

## Evidence for a proximal origin of SARS-CoV-2 in the wildlife trade is lacking

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To understand how pandemics occur and prevent them, the search continues for the proximal origin of SARS-CoV-2 (Bloom et al., 2021; Relman, 2020). Some experts hold that SARS-CoV-2 most likely originated in the wildlife trade via an intermediate host, i.e., an animal was infected by a bat or another animal before transmitting SARS-CoV-2 to a human person (Holmes et al., 2021; Keusch et al., 2022; Lytras et al., 2021; Worobey, 2021; Worobey et al., 2022). Yet, direct evidence of an origin of the virus in Wuhan's wildlife markets remains elusive.

To date, tens of thousands of animals have been sampled and tested by independent groups for SARS-CoV-2 or closely related viruses, and none have been found in the Wuhan markets or its wildlife trade supply (He et al., 2022; Wang et al., 2022; World Health Organization, 2021). The only reports of SARS-CoV-2-like (SC2-like) viruses in the wildlife trade have been limited to pangolin coronaviruses sampled in south China or Southeast Asia (Lam et al., 2020; Liu et al., 2020; Nga et al., 2022; Xiao et al., 2020; Zhang et al., 2020). Chinese investigators reported that they had tested 80,000 animal samples across 31 Chinese provinces, including from the Wuhan Huanan seafood market, Wuhan city, and elsewhere in Hubei province (World Health Organization, 2021). Although the China-World Health Organization (WHO) study did not provide a precise breakdown of these 80,000 samples by species, they reported that these animal samples were collected in 2015-2020 and more than half were from wild animal species. A study in January 2020, which to the best of their ability was able to obtain fifteen raccoon dogs, seven Siberian weasels, three hog badgers, and three muntjacs captured by Wuhan traders for markets including the Huanan seafood market, also did not find evidence of exposure to SARS-CoV-2 or sarbecoviruses using pan-coronavirus nucleic acid testing (Wang et al., 2022). Another study involving 2,595 samples from 1,726 animals (16 species, including

pangolins) collected between 2017 and 2021, across 344 locations in 19 Chinese provinces, reported zero SARS-like viruses (He et al., 2022). To be clear, these studies are not sufficiently extensive to rule out a natural origin of the pandemic virus due to limitations in some of their sampling strategies (e.g., testing domesticated or zoo animals that are unlikely to be the animal source of the pandemic) and testing protocols (e.g., highly specific tests that can only identify SARS-CoV-2 but not necessarily its progenitor or animal variants of the virus). However, the fact remains that searches over almost three years for an original animal source of the pandemic virus have come up empty.

Furthermore, over the years of bat and animal virus surveillance across China, neither viruses closely related to SARS-CoV-2 nor SARS-related coronaviruses (SARSr-CoVs) that can utilize the human ACE2 entry receptor have been detected in Hubei (Latinne et al., 2020; Wang et al., 2022; Wells et al., 2021; Yu et al., 2019). The closest bat virus relatives to SARS-CoV and SARS-CoV-2 have been found in Yunnan, China (e.g., the SARSr-CoVs RaTG13, RmYN02, and RpYN06) and the bordering country of Laos (e.g., SARSr-CoV BANAL-20-52). Even so, scientists studying southern China rural communities reported that SARSr-CoV seroprevalence in human populations was low (only 0.6%) and suggested that spillover is rare (Li et al., 2019). We cannot rule out the possibility that SC2-like viruses that utilize the ACE2 entry receptor exist at an exceedingly low, as yet undetected, frequency in bats in Hubei. Nonetheless, based on existing knowledge, the outstanding question is how a SC2-like virus in a bat from southern China or southeast Asia transformed into the SARS-CoV-2 that emerged in central China in 2019.

