	Cases meeting any of the following criteria: (1) Shortness of breath ( $RR \ge 30$ ) breaths/ min); (2) At rest, oxygen saturation is $\le 93\%$ (3) Arterial partial pressure of oxygen ( $PaO2$ )/ fraction of inspired oxygen ( $FiO2$ ) $\le 300$ mmHg (1 mmHg=O. 133kPa). PaO2/FiO2 should be corrected at high altitudes (over 1000 m) according to the following formula: PaO2/FiO2 × [Atmospheri c pressure (mmHg)/760	Same with V3 Cases meeting any of the following criteria: (1) Respirato ry failure and requiring mechanic al ventilatio n; (2) Shock; (3) With other concomit ant organ failure that requires ICU monitorin g and treatment
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cells is normal or decreases, or the lymphocyte count decreases in the early stage of disease. ] (4) Pulmonary imaging showed significant lesion progression to > 50% within 24 to 48 hours

V7	2020- 03-03	<ul> <li>(1) Travel history or residence history in</li> <li>Wuhan and surrounding areas or other community with reported NCP patient within 14 days before the onset of illness;</li> <li>(2) Contact history with NCP infection person (2019- nCoV nucleic acid positive) within 14 days before the onset of illness;</li> <li>(3) Contact history with patient who had fever or respiratory symptom from Wuhan and surrounding areas or other community with reported NCP patient within 14 days before the onset of illness;</li> <li>(4) Cluster (within two</li> </ul>	<ul> <li>(1) Fever and/or respiratory symptom;</li> <li>(2) With the imaging characteristics of pneumonia mentioned above;</li> <li>(Multiple small patchy shadows and interstitial changes appeared early, and the extrapulmonary bands were obvious.</li> <li>Furthermore, it develops multiple ground glass infiltration and infiltrates in both lungs. In severe cases, pulmonary consolidation may occur, but pleural effusion are rare.)</li> <li>(3) The total number of white blood cells is normal or decreases, or the lymphocyte count is normal</li> </ul>	Have any one of the epidemiol ogical history and have any two of the clinical manifestati ons. Or without obvious epidemiol ogical history, but have three of the clinical manifestati ons.	NA	Suspected cases with one of the following pathogeni c or serologica l evidence: 1. 2019- nCoV nucleic acid is tested positive by real- time fluorescen t RT-PCR; 2. Gene sequence of virus is highly homologo us to known 2019- nCoV. 3. COVID- 19 IgM or IgG is positive in serum, or a significant (>4-fold) rise in COVID- 19 IgG	Same with V5 The clinical symptom s were mild, and there was no sign of pneumon ia on imaging	Same with V4 Cases who have fever or respirator y symptom s, and imaging shows pneumon ia	Adult cases meeting any of the following criteria: (1) Shortness of breath (RR $\geq$ 30 breaths/ min); (2) At rest, oxygen saturation is $\leq$ 93% (3) Arterial partial pressure of oxygen (PaO2)/ fraction of inspired oxygen (FiO2) $\leq$ 300 mmHg (1 mmHg=O. 133kPa). PaO2/FiO2 should be corrected at high altitudes (over 1000 m) according to the following formula: PaO2/FiO2 × [Atmospheri c pressure	Same with V3 Cases meeting any of the following criteria: (1) Respirato ry failure and requiring mechanic al ventilatio n; (2) Shock; (3) With other concomit ant organ failure that requires ICU monitorin g and treatment.
		weeks, in a	or decreases in			concentrat			(mmHg)/760	

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small range such as a family, an office, a classroom in school, 2 or more cases with fever and/or respiratory symptom were detected).	the early stage of disease.	ions between acute and convalesc ent sera.	] (4) Pulmonary imaging showed significant lesion progression to > 50% within 24 to 48 hours
			Child cases meeting any of the following criteria: (1) Shortness of breath (RR $\geq 60$ breaths/min for under 2- month-old, RR $\geq 50$ breaths/min for 2~12 month-old, RR $\geq 40$ for 1~5 year-old, RR $\geq 30$ for older than 5 year-old) except for effect from fever and crying; (2) At rest, oxygen saturation is $\leq 92\%$
(3) Assisted breathing (groaning, wing flaps, triconcave sign), cyanosis, intermittent apnea; (4) Drowsiness and convulsions; (5) Refuse to feed or difficult to feed, with signs of dehydration.

# Annex E4- Analysis of market-related cases and the cluster outbreak in the Huanan Market

### Background

The papers published show that 66% of the 41 confirmed cases in Wuhan before 2 January 2020 were once exposed to the Huanan Market (Huang et al. 2020). Therefore, investigating the possible source of the outbreak in the Huanan Market, the earliest reported epidemic site, is of great significance to the global origin tracing of SARS-CoV-2. This study will focus on the possible source of the Huanan Market epidemic and explore the SARS-CoV-2 transmission mechanism in the market, so as to provide clues for the origin tracing of SARS-CoV-2.

Materials and methods

#### (I) Data source

i Infectious disease epidemic network and epidemiological study

The information of cases is mainly collected through the direct reporting system of infectious disease information network and retrospective questionnaires done by the confirmed cases, including general information such as age, gender and home address, as well as the time of onset, market exposure history, exposure history with other patients, animal exposure history, and other onset and exposure related information.

#### ii Research on Huanan Market

The information of business operators and vendors in Huanan Market was collected from the market administration authority, including:

(a) The information of 678 operators, including the location of stalls, types of stores, types of goods, source of purchase (company name and address), whether the cold chain system and imported goods were involved;

(b) Information on 1,162 vendors, including gender, age, stall locations, types of goods sold, contact history with cold chain system, animals and imported goods.

### (II) Analysis methods

By describing the temporal and spatial distribution characteristics of epidemiology in different populations, comparing the possible association and joint exposure factors of the early cases, and analysing the characteristics of epidemic curve of populations with different market exposure history, the time points of community and market transmission and the possible sources of Huanan Market outbreak are explored; by comparing the morbidity risk of population under different exposures, the epidemic transmission mechanism in the Huanan Market is discussed.

#### Results

#### (I) Market exposure

There were 174 diagnosed cases of COVID-19 with onset date prior to 31 December 2019 (including those retrospectively diagnosed) in Wuhan. 6 cases with unknown market exposure history, 55.4% (93/168) of the cases had a history of market exposure. Among them, 28.0% were only exposed to the Huanan Market, and 22.6% were only exposed to other markets. 26.4% of the early cases were exposed to cold-chain food in markets. The earliest cases had no history of exposure to the Huanan Market, but had a history of exposure to other markets, the history of cold-chain exposure was unknown.

Туре	Yes		No		Total
	n	%	n	%	Total
All market	93	55.4	75	44.6	168
Only Huanan	47	28	121	72	168
Only Other	38	22.6	130	77.4	168
Mixed	8	4.8	160	95.2	168
Cold chain	29	26.4	81	73.6	110

Table 1. Market exposure of early cases.

According to whether the cases had a history of market exposure, the epidemic curves are drawn respectively (Fig. 1). It is found that all the first four known cases had market exposure history; after late December, a large number of cases without market exposure history appeared.



Fig. 1. Epidemic curve of people with and without market exposure history.

Among the cases with market exposure history, 51% (47/93) of the cases were only exposed to the Huanan Market. Further analysis of the cases that were only exposed to other markets found that, except for 9 cases with unknown market exposure history, the remaining 29 cases were related to 27 markets, which were relatively scattered, and no early cases were exposed to the same market as the first case. Therefore, it is necessary to focus on the Huanan Market regarding the role of markets in the Wuhan outbreak.

