

## Testing for SARS-CoV-2 in animals and the environment at the Huanan

### Seafood Market

The 2021 WHO-China report and multiple studies have suggested that the HSM played an important role in SARS-CoV-2 transmission, either through the sale of animals known to be susceptible to SARS-CoV-2 infection, amplification of human transmission (spillover having occurred elsewhere), or as a possible site of spillover via contact with infected animals sold at the market (WHO, 2021c; Liu et al., 2023; Xiao et al., 2021; Pekar et al., 2022; Worobey et al., 2022a; Crits-Christoph et al., 2024).

The HSM was supplied with farmed wildlife from farms located in the prefectures of Tianmen, Xiaogan, Jingmen, Suizhou, Xiangyang, and Huangshi, also at the county level in Jianli, Wuxue and Jingshan - within Hubei Province. Outside Hubei province, source farms were in the provinces of Heilongjiang, Jilin, Shanxi, Henan, Hunan, Jiangxi, Guangdong, Guangxi and Yunnan (WHO, 2021). SAGO's ability to fully understand the significance of the HSM in the origins of SARS-CoV-2 is limited by the fact that no precise information, including on the quantity, species and source locations, of the animals sold at the HSM or other markets in Wuhan has been made available to WHO or SAGO to date or published. It is important to note that the wildlife trade in wet markets is prohibited under Chinese law. As a result, tracing the specific origins of animals sold at the HSM presents logistical and regulatory challenges. These circumstances may also complicate the systematic documentation and sharing of related information with WHO and international partners. It is also important to note that Xiao *et al* reported on animals that were sold at the HSM from May 2017 and November 2019, including 38 live animal species, including racoon dogs (*Nyctereutes procyonoides*), red foxes (*Vulpes vulpes*), mink (*Neovison vison*) and masked palm civets (*Paguma larvata*), all of which are known to be susceptible to SARS-CoV-2 infection (Xiao et al., 2021).

According to information provided to the international team in 2021, it is understood that on 30 December 2019, local Chinese public health authorities entered the HSM market and collected the first environmental samples from the market stalls and environments, and that on 31 December 2019, a joint team consisting of experts from four levels of China CDC conducted the investigation of HSM and initiated epidemiological studies around the market. On midnight 1 January 2020, Chinese authorities,

following standard public health biosafety procedures in response to the outbreak of COVID-19, closed the market for disinfection.

Between 1 January and 30 March 2020, Chinese authorities reportedly conducted interviews with market sellers, collected samples from 457 animals in and around the market (including live rats, mice, stray weasels, cats, and dogs, as well as from the carcasses of sheep, wild boars, bamboo rats, badgers, muntjacs, hedgehogs and rabbits) and 923 environmental samples (e.g., from equipment, stalls and drains) (Liu et al., 2023). Published reports and presentations to SAGO stated that all samples from animals collected at the market tested negative for SARS-CoV-2. However, 73 environmental samples collected from various locations and from surfaces inside the market were RT-PCR positive (Xiao et al., 2021, Worobey et al., 2022a; Liu et al., 2023; Bosco-Lauth et al., 2021a; Crits-Christoph et al., 2023; Wu, 2023). Further analysis by Crits-Christoph *et al.* (2024), using data shared by Liu *et al.* (2023), performed spatial risk mapping based on SARS-CoV-2 RT-PCR and metagenomic sequence detections in swabs from animal cages and other surfaces, as well as samples from sewage drains, pinpointing specific market stalls (Liu et al., 2023; Crits-Christoph et al., 2024). The authors refined the taxonomic comparison of animal genetic material by partially re-assembling sequence-read contigs. At the HSM stalls, mitochondrial DNA hits from several susceptible species to SARS-CoV-2 were detected, including from raccoon dog, hoary bamboo rat, and palm civet, with genetic traces of the former two species particularly abundant across the wildlife market area, and some of these samples containing high amounts of SARS-CoV-2 sequencing reads. Based on mitochondrial gene SNP typing, geographic origins of the detected raccoon dogs were mapped to central or southern China. Liu *et al.* (2023) confirmed presence of bat and pangolin DNA, but subsequent comparisons of detected nucleic acids with the Barcode of Life Data System COX1 gene database by international scientists failed to confirm the presence of bat or pangolin nucleic acids in market samples (Liu et al., 2023; Crits-Christoph et al., 2024). These findings suggest that some animals, particularly raccoon dogs, known to act as *Sarbecovirus* intermediary hosts, were present at the market before its closure on 1 January 2020, though it cannot be concluded that the virus was introduced via these animals (Liu 2023b).