In the absence of an original animal source of SARS-CoV-2, three key observations are commonly brought up in support of the hypothesis that virus originates from the wildlife trade and specifically from an animal-to-human spillover at the Huanan seafood market. First, an early COVID-19 cluster had been detected at the market. However, it remains unclear whether an infected animal or person sparked off the early cluster at the market. For context, the Huanan seafood market is located in a central, densely populated district containing the Wuhan Center for Disease Control and Prevention (CDC), several large hospitals and transit hubs in the city, and the market itself boasts a retail space larger than nine National Football League fields. The Chinese CDC initially announced on January 22, 2020 that the virus most likely transmitted to people via wild animals illegally sold at the Huanan seafood market (Cao and Pan, 2020). Yet it

became clear that several of the earliest known patients had no connection to the Huanan seafood market or any other wildlife market (Huang et al., 2020; World Health Organization, 2021). Furthermore, patients with no connection to the market carried early SARS-CoV-2 variants distinct from the variant observed in the market cluster (Bloom, 2021; World Health Organization, 2020). More precisely, multiple early variants of SARS-CoV-2 had not been detected at or in association with any wildlife market. These non-market variants were more similar to closely related bat SARSr-CoVs than the market variant. Thus, it is hypothesized that the non-market variants likely emerged earlier than the market variant (Bloom, 2021; Huang et al., 2020; World Health Organization, 2021; Yu et al., 2020). Without access to patient records and contact tracing information, it remains impossible to determine who the earliest COVID-19 victims might be and how they were initially exposed to the virus, or how the virus was brought into the Huanan seafood market.

The second piece of evidence in support of a Huanan seafood market origin of the virus is that wild animals considered capable of being infected by and transmitting SARS-CoV-2 to human beings had been sold in Wuhan between May 2017 and November 2019 (Xiao et al., 2021). The sale of these animals is not in dispute. However, it is unclear how many potential intermediate hosts may have been present at the Huanan seafood market in late 2019. The numbers of wild animals on sale in Wuhan were small compared to those in southern Chinese cities when the first SARS-CoV emerged. One reason is that the southern Chinese border is where large numbers of wild animals are traded or trafficked into China (Zhang et al., 2008). For instance, across all seventeen Wuhan stores selling live wild animal species, approximately 38 raccoon dogs, seven hog badgers, ten palm civets, and ten minks had been sold on average per month (Xiao et al., 2021). Wuhan wildlife sales also diminished on a seasonal basis starting in November each year (personal communication with the authors of Xiao X et al. 2021). When employees of the Wuhan and Beijing CDC arrived at the Huanan seafood market at the end of 2019, they reported no live animals (Page and Khan, 2020). Efforts to track down SARS-CoV-2-infected animals in the supply chain to Wuhan have come up empty (World Health Organization, 2021). Animal traders in southern China, who had supplied Wuhan, had their animals bought and farms shut down by authorities in early 2020, reportedly without testing for SARS-CoV-2 (Page et al., 2021). Notably, Xiao X et al. reported that no bats or pangolins had been sold in Wuhan (Xiao et al., 2021). This meant there was no reported sale in Wuhan of live bats, the reservoir of SARSr-CoVs, or live pangolins, the only species in the wildlife trade that has been identified as carrying SC2-like viruses.

The third piece of evidence often used to support an origin of the virus at the Huanan seafood market is that SARS-CoV-2 genetic material and infectious virions were found on surfaces (e.g., doors, floors, sewage, toilets) at the market. However, the presence of infectious SARS-CoV-2 on surfaces does not necessarily indicate the occurrence of natural spillover. Similar environmental contamination with SARS-CoV-2 has been reported in other locations where there were human superspreader events, e.g., the Diamond Princess cruise (Moriarty et al., 2020), or where there are infected persons in isolation, e.g., at a quarantine hotel (Wong et al., 2021). SARS-CoV-2 can persist and remain infectious on various surfaces for days (Lewis, 2021; OCLC, 2021). Moreover, the China-WHO report stated that “no clear clustering with one specific part of the market was apparent as cases were widely distributed”, and the figure on page 95 of their report shows no clear correlation between wildlife product stalls and COVID-19 cases or positive environmental samples (World Health Organization, 2021). The Chinese CDC also recently reported no correlation between positive environmental samples and wildlife stalls (Gao et al., 2022). However, they have not released their sampling scheme at the market, i.e., the number of samples collected at each stall. Problematically, the market was sampled between January and March 2020, only after the virus had already spread widely in Wuhan, outside the city and province, and beyond China. This makes it challenging to know whether the surface contamination at the market was due to infected animals or infected people in the outbreak.

