Science Not Speculation Should Inform the Investigation of the Origin of COVID-19

Summary. Two hypotheses of the origin of the COVID-19 pandemic exist: a natural zoonosis or a laboratory-related event. An investigation of the origin of the COVID-19 pandemic needs to be conducted to a science-based resolution of these competing hypotheses. Until new evidence is found, an investigation of the pathway for the origin of COVID-19 should proceed with the assumption that a laboratory-acquired infection or research-related accident is at least as likely, if not more likely, than a natural zoonotic transmission.

Authors:

Steven C. Quay MD, PhD, Department of Pathology (Formerly), Stanford University School of Medicine, USA; Currently Atossa Therapeutics, Seattle, WA ORCID 0000-0002-0363-7651
Correspondence: Steven@DrQuay.com

Rahul A. Bahulikar, BAIF Development Research Foundation, Pune, India, ORCID 0000-0002-0442-4607

Jean-Michel Claverie, Professor Emeritus of Medicine, Virologist, Aix-Marseille University, France ORCID 0000-0003-1424-0315

Gilles Demaneuf, Engineer and Data Scientist, New Zealand ORCID 0000-0001-7277-9533

Andre M. Goffinet, MD, PhD, Emeritus Professor of Neurobiology, Univ. Louvain Medical School, Belgium

Milton Leitenberg, Senior Research Associate, University of Maryland, School of Public Affairs

Richard A. Muller, Professor Emeritus of Physics, University of California, Berkeley

Nikolai Petrovsky MBBS, PhD. Professor of Medicine, College of Medicine and Public Health, Flinders University, Australia ORCID 0000-0002-1580-5245

Monali C. Rahalkar, Dr. rer. Nat. Scientist D, Agharkar Research Institute, Pune, India, ORCID 0000-0003-0945-4378

H. Lawrence Remmel, Department of Pathology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands ORCID 0000-0002-5742-829X

Günter Theißen, Dr. rer. nat., Professor of Genetics, Matthias Schleiden Institute, Friedrich Schiller University Jena, Germany, ORCID 0000-0003-4854-8692

Two Lancet letters[1] have put forth a plea to use scientific evidence, not speculation, in determining the origin of the COVID-19 outbreak. We agree that only well collected scientific data and rigorous analysis can bring us closer to understanding how the responsible virus, SARS-CoV-2, entered the human population.

We also agree with the four open letters from concerned scientists and science communicators (including some of the signatories of this communication) that have previously carefully laid out the case for a further scientific investigation and provided a road map for such an investigation, including detailed methods to use, the documents to seek, and the skills needed by the investigators to conduct such work.[2]

At this time, the WHO-convened study, with its Terms of reference, and its 313-page report, including Annexes,[3] contains the largest current dataset collected related to the question of the origin of COVID-19. We note that the assessment by the WHO-convened joint study-team of the likelihood of each possible pathway was as follows: "direct zoonotic spillover is considered to be a possible-to-likely pathway; introduction through an intermediate host is considered to be a likely to very likely pathway; introduction through cold/ food chain products is considered a possible pathway; introduction through a laboratory incident was considered to be an extremely unlikely pathway."

We also note that the assessment was immediately qualified by the declarations of Dr Tedros himself, who asserted that, "As far as WHO is concerned, all hypotheses remain on the table and further reiterated that the conclusions were not those of the WHO. The [study] group came from different institutions and different countries, and they're independent. There were only two WHO staff who joined them. They came up with their study."[4] In agreement with Dr. Tedros' statement, available evidence as well as a preliminary re-analysis[5] of the primary data in the study group report and additional scientific studies lead show that both a traditional zoonosis and a laboratory-acquired infection or research-related accident remain fully plausible hypotheses at this stage.[6]

---

[1] Calisher C, Carroll D, Colwell R, et al. Statement in support of the scientists, public health professionals, and medical professionals of China combatting COVID-19. The Lancet, 395:E42-E43, 2020. Lancet 2020 ; Calisher C, Carroll D, Colwell R, et al. Science, not speculation, is essential to determine how SARS-CoV-2 reached humans. The Lancet, 2021. Lancet 2021