The report by Liu *et al.* (2023) confirmed the presence of two SARS-CoV-2 lineages; A and B, in environmental samples from the HSM, and with a higher frequency of lineage B over A, consistent with lineage B's earlier inferred emergence (Liu et al., 2023).

## Detection of SARS-CoV-2 in wild animals in China and South-East Asia

According to the 2021 WHO-China joint report, 2328 samples from wildlife were collected in December 2019 — using non-structured, convenience sampling — all tested negative for SARS-CoV-2. Additionally, 2995 samples from Hubei province (February-March 2020), and 27,000 samples for PCR and 1914 sera were taken from other parts of China also yielded negative for SARS-CoV-2 (Annex C6 Reports Annexes) (WHO, 2021c).

The WHO Secretariat invited a number of scientists from China, including from the Centre for Infection and Immunity Studies, School of Medicine, Sun Yat-Sen University, Guangzhou, China; Chinese National Influenza Center (CNIC), National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention (CDC), China, to present research associated with testing animal samples from Chinese provinces. In these presentations, the invited scientists reported that all testing undertaken in animals in China did not detect the presence of SARS-CoV-2 virus (nucleic acid) or antibodies.

The most detailed of these surveys was presented to SAGO in January 2022 by the Beijing Institute of Genomics, Chinese Academy of Sciences, and Clinical Laboratory Center, National Health Commission (NHC), who reported that by 30 November 2021, 32,479 animal samples had been collected from 18 provinces including Hebei, Inner Mongolia, Liaoning, Jilin, Heilongjiang, Shanghai, Jiangsu, Henan, Hubei, Hunan, Sichuan, Guizhou, autonomous region of Tibet, Gansu, Shaanxi, Jiangxi, Ningxia and Qinghai. The scientists reported that no SARS-CoV-2-related nucleic acid were found in 218 species from 13 orders including Rodentia, Carnivora, Artiodactyla, primates, Lagomorpha, Pholidota and Erinaceidae. Furthermore, 1311 serum samples from livestock and poultry (in Hubei and seven other provinces), and 2837 serum samples from dogs, cats, mink, foxes, and raccoon dogs (collected in Henan, Shandong, Hebei and Hubei in autumn 2020 - precise dates were not included in the presentation) all tested negative for SARS-CoV-2 antibodies. However, further details

about sample sizes, sampling schemes, specific animal species and numbers of animals tested, methodology of laboratory testing, locations of farms, markets, and sources of animals were not provided during this presentation (unpublished information presented to SAGO by Chinese Scientists).

Scientists in the Mekong Delta and South-East Asia (China, Thailand, Myanmar, Cambodia, Lao People's Democratic Republic, and Viet Nam) presented regional coronavirus research in various bat species at a WHO regional technical surveillance workshop held in Bangkok, Thailand (October 2022). Findings from the surveillance activities in Thailand (which is home to one third of all members of the *Rhinolophidae* family) included partial sequences of new species of SARS-CoV-like coronaviruses found in *Rhinolophus* bats. Researchers from Cambodia presented serological investigations which indicated seropositivity to coronaviruses, filoviruses, and henipaviruses across diverse bat populations and specifically in pteropid bats. Some gaps highlighted from these countries were the regional lack of technology and capacity in labs to detect and characterize novel viruses, including a lack of funding to conduct whole genome sequencing of the viruses, as well as biosafety capacities and training. These limitations hamper the ability for researchers to characterise novel SARS-CoV related viruses identified in bats and mammals in the region.