#### (II) Huanan Market exposure

(i) About the Huanan Market

The Huanan Market was located in Jianghan District, Wuhan City, with a total construction area of 50 000 square meters. It was divided into east area and west area by Xinhua Road. The west area mainly dealt in fresh aquatic products, frozen seafood, dried seafood, poultry meat and vegetables, while the east area mainly dealt in frozen livestock meat, fresh prawns and dried seasonings.

There were 678 fixed business operators in the market, with some business operators selling multiple types of goods. In terms of the types of goods sold (Table 2), the market mainly dealt in fresh water aquatic products and seafood, followed by livestock meat and poultry meat; in addition, the market also dealt in vegetables and other goods (including cereals and oils, seasonings, dried fruits, prepackaged food, disposable tableware, hardware and daily necessities). There were 10 stalls selling animals, mainly dealing in meats of domesticated wild animals, such as snakes, bamboo rats and turtledoves.

Types of goods	Number of stalls (N=678)	Composition ratio (%)
Freshwater aquatic products	300	44
Seafood	267	39
Livestock meat	160	24
Poultry meat	115	17
Vegetables	58	8.6
Meat of domesticated animals	10	1.5
Others	178	26

Table 2. The composition of the types of goods in the Huanan Market.

Note: Those selling two or more types of goods at the same time were separately counted in different groups.

(ii) Demographic characteristics of the cases

Most of the market-related cases are occupational population (Table 3), mainly male, mostly between 40 and 60 years old. 94% of the cases related to the Huanan Market were directly exposed. Among them, market vendors and purchasers accounted for 77% of all cases, which was significantly more than passers-by, buyers, deliverymen, visitors, and those who were indirectly exposed to the Huanan Market, indicating that the way and frequency of exposure in the market may be related to the morbidity risk.

		Number of cases (N=55)	Composition ratio (%)
Gender			
	male	37	67
	female	18	33
Age			
	20-	3	5.5
	30-	7	13
	40-	20	36
	50-	16	29
	60-	7	13
	70-	2	3.6
Types of exposure			
Direct exposure	Vendor <sup>a</sup>	30	55
	Purchaser <sup>b</sup>	12	22
	Passer-by <sup>c</sup>	5	9.1
	Buyer <sup>d</sup>	3	5.5
	Deliveryman <sup>e</sup>	2	3.6
	Visitors <sup>f</sup>	1	1.8
Indirect exposure	Contact of the Huanan Market exposed population	2	3.6

Table 3. General information of cases.

<sup>a</sup> Owners who have fixed stalls in the market or their employees;

<sup>b</sup> Purchasing food materials at different stalls for hotels, restaurants, etc.;

<sup>c</sup> Passing by the market without making a purchase;

<sup>d</sup> Community residents who purchase food for their families in the market;

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<sup>e</sup>Delivering certain goods to designated merchants;

<sup>f</sup>Looking for someone in the market, without purchasing.

(III) Analysis of the time points of community transmission and Huanan Market transmission

The cases are further classified according to their history of exposure to the Huanan Market, and the epidemic curve is shown in Figure 2. Among them, cases with a history of exposure to the Huanan Market account for 33% (55/168); the first known case (8 December) had no history of exposure to Huanan Market (none of the patient, family members and other contacts had history of exposure to Huanan Market). The market he visited was RT-Mart supermarket in Jiangxia District, which was more than 20km away from the Huanan Market. After the first case with history of exposure to Huanan Market appeared on December 11, Huanan Market-related cases increased rapidly, and reached a peak (nine cases) on 25 December 2019.



Fig. 2. Epidemic curve of cases with and without history of exposure to Huanan Market

According to the type of association, the cases associated with the Huanan Market are further classified into vendors and visitors. The epidemic curve is shown in Fig. 3. Among them, both of the first two cases were visitors (11 and 12 December); the first vendor case appeared on 13 December and reached a peak on 25 December (7 cases).



Fig. 3. Epidemic curve of cases for vendor and visitors of Huanan Market.

(IV) Analysis of the possibility of virus introduced into the market

(i) Analysis of the possibility of virus introduced into the market by human

Through the analysis of the onset date of vendors, purchasers and other people directly exposed to Huanan Market (Fig. 4), it was found that the onset of purchasers and vendors were the earliest, while the onset of cases exposed by other ways were later than 20 December, indicating that vendors and purchasers deserve more attention in the analysis of the source of the Huanan Market outbreak. The onset of purchasers (12 December) was earlier than that of vendors (13 December), but the difference was only one day, which was within a common incubation period. Therefore, it is impossible to judge whether the purchasers and vendors with the earliest onset are the same generation of cases caused by joint exposure, or the infection of vendors were caused by the virus introduced into the market by purchasers.



Fig. 4. Epidemic curve of cases for directly exposed to Huanan Market.

(ii) Analysis of the possibility of virus introduced into the market through joint exposure

Through the analysis of the exposure information of 2 purchasers and 3 vendors with the earliest onset in the Huanan Market (Table 4), it was found that none of the 5 cases had similar exposure history. Both of the two purchasers were seafood purchasers, but there were no fixed purchasing stalls; 2 of the 3 vendors sold seafood, and further study on the source of their purchase found that no one in other stalls selling seafood from the same source in Huanan Market was infected, so the possibility of infection caused by the exposure of seafood sold in these two stalls was not high. The purchasers with the earliest onset may have had contact with some vendors, but it cannot be ruled out that they have been exposed to other common risk factors, such as asymptomatic infection or contaminated stall environment.

Serial Number	Way of exposure	Date of onset	Types of goods	History of contact with other animals	Travel history	History of case contact
Case 1	purchasing	2019/12/12	seafood	No	No	No
Case 2	vending	2019/12/13	seafood, aquatic products, livestock meat, and frozen poultry meat	No	No	No
Case 3	purchasing	2019/12/17	Seafood	Yes (poultry meat)	Yes	No
Case 4	vending	2019/12/17	Frozen products such as pastry and soy products	No	No	No
Case 5	vending	2019/12/18	Seafood	No	No	No

Table 4. Exposure information of five cases with the earliest onset in Huanan Market

(V) The spread characteristics of the Huanan Market epidemic in early stage

In order to further understand the spread characteristics of the Huanan Market epidemic in early stage, and to understand the impact of different goods exposures, cold chains and other market factors on the spread of the epidemic in the market, we have further analysed 30 vendor cases.

(i) Temporal and spatial distribution of cases in the Huanan Market

The spatial distribution of early cases in the Huanan Market was analysed weekly (Fig. 5). It was found that the cases before 20 December were all distributed in the west area. The first case occurred in the east area in the third week, and the total number of cases in east area was significantly less than those in the west area.



Fig. 5. The spatial distribution of early cases in the market weekly.

### (ii) Types of goods sold by the cases

Among the 30 cases (Fig. 6), 50% (15/30) of the patients dealt in aquatic products, followed by seafood 47% (14/30). This result was consistent with the overall composition of people dealing in different goods in the Huanan Market: 48% and 42% of the vendors in the Huanan Market were involved in aquatic products and seafood business respectively. There were no cases found to be domesticated animal vendors.



Fig. 6. Distribution of vendor cases among stalls selling different food.

Note: since the same vendor sells multiple types of goods at the same time, the same case may belong to different group of exposed goods at the same time.

