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What we know and what we need to know about the origin of SARS-CoV-2

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ABSTRACT

Since the appearance of the first cases of COVID-19 in 2019, an unprecedented number of documents on that disease have been published in a short space of time. The current available information covers a large number of topics related with COVID-19 and/or the coronavirus (SARS-CoV-2) responsible of the disease. However, only a limited number of publications have been focused on a controversial issue: the origin of the SARS-CoV-2. In this paper, the scientific literature on the origin of SARS-CoV-2 has been reviewed. Documents published during 2020 and 2021 (January 1-July 19) in journals that are indexed in PubMed and/or Scopus has been considered. The revised studies were grouped according to these two potential origins: natural and unnatural. The analyses of the conclusions of the different documents here assessed show that even considering the zoonotic hypothesis as the most likely, with bats and pangolins being possibly in the origin of the coronavirus, today's date the intermediate source species of SARS-CoV-2 has not been confirmed yet. On the other hand, some researchers point to an unnatural origin of this coronavirus, but their conclusions are not strongly supported by a clear scientific evidence. Given the tremendous severity of the current pandemic, investigations to establish clearly and definitively the origin of SARS-CoV-2, are basic and essential in order to prevent potential future pandemics of similar nature.

1. Introduction

In recent months, the amount of scientific information published on SARS-CoV-2/COVID-19 has been spectacular and certainly impressive. Never before, in the history of science, so many scientific papers had been published in such a short space of time. They cover a great number of aspects related with SARS-CoV-2/COVID-19. For example, in PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) using as keywords SARS-CoV-2 or COVID-19, the number of documents are 96,816 and 157,630, respectively (July 19, 2021). In turn, a similar query made in Scopus (<https://www.scopus.com/home.uri>) shows (July 19, 2021) 69,607 and 168,851 documents, for SARS-CoV-2 and COVID-19, respectively. In addition, the number of scientific papers on SARS-CoV-2/COVID-19 is dramatically increasing over time. Thus, a similar number of documents than those published throughout the entire 2020 has been already indexed between January–July 2021. The quantity of specific topics that have been investigated is tremendous, covering a great number of medical fields, but also certain areas that, in principle, would be remarkably distant from medical sciences.

In spite of the huge amount of published information, important gaps require still to be investigated. These gaps range from the finding of effective pharmacological treatments for patients with serious COVID-19, or the time of efficacy of the current vaccines and their potential

adverse long-term effects, to other key issues such as to clearly establish the origin of SARS-CoV-2 and the consequent pandemic. Recently, in an Editorial of this Journal (Domingo, 2021) the great importance of knowing the origin of this coronavirus was already highlighted in order to prevent potential future pandemics of similar nature. Obviously, the possible ways of preventively acting should be substantially different depending on the specific origin of the SARS-CoV-2.

With regard to this origin, the available scientific literature is very scarce in comparison to the global information above indicated. PubMed includes only 1675 results on the “Origin of SARS-CoV-2”, but even most of them can be considered as *background noises*, not computable to establish that origin. In fact, no more than 100 documents are more or less directly related with the search term. In turn, using this same search term, only 85 documents were found in Scopus. A part of the available documents in the scientific literature are focused on discussions on the origin of SARS-CoV-2, being some of them not supported by sufficient scientific evidence. Thus, the main purpose of the present paper has been to revise the scientific literature on the origin of SARS-CoV-2, which has been published in journals indexed in PubMed and/or Scopus, in 2020 and 2021 (January 1-July 19). Since the literature suggests basically only two possible origins for the SARS-CoV-2: natural (zoonotic) or laboratory, we next summarized the collected information according to these two potential origins. However, for certain papers,

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and given their specific results and/or their conclusions, the summaries of some studies have been exceptionally included in a category (zoonotic or unnatural) to which in principle would seem not directly corresponding. The studies here reviewed are basically presented following their dates of publications.