While the intermediate host(s) for SARS-CoV-2 has (have) not yet been determined, it is widely accepted that precursor viruses for coronaviruses originate in bats (Zhou et al., 2021). Sixteen bat species have been identified as potential sarbecoviruses carriers (Wu et al., 2022). Shortly after the reports of the first cases of SARS-CoV-2, phylogenetic analyses identified the closest related precursor strains to SARS-CoV-2, in bats detected from China (RaTG13; 2013; 96.1% identity) and later in Lao People's Democratic Republic (BANAL-52; 2020; 96.8% identity) (as seen in Table 2) (WHO, 2024a; Temmam et al., 2022; Zhou et al., 2020b). Wu *et al.* (2022) tested 4270 bats pertaining to different *Rhinolophus* species across China that yielded 146 novel Sarbecovirus genomes. None of the genomes were closely related to SARS-CoV-2 (680 members of the highly relevant species, *R. affinis* were included in the sample). Zhou *et al.* (2021) tested 283 bats from at least five *Rhinolophus* species and also *Hipposideros larvatus* identifying novel Sarbecoviruses but none closely related to SARS-CoV-2 (Zhou et al., 2021). These are discussed further in the genomics section of this assessment.

Variability of virus prevalence and species distribution makes it difficult to define virus endemicity. For instance, Wu *et al.* 2022 noted that the closest relatives to SARS-CoV-2 – BANAL-52, BANAL-103 and RaTG13 – stem from *Rhinolophus malayanus*, *R. pusillus*, and *R. affinis*, respectively (Table 2 summarises the closest known coronavirus relatives to SARS-CoV-2, including their host species, genome similarity, and geographic origin) (Wu et al., 2022). While some of these species may live predominantly outside China, they also exist within China, but few samples have been tested to date. Moreover, since the direct precursors to SARS-CoV-2 may have passed through an intermediate host, bat distribution alone may not indicate the site of spillover. A recent paper (Havens et al., 2025) analysed the hypothesis that zoonotic viruses require adaptation prior to zoonosis to sustain human-to-human transmission. Based on Ebola, Marburg, influenza A, SARS-CoV-1 and SARS-CoV-2, there was no change of selection immediately prior to a host switch. This implies that closely related precursor viruses from bats might have transmitted directly to humans and subsequent evolution occurred in humans. Nevertheless, the currently known precursor viruses are still too distant from SARS-CoV-2 to have been the source of the early cases in Wuhan as discussed under the genomics section.

The search for viral precursors in potential intermediary hosts suffers from similar challenges, based on limited sample size and geographic coverage in currently published studies. For example, He *et al.* (2022) tested 1941 individual game animals (samples collected after February 2020) typically sold in Chinese markets, including from the orders Rodentia, Carnivora, Lagomorpha, Eulipotyphla, and Pholidota (pangolins), but none were reported as positive for *Sarbecovirus* (He et al., 2022). However, in these analyses, only 95 raccoon dogs and 425 masked palm civets were tested – both known susceptible species. Additionally, animals in this study were mostly from natural habitats and not targeting the animal production sites that had been recommended for testing in the 2022 SAGO report (SAGO, 2022). Despite raccoon dog fur farming being legal in China, the sale of raccoon dogs in live animal markets was halted (precise dates were not included in the presentation in which this information was shared), and almost all samples that have been tested were collected after February 2020 (Xiao et al., 2021). No results of surveillance studies on upstream wildlife value chains and wildlife farms have been published or made available to SAGO, following the publication of the WHO-China 2021 report despite requests (WHO, 2021c).

After the SARS epidemic in 2002-2003, Chinese authorities discovered a second animal-to-human spillover of SARS-CoV (referred to as SARS-CoV-1 in the remainder of this report) at the end of 2003 (Wang et al., 2005; Ye et al., 2020), and they ordered the destruction of civet cats and raccoon dogs in the Xinyuan market in Guangdong province. During this process, culled animals were swabbed and tested for SARS-CoV-1, identifying 14 of 18 as positive at the Xinyuan market, as well as identifying antibodies to SARS-CoV-1 in masked palm civets in one of the four supplier farms in Guangdong province.