#### (iii) Morbidity rate of vendors

The morbidity rate of vendors dealing in various types of goods is shown in Table 5. Among them, the morbidity rate of vegetable vendors is the highest, which is 4.6% (5/108), followed by poultry vendors, which is 3.5% (8/230).

The spatial distribution of vegetable vendors and 5 cases in the market is shown in Fig. 7. It can be seen from the figure that these 5 cases had obvious spatial clustering characteristic.

Group	Number of cases	Total	Morbidity (%)
Vegetables	5	108	4.6
Poultry meat	8	230	3.5
Seafood	14	484	2.9
Livestock meat	9	318	2.8
Freshwater aquatic products	15	559	2.7
Others	3	266	1.1
Meat of domesticate animal	0	15	0.0

Table 5. Morbidity rate of vendors by type of sold goods

Note: there were 1162 vendors and 30 confirmed cases in total, so the same case and vendor may belong to different group of sold goods.



Fig.7. Distribution of vegetable stalls and vendor cases

#### (iv) The impact of exposure to cold chain and imported goods on morbidity

In view of previous studies, the cold chain environment is conducive to the survival and spread of the virus, therefore we have analysed the impact of the cold chain exposure history on the spread of virus in the Huanan Market. Table 6 shows the morbidity rates of vendors with different level of cold chain exposure, calculated with merchants and vendors as the analysis unit respectively. The results show that the morbidity rate of stalls with cold chain was significantly higher than stalls without cold chain (5.6% vs. 1.7%), and the morbidity rate of people with cold chain exposure was also higher than people without cold chain exposure (3.3% vs. 1.4%), and the morbidity rate of people exposed to non-imported cold chain are slightly higher than those exposed to imported goods with cold chain (3.4% vs. 1.7%). Figure 8 shows the epidemic curve of vendor cases with and without exposure to cold chains. Among them, the earliest 3 cases all had a history of exposure to cold chain.

		Stalls		Vendors	
Cold chain	Imported 100d	With case	Morbidity (%)	Case	Morbidity (%)
Yes		22	5.6	24	3.3
	Yes	1	3.2	1	1.7
	No	21	5.8	23	3.4
No		5	1.7	6	1.4

Table 6. Morbidity rate of vendors and stalls – by cold-chain.



Fig. 8. Epidemic curve of vendor cases with and without exposure to cold-chain.

#### 4.5 Market environment

Environmental samples were collected from 134 stalls in the Huanan Market for SARS-CoV-2 nucleic acid test (Table 7). It was found that 21 business environments tested positive in the SARS-CoV-2 nucleic acid test, and 7 of them appeared cases. Among the 113 stalls with negative environmental NATs results, 9 of them have confirmed cases. The relative risk of cases in stalls with positive nucleic acid testing results is 4.2 times that of stalls with negative results (RR=4.2, 95%CI: 1.8-10). It can also be seen from Figure 9 that the number of cases in areas with positive environmental nucleic acid testing results is relatively higher.

Environment samples	With case	Morbidity (%)
Positive(n=21)	7	33
Negative(n=113)	9	8.0

Table 7. Morbidity rate of stalls – by environment(n = 134).



Fig. 9. Distribution of the stalls with cases and the results of SARS-CoV-2 environmental nucleic acid test.

#### Discussion

1. There are two possibilities where the epidemic first occurred, and neither the community nor the market could be ruled out.

2. The Huanan Market may not be the first place where the epidemic occurred.

3. There are two possibilities for the introduction of viruses into the Huanan Market, and neither human nor goods could be ruled out.

4. The Huanan Market may act as an "amplifier" in the early stage of the epidemic, and the contaminated environment may be a joint exposure factor leading to the infection of market-related cases.

#### Reference

(1) Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet, 2020, 395(10223):497-506.

# ANNEXF: Animal and environment

Further information on animals and environment

Member	Species affected	Date of first report
Hong Kong SAR	Dog and cat	21/03/2020
Belgium	Cat	28/03/2020
	Feline (tiger, lion, cat),	0(104/2020
United States of America	dog, mink	06/04/2020
Netherlands	Mink	26/04/2020
France	Cat	02/05/2020
	Mink	25/11/2020
Spain	Cat	11/05/2020
	Mink	16/07/2020
Germany	Cat and dog	13/05/2020
Russian Federation	Cat	26/05/2020
Denmark	Mink	17/06/2020
United Kingdom of Great Britain and Northern Ireland	Cat	28/07/2020
Japan	Dog	07/08/2020
	Cat	06/11/2020
South Africa	Puma	11/08/2020
Italy	Mink	30/10/2020
	Cat	09/12/2020
Sweden	Mink	29/10/2020
Chile	Cat	22/10/2020
Canada	Dog	28/10/2020

Table 1. Reported cases of animals infected with SARS-CoV-2, from (1).

	Mink	09/12/2020
Brazil	Cat	29/10/2020
Greece	Mink	16/11/2020
Argentina	Cat and dog	18/11/2020
Lithuania	Mink	30/11/2020
Switzerland	Cat	03/12/2020
United States of America	Gorilla	11/01/2021

Table 2. Animal species infected in laboratory susceptibility studies.

	Species	Susceptibility	Symptom	Transmission capacity
	Ferret	High	Yes, a few	Horizontal transmission
	Rabbit	High	No	No
T in the la	Racoon dog	High	No	Horizontal transmission
Livestock	Cattle	Very low	No	No
	Pig	Very low	No	No
	Poultry (chicken, duck, turkey)	No	No	No
Pets	Cat	High	Yes, some	Horizontal transmission
	Dog	Low	No	No
Wild animals	Pangolin (Malay)	High	No	Horizontal transmission
	Fruit bat	High	No	Horizontal transmission

	North American possums	High	No	Horizontal transmission
	Golden hamster	High	Yes, various	Horizontal transmission
Other animals	Monkeys (Rhesus monkeys, crab-eating monkeys, African green monkeys)	High	Yes, various	Horizontal transmission
	Marmoset	High	No	Horizontal transmission
	Tree shrew	High	No	Unknown



Fig. 1. Electron micrograph of negatively-stained SARS-CoV-2 virions from environmental samples of Huanan market



Fig. 2 Transmission electron micrograph of SARS-CoV-2 cultured from environmental swabbing of Huanan market.

Table 3: List of supplier type, location and animal products for all vendors selling wildlife products at Huanan Market.

To protect private and commercial information, names of companies have in most cases been redacted. Source, Market Authorities.

Serial number for Vendors	Animal type	Sources	
West 8-25 (1012)	Snake	Snake companies, Jingshan county, Hubei Yuchuan town, Wuxue city, Hubei	
	Snake	Special breeding garden, Junshan District, Yueyang city, Hunan	
West 9-38 (1102)	Bamboo rat	Bamboo rat breeding corporation, Nanzhang county, Xiangyang City, Hubei	
	Porcupine		
	Sika deer	Sika deer farms, Shuangyang District, Changchun city, Jilin	
	Porcupine	Porcupine Farms, Suizhou city, Hubei	
West 9-34,36 (1104)	Snake	Snake farms, Jianli county, Hubei	
	Bamboo rat	Bamboo rat farms, Liucheng county, Guangxi,	

