WHO-convened Global Study of the Origins of SARS-CoV-2：

Terms of References for the China Part

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Contents

Background

The current COVID-19 pandemic shows the devastating impact emerging zoonotic diseases can have on societies. As the pandemic continues to unfold, understanding how the epidemic began is essential to prevent 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 (Zhang Z. et al. 2020). Identifying the origin of the virus, however, is a complex task, which requires strong international and multi-sectoral collaboration, and a commitment to leverage expertise, capacity, and work globally.

In February 2020, WHO convened an international meeting of experts to develop and agree on a number of priority research initiatives in all aspects of COVID-19 which included virus origin, virus persistence and animal susceptibility to SARS-CoV-2 (WHO, 2020a). Under this Research and Development blueprint, WHO is also coordinating several international collaborative efforts that contribute to better understanding the origin of the virus. Furthermore, WHO, the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO) agreed through the 73rd World Health Assembly resolution, to galvanize efforts to trace the animal origin of the virus, its route of transmission to humans and possible role of the intermediate host.

Building on the recommendations from the 73rd World Health Assembly, WHO, together with the Government of China, are setting up an international multidisciplinary team to design, support and conduct a series of studies that will contribute to origin tracing work. The work will contribute to improve the understanding of the virus origins, help set up further studies, thereby improving global preparedness and response to SARS-CoV-2 and zoonotic emerging diseases of similar origin. In addition, the international team will also develop study frameworks and materials that may set the ground for origin tracing work elsewhere. The global origin tracing work is therefore not bound to any location and may evolve geographically as evidence is being generated, and hypotheses evolve.

These Terms of References (ToRs) outline the scope of studies, set out the main guiding principles and key expected deliverables for these studies.

Approach

Where an epidemic is first detected does not necessarily reflect where it started. An outbreak of pneumonia of unknown etiology was identified through surveillance in Wuhan, however the possibility that the virus may have silently circulated elsewhere cannot be ruled out. For example, some countries have retrospectively identified cases of COVID-19 weeks before the first case was officially notified through surveillance, and unpublished reports of positive sewage samples could suggest that the virus may have circulated undetected for some time.

The COVID-19 outbreak was first reported in Wuhan, where the SARS-CoV-2 virus was also first identified and characterized. The detection was followed by a rapid exponentially growing outbreak, weeks before other outbreaks were detected elsewhere. Thus, under the framework of global tracing for the origins of SARS-CoV-2, the approach to identifying the source of infection, the source of introduction and possible origins of the virus initially conducted in Wuhan is of importance for further global origin tracing work.

Findings from these initial studies will inform many efforts globally to better understanding the origins of the virus. Ongoing parallel studies, undertaken in a systematic and logical way are essential.

Studies under these ToRs will aim to (i) explore how the circulation of SARS-CoV-2 might have started and (ii) gather evidence from the cluster of cases identified in December 2019 for potential links and clues as to its origin. Studies to address those points will be addressed in Phase 1 of the work. The result of Phase 1 studies will generate hypotheses that lay the ground for Phase 2 studies, which could be conducted elsewhere in China, in neighboring countries and globally. For example, susceptibility studies have demonstrated that several animal species could potentially play the role of intermediary host. This, combined with evidence from Wuhan, could help better guide future work into intermediary animal species in China and elsewhere.

The approach will be open-minded, iterative, not excluding any hypothesis that could contribute to evidence generation and help narrow the focus of research.

Findings from origin studies in China will advance origins tracing in other countries and may lead to similar work elsewhere. WHO will continue to work closely with the World Health Organization of 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, as per 73rd WHA resolution.

Current knowledge supporting origin tracing work

Phylogenetic work

Tens of thousands of genome sequences of SARS-CoV-2 have now been shared through GISAID, a public database (https://gisaid.org), including sequences of early cases from Wuhan and three environmental samples from the Huanan market at the epicenter of the initial reported cases. Phylogenetic analyses help better understand transmission dynamics, particularly how the virus may have evolved over time and how clusters might be related in time and place. For example, it can help identify whether transmission in a new area is related to one or multiple introductions which, combined with epidemiological data, can strengthen the evidence about how the virus spreads and where it originated from (Oude Munnink et al. 2020)

Current findings show that the virus has been remarkable stable since it was first reported in Wuhan, with sequences well conserved in different countries, suggesting that the virus was well adapted to human transmission from the moment it was first detected. This is also corroborated by the epidemiology and transmission patterns seen since the start of the COVID-19 pandemic.

