

The Origins of SARS-CoV-2: A Critical Review

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Since the first reports of a novel SARS-like coronavirus in December 2019 in Wuhan, China, there has been intense interest in understanding how SARS-CoV-2 emerged in the human population. Recent debate has coalesced around two competing ideas: a “laboratory escape” scenario and zoonotic emergence. Here, we critically review the current scientific evidence that may help clarify the origin of SARS-CoV-2.

Evidence supporting a zoonotic origin of SARS-CoV-2

Coronaviruses have long been known to present pandemic risks. SARS-CoV-2 is the ninth documented coronavirus that infects humans and the seventh identified in the last 20 years^{1,2}. All previous human coronaviruses have zoonotic origins, as have the vast majority of human viruses. The emergence of SARS-CoV-2 bears several signatures of these prior zoonotic events. It displays clear similarities to SARS-CoV that spilled over into humans in Foshan, Guangdong province, China in November 2002, and again in Guangzhou, Guangdong province in 2003³. Both these SARS-CoV emergence events were associated with markets selling live animals and involved species, particularly civets and raccoon dogs⁴, that were also sold live in Wuhan markets in 2019⁵ and are known to be susceptible to SARS-CoV-2 infection⁶. Animal traders working in 2003, without a SARS diagnosis, were documented to have high levels of IgG to SARS-CoV (13% overall and >50% for traders specializing in civets⁷). Subsequent serological surveys found ~3% positivity rates to SARS-CoV related (SARSr-CoV) viruses in residents of Yunnan province living close to bat caves⁸, demonstrating regular exposure in rural locations. The closest known relatives to both SARS-CoV and SARS-CoV-2 are viruses from bats in Yunnan, although animals from this province have been preferentially sampled. For both SARS-CoV and SARS-CoV-2, there is a considerable geographic gap between Yunnan and the location of the first human cases, highlighting the difficulty in identifying the exact pathway of virus emergence and the importance of sampling beyond Yunnan.

SARS-CoV-2 also shows similarities to the four endemic human coronaviruses: HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63. These viruses have zoonotic origins and the circumstances of their emergence are unclear. In direct parallel to SARS-CoV-2, HCoV-HKU1, which was first described in a large Chinese city (Shenzhen, Guangdong) in the winter of 2004, has an unknown animal origin, contains a furin cleavage site in its spike protein, and was originally identified in a case of human pneumonia⁹.