	Bamboo rat	Bamboo rat farms, Baojing town, Hunan	
West 9-35,37 (1114)	rabbit	Zhoukou city, Henan	
	Badger	Macheng City, Hubei province	
	Snake	Snake farms, Jianli county, Hubei Snake farms, Yueyang city, Hunan	
	Giant salamander	Giant salamander farms, Hanzhong city, Shaanxi	
	Snake	Shanyang county, Shaanxi	
West 8 26 28 (1122)	Bamboo rat	Bamboo farms, Yongde county, Yunnan	
west 8-30,38 (1122)	Estuarine crocodile	Huangsha market, Guangzhou city, Guangdong	
	Siamese crocodile	Huangsha, Guangzhou	
	Pheasant	Sanliqiao, Huangpi district, Wuhan city, Hubei	
	Giant salamander	Guangzhou city, Guangdong	
	Snake	Xiangyang city, Hubei	
West 6-29,31,33 (1134)	Rabbit	Zhoukou city, Henan	
	Pheasant	Yangxin county, Huangshi city, Hubei	
	Hedgehog	Unknown private breeder	
West 9-31,33 (1138)	Dove	Aquatic product market, Wuhan city, Hubei	
	Duck	Aquatic product market, Wuhan city, Hubei	
	Checken	Aquatic product market, Wuhan city, Hubei	
	Goose	Aquatic product market, Wuhan city, Hubei	

	Rabbit	Zhoukou city, Henan	
	Snake	West 6-29,31,33	
	Frozen mutton	Meat company, Daqing city, Heilongjiang	
East attached street 9- 31,33 (1161)	Sika deer	Sika deer farms, Shuangyang District, Changchun, Jilin	
	Giant salamander	Giant salamander farms, Tianmen city, Hubei	
	Duck	Food company, Jinmen city, Hubei	
	Sheep and goat	taiping town, Zaoyang city, Xiangyang city, Hubei	
	Beef	Beef processing plant, Qiaokou District, Wuhan city, Hubei	
	Siamese crocodile	Crocodile Industries Inc. Guangdong	
East attached street 6 (5148)	Snake	Farms, Xiaogan city, Hubei Special breeding garden, Junshan District, Yueyang city, Hunan	
West 10-29,31 (5149)	Snake	Snake farms, Nanchang, Jiangxi	

### Statement on presence of live mammals in Huanan Market in 2014.

Statement from Professor EC Holmes delivered by email to Dr Peter Daszak on 26 February 2021 re. photographs from (2): "These photographs were taken by me on 29 October 2014 at Huanan Market, Wuhan. I was visiting Wuhan to meet with Prof. ZHANG Yong-Zhen and other collaborators at Wuhan and we all visited the market. We witnessed live animals for sale, including snakes, what I believe are raccoon dogs, and other unidentified rodents. I witnessed an animal that may have been raccoon dog being clubbed to death in front of me."

**Table 4. Testing of fecal samples from 1856 Hubei province bats for coronaviruses.** Above: Table to show number and species of bats sampled and tested for coronaviruses (family-level primers). Below: Details of the bat-CoV sequences identified.

Family	amily Genus Species		The number of samples tested for CoVs	
-	-	Unknown species (For feces)	1115	
Vespertilionidae	Eptesicus	Eptesicus.sp	1	
	Myotis	Myotis ricketti	10	
		Myotis cf. davidii	12	
		Myotis altarium	10	
		Myotis daubentonii	11	
		Myotis.sp	9	
	Pipistrellus	Pipistrellus abramus	32	
		Pipistrellus pipistrellus	12	
	Miniopterus	Miniopterus schreibersi	6	
		Miniopterus fuliginosus	4	
		Hipposideridae.sp	2	
Rhinolophidae	Hipposideros	Hipposideridae pratti	86	
		Hipposideros armiger	205	
	Rhinolophus	Rhinolophus affinis	28	
		Rhinolophus pusillus	7	
		Rhinolophus ferrumequinum	12	
		Rhinolophus pearsonii	8	
		Rhinolophus macrotis Blyth	3	
		Rhinolophus sinicus	165	
		Rhinolophus.sp	118	

Number of samples tested	Number positive for alpha- and beta-CoVs	Number positive for SARSr-CoVs	Sequence identity to SARS coronavirus Tor2 /SARS-COV-2 (nt,based on about 400bp RdRp sequences)
1856	Beta-CoVs (119)	SARS-related CoVs (36) /	SARS coronavirus Tor2 (87%~97%)
		R. ferrumequinum, R. macrotis, R. sinicus, R.pusillus	SARS-COV-2 isolate WIV04 (74‰-90%)
		Bat Hp-betacoronavirus Zhejiang2013 related CoVs (78)	-
	Alpha-CoVs (76)	-	-

# References

(1) OIE. Infection with SARS-CoV-2 in animals. Paris: OIE; 2020.

(2) Zhang YZ, Holmes EC. A genomic perspective on the origin and emergence of SARS - CoV-2. *Cell*,(2020).

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Updated Assessment on COVID-19 ORIGINS

# Updated Assessment on COVID-19 Origins

### **Key Takeaways**

Scope Note: This assessment responds to the President's request that the Intelligence Community (IC) update its previous judgments on the origins of COVID-19. It also identifies areas for possible additional research. Annexes include a lexicon, additional details on methodology, and comments from outside experts. This assessment is based on information through August 2021.

The IC assesses that SARS-CoV-2, the virus that causes COVID-19, probably emerged and infected humans through an initial small-scale exposure that occurred no later than November 2019 with the first known cluster of COVID-19 cases arising in Wuhan, China in December 2019. In addition, the IC was able to reach broad agreement on several other key issues. We judge the virus was not developed as a biological weapon. Most agencies also assess with low confidence that SARS-CoV-2 probably was not genetically engineered; however, two agencies believe there was not sufficient evidence to make an assessment either way. Finally, the IC assesses China's officials did not have foreknowledge of the virus before the initial outbreak of COVID-19 emerged.

After examining all available intelligence reporting and other information, though, the IC remains divided on the most likely origin of COVID-19. All agencies assess that two hypotheses are plausible: natural exposure to an infected animal and a laboratory-associated incident.

- Four IC elements and the National Intelligence Council assess with low confidence that the initial SARS-CoV-2 infection was most likely caused by natural exposure to an animal infected with it or a close progenitor virus—a virus that probably would be more than 99 percent similar to SARS-CoV-2. These analysts give weight to China's officials' lack of foreknowledge, the numerous vectors for natural exposure, and other factors.
- One IC element assesses with moderate confidence that the first human infection with SARS-CoV-2 most likely was the result of a laboratory-associated incident, probably involving experimentation, animal handling, or sampling by the Wuhan Institute of Virology. These analysts give weight to the inherently risky nature of work on coronaviruses.
- Analysts at three IC elements remain unable to coalesce around either explanation without additional information, with some analysts favoring natural origin, others a laboratory origin, and some seeing the hypotheses as equally likely.
- Variations in analytic views largely stem from differences in how agencies weigh intelligence reporting and scientific publications and intelligence and scientific gaps.

The IC judges they will be unable to provide a more definitive explanation for the origin of COVID-19 unless new information allows them to determine the specific pathway for initial natural contact with an animal or to determine that a laboratory in Wuhan was handling SARS-CoV-2 or a close progenitor virus before COVID-19 emerged.

• The IC—and the global scientific community—lacks clinical samples or a complete understanding of epidemiological data from the earliest COVID-19 cases. If we obtain information on the earliest cases that identified a location of interest or occupational exposure, it may alter our evaluation of hypotheses.

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China's cooperation most likely would be needed to reach a conclusive assessment of the origins of COVID-19. Beijing, however, continues to hinder the global investigation, resist sharing information, and blame other countries, including the United States. These actions reflect, in part, China's government's own uncertainty about where an investigation could lead as well as its frustration the international community is using the issue to exert political pressure on China.