Two of the genetically closest known coronaviruses, RaTG13 and RmYN02, were discovered in bat populations in Yunnan province of China (Zhou et al. 2020). RaTG13, which was identified in 2013 shares 96.2% sequence homology with SARS-CoV-2 (Zhou et al. 2020, Li W. et al 2005) while RmYN02, has 93.3 % homology. However, with a genome size of about 30,000, the genetic distance between the genetically closest virus, RaTG13, still corresponds to a difference of nearly 1,200 nucleotides, remaining a distant ancestor of SARS-CoV-2.

Bats are natural reservoirs of coronaviruses, and likely harbor many other coronaviruses not yet discovered. However, while direct spillover of a new virus from bats to humans is possible, zoonoses often stem from exposure to intermediate animal hosts. Research conducted in China and elsewhere since the COVID-19 pandemic began has shown that a range of animals – including wild and farmed species – are susceptible to infection, but when and where SARS-CoV-2 spilled over to humans, and from which animal, remains unknown.

A virus with 92.4 percent homology to SARS-CoV-2 was recently discovered in pangolins (Lam et al. 2020), with 97.4 percent homology in the receptor binding domain (RBD)indicating some possible recent similar or related evolution of these viruses (Andersen et al. 2020)

However, to date the specific reservoir has not been identified and there is no evidence to demonstrate the possible route of transmission from a bat reservoir to human through one or several intermediary animal species (WHO 2020, OIE 2020).

Animal susceptibility and animal surveys

As part of the studies conducted to help identify possible intermediary host animal(s) species for SARS-CoV-2, several susceptibility studies on a range of animal species have been performed or are underway. The initial target species have covered animal species known to have ACE2 receptor cells, believed to be the main entry point for SARS-CoV-2 infection in humans. More than 500 animal species have been identified for possible further studies based on predicted ACE2 affinity to bind SARS-CoV-2 receptor binding domain (FAO 2020b).

So far, susceptibility studies conducted in several countries have shown that domestic cats, ferrets, hamsters and minks are particularly susceptible to infection, and in some cases exhibit morbidity and mortality and can transmit the disease to other animals of the same species (FAO, 2020a). Cats have been found to be easily infected with SARS-CoV-2 and can transmit to other cats. Infected cats were also shown to shed viruses in large quantities while asymptomatic (Shi et al., 2020, Halfmann et al. 2020). The possible role of cats was further suggested by a seroprevalence study in Wuhan in which 13.7% of 105 samples taken during the outbreak from both domestic cats with high exposure risk and stray cats were found positive while 39 samples from mid-2019 were all negative (Zhang Q et al. 2020). Other species have shown different degree of susceptibility to SARS-CoV-2 infection including several species of bats, tree shrews, and different species of non-human primates (FAO 2020) while other species such as chicken, turkeys, ducks and quails were found not to be susceptible. Although overexpression of swine ACE2 in cultured cells supports some degree of viral entry (Zhou et al. 2020). Recent experimental work with direct inoculation, however, has demonstrated that pigs are non-susceptible to the virus (Shi et al. 2020). The susceptibility of pigs warrants further studies.

In February and March 2020, the first cases of infection with SARS-CoV-2 were reported in domestic cats and dogs from household of COVID-19 patients in Hong Kong SAR, China and Belgium. Similar cases of pet cats and dogs have since been reported from several countries (OIE 2020). Other felines such as tigers and lions in a zoo in the USA were also found to be infected with SARS-CoV-2 following contact with an infected pre-symptomatic zookeeper.

A number of outbreaks in mink farms in the Netherlands, Denmark and Spain have shown that minks can spread the disease in farm environment and could potentially establish a new animal reservoir in mink populations for SARS-CoV-2 if not efficiently controlled.