2. Zoonotic origin of SARS-CoV-2

In January 2020, [Torres-López \(2020\)](#) already suggested that the origin of the coronavirus was likely zoonotic. However, no data supported that suggestion. Just at the beginning of the onset of the pandemic (February 3, 2020), the CEO of the Institute for Pure and Applied Knowledge made an online statement indicating that SARS-CoV-2 was most likely constructed via a laboratory recombination ([Lyons-Weiler, 2020](#)). That statement was rapidly questioned through the results of [Hao et al. \(2020\)](#), who experimentally reached an opposite conclusion, not supporting the theory for the formation of the coronavirus in a laboratory. [Hao et al. \(2020\)](#) reported that the particular fragment (1378 bp) in SARS-CoV-2 spike gene was widely spread in naturally existing coronaviruses, and therefore, according to these authors it could not be originated in a laboratory. In March 2020, a review on the zoonotic origins of human coronaviruses, including SARS-CoV-2, was published by [Ye et al. \(2020\)](#), who highlighted the importance of identifying the animal hosts of human coronaviruses that could have direct implication in the transmissions of diseases. In relation to this, [Guo et al. \(2020\)](#) remarked that *several independent research groups have identified that SARS-CoV-2 belongs to β -coronavirus, with highly identical genome to bat coronavirus, pointing to bat as the natural host*. However, in their review-paper these authors concluded that although bat could be the natural host of the new coronavirus, the source(s) and transmission routine(s) of SARS-CoV-2 remained elusive at the time of their review. In a subsequent publication, [Zhang et al. \(2020\)](#) suggested that pangolin species were a natural reservoir of SARS-CoV-2. In turn, [Zhang and Holmes \(2020\)](#) stated that SARS-CoV-2 had undoubtedly a zoonotic origin, reason for which the link to a “wet” market where a variety of mammalian species were available for purchase at the time of the outbreak, should have not mean any surprise. The review of these authors on a genomic perspective on the origin and emergence of SARS-CoV-2 concluded indicating that according to the enormous diversity of viruses in wildlife and their ongoing evolution, the simplest and most cost-effective way to reduce the risk of future outbreaks would be to limit human exposure to animal pathogens as much as possible.

In the same line, but using bioinformatics analyses (BLAST and clustalW), [Dallavilla et al. \(2020\)](#) reported that SARS-CoV-2 was unrelated to known artificial coronaviruses. The data of these authors supported the natural origin of the COVID-19 virus, which probably derived from bats, being possibly then transferred to pangolins before spreading to man. These authors remarked that speculations about the artificial origin of SARS-CoV-2 would be most likely unfounded. The hypothesis that SARS-CoV-2 was originated very probably from bats was also suggested by [Paraskevis et al. \(2020\)](#). These investigators characterized the genetic relationships of the new coronavirus, and searched for putative recombination within the subgenus of sarbecoviruses (viruses related to SARS-CoV and SARS-CoV-2). However, in May 2020, the exact species of bats that could serve as the natural host of SARS-CoV-2 remained still unknown ([Wassenaar and Zou, 2020](#)). Anyhow, [Qiu et al. \(2020\)](#) reported that the direct transmission of the coronavirus from bats to humans was not probable. They predicted that SARS-CoV-2 might tend to utilize ACE2s of various mammals (excepting murines) and some birds (pigeons, for example). According to these authors, this could help to screen the intermediate hosts of SARS-CoV-2. In contrast, [Lauxmann et al. \(2020\)](#) supported the hypothesis on the probability that bats could serve as reservoir hosts for SARS-CoV-2, but the intermediate host was not yet determined. In turn, [Latinne et al. \(2020\)](#) reported the results of a phylogenetic analysis suggesting a likely origin for SARS-CoV-2 in *Rhinolophus* spp. bats.

