Letter to the Editor:
Comment of a critical review about the origins of SARS-CoV-2

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Text

Holmes et al. (2021) contrast two competing hypotheses for the origin of SARS-CoV-2, a zoonotic origin versus a laboratory escape. Rather than evaluating all information available, this review defends the zoonotic origin by turning several formally correct scientific facts into incorrect arguments. Here we examine the arguments, present new elements and conclude that the debate is unresolved.

Formally, the burden of the proof is asymmetrical. As the review notes "the possibility of a laboratory accident cannot be entirely dismissed, and may be near impossible to falsify". To demonstrate that the origin was independent of research, it would be necessary to either discover the proximal virus and host, or to provide comprehensive and well-reasoned forensic evidence that no research activity has ever intervened at any stage: the evolution of the virus sequence, the transport of the virus to Wuhan, and the first transmission events to humans. Conversely, in order to demonstrate that the origin was research-related, it would be sufficient to exhibit just one contribution of a research activity to one of these three stages.

The review refers to the past documented coronaviruses that infected humans, notably during the last 20 years, to stress that all of them have had zoonotic origins, and that both SARS and SARS-CoV-2 emergencies were associated with markets selling live animals. Conversely, the review notes that in the past, "with the exception of Marburg virus, all documented laboratory escapes have been of readily identifiable viruses capable of human infection"; and "no case of laboratory escape has been documented following the sequencing of viral samples".

However, past history provides no convincing argument to refute a research-related origin for SARS-CoV-2. Since a decade, lab practices have changed dramatically: ambitious international projects are funded to detect potentially pandemic pathogens, which involve wide campaigns of sample collection in the field, transporting collected samples to labs, culturing viruses on human cells, testing their infectivity on model animals, and experimental engineering of viral genomes. Viruses collected and stored in labs are now far more numerous, virus sequence modifications are much quicker and easier (Menachery, 2015; Hu, 2017) and some bat coronaviruses can directly infect human cells (Menachery, 2015).

The review notes "there are precedents for laboratory incidents leading to isolated infections and transient transmission chains, including SARS-CoV". But not all previous research-related outbreaks infections have been isolated or transient. Notably, the 1977 influenza pandemic, which originated with a virus sample in a laboratory freezer (Wertheim, 2010), and the 2007 UK Foot and mouth disease virus epidemic, which originated with a leak of infected wastewater from a BSL-4 laboratory (Blakely, 2021, Web Resources), are clear examples of global or regional infections and sustained transmission following research-related spillover. Hantavirus propagation by rats has proven that laboratory leaks are an important source of infection in China (Zhang et al., 2010). The Wuhan Institute of Virology (WIV) Biosafety Level (BSL)-4 has a documented record of safety problems (Rogin, 2020, Web Resources) and recognizes the challenges it has to face (Zhiming, 2019).

The review claims to find "no epidemiological link" with the WIV BSL-4. This is unsurprising since the WHO-China joint mission had neither the mandate nor the means to investigate a possible link with a lab. But more importantly, the review omits than bat coronaviruses were manipulated in BSL-3 and BSL-2 environments, including in the Wuhan Center for Disease Control (CDC) which moved at a 700 m distance from the Huanan market in late 2019 (WHO-China, 2021, p. 119) and in the WIV BSL-2 and BSL-3 labs located in Wuchang Distinct at 15 km from the same market.

Furthermore, the review dismisses a specific laboratory as a potential source because it is a few kilometers from the Wuhan market, yet recognises that “for both SARS-CoV and SARS-CoV-2, there is a considerable geographic gap between Yunnan and the location of the first human cases''. The most closely related viruses to SARS-CoV-2 are found in bats in South-East Asia, and the distant ancestor of SARS-CoV-2 was probably among them. How the virus went from South-East Asia to Wuhan remains unknown, and intermediate animal reservoirs have not yet been found. The review overlooks plausible research-related scenarios to fill this ~1500 km gap, such as the transportation of collected samples from Yunnan to WIV (Zhou, 2020, Addendum) or human infection during field sample collections by the WIV and the CDC, which raise safety concerns (Dou, 2021, Web Resources).

Regarding the SARS-CoV-2 itself, the review examines its adaptation to humans, its furin cleavage site and its CGG codons. These three characteristics initially prompted a laboratory-engineered origin hypothesis, and the review concludes these do not constitute sufficient proof for such a thesis. We note that these three characteristics are compatible with both origin theories, and do not provide fundamental insight.