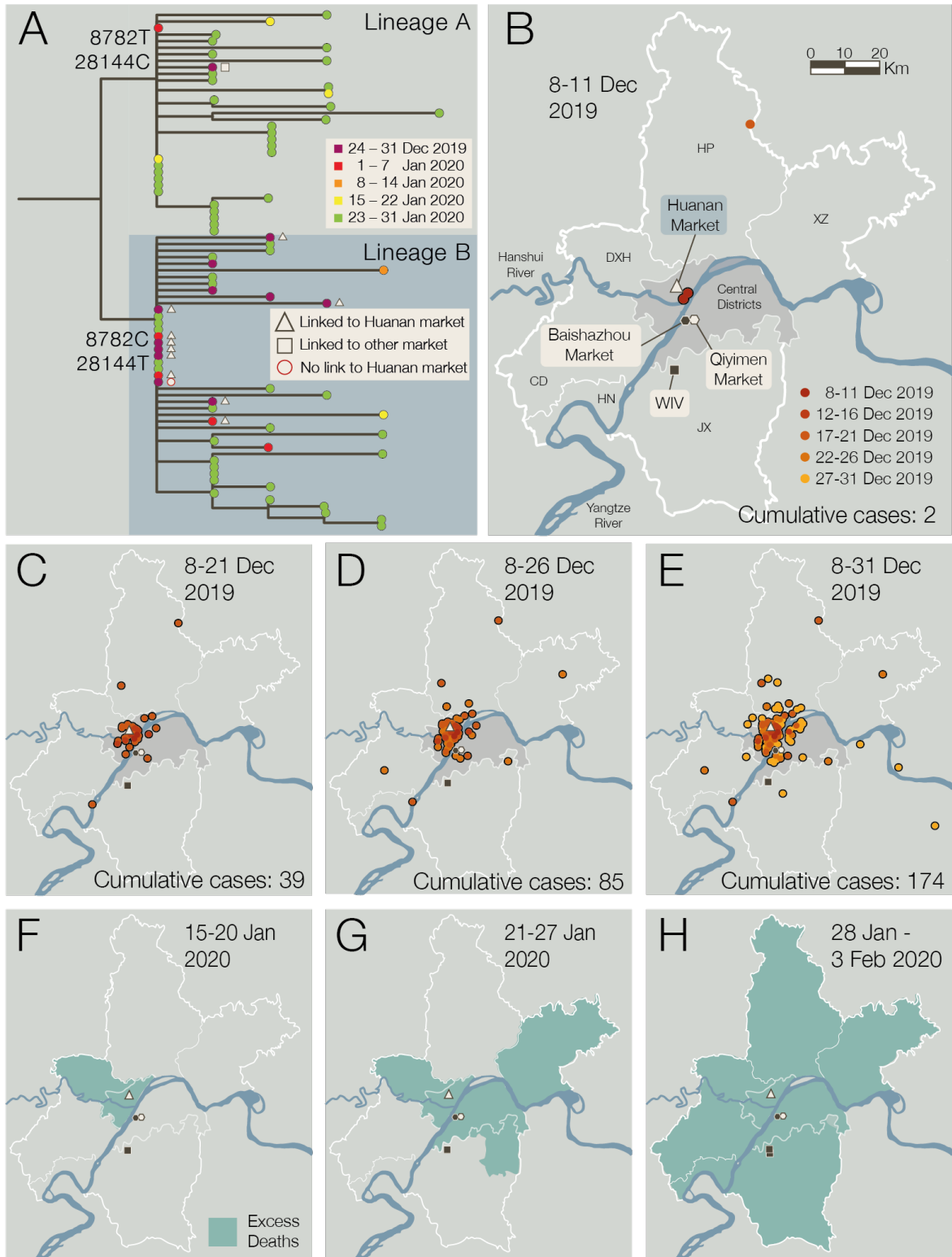


Figure 1 | Phylogenetic and epidemiological data on the early COVID-19 pandemic in Wuhan. (a) Phylogenetic tree of early SARS-CoV-2 genomes sampled from Wuhan during December 2019-January 2020. The split between lineages A and B is labelled with the coordinates and base of the

two differentiating nucleotide mutations. Cases with a known association to the Huanan or other markets are denoted by symbols (reported in ref. 10). **(b)** Map of districts of Wuhan showing the location of markets, the BSL-4 campus of the Wuhan Institute of Virology (where the coronavirus work of Dr. Shi Zhengli is performed) and the earliest known cases. **(c-e)** Location of recorded COVID-19 cases in Wuhan from 8th December to 31st December 2019. Cases with a home address outside of Wuhan city are not shown. **(f-h)** Map of districts of Wuhan indicating the first record of excess deaths due to pneumonia (shaded green) from 15th January 2020. Case and excess death data were extracted and redrawn from figures provided in ref 10. For more details see **supplementary information**.

Based on epidemiological data, the Huanan market in Wuhan was an early and major epicenter of SARS-CoV-2 infection. Two of the three earliest documented COVID-19 cases were directly linked to this market selling wild animals, as were 28% of all cases reported in December 2019¹⁰. Overall, 55% of cases during December 2019 had an exposure to either the Huanan or other markets in Wuhan, with these cases more prevalent in the first half of that month¹⁰. Examination of the locations of early cases shows that most cluster around the Huanan market, located north of the Yangtze river (**Fig. 1a-e**). These districts were also the first to exhibit excess pneumonia deaths in January 2020 (**Fig. 1f-h**). There is no epidemiological link to any other locality in Wuhan, including the BSL-4 campus of the Wuhan Institute of Virology (WIV) located south of the Yangtze and the subject of considerable speculation. Although some early cases do not have a direct epidemiological link to a market¹⁰, this is expected given high rates of asymptomatic transmission and undocumented secondary transmission events, and was similarly observed in early SARS-CoV cases in Foshan³.

During 2019, markets in Wuhan – including the Huanan market – traded many thousands of live wild animals including high-risk species such as civets and raccoon dogs⁵. Following its closure, SARS-CoV-2 was detected in environmental samples at the Huanan market, primarily in the western section that traded in wildlife and domestic animal products, as well as in associated drainage areas¹⁰. While animal carcasses retrospectively tested negative for SARS-CoV-2, these were unrepresentative of the live animal species sold, and specifically did not include raccoon dogs and other animals known to be susceptible to SARS-CoV-2⁵.

