

COMMENTARY

Thoughts & Opinion

The SARS-CoV-2 origin dilemma: Zoonotic transfer or laboratory leak?

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Abstract

The COVID-19 pandemic is responsible for millions of deaths worldwide yet its origin remains unclear. Two potential scenarios of how infection of humans initially occurred include zoonotic transfer from wild animals and a leak of the pathogen from a research laboratory. The Wuhan wet markets where wild animals are sold represent a strong scenario for zoonotic transfer. However, isolation of SARS-CoV-2 or its immediate predecessor from wild animals in their natural environment has yet to be documented. Due to incomplete evidence for a zoonotic origin, a laboratory origin is plausible. The Wuhan Institute of Virology is at the epicenter of the pandemic and their work has included manipulation of wild-type coronavirus to enable infection of human cells. Although stronger evidence supports the zoonotic transfer, inconclusive reports maintain the laboratory leak hypothesis alive. It is imperative to reach a factual conclusion to prevent future pandemics.

KEYWORDS

COVID-19 origin, laboratory leak, SARS-CoV-2, zoonotic transfer

Intensive molecular virology and epidemiological studies associated with the COVID-19 causative agent are currently ongoing, with findings highly relevant both for the scientific community and humanity. In March 2020, the Director-General Dr Tedros Adhanom Ghebreyesu of the World Health Organization (WHO) declared the coronavirus (CoV) disease 2019 (COVID-19) outbreak a pandemic [<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen>]. The COVID-19 was first detected in Wuhan City, Hubei Province, China, and subsequently spread worldwide.^[1] Previously named the 2019-novel coronavirus (2019-nCoV) and colloquially known just as coronavirus, the causative agent for this disease was officially called Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV), centered on phylogenetic analysis, determination of the viral nucleotide, and protein sequence

[[https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-\(covid-2019\)-and-the-virus-that-causes-it](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it), accessed 09/15/21]. So far, SARS-CoV-2 has killed more than 739,778 in the USA and 4,888,743 worldwide [<https://www.covidvisualizer.com>, accessed 10/13/21]. However, to date, October 2021, the origin of the SARS-CoV-2 remains unclear.

There are two main potential scenarios of how SARS-CoV-2 infected humans initially: first, a zoonotic transfer from wild animals, most likely bats or an intermediate animal; and second, a leak of this pathogen from a virology research lab. Interestingly, these two potential scenarios were predicted in 2007 by Cheng et al. who alerted the world in a review paper that “The presence of a large reservoir of SARS-CoV-like viruses in horseshoe bats, together with the culture of eating exotic mammals in southern China, is a time bomb.”^[2] In the

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same paper, the authors mention that “The possibility of the reemergence of SARS and other novel viruses from animals or laboratories and therefore the need for preparedness should not be ignored.”^[2] “Animals or laboratories” from this last sentence have become quite relevant in the current state of the ongoing WHO investigation, since both scenarios are plausible.

The scenario that the virus originated from wild animals either directly or via an intermediary host was presented early on during the pandemic by the Chinese virology research team of the Wuhan laboratory. Their argument is that the SARS-CoV-2, the causative agent for COVID-19 and other unknown viruses could be associated with everyday activities at the Wuhan wet markets. Live wild animals are sold for food or as pets and have no origin or quarantine certificate to guarantee hygiene. Stores at wet markets usually offer on-site butchering services with questionable sanitary practices,^[3] where handling of contaminated products poses a risk of human infection. There is a low probability of a pathogen like a potential novel virus being transferred to humans if the infected animal is well-cooked before being consumed. However, there could be a risk of transfer if a contaminated product is ingested raw or poorly cooked. In addition, humans can become infected by respiratory droplets or aerosols expelled by infected animals in the market. The coronavirus with the closest genome to the SARS-CoV-2 is RaTG13 (BtCoV/4991), which shares 96.2% sequence homology with the novel SARS-CoV-2 virus and was first identified in horseshoe bats (*Rhinolophus affinis*) at Yunnan, south China,^[4] hence the idea of a natural transfer from animals to humans. It can be argued that the Huanan market in Wuhan was the source for the SARS-CoV-2 since 73 environmental samples out of 923 tested positive for the virus, although if it was introduced by contaminated products, infected animals, or infected people cannot be determined [<https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-sars-cov-2-china-part>, accessed 09/15/21]. Some evidence indicates that the first identified coronavirus-infected person never visited this market.^[4] Additionally, no one has been able to find the SARS-CoV-2 or a sufficiently closely related virus in wild animals.^[5,6] Currently, humans are the dominant host species for the SARS-CoV-2.