[2] C.D. Butler, B. Canard, H. Cap, et al. Reaction to the China-WHO joint press conference on 9 Feb 2021, (March 4, 2021). Open Letter 1 ; C.D. Butler, H. Cap, J-M Claverie, et al. Reaction to the China-WHO joint study team report, (April 7, 2021). Open Letter 2 ; C.D. Butler, H. Cap, J-M Claverie, et al. Open Letter to the World Health Organization and the Members of its Executive Board ahead of the World Health Assembly (May 2021), (April 30, 2021). Open Letter 3 ; Adadi, R. Bahulikar, C.D. Butler, J-M Claverie, et al. Call for a Comprehensive Investigation of the Origin of SARS-CoV-2, if Possible with Chinese Government Participation, (June 28, 2021). Open Letter 4.

[3] A WHO Terms of Reference for SARS-CoV-2 Origin Study; B WHO Study Report on SARS-CoV-2 Origin March 2021 ; C WHO Study Report Annexes.

[4] See Science Magazine, 18 June 2021.

[5] A - Quay S. OPEN LETTER TO THE WHO: Evidence that is Undisputed Favoring Lab Origin of COVID Needs to Be Acknowledged. Preprint, Zenodo, March 2021 ; B - Quay S. A Bayesian analysis concludes beyond a reasonable doubt that SARS-CoV-2 is not a natural zoonosis but instead is laboratory derived. Preprint, Zenodo, January 2021

[6] See for instance this recent summary of the situation by Stanford professor David Relman.

Based on this information, we address here some limitations of these two Lancet letters, and, where appropriate, a recent preprint entitled, "The Origins of SARS-CoV-2: A Critical Review," by Holmes et al.[7,8]

Wrong Focus on Biosafety Level-4 Research

Neither Holmes et al nor the two Lancet letters acknowledge the now well documented fact that bat coronavirus research was conducted at BSL-2 and BSL-3 levels in various labs in Wuhan, including the Wuhan Institute of Virology (WIV), and never at BSL-4.[9] Respected Western scientists, including a signatory of the first Lancet letter, have since expressed their dismay at this low level of biosafety. Holmes et al. describe the WIV BSL-4 site in Wuhan, but this is irrelevant to a discussion of possible lab origins.

No acknowledgement of a possible field research-related scenario

Neither Holmes et al. nor the two Lancet letters properly consider the various research related scenarios.[10] In particular they fail to consider the possibility of an infection of a Wuhan based personnel on a bat sampling trip, for which there is abundant evidence that limited PPEs were used (despite the declarations of the Annex D7 of the China-WHO report). Contrary to what Holmes et al assert, such a scenario is highly parsimonious; it would explain the location (Wuhan), the absence of infection at the source (possibly some cave in Yunnan), the absence of such virus in a lab to date (as it may well never have been encountered until that fateful sampling trip) and could have involved an asymptomatic personnel making detection much more difficult.

No proper consideration to the location or virus factor

Neither Holmes et al nor the two Lancet letters pay proper attention to the fact that the outbreak started in Wuhan, China. Considerations of a purported link to wildlife markets and transport hubs fail to acknowledge that there are at least 110 cities in China with more than one million residents, transport hubs, and wildlife markets. There is nothing unique to Wuhan in having these three, commonly found urban features. What is unique to Wuhan is the WIV, the world's leading institution in coronavirus field collection, and laboratory gain-of-function research.