[Zhang et al. \(2020\)](#) reinvestigated published data from pangolin lung samples from which SARS-CoV-like CoVs had been detected. These authors found genomic and evolutionary evidence of the occurrence of a SARS-CoV-2-like CoV (named Pangolin-CoV) in dead Malayan pangolins, concluding that pangolin species would be a natural reservoir of SARS-CoV-2-like CoVs. The great importance of clearly establishing the zoonotic origin of SARS-CoV-2 for the control and prevention of the pandemic was stated in an Editorial of the journal Cell in May-2020 ([Wong et al., 2020](#)). In June 2020, [Wang and Liao \(2020\)](#) published a review focused -among other issues-on consolidating and discussing the probable origin and genetic features of SARS-CoV-2. After assessing a series of data and information, it was concluded that -at that time-the exact origin of the virus was still highly debatable. Then, there was no evidence that the bat came from Wuhan seafood wholesale market. In subsequent studies, [Lau et al. \(2020\)](#) reported that the genome of SARS-CoV-2 was closest to that of SARS-related CoVs from horseshoe bats, and its receptor-binding domain was closest to that of pangolin viruses, while [Han \(2020\)](#) stated that the origin of SARS-CoV-2 remained still being mysterious. Nevertheless, [Vilcek \(2020\)](#) reported that the analysis of the SARS-CoV-2 genome (especially the S gene) indicated that natural evolutionary process between a bat-CoV and a pangolin-CoV (or another animal coronavirus) could have been important in the origin of SARS-CoV-2. New analyses indicated that SARS-CoV-2 was not a recombinant virus.

[Tang et al. \(2020\)](#) conducted a study on the origin and continuing evolution of SARS-CoV-2. Although only a 4 % of variability in genomic nucleotides between SARS-CoV-2 and a bat SARS-related coronavirus (SARSr-CoV; RaTG13) was observed, the difference at neutral sites was 17 %. It suggested a divergence between the two viruses, which was much larger than that previously estimated. Therefore, these results suggested that the development of new variations in functional sites, in the receptor-binding domain (RBD) of the spike, seen in SARS-CoV-2 and viruses from pangolin SARSr-CoVs, were likely to be caused by natural selection besides recombination. In August 2020, [Mawolo et al. \(2020\)](#) published a review on “the origins of coronaviruses” in general, but the information on SARS-CoV-2 was very limited. A recommendation of the authors was that in order prevent potential next outbreaks, strong vigilance in long-term coronavirus surveillance studies carried out in bats and pangolins (as well as in other wildlife and livestock) is necessary. The potential zoonotic sources of SARS-CoV-2 were also assessed by [Jo et al. \(2021\)](#), who based on the existence of genetically related viruses existing in *Rhinolophid* bats and pangolins, extrapolated that potential to carnivores, including domestic cats, ferrets and minks. These species would be particularly susceptible to SARS-CoV-2, in contrast to poultry and other animals reared as livestock like cattle and swine. However, [Lundstrom et al. \(2020\)](#) based on the assumption that laboratory manipulation was highly unlikely for the origin of SARS-CoV-2, the possibilities would comprise either natural selection in animal host before zoonotic transfer, or natural selection in humans following zoonotic transfer. For the second possibility, the authors hypothesized that the SARS-CoV-2 genome adapted during human-to-human transmission. Taking into account the available data, the isolated SARS-CoV-2 genomes would derive from a common origin. On the other hand, [Phillis \(2020\)](#) reported how the zoonotic origins of SARS-CoV-2 would ensure its survival as a human disease, while in a review published in November 2020 by [Wang MY et al. \(2020\)](#), the zoonotic origin (bat) of SARS-CoV-2 was again suggested.

