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# Is Gain of Function a Reliable Tool for Establishing Sars-Cov-2 Origin?

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## Abstract

The present opinion article, while dealing with the debated origin of "Severe Acute Respiratory Syndrome-Coronavirus-2" (SARS-CoV-2), the betacoronavirus responsible for "Corona Virus Disease-2019" (COVID-19), provides a speculative insight into the so-called "gain of function" (GOF), a process resulting in the acquirement of new phenotypic features on behalf of the viral pathogen. More in detail, a GOF-related phenomenon leading to increased SARS-CoV-2 virulence and/or transmissibility—as clearly exemplified by the "delta" and the "omicron" as well as by other "variants of concern"—would not necessarily imply that viral genetic manipulations made in the laboratory are its exclusive drivers, provided that GOF may also occur as a consequence of a natural selection process. In order to gain a better understanding of SARS-CoV-2 GOF and GOF-associated phenomena, an in-depth knowledge of the complex viral-host interaction dynamics is absolutely needed, while also paying special attention to the human-animal-viral ecological interfaces within an "ad hoc" multidisciplinary, "holistic", scientific evidence-based and "One Health"-based approach.

## Keywords

SARS-CoV-2, COVID-19, Gain of Function, Viral Evolutionary Phylogeny, Viral Host Range, Viral Spillover, Viral Spillback, One Health

## 1. Introduction

Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2), the seventh known human coronavirus and the fifth hitherto characterized betacoronavirus in mankind, has been recognized since over two years as the sarbecovirus pathogen responsible for CoronaVirus Disease-2019 (COVID-19). Based upon the

official data released each day by the World Health Organization (WHO), approximately 420 million COVID-19 cases have been hitherto recorded worldwide, with almost 6 million human lives having been additionally taken away by SARS-CoV-2 on a global scale (official data released by WHO on February 21, 2022).

The origin of SARS-CoV-2 represents a hot topic and a highly debated scientific issue.

Within this challenging and intriguing context, an "artificial origin hypothesis", presumably resulting from laboratory recombination, was originally raised based upon the occurrence of a unique insertion sequence, 1378 bp-long, located in the middle of SARS-CoV-2 spike (S) glycoprotein gene, which did not match with those from other coronaviruses (James Lyons-Weiler, 2020, personal communication). Subsequent studies showed, however, that the aforementioned SARS-CoV-2 S gene sequence was largely available in coronaviruses from natural sources, thereby allowing the aforementioned "artificial origin hypothesis" to be set aside, if not ruled out [1]. Notwithstanding the above, the laboratory origin hypothesis of SARS-CoV-2 has recently drawn additional attention, with this renewed "flame of interest" being apparently justified by the so-called "gain of function" (GOF). This is a process resulting in the acquirement of new phenotypic characters, as a consequence of the manipulations of the viral genetic make-up artificially made in the laboratory. Within the aforementioned scenario, there is a question which I believe to be of crucial relevance: is the "laboratory of virology" or, more precisely, was the "Institute of Virology of Wuhan" (IVW) the site where SARS-CoV-2 originated, based upon a GOF-related phenomenon involving one or more "cousin" (and closely related) beta coronaviruses like those naturally infecting Rinolophus bats (RATG13, RmYN02), which had been previously identified and genetically characterized by the researchers working at the IVW? And, still noteworthy, may a GOF-associated/related process also occur in nature, with one or more viral "genetic/molecular signatures" testifying its development (which would also apply to artificial/laboratory conditions)?

In this respect, while it should be duly emphasized, on one side, that 14 years were needed to trace back the origin of the SARS-CoV epidemic in 2002-2003 from bats to humans (via an intermediate host, most likely represented by civets) [2], recent studies have found, on the other side, that a coronavirus (RmYN02) from bats living in Southern China might be more closely related to SARS-CoV-2 than a previously identified bat coronavirus (RATG13) [3]. Three new bat coronaviruses (BANAL-52, BANAL-103, BANAL-236) have been additionally identified in Laos, with BANAL-52 sharing 96.8% genomic sequence homology with SARS-CoV-2 and with these newly characterized agents harbouring an angiotensin-converting enzyme-2 (ACE-2) S glycoprotein receptor-binding domain (RBD) almost identical to that of SARS-CoV-2, thus being potentially capable to infect human cells [4].

## 2. The Origin of the SARS-CoV-2 Pandemic

Noteworthy, the SARS-CoV-2 genome is made of approximately 30,000 nucleotides, with each viral replication cycle implying the occurrence of an average of 1 mutation/10,000 bases. There are, of course, different types of mutations, some "silent", some "non-silent", some "disadvantageous" (against which "purifying, or negative selection" operates), some other ones "advantageous" (in favour of/toward which "Darwinian, or positive selection" operates).