The initial investigation of the Wuhan wet market by the WHO team conducted from January 14th to February 10th, 2021, provided inconclusive results related to the SARS-CoV-2 origin. The team suggested that pangolins (*Pholidota*) should be considered as a potential intermediate host of the SARS-CoV-2. However, as with bats, viruses isolated from pangolins are evolutionarily distant from SARS-CoV-2 [<https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-sars-cov-2-china-part>, accessed 09/15/21]. In addition, a study conducted by Xiao et al. between May 2017 and November 2019 before the onset of the SARS-CoV-2 outbreak, confirmed that no trade of bats or pangolins took place at the Wuhan market.^[3] This indicates that the Wuhan market may not be the origin of the global pandemic. It is possible that the zoonotic transmission of SARS-CoV-2 to humans occurred through an intermediate animal: bats → Intermediate animal(s) → humans. In a study performed in the Netherlands from April to June 2020, Oude Munnink et al. reported SARS-CoV-2 infections in captive minks and humans working or living on 16 mink farms. SARS-CoV-2

infections were identified in 66 out of 97 (68%) of the workers and their intimate contacts.^[7] Very likely, humans initially introduced the virus to the farms and then, animal-to-human transmission occurred. Thus, supporting the idea of a zoonotic transmission. Hence, constant vigilance of minks and other animal-related infections should be implemented to avoid fur production becoming a reservoir for future spillover of SARS-CoV-2 or other viruses to humans [<https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-sars-cov-2-china-part>, accessed 9/15/21]. Regardless of the transmission route, to support the animal transfer theory, it is imperative to isolate a potential immediate predecessor of the SARS-CoV-2 virus and/or isolate the SARS-CoV-2 from a wild animal in its natural environment.

Given the incomplete evidence for a zoonotic origin of the SARS-CoV-2, the alternative hypothesis of a laboratory origin has been considered plausible by some. The first COVID-19 human case in the world was detected in the Chinese city of Wuhan, where the Wuhan Institute of Virology (WIV) has supported research on coronaviruses from bat origins for many years. The WIV has a biosafety level-4 (BSL-4) laboratory, the highest level of biocontainment, allowing the institute to work with the most hazardous agents. In one study conducted in these laboratories, a virus that was isolated from horseshoe bats (Severe Acute Respiratory Syndrome (SARS)-like CoVs; SL-CoV S) was genetically modified in the lab by changing “a minimal insert region (amino acids 310 to 518)” at the N terminus of the spike protein (S).^[8] This modification in the S protein of the SL-CoV S transformed this wild-type coronavirus from human cells being resistant to becoming susceptible to infection via the viral mutated S-protein binding to the angiotensin-converting enzyme 2 (ACE2) receptor. In addition, specific genetic characteristics of SARS-CoV-2 that involve binding to ACE2, the furin cleavage site, and the ganglioside-binding domain of the S protein move away from evolutionary adaptation, according to Segreto et al. and thus, favor the laboratory origin hypothesis.^[9] Moreover, some lab-leak supporters affirm that a rare occurrence of a specific genetic codon sequence, CGG, one of the six codons for arginine, is a sign that the SARS-CoV-2 potentially originated in a laboratory.^[10] A rare characteristic is the tandem arginine doublet, encoded by the low-frequency CGG-CGG codons occurrence. Only 2 out of 42 arginines in the SARS-CoV-2 spike protein are encoded by the CGG codon.^[10,11] Moreover, those two arginines are located in the PRRA furin cleavage site. Surprisingly, the CGG-CGG codons in SARS-CoV-2 are not found in any of the furin cleavage sites in other viral proteins expressed by a wide range of viruses.^[10,11] However, the CGG codon is found in human coronaviruses. It encodes nearly 5% of the arginines in SARS-CoV-2 and close to 3% of the arginines in SARS-CoV. In addition, recent analyses have found recurring natural mutations in the viral spike protein that increase their fitness, infectivity, and ACE2 binding.^[12] Furthermore, furin cleavage sites are commonly found in spike proteins of other coronavirus and the out-of-frame insertion of the one found in SARS-CoV-2 can be explained by straightforward evolutionary processes.^[12] Similarly, the conservation of the CGG codon among the more than 2 million SARS-CoV-2 genomes sequenced to date indicates a natural functional role, or it would have been selected against throughout the evolution of the virus.^[12]

Some controversy also derived from a report published on February 6th, 2020, of insalubrious incidents involving a researcher in China that included bats attacking him, and bat's blood touching his skin, which highlights improper laboratory practices [https://img-prod.tgcom24.mediaset.it/images/2020/02/16/114720192-5eb8307f-017c-4075-a697-348628da0204.pdf, accessed 09/17/21]. This report was later retracted after the Chinese authorities insisted no lab accident had taken place related to the COVID-19 pandemic. On May 23rd, 2021, it was reported that the US intelligence informed about three scientists from WIV becoming sick in November 2019, requiring hospitalization due to COVID-19 pneumonia-like symptoms [https://www.wsj.com/articles/intelligence-on-sick-staff-at-wuhan-lab-fuels-debate-on-covid-19-origins-11621796228, accessed 09/17/21]. Disclosure of the particulars on the health conditions and fate of the three researchers involved was not provided. However, after a 90-day investigation into SARS-CoV-2 origin, the US Intelligence Community, comprised 18 organizations [https://www.dni.gov/index.php/what-we-do/members-of-the-ic, accessed 09/17/21], reported inconclusive findings on the SARS-CoV-2 origin on August 27th, 2021. They did conclude however, that "SARS-CoV-2 wasn't weaponized and was unlikely to be engineered." [https://www.nature.com/articles/d41586-021-02366-0, accessed 09/17/21]. In addition, epidemiological modeling places the first case of SARS-CoV-2 back to mid-October to mid-November of 2019 and the number of infected people needed to result in hospitalization of patients are incompatible with the observed data.^[13] Regardless, reported viral transformations that occurred at the WIV are currently crucial and should be investigated because the origin of SARS-CoV-2 is elusive.