These preliminary results demonstrate that different animal species in regular contacts with humans are susceptible to infection with SARS-CoV-2 and could serve as intermediate animal host species or could establish new reservoirs for the virus and new sources for spill-over events into the human population.

Persistence of the virus on food and surfaces

Recent outbreaks in markets as well as food processing plants in USA, Germany, Thailand, India, Brazil, Spain, China and Ireland have raised questions about the potential role of food products as a vehicle of transmission for SARS-CoV-2. Preliminary results from the Xinfadi market outbreak in Beijing suggest that food items, as other surfaces and objects, might be contaminated by the virus. But there is no evidence that contaminated food items may have contributed to transmission. Furthermore, shrimps and salmon packages were found positive for SARS-CoV-2 RNA in recent surveys in China. In experimental studies on the survival of SARS-CoV-2 virus on different surfaces, the virus can remain viable for up to 72 hours on different surfaces (WHO, 2020c), and recent results further suggest virus viability on meat products (Fisher et al., 2020). However, while this suggests that transmission may occur from contaminated surfaces or items, there is currently no evidence for foodborne transmission.

While there is currently no evidence that food is a likely source or route of transmission for the virus, it is necessary to coordinate and cooperate globally to share data and evidence to further clarify the potential role of food in the spread of COVID-19 virus.

The early cluster in Wuhan

Wuhan is a city with a good surveillance system, and while the outbreak was first identified in Wuhan, it is possible that it started elsewhere and circulated unnoticed. As the information is scarce, there are limited hypotheses about how the outbreak might have started in Wuhan. It may have started from an infected individual contaminated elsewhere, from contact with an infected animal, or less likely through contact with contaminated products.

The early cases in Wuhan are thought to have occurred in early December, and preliminary information from surveillance data of severe pneumonia suggest no unusual cluster or departure from trends in the weeks and months preceding the first reported case in Wuhan.

Retrospective review of cases identified a total of 124 confirmed cases with onset date in December 2019, 119 of whom were from Wuhan and 5 others from Hubei or other provinces, but all with travel links to Wuhan during the period of exposure. A study of 41 initially identified confirmed cases showed that 70% of the cases had a link to the Huanan market (Huang et al, 2020), but detailed exposure factors within the market and elsewhere remain unclear.

The Huanan wholesale market is a large market (653 stalls and more than 1180 employees) mainly supplying seafood products but also fresh fruits and vegetables, meat, and live animals. In late December 2019, 10 stalls operators were trading live wild animals including chipmunks, foxes, racoons, wild boar, giant salamanders, hedgehogs, sika deer, among others. Farmed, wild and domestic animals were also traded at the market including snakes, frogs, quails, bamboo rats, rabbits, crocodiles, and badgers. The market was closed on 1 January 2020, and several investigations followed, including environmental sampling in the market, as well as sampling of frozen animal carcasses at the market. Of the 336 samples collected from animals, none were PCR positive for SARS-CoV-2, whereas 69 out of 842 environmental samples were positive by PCR for SARS-CoV-2. Sixty- one of those (88%) were from the western wing of the market. Of these, 22 samples were from 8 different drains and sewage, and 3 viruses were isolated, sequenced and shared on GISAID. These were virtually identical to the patient samples collected at the same time (>99.9 % homology).

In the absence of analytical epidemiological study among vendors and shoppers, and without detailed mapping of exposure factors at the market over the exposure period, type of products and animals sold, the proportion of animals and commodities that were available for testing, interpreting laboratory sampling results remains difficult.

As such, it remains unclear whether the market was a contamination source, acted as an amplifier for human-to-human transmission, or a combination of those factors. In addition, there is limited information about potential risk factors outside of the market, such as occupation, travel history, or other were identified among the first diagnosed cases. A better understanding of potential risk factors through in-depth epidemiological investigations is critical in orienting the nature and geographic focus of other studies. For example, if the market as acted primarily as a common source of contamination through animal or non-animal products, the focus of subsequent studies should be on source tracing of such products, whereas if evidence points towards the market as enabler of human-to-human transmission, source tracing would need to focus elsewhere.

No unusual clusters of cases or deaths were reported elsewhere in China prior to detecting the cases in Wuhan, and clinical surveillance of farmed animals since 2019 have shown no departure from trends.