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# IC Assessments of COVID-19 Origins



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### Introduction

The IC has prepared several assessments examining the origins of COVID-19. Analysts have focused on whether SARS-CoV-2, the causative virus of COVID-19, was genetically engineered—particularly as a biological weapon—was transmitted to humans naturally or transmitted due to a laboratory-associated incident, perhaps during sampling or experimentation. China's reaction to and handling of the pandemic have given analysts insights into these issues, but Beijing's actions have also impeded the global scientific community and our ability to confidently determine how the virus first infected humans.

# SARS-CoV-2 Probably Not a Biological Weapon

The IC assesses China did not develop SARS-CoV-2 as a biological weapon.

• We remain skeptical of allegations that SARS-CoV-2 was a biological weapon because they are supported by scientifically invalid claims, their proponents do not have direct access to the Wuhan Institute of Virology (WIV), or their proponents are suspected of spreading disinformation. [*See appendix B.*]

## Most Analysts Assess SARS-CoV-2 Not Genetically Engineered

Most IC analysts assess with low confidence that SARS-CoV-2 was not genetically engineered. Their assessment is based on technical analysis of SARS-CoV-2 and the IC's growing understanding of traits and the potential for recombination in other coronaviruses. Two agencies believe there is not sufficient evidence to make an assessment either way.

• As of August 2021, we still have not observed genetic signatures in SARS-CoV-2 that would be diagnostic of genetic engineering, according to the IC's understanding of the virus. Similarly, we have not identified any existing coronavirus strains that could have plausibly served as a backbone if SARS-CoV-2 had been genetically engineered.

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 Our growing understanding of the similarities of SARS-CoV-2 to other coronaviruses in nature and the ability of betacoronaviruses—the genus to which SARS-CoV-2 belongs—to naturally recombine suggests SARS-CoV-2 was not genetically engineered. For instance, academic literature has noted that in some instances betacoronaviruses have recombined with other viruses in nature and that furin cleavage sites (FCS)—a region in the spike protein that enhances infection—have been identified in naturally occurring coronaviruses in the same genetic location as the FCS in SARS-CoV-2. This suggests that SARS-CoV-2 or a progenitor virus could have acquired its FCS through natural recombination with another virus.

IC analysts do not have higher confidence that SARS-CoV-2 was not genetically engineered because some genetic engineering techniques can make modifications difficult to identify and we have gaps in our knowledge of naturally occurring coronaviruses.

- Some genetic engineering techniques may make genetically modified viruses indistinguishable from natural viruses, according to academic journal articles. For instance, a 2017 dissertation by a WIV student showed that reverse genetic cloning techniques—which are standard techniques used in advanced molecular laboratories—left no trace of genetic modification of SARS-like coronaviruses.
- It will be difficult to increase our confidence that the distinguishing features in SARS-CoV-2 emerged naturally without a better understanding of the diversity of coronaviruses in nature and how often recombination occurs during co-infection of multiple coronaviruses within a particular host. For example, academic literature has indicated that a FCS had previously been inserted into SARS-CoV-1, the causative agent of SARS, complicating differentiation of how such a feature may have appeared.

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## Closest Known Relatives of SARS-CoV-2, as of August

As of August, the closest known whole genome match to SARS-CoV-2—around **96 percent** identical—is **RaTG13**, a coronavirus collected from a bat in 2013 by the Wuhan Institute of Virology (WIV), according to academic literature. Scientific literature examining the genome of SARS-CoV-2 has identified at least some similarities to those of other naturally occurring coronaviruses in bats and pangolins, but an immediate precursor virus strain and animal reservoir have not been identified.

VIRUS NAME	PERCENT IDENTITY TO COVID-19 VIRUS	YEAR COLLECTED	LOCATION COLLECTED	ANIMAL COLLECTED
RaTG13	96.2	2013	Yunnan Province, China	Bat
RpYN06	94.5	2020	Yunnan Province, China	Bat
RmYN02	93.3	2019	Yunnan Province, China	🕨 Bat
RShSTT200	92.7	2010	Cambodia	Bat
RacCS203	91.5	2020	Thailand	Bat
PrC31	90.7	2018	Yunnan Province, China	🕨 Bat
Pangolin-CoV Guangdong	90.1	2019	Guangdong Province, China	Pangolin
ZC45	88.1	2015	Zhejiang Province, China	🝆 Bat
ZXC21	88.0	2015	Zhejiang Province, China	Bat
Guangxi pangolin-CoV	85.5	2017, 2018	Guangxi Zhuang Autonomous Region, China	Arr Pangolin
Rc-0319	79.2	2013	Japan	🝆 Bat
RaTG15	77.6	2015	Yunnan Province, China	🕨 Bat

• The WIV previously created chimeras, or combinations, of SARS-like coronaviruses, but this information does not provide insight into whether SARS-CoV-2 was genetically engineered by the WIV.

No IC analysts assess that SARS-CoV-2 was the result of laboratory adaptation, although some analysts do not have enough information to make this determination. Repeated passage of a closely related virus through animals or cell culture—which we consider laboratory adaptation and not genetic engineering—could result in some features of SARS-CoV-2, according to publicly available information. However, it probably would take years of laboratory adaptation using the appropriate cell types and a virus that is more closely related to SARS-CoV-2 than ones currently known to generate the number of mutations separating SARS-CoV-2 from any known coronavirus strains, judging from scientific journal articles. Such processes would require differentiation and maintenance of primary cells and the development of appropriate animal models.

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# China's Lack of Foreknowledge of SARS-CoV-2

The IC assesses China's officials probably did not have foreknowledge that SARS-CoV-2 existed before WIV researchers isolated it after public recognition of the virus in the general population. Accordingly, if the pandemic originated from a laboratory-associated incident, they probably were unaware in the initial months that such an incident had occurred.

• Early in the pandemic, the WIV identified that a new virus was responsible for the outbreak in Wuhan. It is therefore assessed that WIV researchers pivoted to COVID-19-related work to address the outbreak and characterize the virus. These activities suggest that WIV personnel were unaware of the existence of SARS-CoV-2 until the outbreak was underway.

## Two Plausible Hypotheses of Pandemic Origin

IC analysts assess that a natural origin and a laboratoryassociated incident are both plausible hypotheses for how SARS-CoV-2 first infected humans. Analysts, however, disagree on which is more likely, or whether an assessment can be made at all, given the lack of diagnosticity of the available information. Most agencies are unable to make higher than low confidence assessments for these reasons, and confidence levels are tempered by plausible arguments for the opposing hypothesis. For these hypotheses, IC analysts consider an exposure that occurs during animal sampling activity that supports biological research to be a laboratoryassociated incident and not natural contact. What follows is a look at the cases that can be made for these competing hypotheses.

# The Case for the Natural Origin Hypothesis

Some IC analysts assess with low confidence that the first human COVID-19 infection most likely was caused by natural exposure to an animal that carried SARS-CoV-2 or a close progenitor virus—a virus that would likely be more than 99 percent similar to SARS-CoV-2. Four IC elements, the National Intelligence Council, and some analysts at elements that are unable to coalesce around either explanation are among this group. Analysts at these agencies give weight to China's officials' lack of foreknowledge and highlight the precedent of past novel infectious disease outbreaks having zoonotic origins, the wide diversity of animals that are susceptible to SARS-CoV-2 infection, and the range of scenarios-to include animal trafficking, farming, sale, and rescue—in China that enable zoonotic transmission. Although no confirmed animal source of SARS-CoV-2 has been identified, to include a reservoir or intermediate species, analysts that assess the pandemic was due to natural causes note that in many previous zoonotic outbreaks, the identification of animal sources has taken years, and in some cases, animal sources have not been identified.