An analysis of the relationship between the location of the Wuhan hospitals with early admission (December 1, 2019 to January 10, 2020) and the catchment zone of the Wuhan Metro subway system establishes that the pandemic began along Line 2 of the Metro (a likelihood of 1 in 68,500 this occurred by chance). Because Line 2 has stations at the Wuhan Institute of Virology, the Huanan Seafood Market, the high-speed rail system to reach all points of China within hours and the international airport, with non-stop destinations in the US, Europe, and the Middle East, the

[7] Holmes et al. The Origins of SARS-CoV-2: A Critical Review. Holmes et al. 2021 Zenodo pre-print

[8] Another detailed review of Holmes et al., entitled, "A response to "The Origins of SARS-CoV-2: A Critical Review" was also recently published.: Chan A. Response to Holmes et al. Medium, 2021.

[9] See for instance Shi Zhengli answer to Science Magazine: 'The coronavirus research in our laboratory is conducted in BSL-2 or BSL-3 laboratories.' or the classification of pathogens as per the National Virus Resource Centre affiliated to the WIV.

[10] See Annex A of the 3rd open letter for a description of the research-related scenarios.

pandemic has been said to have started and been amplified by the "Wuhan Line 2 COVID Conduit."[11]

There is also no acknowledgement that, of the 209 possible viral diseases to cause an outbreak in Wuhan, COVID-19 was a SARS-like coronavirus. Before 2019, 60% of all publications in the world on SARS-like coronaviruses came from the Wuhan Institute of Virology[12] (all of these were done at BSL-2 or BSL-3 level).

No pre-pandemic COVID-19 clinical cases

In Andersen et al.[13] the authors speculated on the timing of the acquisition of the polybasic cleavage site in a progenitor virus that had been pre-adapted to the human ACE2 receptor. In doing so they wrote:

> "Hence, this scenario presumes a period of unrecognized transmission in humans between the initial zoonotic event and the acquisition of the polybasic cleavage site. Sufficient opportunity could have arisen if there had been many prior zoonotic events that produced short chains of human-to-human transmission over an extended period. Studies of banked human samples could provide information on whether such cryptic spread has occurred."

Previous human SARS-like coronavirus exposure in Wuhan would have been surprising. A 2018 study[14] by Daszak, Shi, and colleagues used blood bank specimens from Wuhan as a control for a study in Southern China of coronavirus seroconversion in people living in close proximity to large bat caves. The stated reason was the "much lower likelihood of exposure to bats due to its urban environment." All Wuhan specimens were negative.

The WHO Report documents three independent studies of human blood-derived specimens, totaling over 9000, drawn before January 2020, that were tested for serological evidence of COVID-19 antibodies, and all were found to be negative.[15] No cryptic spread was found. A seropositivity of 2.2% in specimens from patients collected from January to April 2020 documented the sensitivity of the assay used. A similar analysis for the previous zoonotic coronavirus epidemics, SARS-CoV-1 and MERS, found about 0.6% community resident pre-epidemic seroconversion and up to 19.9% in supply chain workers.[16] The WHO data establishes

[11] Quay, S. Where Did the 2019 Coronavirus Pandemic Begin and How Did it Spread? The People's Liberation Army Hospital in Wuhan China and Line 2 of the Wuhan Metro System Are Compelling Answers. October 28, 2020. Zenodo Preprint

[12] Quay S. Personal communication.

[13] Andersen, K.G., Rambaut, A., Lipkin, W.I. et al. The proximal origin of SARS-CoV-2. Nat Med 26, 450–452 (2020). Andersen et al Proximal Origin paper

[14] Wang N, Li SY, Yang XL, et al. Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. Virol Sin. 2018 Feb;33(1):104-107. doi: 10.1007/s12250-018-0012-7.

[15] Reference 4B, p. 50.

[16] MERS pre-epidemic seroconversion was 2.3% in shepherds and 3.6% in slaughterhouse workers MERS seroconversion; SARS-CoV-1 pre=epidemic seroconversion among wild animal workers was 19.9%. SARS-CoV-1 seroconversion; MERS and SARS-CoV-1 pre-epidemic seroconversion in community residents of epidemic outbreaks averaged 0.6% (Reference 4-B, p. 66).

that the incidence of COVID-19 in the community before December 2019 must be less than 0.03% (95% CI, Upper Bound).