From January to September 2004, breeding facilities for masked palm civets in 12 Chinese provinces supplying animals to the Xinyuan market were systematically visited and the animals tested – results from 1107 samples tested negative (Kan et al., 2005). Detected viruses were sufficiently characterized to enable the retrospective reconstruction of genomic changes that occurred during the emergence of SARS in 2002 in humans. As of June 2025, no additional data outside of the information provided in the 2021 WHO-China report (WHO, 2021c) (refer to pages 99-106) has been made available to SAGO or WHO on SARS-CoV-2 testing conducted on raccoon dogs and other susceptible species that were commercially bred and had trade links to the HSM, or any markets in Wuhan city, Hubei Province or within China, prior to December 2019.

Also, data has also not been made available from SAGO-recommended studies (SAGO 2022), including the systematic random sampling of animal farms, hunters, animal traders, markets or market sellers to and from the HSM. There is a need for more information, particularly from source farms trading animals to wet markets, in order to determine the existence of early circulation and spillover (WHO, 2022).

## Animal infection experiments and reverse zoonoses

Reverse zoonosis of SARS-CoV-2 from humans to animals has been documented in wild, farmed, zoo, and domestic animal species. As of October 2023, the World Organisation for Animal Health (WOAH) reported 775 outbreaks in animals, across 29 species in 36 countries (World Organisation for Animal Health, 2023). Susceptible species to SARS-CoV-2 infection belong to the families: Bovidae, Canidae, Cebidae, Cercopithecidae, Cervidae, Cricetidae, Felidae, Hominidae, Hyaenidae, Mustelidae,

Procionidae, Viverridae, Hippopotamidae, Myrmecophagidae, Atelidae, Rhinocerotidae, Suidae, Agamidae, Phasianidae, Anatidae, Castoridae, Muridae, Chlamyphoridae, Leporidae, Vespertilionidae, Sciuridae, Didelphids and Procyonidae, with spillover potential from one animal species to another. (Food and Agriculture Organization of the United Nations, 2023) (Annex 1 Table 1). In addition, laboratory experiments have been conducted on animals to identify potential susceptible species that could be considered in search of the SARS-CoV-2 reservoir and/or have the potential to create new SARS-CoV-2 variants (Food and Agriculture Organization of the United Nations, 2023) (Annex 1 Table 2).

Although most SARS-CoV-2 zoonotic transmission documented has been from humans to animals, some transmission has included spread among animals, and spillback to humans (Zhou and Shi, 2021; Yen et al., 2022). Farmed mink exhibit both symptomatic and asymptomatic SARS-CoV-2 infections and have been affected by large outbreaks before mass culling in several European countries in 2020 (Pomorska-Mól et al., 2021). In Denmark, culling of mink was recommended following the discovery of the “cluster 5” SARS-CoV-2 variant, which infected 12 people during November 2020 (EFSA Panel on Animal Health and Welfare et al., 2023). Outbreaks on mink farms in Poland were linked to a two-year-old human strain of the B.1.1.307 lineage that carried mutations typical of mink adaptation, suggesting undetected circulation in a previously unknown animal reservoir (Domańska-Blicharz et al., 2023). SARS-CoV-2 transmission from hamsters to humans was also documented in China, Hong Kong Special Administrative Region (SAR) when infected animals transmitted the virus to a pet shop owner, as well as probable cat to veterinarian transmission in Thailand (Yen et al., 2022 ; Chandler et al., 2021; Kuchipudi et al., 2022 ; Marques et al., 2022 ; Hale et al., 2022; Palermo et al., 2022 ; Caserta et al., 2023).

Another example is white-tailed deer (*Odocoileus virginianus*), where SARS-CoV-2 has spread widely. The Animal and Plant Health Inspection Service (APHIS) reported virus in 12.2% and antibodies in 31.6% of white-tailed deer in the USA (APHIS, 2023) – which thus could act as a reservoir for nearly extinct SARS-CoV-2 variants. (Chandler et al., 2021; Kuchipudi et al., 2022 ; Marques et al., 2022; Hale et al., 2022; Palermo et al., 2022; Caserta et al., 2023).