- These analysts assess that WIV's activities in early 2020 related to SARS-CoV-2 are a strong indicator that the WIV lacked foreknowledge of the virus.
- They also see the potential that a laboratory worker inadvertently was infected while collecting unknown animal specimens to be less likely than an infection occurring through numerous hunters, farmers, merchants, and others who have frequent, natural contact with animals.
- Given China's poor public health infrastructure and the potential for asymptomatic infection, some analysts that lean towards a natural origin argue that China's infectious disease surveillance system would not have been able to detect the SARS-CoV-2 exposure as quickly as a suspected exposure in a laboratory setting.

## History of Zoonotic Pathogen Emergence, Conditions in China Ripe for Zoonotic Spillover

Analysts that find the natural zoonotic spillover hypothesis the most likely explanation for the pandemic also note the wide diversity of animals that are susceptible to SARS-CoV-2 infection, range of scenarios—to include animal trafficking, farming, sale, and rescue—in China that would enable zoonotic

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# **Comparing COVID-19 Pandemic to Past Select Viral Zoonotic Outbreaks**

Location of Emergence		Asymptomatic Infection Common	Reservoir Species and Year Identified	Probable Intermediate Species and Year Identified	
<b>COVID-19</b> (2019– Present)	China	Yes	Unknown	Unknown	
<b>Ebola</b> (2014-16)	Guinea	No (Probably)	Bats (Probably); N/A	Nonhuman primate (Probably); N/A	
<b>MERS</b> (2012)	Saudi Arabia, Jordan	Yes	Bats (Probably); N/A	Dromedary camels; 2013	
<b>SARS</b> (2002-04)	China	No (Probably)	Horseshoe bats 2016	; Masked palm civets and Raccoon dogs (Possibly); 2003	
<b>Nipah</b> (1998-99)	Malaysia	Yes	Fruit bats; 1999	Pigs; 1998	
<b>HIV-1</b> ª (1970s– Present)	Democratic Republic of Congo (Probably)	No (Probably)	Chimpanzees (Probably); 1999	N/A	

transmission, and precedent of novel human infectious disease outbreaks originating from zoonotic transmission. Previous human coronavirus outbreaks, to include SARS-CoV-1 and Middle East Respiratory Syndrome coronavirus (MERS-CoV), occurred naturally and were linked to animal reservoirs with zoonotic transmission to humans, according to scientific literature.

- Extensive wildlife and livestock farming, wildlife trafficking, and live animal markets in China and historically lax government regulation—and even promotion—of these activities increase the probability that initial transmission occurred along one of these routes.
- Academic literature has revealed Wuhan markets sold live mammals and dozens of species—including raccoon dogs, masked palm civets, and a variety of other mammals, birds, and reptiles—often in poor conditions where viruses can jump among species, facilitating recombination events and the acquisition of novel mutations. SARS-CoV-2 can infect a range of mammals, including cats, dogs, pangolins, minks, raccoon dogs, and a variety of wild and domestic animals, according to academic literature.
- Wider Hubei Province has extensive farming and breeding of animals that are susceptible to SARS-CoV-2, including minks and raccoon dogs.

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These analysts note that there is a precedent for viral vectors to travel long distances in China and cause infection elsewhere because of transportation and trade nodes, thereby widening and complicating the search for the specific zoonotic spillover incident. For instance, the bat coronavirus that is currently the closest known relative to the original SARS-CoV-1 was identified in Yunnan Province, even though the first SARS outbreak detected in humans occurred in Guangdong Province, hundreds of kilometers away.

### The Case for the Laboratory-Associated Incident Hypothesis

One IC element assesses with moderate confidence that COVID-19 most likely resulted from a laboratoryassociated incident involving WIV or other researchers-either through exposure to the virus during experiments or through sampling. Some analysts at elements that are unable to coalesce around either explanation also assess a laboratory origin with low confidence. These analysts place emphasis on academic articles authored by WIV employees indicating that WIV scientists conducted research on other coronaviruses under what these analysts consider to be inadequate biosafety conditions that could have led to opportunities for a laboratory-associated incident. These analysts also take into account SARS-CoV-2's genetic epidemiology and that the initial recorded COVID-19 clusters occurred only in Wuhan-and that WIV researchers who conducted sampling activity throughout China provided a node for the virus to enter the city.

### WIV Research Includes Work With Animals That Carry Relatives of SARS-CoV-2

The analysts that find the laboratory-associated origin theory most likely assess that WIV researchers' inherently risky work with coronaviruses provided numerous opportunities for them to unwittingly become infected with SARS-CoV-2. Although the IC has no indications

#### WIV Illnesses in Fall 2019 Not Diagnostic

The IC assesses that information indicating that several WIV researchers reported symptoms consistent with COVID-19 in autumn 2019 is not diagnostic of the pandemic's origins. Even if confirmed, hospital admission alone would not be diagnostic of COVID-19 infection.

that WIV research involved SARS-CoV-2 or a close progenitor virus, these analysts note that it is plausible that researchers may have unwittingly exposed themselves to the virus without sequencing it during experiments or sampling activities, possibly resulting in asymptomatic or mild infection. Academic literature indicates that WIV researchers conducted research with bat coronaviruses or collected samples from species that are known to carry close relatives of SARS-CoV-2.

- Based on currently available information, the closest known relatives to SARS-CoV-2 in bats have been identified in Yunnan Province, and researchers bringing samples to laboratories provide a plausible link between these habitats and the city.
- These analysts also note that China's investigations into the pandemic's origin might not uncover evidence of a laboratory-associated incident if it involved only a small number of researchers who did not acknowledge or have knowledge of a potential infection.

### Biosafety Conditions for Specific Work Could Have Led to an Incident

The analysts that assess COVID-19 most likely originated from a laboratory-associated incident also place emphasis on information suggesting researchers in China used biosafety practices that increased the risk of exposure to viruses. Academic publications suggest that WIV researchers did not use adequate biosafety precautions at least some of the time, increasing the risk of a laboratory-associated incident.

## The Role of the Huanan Seafood Wholesale Market

Some scientists and China's public health officials have shifted their view on the role of the Huanan Seafood Wholesale Market in the pandemic since early 2020. Some now view the market as a potential site of community spread rather than where the initial human infection may have occurred.

- On January 1, 2020, China's security authorities shut down the market after several workers fell ill in late December 2019. China focused early source tracing on the market and Hubei Province; association with the market was included as part of the early case definition.
- In January 2020, a scientific article that described clinical features of initial COVID-19 infections in China found that some COVID-19 patients did not have any known association with the market. Furthermore, there continues to be conflicting data with some academic articles and preprints noting that phylogenetic analysis of the available data on the earliest cases suggests that the progenitor virus may not have originated from the market.

## China's Transparency Key to Determining COVID-19 Origin

The IC judges that closing persistent information gaps on the origins of COVID-19 is very likely to require greater transparency and collaboration from Beijing. The scientific community lacks technical data on a reservoir species, possible intermediate species, and closer relatives to SARS-CoV-2.

Data and Samples From Initial Cases: The global scientific community does not know exactly where, when, or how the first human infection with SARS-CoV-2 occurred. It lacks a complete picture of the initial cases in Wuhan—or potentially elsewhere in China—that would allow it to better understand potential sources of infection or conduct phylogenetic analysis that would help validate both hypotheses.