The review claims that "no sequence has been identified that could have served as precursor" and there is "no mention of a SARS-CoV-2-like virus in any prior publication or study from the WIV". But labs rarely publish virus sequences in real time; years of delay are usual. For instance, the bat virus RaTG13 was collected in 2013, partially sequenced in 2016, fully sequenced in 2018, and its full sequence was published in February 2020 (Zhou, 2020 and Addendum). It is impossible to check if other sequences might have served as precursors, since the WIV database 'batvirus.whiov.ac.cn', which was established to share data to prevent outbreaks, is no longer accessible (Tang, 2019, Web Resources). In addition, synthetic viruses have been constructed based on consensus genomes from strains having 5% differences (Becker, 2008). Such lab-constructed consensus viruses would not closely resemble any existing virus.

The review finds "no logical reason why an engineered virus would utilize such a suboptimal furin cleavage site". However, evolution during passages in cell culture or model animals could explain a selection of features which, although not theoretically optimal, may yet contribute to the widespread of SARS-CoV-2, for instance by maintaining efficient cell entry while evading immune surveillance (Shang, 2020). Similarly, by stating that "the SARS-CoV-2 furin site is also lost under standard cell culture conditions involving [monkey] Vero E6 cells", the review overlooks other mammalian cell systems used at WIV (Ge et al., 2013) where the furin cleavage site is retained (Shang et al., 2020).

The review underlines SARS-CoV-2's "low pathogenicity in commonly used laboratory animals" and lack of "rodent adaptation". This is true for wild-type mice but not for "humanized" mice, genetically modified to produce the human ACE2 receptor, which have been used at WIV (Baric, 2021, Web Resources).

The review notes there is "no evidence of genetic markers one might expect from laboratory experiments". However, techniques enabling "seamless" viral sequence modification have existed for over a decade and are commonly used (Becker, 2008; Menachery, 2015). Moreover, research-related scenarios do not necessarily entail genetic manipulation, for instance infection during field collection or passage experiments on cultured cells or humanized animals.

The review states that there is no evidence of prior WIV research “involving the artificial insertion of complete furin cleavage sites into coronaviruses." However, since fifteen years (Follis, 2006) several labs have published experiments inserting human-adapted cleavage sites in coronaviruses.

Key documents disclosed a few days before and after the review publication (Daszak 2021, Web Resources) report the construction at WIV of novel chimeric SARS-related coronaviruses that combined the spike gene of one SARS-related coronavirus with the genomic backbone of another SARS-related coronavirus, that efficiently infected and efficiently replicated in human airway cells, and that exhibited 10,000 times higher viral growth and 2 to 4 times higher lethality in humanized mice (which were grown in two BSL3 laboratories: WIV and Wuhan University). Expanding this program of constructing novel chimeras was funded in 2019. The “Defuse” project proposed to synthesize consensus genomes from a panel of closely related coronavirus strains, and to insert cleavage sites in the Spike protein of SARS-related viruses; it has been submitted in 2018 to a military agency and rejected for safety concerns (Daszak, 2018, Web Resources). Together, these documents show that above considerations are not speculation, and make it imperative to keep open the debate about research of concern.

After the review publication, Temmam et al. (2021, Web resources) described bat viruses sampled in North Laos, which are able to infect human cells and have a receptor binding domain much closer to SARS-CoV-2 than previously known animal coronaviruses. This suppresses the need for intermediate host species but does not solve the geographical and sequence gaps. This discovery does thus enforces neither the zoonotic nor the research-related hypotheses, but probably brings us a bit closer to the proximal virus in either cases.

An important merit of the review is its endorsement of open scientific debate. Such debate should distinguish facts from interpretations and opinions (Bloom et al., 2021; van Helden et al., 2021; van Kerkhove et al., 2021). In view of the possible scenarios, and of the lack of conclusive arguments in favor of one or another, both zoonotic emergence and research-related incidents are plausible and neither can be discarded with present data. Since investigations of research-related scenarios are feasible (Pilch 2020; Adadi 2021, Web Resources), we expand the review’s conclusion by adding four words (in italics): "Failure to comprehensively investigate the zoonotic and research-related origin hypotheses through collaborative and carefully coordinated studies would leave the world vulnerable to future pandemics."

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Declaration of interest

FG and VCO are collaborating on a research project entitled "Elucidating the proximal origin(s) of the SARS-Cov2" (Labex WhoAmI, Excellence Initiative, University of Paris, September 2021-August 2023). CDB, ED, GT, JvH, JH, BK, SM, HK, JMC, FC, MCR, RAB, ML, RHE have no conflict of interest.

Web Resources

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