The earliest split in the SARS-CoV-2 phylogeny defines two lineages - denoted A and B¹¹ - that likely circulated contemporaneously (**Fig. 1a**). Lineage B, which became dominant globally, was observed in early cases linked to the Huanan market and environmental samples taken there, while lineage A contains a case with exposure to other markets (**Fig. 1a,b**) as well as with later cases in Wuhan and other parts of China¹⁰. This phylogenetic pattern is consistent with the emergence of SARS-CoV-2 involving one or more contacts with infected animals and/or traders, including multiple spill-over events, as potentially infected or susceptible animals were moved into or between Wuhan markets via shared supply chains and sold for human consumption⁵. The potential emergence of SARS-CoV-2 across multiple markets again mirrors SARS-CoV in which high levels of infection, seroprevalence and genetic diversity in animals were documented at both the Dongmen market in Shenzhen^{4,12} and the Xinyuan market in Guangzhou^{13,14}.

Viruses closely related to SARS-CoV-2 have been documented in bats and pangolins in multiple localities in South-East Asia, including in China, Thailand, Cambodia, and Japan^{15,16}, with serological evidence for viral infection in pangolins for more than a decade¹⁷. However, a significant evolutionary gap exists between SARS-CoV-2 and the closest related animal viruses: their genetic distances of approximately 4% (~1,150 mutations) equates to decades of evolutionary divergence¹⁸. Widespread genomic recombination also complicates the assignment of which viruses are closest to SARS-CoV-2. Although *Rhinolophus* bat virus RaTG13 collected in Yunnan has the highest average genetic similarity to SARS-CoV-2, a history of recombination means that three other bat viruses – RmYN02, RpYN06 and PrC31 – are closer in most of the virus genome (particularly ORF1ab) and thus share a more recent common ancestor with SARS-CoV-2^{15,16,19}. None of these closer viruses were collected by the WIV. This demonstrates beyond reasonable doubt that RaTG13 is not the progenitor of SARS-CoV-2, with or without laboratory manipulation or experimental mutagenesis.

Although no bat reservoir nor intermediate animal host for SARS-CoV-2 has been identified to date, initial cross-species transmission events are very likely to go undetected. Most SARS-CoV-2 index case infections are unlikely to have resulted in sustained onward transmission²⁰ and only a very small subset of spillover events from animals to humans result in major outbreaks. Indeed, the animal origins of many well-known human pathogens, including Ebola virus, Hepatitis C virus, poliovirus, and the coronaviruses HCoV-HKU1 and HCoV-NL63, are yet to be identified,

while it took over a decade to discover bat viruses with >95% similarity to SARS-CoV and able to use hACE-2 as a receptor²¹.

Could SARS-CoV-2 have escaped from a laboratory?

There are precedents for laboratory incidents leading to isolated infections and transient transmission chains, including SARS-CoV²². Aside from the 1977 A/H1N1 influenza pandemic that likely originated from a large-scale vaccine challenge trial²³, there are no documented examples of human epidemics or pandemics resulting from research activity.

The emergence of SARS-CoV-2 differs markedly from documented laboratory escapes that, with the exception of Marburg virus²⁴, have been of readily identifiable viruses capable of human infection and associated with sustained work in high titer cultures²⁵⁻²⁷. No previous epidemic has been caused by the escape of a novel virus and there is no data to suggest that the WIV—or any other laboratory—were working on SARS-CoV-2, or any virus close enough to be the progenitor, prior to the COVID-19 pandemic. Viral genomic sequencing without cell culture, which was routinely performed at the WIV, represents a negligible risk as viruses are inactivated during RNA extraction²⁸ and no case of laboratory escape has been documented following the sequencing of viral samples.

Known laboratory outbreaks have been traced to both workplace and family contacts of index cases and to the laboratory of origin^{25-27,24}. Despite extensive contact tracing of early cases during the COVID-19 pandemic, there have been no reported cases related to any laboratory staff at the WIV and all staff in the laboratory of Dr. Shi Zhengli were reported to be seronegative for SARS-CoV-2 when tested in March 2020¹⁰. During a period of high influenza transmission and other respiratory virus circulation²⁹ reports of illnesses would need to be confirmed as caused by SARS-CoV-2 to be relevant. Epidemiological modeling suggests that the number of hypothetical cases needed to result in multiple hospitalized COVID-19 patients prior to December 2019 is incompatible with observed clinical, genomic, and epidemiological data²⁰.