A corollary dataset came from a multi-disciplinary clinical team that reviewed 76,253 episodes of fever, influenza-like illness, acute respiratory infection, or pneumonia unspecified that had presented to Wuhan health institutions in individuals of all ages in the period from 1 October to 10 December 2019. Following initial review by the health institutions, only 92 of the 76,253 episodes were considered to have an illness clinically compatible with SARS-CoV-2 infection. Following further review by the external multidisciplinary clinical team, all these cases were assessed not to be cases of SARS-CoV-2 infection.[17] These data establish the upper limit of the incidence of COVID-19 in Wuhan residents in the fall of 2019 at 0.004%.

As noted by Holmes et al. "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 civets). Subsequent serological surveys found ~3% positivity rates to SARS-CoV related (SARSr-CoV) viruses in residents of Yunnan province living close to bat caves, demonstrating regular exposure in rural locations."

No hypothesis has been offered to explain the lack of any evidence of pre-pandemic seroconversion anywhere in the community.

No intermediate animal host has been implicated

Approximately 70% of all initial cases are reported to have had a relationship to the Huanan Seafood Wholesale Market in Wuhan.[5] In noting this fact Andersen et al. wrote:

> "As many early cases of COVID-19 were linked to the Huanan market in Wuhan, it is possible that an animal source was present at this location. For a precursor virus to acquire both the polybasic cleavage site and mutations in the spike protein suitable for binding to human ACE2, an animal host would probably have to have a high population density (to allow natural selection to proceed efficiently) and an ACE2-encoding gene that is similar to the human ortholog."

In an effort to find the intermediate host predicted by Andersen "to have a high population density," an extensive investigation[18] was conducted. 457 animals from 18 species from the Huanan market, warehouses related to the market, and other markets were tested for the virus. 616 animals (10 species) from suppliers to the market were tested. 1864 wild animals from 27 species (including pangolins, civet cats and *Rhinolophus affinis* bats) from Southern China of the type found in the market were tested. Domestic animals from 31 provinces were tested for antibodies (11,708 samples) or nucleic acid by PCR (12,092 samples). In total, over 80,000 specimens from 209 different species were tested, and all were negative for evidence of infection by antibody serology and/or nucleic acid PCR. Each of these sampling exercises is sufficiently large to permit

[17] Reference 3B, p. 49.
[18] Reference 2B, p. 92.

a conclusion that the population of SARS-CoV-2 infected animals in each population must be significantly less than 1.0% to produce the results observed.

A study of 1055 imported cold chain food products to the Huanan market were also all negative by PCR testing.[19] Additionally 21 of the 22 stalls where some vendor(s) contracted COVID-19 were only selling domestic, not imported, frozen products.

For SARS-CoV-1 and MERS, when human cases were found to be associated with markets, animal testing in those markets found a high level of infection (about 80%) in the respective intermediate hosts and a detectable level (about 10%) in farms in the supply chain.[20] Market workers were seropositive at high levels (10-40%) and the virus could be recovered from multiple markets for many months.[21]

Furthermore, given the known high susceptibility of felines to infection with SARS-CoV-2 if live animals being sold in the market had been infected with SARS-CoV-2 then this would be expected to have transmitted just as readily to local cat populations around the market as human populations. No such evidence of feline infections was found.

Holmes et al.[4] describe the situation concerning SARS-CoV-2 and market emergence here:

> "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 and the Xinyuan market in Guangzhou."

While we agree with Holmes et al.[4] in their characterization of SARS-CoV, for the SARS-CoV-2 outbreak the lack of evidence of animal infection, lack of seroprevalence in market workers or anyone in the community pre-epidemic, and the genetic purity (*infra)* of the virus isolated is in fact the mirror opposite situation to the first SARS outbreak. We can find no scientific justification within the Holmes et al.[4] pre-print for what they say about SARS-CoV-2.