It is easy to assume that if the current COVID-19 pandemic was initiated from a zoonotic transmission incident, additional human infections would emerge from the same animal source, most likely occurring at the same geographic location or its surroundings. It is a matter of time to determine whether the SARS-CoV-2 variants evolving in humans, like the delta variant first identified in India, will affect wild animal species by reverse zoonosis; human → animals transmission.

At present, there is stronger evidence supporting a zoonotic transfer. However, lack of transparency has given way to maintain the laboratory leak hypothesis alive. Because of the implications, the two scenarios should remain on the table, and additional vigorous investigations are needed to reach a conclusion about the origin of SARS-CoV-2. More than likely, it will take some time before the knowledge of the origin of SARS-CoV-2 will be elucidated. Hence the faster we move to reach that conclusion, the better. The world is demanding and waiting for a factual answer because, once clarified, this knowledge can help prevent future pandemics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

- Liu, Y.-C., Kuo, R.-L., & Shih, S.-R. (2020). COVID-19: The first documented coronavirus pandemic in history. *Biomedical Journal*, 43(4), 328–333.
- Cheng, V. C. C., Lau, S. K. P., Woo, P. C. Y., & Yuen, K. Y. (2007). Severe Acute Respiratory Syndrome Coronavirus as an Agent of Emerging and Reemerging Infection. *Clinical Microbiology Reviews*, 20(4), 660–694.
- Xiao, X., Newman, C., Buesching, C. D., Macdonald, D. W., & Zhou, Z.-M. (2021). Animal sales from Wuhan wet markets immediately prior to the COVID-19 pandemic. *Scientific Reports*, 11(1), <https://doi.org/10.1038/s41598-021-91470-2>.
- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, 395(10223), 497–506.
- Mallapaty, S. (2020). Animal source of the coronavirus continues to elude scientists. *Nature*, <https://doi.org/10.1038/d41586-020-01449-8>.
- Maxmen, A., & Mallapaty, S. (2021). The COVID lab-leak hypothesis: what scientists do and don't know. *Nature*, 594(7863), 313–315.
- Oude Munnink, B. B., Sikkema, R. S., Nieuwenhuijse, D. F., Molenaar, R. J., Munger, E., Molenkamp, R., van der Spek, A., Tolsma, P., Rietveld, H., Brouwer, M., Bouwmeester-Vincken, N., Harders, F., Hakze-van der Honing, R., Wegdam-Blans, M. C. A., Bouwstra, R. J., GeurtsvanKessel, C., van der Eijk, A. A., Velkers, F. C., Smit, L. A. M., Stegeman, A., van der Poel, W. H. M., & Koopmans, M. P. G. (2021). Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science*, 371(6525), 172–177.
- Ren, W., Qu, X., Li, W., Han, Z., Yu, M., Zhou, P., Zhang, S.-Y., Wang, L.-F., Deng, H., & Shi, Z. (2008). Difference in Receptor Usage between Severe Acute Respiratory Syndrome (SARS) Coronavirus and SARS-Like Coronavirus of Bat Origin. *Journal of Virology*, 82(4), 1899–1907.
- Segreto, R., Deigin, Y., McCairn, K., Sousa, A., Sirotkin, D., Sirotkin, K., Couey, J. J., Jones, A., & Zhang, D. (2021). Should we discount the laboratory origin of COVID-19?. *Environmental Chemistry Letters*, 19(4), 2743–2757.
- Romeu, A., & Olle, E. SARS-CoV-2 origin: An affair of codons?. Preprints 2021, 2021060121 (doi: 10.20944/preprints202106.0121.v1).
- Romeu, A., & Olle, E. SARS-CoV-2 and the secret of the furin site. Preprints 2021, 2021020264 (doi: 10.20944/preprints202102.0264.v1).
- Holmes, E. C., Goldstein, S. A., Rasmussen, A. L., Robertson, D. L., Crits-Christoph, A., Wertheim, J. O., Anthony, S. J., Barclay, W. S., Boni, M. F., Doherty, P. C., Farrar, J., Geoghegan, J. L., Jiang, X., Leibowitz, J. L., Neil, S. J. D., Skern, T., Weiss, S. R., Worobey, M., Andersen, K. G., Garry, R. F., & Rambaut, A. (2021). The origins of SARS-CoV-2: A critical review. *Cell*, 184(19), 4848–4856.
- Pekar, J., Worobey, M., Moshiri, N., Scheffler, K., & Wertheim, J. O. (2021). Timing the SARS-CoV-2 index case in Hubei province. *Science*, 372(6540), 412–417.

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