The WIV possesses an extensive catalogue of samples derived from bats and has reportedly successfully cultured three SARS-CoVs from bats, all of which are genetically distinct from SARS-CoV-2³⁰⁻³². These viruses were isolated from fecal samples through serial amplification in

VeroE6 cells, a process that consistently results in the loss of the SARS-CoV-2 furin cleavage site³³⁻³⁹. It is therefore highly unlikely that these techniques would result in the isolation of a SARS-CoV-2 progenitor with an intact furin cleavage site. No published work indicates that other methods, including the generation of novel reverse genetics systems, were used at the WIV to propagate infectious SARSr-CoVs based on sequence data from bats. Gain-of-function research would be expected to utilize an established SARSr-CoV genomic backbone, or at a minimum a virus previously identified via sequencing. However, past experimental research using recombinant coronaviruses at the WIV has used a genetic backbone (WIV1) unrelated to SARS-CoV-2³² and SARS-CoV-2 carries no evidence of genetic markers one might expect from laboratory experiments⁴⁰. There is no rational experimental reason why a new genetic system would be developed using an unknown and unpublished virus, with no evidence nor mention of a SARS-CoV-2-like virus in any prior publication or study from the WIV^{32,41,42}, no evidence that the WIV sequenced a virus that is closer to SARS-CoV-2 than RaTG13, and no reason to hide research on a SARS-CoV-2-like virus prior to the COVID-19 pandemic. Under any laboratory escape scenario SARS-CoV-2 would have to have been present in a laboratory prior to the pandemic, yet no evidence exists to support such a notion and no sequence has been identified that could have served as a precursor.

A specific laboratory escape scenario involves accidental infection in the course of serial passage of a SARSr-CoV in common laboratory animals such as mice. However, early SARS-CoV-2 isolates were unable to infect wild-type mice⁴³. While murine models are useful for studying infection *in vivo* and testing vaccines, they often result in mild or atypical disease⁴⁴⁻⁴⁸. These findings are inconsistent with a virus selected for increased pathogenicity and transmissibility through serial passage through rodents. Although SARS-CoV-2 has since been engineered⁴⁹ and adapted by serial passage⁵⁰⁻⁵², specific mutations in the spike protein, including N501Y, are necessary for such adaptation in mice^{51,52}. Notably, N501Y has arisen convergently in multiple SARS-CoV-2 variants of concern in the human population, presumably being selected to increase ACE2 binding affinity⁵³⁻⁵⁶. If SARS-CoV-2 resulted from attempts to adapt a SARSr-CoV for study in animal models, it would likely have acquired mutations like N501Y for efficient replication in that model, yet there is no evidence to suggest such mutations existed early in the pandemic. Both the low pathogenicity in commonly used laboratory animals and the absence of genomic markers associated with rodent adaptation indicate that SARS-

CoV-2 is highly unlikely to have been acquired by laboratory workers in the course of viral pathogenesis or gain-of-function experiments.

Evidence from genomic structure and ongoing evolution of SARS-CoV-2

Considerable attention has been devoted to claims that SARS-CoV-2 was genetically engineered or adapted in cell culture or “humanized” animal models to promote human transmission⁵⁷. Yet, since its emergence, SARS-CoV-2 has experienced repeated sweeps of mutations that have increased viral fitness^{58,59}. The first clear adaptive mutation, the D614G substitution in the spike protein, occurred early in the pandemic^{60,61}. Recurring mutations in the receptor binding domain of the spike protein, including N501Y, K417N/T, L452R, and E484K/Q—constituent mutations of the variants of concern—similarly enhance viral infectivity^{54,55,62} and ACE2 binding^{53,63}, refuting claims that the SARS-CoV-2 spike protein was optimized for binding to human ACE2 upon its emergence⁵⁶. Further, some pangolin-derived coronaviruses have receptor binding domains that are near-identical to SARS-CoV-2 at the amino acid level^{40,64} and bind to human ACE2 even more strongly than SARS-CoV-2, showing that there is capacity for further human adaptation⁶⁵. SARS-CoV-2 is also notable for being a host generalist virus⁶⁶, capable of efficient transmission in multiple mammalian species, including mink, tigers, cats, gorillas, dogs, raccoon dogs, ferrets, and large outbreaks have been documented in mink with spill-back to humans⁶⁷ and to other animals⁶⁸. Combined, these findings show that no specific human “pre” adaptation was required for the emergence or early spread of SARS-CoV-2, and the claim that the virus was already highly adapted to the human host⁵⁷, or somehow optimized for binding to human ACE2, is without validity.