The Holmes et al.[4] review emphasizes that the search for an intermediate animal host needs to be focused on the Wuhan markets and their supply chains. This search of the Wuhan markets and their supply chains has been completed and no intermediate host was found, nor even peripheral evidence that an intermediate host may have existed.

Holmes et al.[4] list many potential animal hosts for SARS-CoV-2 in their review. As documented in the WHO Report, all of the animal hosts they propose were among the 209 species tested and all were found to be negative.

---

[19] Reference 2B, p. 107; Annex E4.

[20] Tu C, Crameri G, Kong X, et al. Antibodies to SARS coronavirus in civets. Emerg Infect Dis. 2004 Dec;10(12):2244-8. doi: 10.3201/eid1012.040520. SARS Antibodies in Civets.

[21] Hu S, Shi Z-L. Investigation of Animal Reservoir(s) in SARS-CoV. Y. Lu et al. (eds.), Emerging Infections in Asia. Springer Science+Business Media, LLC 2008, pp 57-73.

Circulation in an intermediate animal host could not have occurred before the first clinical cases

The time to Most Recent Common Ancestor (tMRCA), determined by a phylogenetic analysis, is one method in zoonotic research to estimate the time when a virus was first transmitted into the intermediate host population and began to develop a background of genetic diversity. This is typically well before the human epidemic spread and even before the first animal-to-human transmission. For example, with MERS it was determined that the virus had entered the camel populations of the Middle East about two years before the first human clinical cases became known.[22]

The SARS-CoV-2 phylogeny is of course different. A universal observation of the SARS-CoV-2 genome was its lack of mutational diversity at the beginning. The WHO and Drs. Baric, Rambaut, Holmes, and even Shi[23] have all commented on this. The WHO has written:

> "All the published genetic sequences of SARS-CoV-2 isolated from human cases are very similar, suggesting that the start of the outbreak resulted from a single point introduction in the human population around the time that the virus was first reported in humans in Wuhan, China. The analyses of the published genetic sequences further suggest that the spillover from an animal source to humans happened during the last quarter of 2019.
>
> "Current findings show that the virus has been remarkably 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."

Holmes and Rambaut wrote:

> "Because of the early sampling and genome sequencing of COVID-19 cases in China, especially in Hubei province, it appears that the 'root sequence' of SARS-CoV-2 is known. Many of the genomes from the earliest sampled cases are genetically identical and hence also probably identical to the most recent common ancestor of all sampled viruses. This occurrence is different to previous viruses and epidemics." [Emphasis added].

[22] Lau SKP, Wong ACP, Lau TCK, Woo PCY. Molecular Evolution of MERS Coronavirus: Dromedaries as a Recent Intermediate Host or Long-Time Animal Reservoir? Int J Mol Sci. 2017 Oct 16;18(10):2138. doi: 10.3390/ijms18102138. PMID: 29035289; PMCID: PMC5666820. Molecular Evolution of MERS Coronavirus

[23] WHO statements: WHO Statement 23 Apr 2020; Baric R: "early strains identified in Wuhan, China, showed limited genetic diversity, which suggests that the virus may have been introduced from a single source." Emergence of a Highly Fit SARS-CoV-2 Variant. N Engl J Med 2020; 383:2684-2686 DOI: 10.1056/NEJMcibr2032888. NEJM; Shi, Z-L: On January 23, 2020 Dr. Shi wrote in the draft of her paper: "The almost identical sequences of this virus in different patients imply a probably recent introduction in humans…" 23 Jan 2020 draft pre-print. By February 3, 2020, when the final version of this paper was published, this sentence had been deleted. Zhou, P., Yang, XL., Wang, XG. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273 (2020). Final paper; Rambaut, A., Holmes, E.C., O'Toole, Á. et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol 5, 1403–1407 (2020). Rambaut & Holmes et al paper

Holmes et al.[4] updated the early genomic clade analysis in their recent review, establishing that two clades were circulating early. They fail to point out, however, that these clades differ by only two base substitutions. All SARS-CoV-2 samples taken from any source collapse phylogenetically into a tMRCA in approximately November 2019. This includes all human clinical cases, regardless of their relationship to any market, all environmental specimens from any market tested, any surface specimens from any market, etc.