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**Information That Would Confirm Natural Outbreak:** Searching for a natural reservoir or potential intermediate host requires collecting, isolating, and sequencing viruses from samples taken from potential host species and environments to search for viruses related to SARS-CoV-2, endeavors that require international collaboration, resources, and time.

- Information that the earliest confirmed COVID-19 cases were in individuals or families who spent time in rural regions or who were involved in animal trade or environments that facilitate close human-to-animal interactions could indicate that the virus was circulating within an animal reservoir and a zoonotic spillover event caused the first COVID-19 case in humans.
- However, some transmission pathways are fleeting, meaning an animal acquires a virus and evidence of infection vanishes, particularly if the animals are reared and harvested for agricultural or commercial purposes.

**Information That Would Confirm Laboratory-Associated Incident:** China's coronavirus research or related information from origins investigations by Beijing or international organizations could provide clear indications of a laboratory-associated incident or at least yield some new insights.

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## WIV's Publicly Available Coronavirus Research

IC analysts are examining published research from China for any indicators that would inform our understanding of COVID-19's origins. The WIV and other research groups in China published coronavirus articles in 2020 and 2021, including the discovery of the closest known relative of SARS-CoV-2, but at least some relevant data on coronaviruses of interest has either been unavailable or has not been published.

Although the WIV described the sampling trip to the mineshaft in Mojiang in Yunnan Province where it collected RaTG13 in 2016, it did not explicitly state that RaTG13 was collected from that mine until 2020. Similarly, the WIV collected eight other coronaviruses from the same mine in 2015 that it did not fully disclose until 2021. In some of these instances, however, the WIV has described unpublished work in webinars and interviews prior to publishing.

### **China Likely To Impede Investigation**

The IC judges they will be unable to provide a more definitive explanation for the origin of COVID-19 unless new information allows them to determine the specific pathway for initial natural contact with an animal or to determine that a laboratory in Wuhan was handling SARS-CoV-2 or a close progenitor virus before COVID-19 emerged.

- For instance, Beijing limited the World Health Organization (WHO) investigation team's access to sites.
- In late July, China denounced a WHO plan for future investigations into COVID-19 origins, claiming that the proposal for future investigations was politicized. China's officials publicly rebuked the WHO's plans for a future study of labs in China,

saying Beijing would not allow the WHO to engage in the "conspiracy theory."

China is also pushing its narrative that the virus originated outside China.

- Public statements from China's Government have continued to claim the virus originated from imported frozen food, an extremely unlikely theory.
- China's Government continues to spread allegations that the United States created or intentionally spread SARS-CoV-2 to divert attention away from Beijing.

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## **Annex A: Definitions**

**Antibody:** A protein produced during an immune response to a part of an infectious agent called an antigen.

**Backbone:** A genetic sequence used as a chassis upon which to build synthetic constructs, such as those used for cloning, protein expression, and production.

**Biological weapon:** A weapon that uses bacteria, viruses, toxins, fungi, and biochemical/biomolecule agents that can cause death or injury to humans, plants, or animals or destroy materials.

**Biosafety:** The application of knowledge, techniques, and equipment to prevent personal, laboratory, and environmental exposure to potentially infectious agents or biohazards. Four **Biosafety levels (BSL)** define the containment conditions under which biological agents can be safely manipulated. These standards range from moderate safety requirements for low-risk agents (BSL-1), to the most stringent controls for high-risk agents (BSL-4). China's standards range from P1–4.

**Biosecurity:** The protection, control of, and accountability for biological agents, toxins, and biological materials and information to prevent unauthorized possession, loss, theft, misuse, diversion, and accidental or intentional release.

**Coronavirus:** A common type of virus that can infect humans and/or animals. The human illness caused by most coronaviruses usually last a short time and presents symptoms consistent with the "common cold," such as a runny nose, sore throat, cough, and a fever.

**COVID-19:** An infectious disease caused by the **SARS-CoV-2** virus, which is a betacoronavirus.

**Diagnostic information:** Information that allows IC analysts to distinguish between hypotheses—in this case, the laboratory origin and natural origin theories.

**DNA (deoxyribonucleic acid):** A molecule that carries an organism's genetic blueprint for growth, development, function, and reproduction.

**Epidemiology:** The study of the distribution and determinants of health-related events in specified populations, and the application of this study to prevent and control health problems.

**Furin cleavage site (FCS):** A region in the spike protein of SARS-CoV-2 that enhances infection.

**Gain-of-function:** The IC considers this as a research method that involves manipulating an organism's genetic material to impart new biological functions that could enhance virulence or transmissibility (e.g., genetically modifying a virus to expand its host range, transmissibility, or severity of illness). The IC assesses that genetic engineering, genetic modification, and laboratory-adaptation can all be used for gain-of-function experiments, but are not inherently so. We address both genetic engineering and laboratory-adaptation in the body of this assessment; the IC is unaware of an agreed, international definition.

**Genetically engineered or genetically modified viruses** are intentionally altered, created, or edited using biotechnologies, such as Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR), DNA recombination, or reverse genetics. These viruses have intentional, targeted edits to the genome designed to achieve specific results, but unintentional genomic changes may also occur.

**Genome:** The genetic material of an organism. It consists of DNA (and sometimes RNA for viruses).

**Genome sequencing:** The process of determining the DNA or RNA sequence of an organism's genome, or its "genetic code." An organism's genetic code is the order in which the four nucleotide bases—adenine, cytosine, guanine, and thymine—are arranged to direct the sequence of the 20 different amino acids in the proteins that determine inherited traits.

**Intermediate species/host:** An organism that can be infected with a pathogen from a resevoir species and

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passes the pathogen to another host species; infection is not sustained in this population.

Laboratory-adapted viruses have undergone natural, random mutations through human-enabled processes in a laboratory—such as repeated passage through animals or cells—that put pressure on the virus to more rapidly evolve. Specific changes to the viral genome are not necessarily anticipated in these processes, though the virus can be expected to gain certain characteristics, like the ability to infect a new species. This is a common technique used in public health research of viruses. We consider directed evolution to be under laboratory adaptation.

Laboratory-associated incidents include incidents that happen in biological research facilities or during research-related sampling activities.

**Molecular biology:** Study of the molecular basis of activities in and between cells. This includes techniques to amplify or join genetic sequences.

**Naturally occurring viruses** have not been altered in a laboratory. Viruses commonly undergo random mutations as part of the evolutionary process and can continue to change over time; mutations may enable a virus to adapt to its environment, such as evading host immune responses and promoting viral replication.

**Outbreak:** A sudden increase in occurrences of a disease in a particular time and place. Outbreaks include **epidemics**, which is a term that is reserved for infectious diseases that occur in a confined geographical area. **Pandemics** are near-global disease outbreaks.

**Pangolin:** An African and Asian mammal that has a body covered in overlapping scales. Pangolins are a natural reservoir of coronaviruses and researchers are investigating their potential role as an intermediate host for SARS-CoV-2.

**Pathogen:** A bacterium, virus, or other microorganism that can cause disease.

**Phylogenetics:** The study of the evolutionary relationships among groups of organisms.

**Progenitor virus:** A virus that is closely related enough—probably more than 99 percent—to SARS-CoV-2 to have been its direct ancestor or plausible immediate origin of the outbreak. The closest known relative to SARS-CoV-2 is only around 96 percent similar; to put this into context, humans and chimps are around 99 percent similar, demonstrating the significant differences even at this similarity.

**RaTG13:** A coronavirus with the closest known whole genome to SARS-CoV-2, although it is widely believed to not be a direct ancestor of SARS-CoV-2.