The genesis of the polybasic (furin) cleavage site in the spike protein of SARS-CoV-2 has been subject to recurrent speculation. Although the furin cleavage site is absent from the closest known relatives of SARS-CoV-2⁴⁰, this is unsurprising as the lineage leading to this virus is poorly sampled and the closest bat viruses have divergent spike proteins due to recombination^{15,16,18}. Furin cleavage sites are commonplace in other coronavirus spike proteins, including some feline alphacoronaviruses, MERS-CoV, most but not all strains of mouse hepatitis virus, as well as in endemic human betacoronaviruses such as HCoV-OC43 and HCoV-HKU1⁶⁹⁻⁷¹. A near identical nucleotide sequence is found in the spike gene of the bat coronavirus HKU9-1⁷², and both SARS-CoV-2 and HKU9-1 contain short palindromic sequences immediately upstream of this sequence that are indicative of natural recombination break-points via template switching⁷². Hence, simple

The SARS-CoV-2 furin cleavage site (containing the amino acid motif RRAR) does not match its canonical form (R-X-R/K-R), is suboptimal compared to those of HCoV-HKU1 and HCoV-OC43, lacks either a P1 or P2 arginine (depending on the alignment), and was caused by an out-of-frame insertion (**Fig. 2**). The RRAR and RRSR S1/S2 cleavage sites in feline coronaviruses (FCoV) and cell-culture adapted HCoV-OC43, respectively, are not cleaved by furin⁶⁹. There is no logical reason why an engineered virus would utilize such a poor furin cleavage site, which would entail such an unusual and needlessly complex feat of genetic engineering. The only previous studies of artificial insertion of a furin cleavage site at the S1/S2 boundary in the SARS-CoV spike protein utilized an optimal 'RRSRR' sequence in pseudotype systems^{73,74}. Further, there is no evidence of prior research at the WIV involving the artificial insertion of complete furin cleavage sites into coronaviruses.

The recurring P681H/R substitution in the proline (P) residue preceding the SARS-CoV-2 furin cleavage site improves cleavage of the spike protein and is another signature of ongoing human adaptation of the virus⁷⁵. The SARS-CoV-2 furin site is also lost under standard cell culture conditions^{34,76}, as is true of HCoV-OC43⁷³. The presence of two CGG codons for arginines in the SARS-CoV-2 furin cleavage site is similarly not indicative of genetic engineering⁷⁷. Although the CGG codon is rare in coronaviruses, it is observed in SARS-CoV, SARS-CoV-2 and other human coronaviruses at comparable frequencies⁷⁷. Further, if low-fitness codons had been artificially inserted into the virus genome they would have been quickly selected against during SARS-CoV-2 evolution, yet both CGG codons are more than 99.8% conserved among the >1,800,000 near-complete SARS-CoV-2 genomes sequenced to date, indicative of strong functional constraints (**supplementary information, Table S1**).

Conclusions

As for the vast majority of human viruses, the most parsimonious explanation for the origin of SARS-CoV-2 is a zoonotic event. The documented epidemiological history of the virus is comparable to previous animal market-associated outbreaks of coronaviruses with a simple route for human exposure. The contact tracing of SARS-CoV-2 to markets in Wuhan exhibits striking similarities to the early spread of SARS-CoV to markets in Guangdong, where humans infected early in the epidemic lived near or worked in animal markets. Zoonotic spillover by definition selects for viruses able to infect humans. The laboratory escapes documented to date

have almost exclusively involved viruses brought into laboratories specifically because of their known human infectivity.

There is currently no evidence that SARS-CoV-2 has a laboratory origin. There is no evidence that any early cases had any connection to the WIV, in contrast to the clear epidemiological links to animal markets in Wuhan, nor evidence that the WIV possessed or worked on a progenitor of SARS-CoV-2 prior to the pandemic. The suspicion that SARS-CoV-2 might have a laboratory origin stems from the coincidence that it was first detected in a city that houses a major virological laboratory that studies coronaviruses. Wuhan is the largest city in central China with multiple animal markets and is a major hub for travel and commerce, well connected to other areas both within China and internationally. The link to Wuhan therefore more likely reflects the fact that pathogens often require heavily populated areas to become established²⁰.

We contend that there is substantial body of scientific evidence supporting a zoonotic origin for SARS-CoV-2. While the possibility of a laboratory accident cannot be entirely dismissed, and may be near impossible to falsify, this conduit for emergence is highly unlikely relative to the numerous and repeated human-animal contacts that occur routinely in the wildlife trade. Failure to comprehensively investigate the zoonotic origin through collaborative and carefully coordinated studies would leave the world vulnerable to future pandemics arising from the same human activities that have repeatedly put us on a collision course with novel viruses.

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