To this day, the available epidemiological and genetic data cannot determine whether the Huanan seafood market was the site of an early human superspreader event (a person brought the virus into the market and transmitted it to others) or the site of natural spillover (Bloom, 2021; Courtier-Orgogozo and de Ribera, 2022; Gao et al., 2022; Huang et al., 2020; World Health Organization, 2021; Yu et al., 2020; Zhan et al., 2020). To complicate matters, the identification of early cases suffered from bias due to the initial assumption that the virus had spilled over from illegal wildlife sold at the Huanan seafood market and the delay in acknowledging human-to-human transmission of the virus until late January 2020. Chinese investigators specifically reported that, after detecting four cases at a hospital near the market, beginning on Dec 30, 2019 “city-wide case screening was conducted targeting people with... exposure history with Huanan market” and that they “continued epidemiology surveillance at several hospitals (close to Huanan market), Huanan market and the neighborhood of Huanan

market” (World Health Organization, 2021). This meant that the earliest known cases either had to be linked to the market or, if unlinked to the market, be identified near the market. Since it was not acknowledged that the virus was transmitting from human to human, investigators at the time had not been searching for early victims unlinked to or far away from the Huanan seafood market. The early case definition included a link to the market and this bias was only removed on January 18, 2020 when human to human transmission was finally acknowledged by the authorities (The-nCoV Outbreak Joint Field Epidemiology Investigation Team and Li, 2020; World Health Organization, 2021). By this time, early cases or clusters (occurring December 2019 or earlier) that were unlinked to the market or located outside of its district would most likely have been missed. No amount of filtering or re-analysis of early case data can retrospectively discover these undetected cases (Bahry, 2022). This lack of information on early cases hinders the determination of the original source of the virus.

### **Comparing the origin tracking of SARS-CoV and SARS-CoV-2**

The absence of a proximal animal source of SARS-CoV-2 two years post-outbreak stands in contrast to the timeline of the first SARS-CoV outbreaks (see Table 1 for key differences between the two series of events). Both viruses are “generalist” viruses that have been found to transmit across and infect a large range of host species (Guan et al., 2003; Kutter et al., 2021; Li et al., 2006; MacLean et al., 2021; Martina et al., 2003; Wang M. et al., 2005). Both viruses have also been hypothesized to have been transmitting for some weeks to months before their respective detected human outbreaks based on the available epidemiological and genetic evidence that will be discussed in this section.

SARS-CoV broke out twice in Guangdong province, in late 2002 and again in late 2003. Both times, Chinese investigators rapidly tracked down early human cases in the province, likely animal sources of the virus, and a well substantiated path for SARS-CoV to have been introduced into human beings via infected animals. Based on the genetic data, each SARS-CoV outbreak was determined to have stemmed from separate zoonotic spillover events (Wang et al., 2006). A considerable portion of the index patients were restaurant employees, clients, or other food handlers, but not market traders who were found to possess a high level of pre-existing immunity to SARS-CoVs due to frequent occupational exposure to these viruses (Centers for Disease Control and Prevention, 2003; Xu et al., 2004). This spurred Chinese