In December 2020, various papers concluded assuming/suggesting the zoonotic origin of SARS-CoV-2. [Huang et al. \(2020\)](#) examined computationally the ACE2 protein usage of SARS-CoV-2 for 285 vertebrates, by modeling the binding energy between the SARS-CoV-2 spike receptor-binding domain (S-RBD) and host ACE2. It was found that the binding data correlated well with the reported experimental studies, being perfectly possible to distinguish the effective ACE2 receptors from the less effective ones. The results of that investigation revealed that many mammals -some primates, rodents and carnivores, rather than

non-mammal species could serve as intermediate hosts of SARS-CoV-2. The detailed structural modeling also supported pangolins as possible intermediate hosts with molecular-level insights. In an evolutionary analysis combining viral and host phylogenies (Lopes et al., 2020), it was also concluded that pangolin had become an opportune host to intermediate bat-to-human SARS-CoV-2 jump and entry. In turn, after collecting and analyzing 29,452 available coronavirus genomes, including 26,312 genomes of SARS-CoV-2 strains, Zhu et al. (2020) indicated that bats might provide a pool of genetic diversity for the origin of SARS-CoV-2.

In a different way, Li et al. (2020) employed “supertree methods” for phylogenetic analysis of SARS-CoV-2, aimed at finding out the origin and evolution of SARS-CoV-2 through phylogenetic supertree analysis. Matrix Representation Parsimony (MRP) supertree construction is a popular technique in the systematic biology community for combining phylogenetic information into a single output tree. The MRP pseudo-sequence supertree provided more information on the SARS-CoV-2 evolution inference relative to the normal phylogenetic tree based on full-length genomic sequences. In a review-article on the genome, structure, receptors and origin of SARS-CoV-2, and specifically regarding the origin of the coronavirus, Wang S et al. (2020) revised the literature about bats, pangolins, snakes, turtles, minks, ferrets, cats and dogs. In general terms, it was found that the SARS-CoV-2-related disease outbreak proved the existence of virus reservoirs in wild and domesticated animals, arguing also for continuous surveillance and early warning programs.

In January 2021, Hassanin et al. (2021) reviewed the data published in 2020 on COVID-19 and analyzed new genetic data on pangolins to better understand how the SARS-CoV-2 virus was transmitted to humans. Available data for sarbecoviruses were reviewed in order to propose all possible hypotheses on the origin of COVID-19. Among these, the involvement of direct transmission from horseshoe bats to humans, the indirect transmission via pangolins or another animal, with interspecies contamination between either wild animals and/or animals kept in cage, were reviewed. Based on the examined information, the authors suggested that some pangolins were contaminated in captivity –either by other pangolins or by another species to be determined– suggesting that illegal trade of living wild mammals was at the origin of the COVID-19. However, the authors clearly remarked that this hypothesis should be still validated, founding a virus almost identical (no less than 99 %) to SARS-CoV-2 in animals sold in wet markets. According to these authors, not only pangolins, but also other mammals –such as small carnivores– would be candidates. With respect specifically to pangolins, Makarenkov et al. (2021) provided arguments supporting the hypothesis of the SARS-CoV-2 origin, according to which, the SARS-CoV-2 genome was a chimera of the RaTG13 and pangolin coronaviruses. Moreover, the results of their horizontal gene transfer and recombination analysis suggested that SARS-CoV-2 could not only be a chimera virus resulting from recombination of the bat RaTG13 and pangolin coronaviruses, but also a close relative of the bat CoV ZC45 and ZXC21 strains. Anyhow, this would reassert that a pangolin might be an intermediate host of SARS-CoV-2. This coronavirus is most closely related –by average genetic distance– to two coronaviruses that were isolated from bats: RaTG13 and RmYN02. Notwithstanding, there is also a segment of high amino acid similarity between human SARS-CoV-2 and a pangolin-isolated strain (GD410721) in the receptor-binding domain of the spike protein (Wang H et al., 2020). This also supports the zoonotic origin of SARS-CoV-2 (Ghareeb and Ramadhan, 2021; Santos-López et al., 2021), although at the time of those publications, the host species involved were not clearly identified (Di Teodoro et al., 2021; Malaiyan et al., 2021).