Therefore, based upon the aforementioned mutational events, SARS-CoV-2 could have originated under natural conditions from a betacoronavirus "ancestor" like the herein dealt bat coronaviruses, all of which have been found to share with it over 96% genome sequence homology [3] [4]. Further mutations of the SARS-CoV-2 genetic make-up could have led the virus to develop a growing number of "variants of concern" (VOC) and/or "variants of interest" (VOI) circulating in many Countries, including the "alfa" (formerly "English", alias "B.1.1.7"), "beta" (formerly "South African", alias "B.1.351"), "gamma" (formerly "Brazilian", alias "P.1"), "delta", "delta plus" and "kappa" (formerly "Indian", alias "B.1.617.1", "B.617.2" and "B.1.617.3"), alongside the more recently identified "lambda" (alias "C.37"), "mu" (alias "B.1.621"), C.1.2 and R.1 and, above all, the highly transmissible/contagious "omicron" (alias "B.1.529") VOC (with its hitherto recognized "BA.1", "BA.1.1", "BA.2" and "BA.3" subvariants).

## 3. Cluster 5

Among the SARS-CoV-2 VOC deserving special interest, there is the one named "cluster 5", which is characterized by the "Y453F" mutation within the viral S glycoprotein's RBD. As a matter of fact, differently from all the aforementioned (as well as from all the other hitherto characterized) VOC and VOI, all of which developed in mankind, the "cluster 5" VOC emerged more than one year ago in intensely bred mink from The Netherlands and Denmark, where approximately 17 million animals had to be killed as a consequence of the public health hazard posed by them. Indeed, once acquired from infected humans (viral spillover), SARS-CoV-2 was shown to evolve into the "cluster 5" variant inside the body of mink, which were additionally shown to "return" the mutated virus to humans (viral spillback) [5]. The growing emergence of SARS-CoV-2 variants, now defined with the Greek alphabet letters, coupled with the progressively expanding list of SARS-CoV-2-susceptible species under both natural and experimental conditions [5], as recently highlighted by white-tailed deer (*Odocoileus virginianus*) [6] [7] [8], could represent a sort of "vicious circle" supporting, in turn, the development of additional VOC and/or VOI of the virus [7], a number of which could escape, more or less consistently, the immunity conferred by SARS-CoV-2 infection and/or by the currently available anti-SARS-CoV-2 vaccines [9] [10]. How not to deem this a GOF-related phenomenon, in a similar fashion to the genetic mutations progressively acquired by SARS-CoV-2 at the S glycoprotein RBD (and, to a much lesser extent, at the nucleoprotein/N gene) levels, a

number of which are known to render the viral agent more infectious [11]?

## 4. Human VOC in Animals

Interestingly enough, a naturally occurring case of infection by the "alfa" (B.1.1.7) VOC of the virus has been recently documented in a domestic cat from North-Western Italy, which most likely caught the infection from a COVID-19-affected owner (Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, unpublished observations). Still noteworthy, infection by the B.1.1.7 SARS-CoV-2 variant has been additionally reported in two cats and in one dog from France with suspected myocarditis and whose owners had shown COVID-19-associated respiratory symptoms 3 - 6 weeks before [12]. Still noteworthy, a very recent "delta" VOC infection's cluster has been additionally reported in pet hamsters from Hong Kong, which likely spread thereafter the virus to humans. This would consequently represent the second documented occurrence of SARS-CoV-2 transmission from animals to people after that involving intensely bred/reared mink in The Netherlands and Denmark [13].

In consideration of what above, why not taking into account the possibility of vaccinating (also) animals against SARS-CoV-2, with particular emphasis on those living in close contact with humans and, especially, on intensely reared species like mink and pigs, or on wild species with a marked social behaviour like white-tailed deer [5] [7]?

To this aim as well as in order to adequately counteract, to prevent from taking place and/or to foresee the occurrence of similar catastrophic events in the next future, a scientific evidence-based, coupled with an "holistic", multidisciplinary, and "One Health-based" approach, should be simultaneously adopted, provided that—as the dramatic SARS-CoV-2 pandemic/syndemic reminds us—human, animal and environmental health are tightly and mutually connected to each other.

Despite all the above, no veterinarians have been appointed yet as members of the "Italian COVID-19 Scientific Committee" (popularly known with the acronym "CTS"), after two years since its official inception [14]!

## 5. Summary

In summary, when dealing with the hotly debated topic of SARS-CoV-2 origin, we should firmly keep in mind that GOF is not diriment about the "laboratory" versus the natural origin of the virus, with the latter being consistently supported, in any case, by the close genomic relatedness of a number of sarbecoviruses (more or less) recently identified in bats from China and Laos [3] [4].

"Ad hoc" experimental studies on suitable animal models, coupled with "ex vivo" and "in vitro" experiments on human and animal "organoids" as well as on specifically engineered cell cultures expressing either the human or the (SARS-CoV-2-susceptible) animal viral receptor molecule, are urgently needed.

In conclusion, while a natural origin appears to be much more plausible and

justified for SARS-CoV-2, from the biological and epidemiological standpoints, as compared to the "artificial" or "laboratory" viral origin, much more experimental work is still needed to assess the natural source(s) from which SARS-CoV-2 spilled over into mankind, thereby giving rise to the dramatic CoViD-19 pandemic experienced by the entire world in the last two years.

## Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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