investigators to sample animals at live animal markets or restaurants, where live animals were often housed until being prepared for consumption. Their approach successfully identified a variety of SARS-CoV-positive animals, including palm civets (Guan et al., 2003; Kan et al., 2005; M. Wang et al., 2005; Wang M. et al., 2005). In the first outbreak, animals carrying SARS-CoV were detected via RT-PCR, virus isolation, and neutralizing antibody tests, and reported to the public by May 2003 (Guan et al., 2003). This tracking of animal hosts was rapid considering the relatively less advanced surveillance and virus characterization technology and expertise at the time. The first SARS-CoV patient isolate had only been obtained in March 2003, and the SARS-CoV genome was only sequenced in April 2003 (Centers for Disease Control and Prevention, 2013; Guan et al., 2003). In the second SARS-CoV outbreak, animal sources of SARS-CoV were again swiftly identified at the workplace of an index patient and at an animal market. Within a few days of diagnosing an index patient, samples had been collected from animals and coworkers at her workplace (a restaurant) and confirmed to be positive for SARS-CoV RNA and/or antibodies (Kan et al., 2005; M. Wang et al., 2005; Wang M. et al., 2005).

In addition, a May 2003 survey of 508 market traders in Guangdong exhibited higher seroprevalence (13%) against SARS-CoV compared to control populations: 1.2% in healthy adults at a clinic for routine physical examinations, and 2.9% in healthcare workers involved with SARS control (Centers for Disease Control and Prevention, 2003; Xu et al., 2004). Among the traders who had primarily sold palm civets, 72.7% had tested positive for IgG antibody to SARSr-CoV, although none had been diagnosed with SARS or atypical pneumonia during the outbreak (Centers for Disease Control and Prevention, 2003). These findings collectively and directly pointed to a proximal zoonotic origin for SARS-CoV and indicated that SARSr-CoVs were prevalent in the Guangdong wildlife trading community prior to the emergence of SARS-CoV (Cheng et al., 2007; Hu and Shi, 2008; Wang et al., 2006). By mid-2005, scientists had traced SARSr-CoVs to bats, which were determined to be the natural reservoir of SARSr-CoVs (Li et al., 2005; Wang et al., 2006). A decade later, the closest bat virus relatives (~96% genome match) to SARS-CoV were found close to Kunming, Yunnan, far away from Guangdong (Hu et al., 2017). The scientists tracking these viruses reported that there had been a farm near these bat habitats that supplied civets to Guangdong at the time of the SARS-CoV outbreak (The Paper, 2017).

Curiously, two studies published in 2005 and 2007 reported the discovery of the 2003 epidemic SARS-CoV in civets in Hubei province (Hu et al., 2005; Liu et al., 2007). The focus of the 2005 study was the development of a new RT-PCR assay for SARS. Using this method, the authors detected low copy numbers of SARS-CoV in all seven of the civet samples they tested. The authors did not isolate the virus from civet samples, test the civets for antibodies, or sequence the SARS-CoV amplicons derived from the samples. Without these measures to validate their finding, it is difficult to know whether these were true positives. The 2007 study reported obtaining three complete SARS-CoV spike sequences from Hubei farmed civets which were a 99.5-99.8% nucleotide match to the Urbani SARS-CoV spike sequence – these 2007 civet CoV spike sequences were even more similar to the human SARS-CoV spike than the SARS-CoV that had infected civets in 2003 in Guangdong markets. The authors did not provide any sample history or even the year of sample collection. To our knowledge, their finding was not followed up upon in the scientific literature. Due to the lack of validation and sample history, it is unclear how the 2003 epidemic SARS-CoV virus appeared in Hubei civets in both studies when the virus' bat reservoir and the human outbreak were both in southern China.