Wacharapluesadee et al. (2021) carried out studies in Southeast Asia focusing on bats and pangolins, because SARS-CoV-2-related viruses had been previously detected in these animals. Molecular and serological evidence of SARS-CoV-2 related coronaviruses, actively circulating in bats in Southeast Asia, were found. Although the authors highlighted

that the origin of the virus remained unresolved, their results extended the geographic distribution of genetically diverse SARS-CoV-2 related coronaviruses from Japan and China to Thailand over a 4800-km range. Authors of successive publications (Banerjee et al., 2021; Gryseels et al., 2020; Hu et al., 2021) have also remarked the importance of the need of unraveling the origin and transmission route of the coronavirus. With respect to the possible geographic origin of SARS-CoV-2, recently Pei and Yau (2021) selected bat coronavirus RaTG13 as reference, and determined which SARS-CoV-2 genome sequence had the closest distance to bat coronavirus RaTG13. It was concluded that based on the rank of distances, before the outbreak at Wuhan, it was probable that SARS-CoV-2 was already present in various countries, including France, India, the Netherlands, United Kingdom and USA. Doubtless, intercontinental flights may have considerably favored the spread of the coronavirus worldwide. For Pei and Yau (2021), to assume that Wuhan was the first place of human-to-human SARS-CoV-2 transmission would be extremely unlikely.

3. Unnatural origin of SARS-CoV-2

While in generalist media and on social networks, there have been all sorts of controversial hypotheses about the unnatural origin of SARS-CoV-2, the scientific literature on a potential non-zoonotic origin of this coronavirus is certainly scarce. The available information on that hypothesis is next summarized. Haider et al. (2020) even questioned the term zoonosis to designate the possible origin of SARS-CoV-2. These authors indicated that the WHO defines a zoonosis as *any infection naturally transmissible from vertebrate animals to humans*. Thus, COVID-19 –caused by SARS-CoV-2– was classified as a zoonotic disease. However, according to Haider et al. (2020) this classification would have been premature because no animal reservoir had been found. Since in the Wuhan market no virus was isolated directly from animals and no animal reservoir was detected, the authors suggested that COVID-19 should be re-classified as an *emerging infectious disease (EID)* of probable animal origin.

A few months ago, Segreto and Deigin (2021) called the attention on the following issue: almost all scientific papers published to date suggested that SARS-CoV-2 had a natural origin. The only scientific paper detected by these authors, in which a laboratory origin might be considered as possible, was focused on serial passage as the technique that could justify SARS-CoV-2 special adaptation to human cells (Sirotkin and Sirotkin, 2020). Segreto and Deigin (2021) described how the two main SARS-CoV-2 features: a) the presence of a furin cleavage site missing in other coronaviruses of the same group, and b) a receptor binding domain optimized to bind to human cells, could be the result of laboratory manipulation techniques (such as site-directed mutagenesis). The acquisition of both unique features by SARS-CoV-2 –more or less simultaneously– would be less likely to be natural, or caused only by cell/animal serial passage. Strong statements were done by Segreto and Deigin (2021) in their conclusions. They indicated that an artificial origin of SARS-CoV-2 was not a baseless conspiracy theory that must be condemned. Consequently, researchers had the responsibility to consider all possible causes for the origin of SARS-CoV-2. These authors also remarked that special attention should be paid to strains of coronaviruses, which were generated in virology laboratories, but on which there are not available publications. In addition, the same authors, together with other co-workers (Segreto et al., 2021), wrote an Editorial article on the potential laboratory origin of COVID-19. It was concluded that while a natural origin of SARS-CoV-2 was possible and the search for a potential host in nature should continue, the amount of peculiar genetic features identified in the genome of SARS-CoV-2 did not rule out a possible *gain-of-function origin*, which should be therefore discussed in an open scientific debate. In a review-article, Kaina (2021) discussed possible scenarios of SARS-CoV-2 evolution. Particular emphasis was given to the hypothesis that the coronavirus might have unintentionally emerged through routine culture, or *gain-of-function experiments*, in a

laboratory. There, it would have been optimally adapted to human cells, which could have caused cryptic infections among workers, who finally spread the coronavirus. One of the most relevant conclusions of that paper (Kaina, 2021) was this: “Even though evidence is lacking that the virus was intentionally genetically engineered, the possibility that the virus could have emerged unintentionally through laboratory experiments should lead to a rethinking of the need for gain-of-function experiments aimed at enhancing the pathogenicity of a disease-causing agent”. The zoonotic origin of the SARS-CoV-2 has been also recently questioned by Balaram (2021), considering the basis of unusual sequence signatures in the spike protein, as well as the absence of evidence for an intermediate animal host.