The WHO Report states: "the time to most recent common ancestor (tMRCA) inferred by more than 10 groups using different approaches is similar: between mid-November and mid-December 2019." This is also the timing of the clinical outbreak, meaning this data establishes that SARS-CoV-2 was unlikely to have been circulating in an intermediate host for any measurable time before the clinical outbreak.

For SARS-CoV-2, the observation that the timing of the first human cases and the tMRCA are the same means there is no measurable temporal period of intermediate host infection. All human cases are traced to a presumed single animal-to-human transmission.[24] For MERS and SARS-CoV 50% or more of early cases were independent animal-to-human transmissions, with the posterior diversity and a tMRCA years before as the evidence for a natural zoonosis.

In the laboratory, experiments using stored, aliquoted virus samples makes the use of molecular clock estimates unreliable; for example, a year's worth of experiments could be performed with no apparent base substitutions by using aliquots of the virus, and codon re-optimization when a virus is grown in cells of a different species to the original host may also accelerate virus evolution compared to its mutation rate in a stable host.

Two additional genomic signatures of SARS-CoV-2 are worth noting at this point. The polybasic cleavage site and its unusual -CGG-CGG- coding sequence, and the evidence of pre-adaption to the human host, whether looking at the entire genome or just the receptor binding domain of the Spike Protein.[25]

Because the subgenera of sarbecovirus to which SARS-CoV-2 belongs has neither furin cleavage sites (of any sequence) or -CGG-CGG- dimer codons, a recombination with a non-sarbecovirus needs to be proposed. Field data from Drs. Shi and Daszak documenting a lack of betacoronavirus co-infection that includes sarbecoviruses[26] and furin site containing viruses from other subgenera

[24] The earliest SARS-CoV-2 sequences in GiSAID from China were collected (n=259) and compared to the GenBank Reference Sequence (Ref Seq 1798174254). The substitution rate per year was calculated using the number of changes from the reference sequence and the time between sampling dates. The established human-to-human intrinsic rate of SARS-CoV-2 nt substitution was taken to be 17.9 with a confidence interval of 12.0 to 20.9 nt per year. Emergence of genetic diversity and recurrent mutations in SARS-CoV-2. No sequences were found that had a substitution rate greater than 20.9 nt per year, as would be characteristic of an independent animal-to-human transmission with posterior diversity.

[25] Zhan SH, Deverman BE, Chan YA. SARS-CoV-2 is well adapted for humans. What does this mean for re-emergence? bioRxiv 2020.05.01.073262; doi: SARS1 vs. SARS-CoV-2 whole genome SNV evolution; Starr et al. Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding 2020, Cell 182, 1295–1310 September 3, 2020.Bloom laboratory yeast display

[26] Latinne, A., Hu, B., Olival, K.J. et al. Origin and cross-species transmission of bat coronaviruses in China. Nat Commun 11, 4235 (2020). Daszak & Shi Co-Infection paper They sampled 187 *Rhinolophidae Sp.* bats and

and the transcription regulatory site incompatibility between subgenera reduce significantly the already low likelihood of SARS-CoV-2 acquiring this critical feature in nature.[27] Extensive prior work in many laboratories synthetically inserting furin cleavage sites with the effect of changing the pathogenesis of the virus show a clear historical precedent for a laboratory route to this SARS-CoV-2 feature. Serial passage in cells of different species is also a well-established method for host adaptation to humans in coronavirus biology.[28]

The evidence of pre-adaption to a human or human-like host must be juxtaposed with the failure of finding any evidence of pre-epidemic circulation in humans or in any of 209 different animal species.

Despite a plea to not speculate, the Holmes et al. note contains many arguments that are just that. One for example, is the notion that because wildtype mice are difficult to infect with SARS-CoV-2 one could speculate the virus was not derived from a laboratory. This ignores many published papers, including by Baric and Shi in early 2020,[29] of the use of humanized ACE2 transgenic mice for SARS-CoV-2 research, mice which the virus infects so well it is often lethal, and displays CNS infection.