Another difference between the SARS-CoV and SARS-CoV-2 outbreaks is that there was no evidence of frequent exposure to SARSr-CoVs in the Wuhan animal trading community prior to the pandemic. In the 2003 SARS epidemic, market workers and animal traders had been underrepresented among confirmed cases, and the traders who tested positive for SARS-CoV antibodies did not report experiencing atypical pneumonia during the outbreak period, suggesting asymptomatic infection with either SARS-CoV or closely related viruses (Centers for Disease Control and Prevention, 2003; Xu et al., 2004). However, in the SARS-CoV-2 outbreak in Wuhan, market workers were highly represented among symptomatic, hospitalized index cases (Zhou et al., 2020). It is unclear whether wildlife traders or handlers at the Huanan seafood market or other Wuhan markets who were not identified as COVID-19 cases were tested for antibodies to SC2-like viruses. A 2015 survey of 240 blood donors in Wuhan found that none had antibodies against SARSr-CoVs, in comparison to the 2.7% seropositivity (6 people) observed in 218 Yunnan residents living close to bat colonies, where SARSr-CoVs had been found (Wang et al., 2018). A survey of 640 throat swabs collected between October, 2019 to January, 2020 from Wuhan patients with influenza-like illness found that only samples in January had tested positive for SARS-CoV-2 (Kong et al., 2020). SARS-CoV-2 was reportedly not identified in the retrospective testing of 4,500 research project human samples from the second half of 2019 at various hospitals in Hubei (including Wuhan) and other provinces, nor did

any of 43,850 samples from 32,484 healthy blood donors in Wuhan between September and December 2019 test positive for SARS-CoV-2-specific antibodies (Chang et al., 2022; World Health Organization, 2021). Collectively, these observations suggest that SARS-CoVs were unlikely to have been circulating at a detectable level in Wuhan or its animal trading community prior to the pandemic. There is no data to suggest SARS-CoVs were considered likely to spillover from animals into people in Wuhan.

The comparison between the origin investigations of the SARS-CoV and SARS-CoV-2 outbreaks should serve as a reminder that it is possible to identify the transmission route of a pandemic pathogen quickly, as was done for the 2003 SARS outbreak. However, each outbreak is different, and finding the origin of each new outbreak has unique challenges. It is possible that SARS-CoV-2, despite being a highly transmissible pathogen that can infect a wide range of mammalian species, only existed in the wildlife trade at a low undetectable level or was transmitted directly from bats to people who then traveled to Wuhan without seeding outbreaks anywhere else. Nonetheless, the following three major questions pertinent to a wildlife trade origin can be investigated: (i) Which species of wild animals and how many were on sale at the Huanan seafood market in late 2019? Data relevant to this question exist but have not been made available (Xiao et al., 2021). (ii) Are there data or samples collected from the farms in southern China that had supplied animals to Wuhan, which had been reportedly shut down by the authorities without testing for SARS-CoV-2 (Page et al., 2021)? (iii) Beyond pangolins, are there signs of SC2-like viruses in any other animal from the wildlife trade?

## **CONCLUSIONS**

The announcement by the Chinese authorities in early 2020 that SARS-CoV-2 was likely from animals sold at the Huanan seafood market and the rapid release of four preprints describing Guangdong pangolin CoV sequences fueled support for the hypothesis that SARS-CoV-2 had spilled over from the wildlife trade. However, since then, international efforts have failed to identify a proximal animal source or a direct precursor of SARS-CoV-2. No SC2-like viruses have been found in the wildlife trade supply to Wuhan or in bats in Hubei province. Virus tracking experts in Wuhan have not been able to find the types of evidence for a natural origin that were so quickly obtained in the case of the 2003 SARS outbreak (Table 1). Under these circumstances where definitive evidence has yet to be found and investigations have been



hindered (see panel for recommendations for tracking the next coronavirus outbreak), it would be premature to declare the origin of the pandemic solved or to claim that the scientific evidence for a natural spillover origin at the Huanan seafood market is overwhelming. Recent reports organized by the WHO Scientific Advisory Group for the Origins of Novel Pathogens (SAGO) and the Lancet on the origin of SARS-CoV-2 are correctly focused on seeking out more information from parties in or outside of China before arriving at a conclusion (World Health Organization, 2022; Sachs et al., 2022).