Overall, very little is currently known about how, where and when the virus started circulation in Wuhan. Preliminary studies have not generated credible leads to narrow the area of research, and studies will therefore focus on developing comprehensive study plans to help generate hypotheses on how the outbreak may have started in Wuhan.

Implementation plan

Short term studies (Phase 1) will be conducted to better understand how the virus might have started circulating in Wuhan.

Building on the findings of these short-term studies, and the scientific literature, longer term studies will be developed (Phase 2).

The framework and methodological approaches implemented in China could also be used to study the virus origins elsewhere, if warranted. For example, methods for serological surveys among population groups potentially exposed to animal hosts can be standardized for comparison.

Phase 1: Short term studies

a) Descriptive Epidemiological Study

1. In-depth reviews of hospital records for cases compatible with COVID-19 before December. This may include the high resolution-CT images of patients with pneumonia of unknown etiology will be undertaken for any COVID-19-like pneumonia, and stored samples will be tested, if available.
2. 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.
3. Review of trends of all-cause mortality, and review death registers for specific causes of death compatible with COVID-19.
4. In-depth interviews and reviews of currently identified early 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 exposure, market exposure, animal contact, characterizes of their social contacts at the time and others. The process will likely be iterative.
5. Serological studies based on stored blood/serum samples collected in weeks and months before December 2019. Consideration should be given to population group of focus (e.g. groups at higher risk of exposure such as frontline workers, laboratory workers, farm workers). Additionally, the work may include targeted serological testing on stored serum samples of suspect COVID-19 patients and suspect deaths identified through retrospective reviews.

b) Analytical Epidemiology Study

1. Design and undertake a **comprehensive epidemiological study** to test whether specific epidemiological factors are associated with COVID-19 in the early part of the outbreak (for cases reported or retrospectively identified in 2019). The approach will include:
    a. The development of an appropriate **study design**. Approaches such as case-cohort designs, where exposures among cases are compared to that of a random sample of the population, or variations of that, may be particularly well suited. The design may also include an approach to explore exposures within the Huanan market in more detail.
    b. **The implementation of the study.** The study will be conducted with highly trained staff, appropriate interpreters, use of electronic data capture tools, and adequate data management and advanced analysis.
2. **Develop additional studies as needed and as informed by 1 and 2 above.** For example, set up serological studies within specific population groups, as informed by abovementioned studies.

c) Animals, Products and Environmental Study

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 domestic animals) and stalls (for all types of goods) present at the Huanan market.
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.
3. Testing of frozen sewage samples for evidence of circulation prior to December 2019.
4. Other studies and approaches, as appropriate.

Some of the abovementioned work may already be partially done or documented by the time the international team initiates its work, and the study will therefore build on existing information and augment, rather than duplicate, ongoing or existing efforts.

The international team will ensure that the scientific framework developed in China can be applicable and replicated in other settings, should there be a need to do so.

Expected outcomes from the Phase 1 studies

A range of deliverables are expected from the short-term studies. These include:

1. Detailed reports for the various studies.
2. Identification of key knowledge gaps.
3. Formulation of working hypotheses
4. Development of plans for longer term studies, as appropriate

Phase 2: Longer term plans

The international team will develop plans for detailed longer-term studies building on the outcome of the Phase 1 short-term studies and any new relevant national or international scientific evidence generated. These will help better define the scope of the Phase 2 studies, both in terms of their geographic focus, animal species to look at, and scientific approach, and will support the development of plans for global origin tracing.

These studies may include the following:

1) in-depth epidemiologic, virologic, serologic assessments in humans in specific geographic areas or specific settings as informed by the initial short-term studies and other international findings
2) in-depth epidemiologic, virologic, serologic studies among animal populations before and after the outbreak (potential hosts and reservoirs) in targeted geographic areas
3) more detailed studies, depending on initial results from the short-term studies

Team composition

The international team will provide a wide range of expertise, from China and several other countries, ensuring a breadth of scientific expertise.

The team will include experts in public health and animal health and human-animal interface, epidemiology, virology, genomics, environmental health, food safety, among others, both nationally and internationally, including academia, scientists from government organizations, and scientists at WHO, OIE and FAO.