Completely contrary to the above suggestions/conclusions, Andersen et al., (2020) published a Letter to the Editor of Nature Medicine, in which the authors reviewed what could be deduced (at that time) on the origin of SARS-CoV-2 from comparative analysis of genomic data. The authors analyzed three theories on the origins of SARS-CoV-2: 1) natural selection in animal host before zoonotic transfer, 2) natural selection in humans following zoonotic transfer, and 3) selection during passage (possibility of an inadvertent laboratory release of the coronavirus). According to these authors, their analyses clearly showed that SARS-CoV-2 was not a laboratory construct, or a purposefully manipulated virus. Anyway, this Letter was published in April 2020, just at the beginning of the pandemic, when the authors already indicated that more scientific data could swing the balance of evidence to favor a certain hypothesis over others. Recently, Colombo and co-workers (2021) demonstrated through analysis of restriction site (RS), that SARS-CoV-2 did not contain peculiar RS or other markers that suggested a manipulation deriving from the recombinant viruses known in the scientific literature.

New information has been added to the debate on the origin of SARS-CoV-2 just a few weeks ago. Deigin and Segreto (2021) noticed that RaTG13, MP789, and RmYN02 are among the coronaviruses most closely related to SARS-CoV-2 identified so far, and their existence became known only after the beginning of the current pandemic. Considering the criticisms expressed by various researchers on these genome sequences and related papers, alternative analyses based only on sequences released before the beginning of the pandemic should be taken into account when drawing conclusions on the origin of SARS-CoV-2. These authors have proposed that the review process of all papers describing SARS-CoV-2's closest relatives, which could contribute to identify the origin of SARS-CoV-2 should be public. It would allow an open and critical evaluation by the entire scientific community.

4. Summary and conclusions

The analysis of the above reviewed publications does not allow yet drawing definitive and conclusive conclusions about the origin of SARS-CoV-2. Most papers here reviewed point in the direction of a zoonotic origin, an origin in which horseshoe bats from the species *Rhinolophus affinis* seemed to be a natural reservoir, while pangolins (*Manis javanica*) could be an intermediate host of the coronavirus (Ahmed et al., 2021; Barh et al., 2020; do vale et al., 2021; Lau et al., 2020). However, even considering the zoonotic hypothesis as the most likely, today, the intermediate source species of SARS-CoV-2 has not been confirmed. On the other hand, although some researchers clearly point to an unnatural origin of SARS-CoV-2, their conclusions are not strongly based on a strict scientific evidence, being largely speculative. Therefore, in July 2021, and according to the revised available scientific literature, the origin of SARS-CoV-2 is not clearly defined yet, being still in the field of the hypotheses. An interesting point to take into account is the variation of SARS-CoV-2 in genome due to its mutations, as shown during the Covid-19 pandemics with the dynamics and spread of some virus variants. The high tendency to mutations may either hamper/help to investigate the origin of SARS-CoV-2.

With respect to the hypotheses on the origin of this coronavirus,

some of them are more likely than others, but they are hypotheses after all. In relation to this, in March 2021, the WHO already stated that all hypotheses remained open (<https://www.who.int/news/item/30-03-2021-who-calls-for-further-studies-data-on-origin-of-sars-cov-2-virus-reiterates-that-all-hypotheses-remain-open>), and made a call for further investigations to establish clearly and definitively the origin of SARS-CoV-2. This is a basic and essential issue for the prevention of potential future pandemics of similar nature, which could appear -depending on the origin (zoonotic)- in a few years, months, or even weeks. Therefore, there is no time to waste on this fundamental and critical research topic.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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