### **Recommendations for tracking the next coronavirus outbreak**

1. Create a protocol and agreement for international investigators to visit the site of the outbreak and collect data in the early days
2. Do not set overly narrow patient/case definitions, e.g., based on symptoms or location, which could lead to ascertainment bias and investigators overlooking early clusters
3. Publish complete datasets and methodology to help understand potential sampling biases and the types of data collected
4. Identify, secure and check repositories of animal or human samples, e.g., from regions with frequent transit or trade with the site of outbreak, for signs of early undetected spread
5. Mandate preprinting and pre-publication raw data deposition (can be embargoed in online databases until the time of publication), alongside an open peer review process, when studies present novel data that are relevant to the outbreak, e.g., early sequences, early case data, wildlife trade data; submissions from the research community that identify errors or missing data in these studies should be incentivized and published in a timely manner
6. Establish secure communication channels for scientists, healthcare workers, journalists and other investigators or witnesses to transmit emerging information to global networks
7. Establish international data sharing platforms for the surveillance and discovery of novel pathogens, accompanied by mechanisms to compel the timely deposition of data describing new pathogen sequences
8. Create a protocol and agreement for international investigators to access full datasets deposited in international databases for the purposes of investigating outbreaks; any alteration, suppression, or deletion of such data should be tracked, and these records made available to investigators

**Table 1. A comparison of the origin tracking of SARS-CoV versus SARS-CoV-2.**

	<b>SARS-CoV</b>	<b>SARS-CoV-2</b>
<b>Host range</b>	Both are “generalist” viruses that can infect and/or transmit across a wide range of mammalian host species.	
<b>Index patients in the detected outbreak</b>	The earliest cases were known to have directly handled or were exposed to live animals; largely restaurant employees, clients, or other food handlers, but not market traders (see below on serological evidence).	Largely vendors at the Huanan seafood market; other early cases had no links to markets or the wildlife trade.
<b>Infected animals</b>	Infected market animals such as palm civets and a raccoon dog were reported by May 2003 (the virus had been isolated from a patient in March and sequenced in April). When the virus appeared again in early 2004, infected civets were again quickly found at the workplace (a restaurant) of an index patient and at a market.	No infected wildlife has been reported at Wuhan markets, in the supply chain, or other sites in Hubei province. Investigators reported that no live wild mammals were found when they sampled the Huanan seafood market on January 1, 2020.
<b>Serological evidence</b>	Guangdong market traders had high seroprevalence against SARS-CoV compared to control human populations; none of the market traders had been diagnosed with SARS or atypical pneumonia during the outbreak, suggesting the circulation of SARSr-CoVs in the Guangdong wildlife trade community.	It has not been reported whether Wuhan market traders or suppliers were tested for antibodies against SARSr-CoVs. There is no data yet to suggest that SARSr-CoVs were considered likely to spillover from animals into people in Wuhan.

<p><b>Bat virus reservoir</b></p>	<p>SARSr-CoVs were traced to bats by mid2005. The closest genome match to SARS-CoV (96%) was found in bats near Kunming, Yunnan as reported in 2017. Investigators reported that there had been a farm near these bat habitats that had supplied civets to Guangdong at the time of the SARS-CoV outbreak.</p>	<p>The closest genome match to SARS-CoV-2 (96.2%) had been discovered in bats near Kunming, Yunnan as reported in 2016. Another close match (96.8%) was found in bats in Laos and reported in 2021.</p>
<p><b>Summary</b></p>	<p>Collectively, substantial evidence of frequent exposure to SARSr-CoVs in the Guangdong animal trading community and a clear conduit from bats in Yunnan to markets in Guangdong, plus direct evidence of original animal-to-human transmission of SARS-CoV.</p>	<p>No evidence of frequent exposure to SARSr-CoVs in the Wuhan animal trading community prior to the SARS-CoV-2 outbreak. No direct evidence of original animal-to-human transmission of SARS-CoV-2.</p>

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