**Resevoir species/host:** An organism that harbors a pathogen, which is endemic within the population.

**RNA (ribonucleic acid):** A molecule essential for gene coding, decoding, regulation, and expression. Certain viruses use RNA as a genetic blueprint.

**Transmissibility:** The measure of new infections initiated by an existing infection.

**Virus:** A replicating piece of genetic material—DNA or RNA—and associated proteins that use the cellular machinery of a living cell to reproduce.

Wet market: A market where fresh food and live and dead animals, including wildlife, are sold.

**Zoonosis:** An infection or a disease that is transmissible from animals to humans under natural conditions. A **zoonotic pathogen** may be viral, bacterial, or parasitic, and can sometimes be transmitted through insects, such as mosquitoes.

**Zoonotic spillover:** An initial infection or disease that is caused by contact between an animal and human under natural conditions.

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# Annex B: IC Examination of Open-Source Theories

IC analysts have examined a number of open-source articles from a variety of sources that have raised theories about SARS-CoV-2 and COVID-19's origin. The IC assesses that these theories generally do not provide diagnostic information on COVID-19 origins, and in some cases, are not supported by the information available to us. However, several have drawn on insightful methods or identified potential leads.

# Theory of Abnormal Activity at the WIV in Fall 2019

The IC assesses that an assessment about abnormal activity at the WIV in fall 2019 lacks support and does not offer diagnostic insight. The Multi-Agency Collaboration Environment (MACE) published a report assessing that the pandemic began in October 2019 because of a release at the WIV.

• Although the methodology is insightful, the IC has concerns with the small data set and analytic rigor used to derive the group's findings, and our review of information directly contradicts some of its findings.

# Theory That SARS-CoV-2 Was a Biological Weapon

The IC assesses that public claims from a Hong Kong virologist that Beijing created SARS-CoV-2 as a biological weapon are inconsistent with available technical information on coronaviruses. We assess that the articles contain several technical inaccuracies and omit key data points.

• Since September 2020, a virologist who worked in a WHO-affiliated laboratory in Hong Kong has publicly stated that Beijing created SARS-CoV-2 from bat coronaviruses and that China's researchers intentionally released it. The scientific community did not peer review these articles and some publicly rejected the articles' claims as scientifically unsound.

### Theory That SARS-CoV-2 Was Genetically Engineered

The IC assesses that public claims that some distinguishing features in SARS-CoV-2 are the result of genetic engineering are not diagnostic of genetic engineering. The IC has been evaluating how SARS-CoV-2 could have developed these features and notes that the furin cleavage site (FCS)—a region in the spike protein that enables infection and has been the topic of open-source debate—can also be consistent with a natural origin of the virus.

We do not fully understand the diversity of natural coronaviruses or how often they recombine, suggesting that there are plausible natural means by which these features in SARS-CoV-2 could have emerged beyond what we currently understand.

- For example, the author of an article in April notes the SARS-CoV-2's FCS is unique among known betacoronaviruses. The author argues that such features are rare and so well-adapted for human infection that they are more likely emerged from laboratory work than from natural selection.
- Although an IC review of scientific literature has indicated that no known betacoronaviruses in the same subgenus have this FCS in the same region of the spike protein as SARS-CoV-2, similar FCSs are present in the same region of the spike protein as other naturally occurring coronaviruses, according to scientific articles.

We also do not find credible a now-withdrawn preprint article from two Indian educational institutes posted in January 2020 that asserted SARS-CoV-2 was genetically engineered using sequences from the human immunodeficiency virus. We assess it is unlikely that scientists would have chosen to intentionally engineer the specific sequences that were the focus of the scientific article.

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# Theory That SARS-CoV-2 Originated Outside China

We are aware of scientific studies claiming to have found SARS-CoV-2 viral fragments or antibodies in samples taken before November 2019 outside China. However, technical flaws in some of these studies, uncertainties in the methodologies, and in some cases, the lack of a credible review process make us skeptical of their utility in determining the pandemic's origin.

- We assess that the first cluster of confirmed COVID-19 cases arose in Wuhan, China, in late 2019, but we lack insight—and may never have it—on where the first SARS-CoV-2 infection occurred. Although all of the earliest confirmed cases of COVID-19 were documented in China's Hubei Province, where Wuhan is located, according to Western and China's press reports, it is plausible that a traveler came in contact with the virus elsewhere and then went to Wuhan.
- We continue to monitor scientific publications and discuss these issues with experts. Even if the virus is found to have existed outside China before the Wuhan outbreak, credible evidence of human infection would also be necessary to determine if the first COVID-19 outbreak began there.
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#### Annex C: IC Approach to 90-Day Study

The NIC collaborated closely with the National Counterproliferation Center (NCPC), the National Intelligence Management Council (NIMC), IC agencies, and other USG entities and departments on this assessment. The IC kicked off the 90-day study by outlining the core intelligence questions that would be addressed over lines of effort—collection and analysis. These questions included:

- Did the outbreak begin through contact with infected domestic or wild animals or was it the result of a laboratory-associated incident?
- Was the virus genetically engineered?
- Is SARS-CoV-2 a biological weapon?

**Collection:** At the kick-off meeting for the 90-day study, the IC discussed core intelligence gaps to drive collection moving forward.

**Analysis:** The NIC had two separate structured analytic exercises to discuss both the underlying reporting and to strengthen argumentation moving into the drafting phase. Analysts at individual agencies also pursued various structured analytic techniques to build their own assessments.

- During a two-day-long in-person IC-wide **Analysis** of **Competing Hypothesis (ACH)** analytic exercise in June, analysts determined whether existing reporting was consistent or inconsistent with information in individual reports. This exercise allowed analysts to determine that most reporting was consistent with both hypotheses and the reporting that was inconsistent was deemed to be not credible.
- Before the start of drafting, the NIC hosted an ICwide **Team A/Team B** analytic exercise to explore how the IC could strengthen either hypothesis through a debate style format. Agencies pulled from these conversations—along with the work conducted during and before the study—to solidify their consensus positions.

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### Annex D: Outside Review

The NIC conducted four rounds of outside review of the draft assessment. These sessions provided valuable feedback that we incorporated into the assessment. The NIC made some organizational changes in response to comments; comments included:

- Emphasize points of agreement.
- Provide additional definitions in the lexicon and ensure technical or intelligence jargon is explicitly explained.

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#### **Annex E: Questions**

Answers to the following questions would help us better evaluate hypotheses related to the origins of COVID-19:

What additional information—to include timing, location, relevant animal exposures, occupational information, and clinical samples—is there on the earliest cases of COVID-19?

How were early cases investigated? What questions or tools were utilized for tracing contacts and contacts of those contacts?

What direct or indirect indicators of COVID-19 clusters is China aware of from early in the outbreak? This may include things like hospital occupancy rates or efforts to triage medical care outside of hospital facilities.

What insight can China provide on the search for the reservoir and potential intermediate species of the COVID-19 virus?

What insight can China provide on the search for the identification of a progenitor virus? Have any leading candidates or regions for spillover been identified?

What information, data, and/or samples does China have on wildlife or other animals present in the following markets in Wuhan:

- Huanan Seafood Wholesale Market
- Qiyimen Live Animal Market
- Baishazhou Market
- Dijiao Outdoor Pet Market

What information, data, and/or samples does China have on wildlife present in the other markets, wildlife rescue centers, and/or farms in Wuhan, across Hubei, in neighboring provinces, or in locations where live animals in Hubei Province are sourced from?