The final composition of the international team should be agreed by both China and WHO.

Ways of working

A program of work will be developed for the international team to operate as follows:

- The team will initially engage on the development of study protocols, and study materials.
- Regular interaction will be maintained to share scientific findings related to origin tracing from China and elsewhere
- Contribute to the delivery of Phase 1 studies where needed
- Undertake a visit to China of the entire team, at an appropriate time
- Develop longer term study plans (Phase 2).

References

Andersen, K.G., Rambaut, A., Lipkin, W.I. *et al.*The proximal origin of SARS-CoV-2. *Nat Med***26***Med***26,** 450–452 (2020). https://doi.org/10.1038/s41591-020-0820-9

FAO (2020a) El Masry, I., von Dobschuetz, S., Plee et al. 2020. *Exposure of humans oranimals to SARS-CoV-2 from wild, livestock, companion and aquatic animals: Qualitative exposure assessment.* FAO animal production and health, Paper 181. Rome, FAO. https://doi.org/10.4060/ca9959en

FAO (2020b) El Masry, I., von Dobschuetz, S., Plee, et al. 2020. Exposure of humans or animals to SARS-CoV-2 from wild, livestock, companion and aquatic animals. Supplementary tables on susceptibility of animals to SARS-CoV-2 based on predicted ACE2 affinity to bind SARS-CoV-2 receptor binding domain – July 2020. Supplementary to FAO Animal Production and Health Paper 181. Rome. FAO.

Fisher D. et al (2020) The risk of cross-border movement of SARS-CoV-2 on frozen and refrigerated fish and meat. *JAMA, in press.*

Guan Y, Zheng BJ, He YQ, et al. (2003) Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science*. 2003;302(5643):276-278. doi:10.1126/science.1087139

Halfmann PJ., Hatta M, Chiba S. (2020) Transmission of SARS-CoV-2 in Domestic Cats. *New England Journal of Medicine.* doi: 10.1056/NEJMc2013400

Huang C, Wang Y, Li X, et al. (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in Lancet. 2020 Jan 30;:]. *Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5

Lam, T T-Y, et al. (2020)Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. *Nature.* Vol 583, 9 July 2020.

Li H.,Mendelsohn E, Zong C et al., (2019) Human-animal interactions and bat coronavirus spillover potential amongrural residents in Southern China *Biosafety and Health* 1 84–90 http://dx.doi.org/10.1016/j.bsheal.2019.10.004

Li W., Shi Z, Yu M, (2005) Bats are Natural Reservoirs for SARS-like Coronaviruses, *Science* 2005: Vol. 310, Issue 5748, pp. 676-679 doi: 10.1126/science.1118391

Oude Munnink B.B., NieuwenhuijseDNieuwenhuijseD.F., Stein, M. *et al.*((2020) Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands. *Nat Med* (2020). https://doi.org/10.1038/s41591-020-0997-y

OIE,2020: https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/

Shi J, Wen Z, Zhong G, et al. (2020) Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science*. 2020;368(6494):1016-1020. doi:10.1126/science.abb70

Wang N. et al., (2018), Serological evidence of bat SARS-related coronavirus infection in humans, China, *Virol. Sin*. 33 (2018) 104–107. https://doi.org/10.1007/s12250-018-0012-7.

WHO (2020a) R&D blueprint.

WHO (2020b). Origin of SARS-CoV-2 https://apps.who.int/iris/bitstream/handle/10665/332197/WHO-2019-nCoV-FAQ-Virus_origin-2020.1-eng.pdf

Zhang Z, Xiao K, Zhang X, Roy A, Shen Y. Emergence of SARS-like coronavirus in China: An update. *J Infect*. 2020;80(5):e28-e29. doi:10.1016/j.jinf.2020.03.010

Zhang Q, Zhang H, Huang K, et al. SARS-CoV-2 neutralizing serum antibodies in cats: a serological investigation. 2020 bioRxiv doi: https://doi.org/10.1101/2020.04.01.021196

Zhou, P., Yang, X., Wang, X. *et al.*A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature***579,** 270–273 (2020). https://doi.org/10.1038/s41586-020-2012-7