Many scientists and the leadership of the WIV have claimed that the WIV was not conducting research on SARS-CoV-2 or any related coronavirus. They have also asserted that no one from the WIV became ill in the fall of 2019 or at any later time. This claim is challenged by an analysis of how very unlikely it would be for a facility in Wuhan with up to 590 employees to have no seroconversions, given the incidence of seroconversions of the Wuhan residents at the time was over four percent.[30] In any case, these are personal assertions, not scientific observations. To be included in a science-based analysis, independent verification is required. Such claims should not have been relied upon by Calisher et al (2021) and the review by Holmes et al.

---

determined subgenera beta coronavirus infections. All 187 were infected with sarbecoviruses but there were no co-infections of other beta coronavirus subgenera. [Supplemental material, pp. 17-8.]

[27] Yiyan Yang, Wei Yan, A Brantley Hall, Xiaofang Jiang, Characterizing Transcriptional Regulatory Sequences in Coronaviruses and Their Role in Recombination, Molecular Biology and Evolution, V. 38, Issue 4, April 2021, pp. 1241–1248, Subgenera TRS & Recombination ; Baric et al. use bespoken TRS changes and create recombination-resistant vaccine candidates: Graham, R.L., Deming, D.J., Deming, M.E. et al. Evaluation of a recombination-resistant coronavirus as a broadly applicable, rapidly implementable vaccine platform. Commun Biol 1, 179 (2018). Recombination resistant vaccine candidate via TRS modification.

[28] Cockrell AS, Leist SR, Douglas MG, Baric RS. Modeling pathogenesis of emergent and pre-emergent human coronaviruses in mice. Mamm Genome. 2018 29: (7-8):367-383. doi: 10.1007/s00335-018-9760-9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6132729/

[29] Jiang RD, Liu MQ, Chen Y, et al. Pathogenesis of SARS-CoV-2 in Transgenic Mice Expressing Human Angiotensin-Converting Enzyme 2. Cell. 2020 182:50-58.e8. doi: 10.1016/j.cell.2020.05.027. Baric & Shi SARS-CoV-2 Transgenic Mouse Model

[30] Quay S, Demaneuf G. An analysis of the results of routine employee testing for SARS-like infections within the WIV and other Wuhan labs raises serious issues about their validity. Researchgate, 2021. WIV COVID-19 Seroconversion Analysis

There was also no mention in Holmes et al 2021 and Calisher et al 2021 about an important clue which connects the Mojiang mineshaft, Yunnan with a lethal pneumonia in six miners and the bat coronavirus, RaTG13, the closest genomic relative of SARS-CoV-2. In 2012, six men went to clean an abandoned mineshaft infested with six species of bats, including horseshoe bats. All of them suffered from pneumonia, including four deaths, and their complications on retrospective analysis were similar to COVID-19.[31] The coincidence between the 2012 illness in Mojiang miners, the subsequent samplings, and finding the nearest SARS-CoV-2 relative from this same mine warrants further inquiry.

Conclusion

An investigation of the origin of the COVID-19 pandemic needs to be conducted to a science-based resolution of competing hypotheses. There is currently no evidence to support the hypothesis this was a natural zoonotic infection of the type previously seen in for example the SARS CoV outbreak. Until new evidence is found, an investigation of the pathway for the origin of COVID-19 should proceed with the assumption that a laboratory-acquired infection or laboratory accident is at least as likely, if not more likely, than a natural zoonotic transmission.

[31]Rahalkar MC and Bahulikar RA (2020) Lethal Pneumonia Cases in Mojiang Miners (2012) and the Mineshaft Could Provide Important Clues to the Origin of SARS-CoV-2. Front. Public Health 8:581569. doi: 10.3389/fpubh.2020.581569 Mojiang Miners Pneumonia