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The Animal Origin Of SARS-Cov-2, The Human Spill-Over and The Recurrent Saga of The Man-Made Virus

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How the scientific evidence gets rid of the man-made theory on SARS-CoV-2

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The novel pneumonia infection (Covid-19) caused by coronavirus 2 of severe acute respiratory syndrome (SARS-CoV-2) emerged with the first cases in China in late 2019 and quickly spread around the world causing a pandemic with unprecedented disruption of social interaction, health systems and badly hit all economies. As of 1 May 2020, 3.214.256 cases of COVID-19 have been reported, including 232570 deaths. Italy, my country, reported the highest number of deaths in the European Union: 27, 967.

Despite the massive research efforts of the scientific community around the globe, unfortunately the understanding of Covid-19 infection suffers from many sources of uncertainty in different domains (eg. epidemiology, virology, medicine, etc.), to name just a

few: the original animal reservoir of SARS-CoV-2, its intermediate host, the route of transmission to humans, the virus mutations impacting the pathogenicity, the human immune system handling the infection, efficiency of antibody response, the potential transmission via food and domestic animals. From the medical side there are many ongoing clinical randomised trials undertaken in different countries aimed to evaluate and confirm the safety and effectiveness of the current therapeutic regimes differentiated according to the stage of the infection and the presence of co-morbidities of patients suffering from severe symptoms, notably those related to the interstitial pneumonia and further pathological complications. Covid-19 is a new infection and the time is the key for understanding how it will evolve, the success of the drugs, their safety, the length of total recovery and the potential occurrence of long-term neurological disorder sequalae.

But, as a veterinarian, while acknowledging the importance of the above cited aspects, my inquisitiveness turns to the likely animal origin of the virus, the direct spill-over to humans from the animal reservoir (eg bats), or through an intermediate host, and considering the ability of SARS-CoV-2 to infect some domestic animals, the possibility that animals in proximity to humans, as pets, might spread the disease in human communities.

Alongside all that, I would also like to write on something that became a recurrent and boring topic pervading the infodemic web and unfortunately catching many naive professionals out there. I am referring to the number of conspiracy visions or bioweapon theories claiming that SARS-CoV-2 emerged through a laboratory

manipulation of a related SARS-CoV-like coronavirus. This will be addressed in the third part of this contribution.

The natural origin of SARS-CoV-2

To briefly recall the story: at the end of December 2019, the beginning of the Wuhan outbreak, scientists based on the genomic sequence of the virus isolated from the first human cases of atypical pneumonia hypothesized that the wild fauna, probably bats, constituted the initial reservoir of the SARS-CoV-2, that subsequently spilled over to humans, perhaps using a different intermediate host in the process. Indeed, the phylogenetic analysis of SARS-CoV-2 showed that while the virus is genetically homogenous but distinct from coronaviruses that cause SARS and MERS, it also shares a high level of genetic similarity (96.3%) with the coronavirus RaTG13, which was obtained from a bat of the genus Rhinolophus, species Rhinolophus affinis in Yunnan in 2013. Hence RaTG13-like viruses are most likely the reservoir, but not the immediate sources of the SARS-CoV-2.

Later on, it come out that despite RaTG13 coronavirus remains the closest to SARS-CoV-2 across the genome, some pangolin (Manis javanica) CoVs does not match the whole genome of SARS-CoV-2, but exhibit strong similarity to SARS-CoV-2 in the RBD (Receptor Binding Domain). It is worth recalling that pangolins are endangered mammals and the most trafficked on earth due to their soft scales used in winemaking, and in some traditional medicines often for male virility. The RBD, which is located in the S (spike) protein present on the surface or envelope of the virus, is the key

element for beta-coronavirus (like SARS-CoV-2) for entering into host cells and the mutations in coronavirus's RBD affects its infection and cross-species capability. Recently, researchers from the South China Agricultural University examined more than 1,000 wild animal samples and found a 99% match between pangolin coronavirus and SARS-CoV 2 sequences. This similarity suggests that by the mechanism of viral recombination, a piece of the pangolin CoV was transferred into a bat CoV, making a brand-new virus and the pangolins have the potential to act as the intermediate host of SARS-CoV-2. This latter fact, consistent with the optimization of the S protein for binding to human-like ACE2, clearly support the origin of SARS-CoV-2 as result of natural selection — this will be later explained. Against the background of scientific evidence on the role of the pangolin in the outbreak, there are conflicting opinions among scientists: while some support the evidence of the above studies, others question the direct jump of the virus from pangolins to humans, noting differences between the pangolin and human viruses and the unclear path established from bats to pangolins.

The two-steps process of the natural selection

Like SARS and MERS, the animal-human spill-over of SARS-CoV-2, appears to be a result of two-steps in the process of natural selection. The first steps took place in an animal host (Rhinolophus affinis bat or Malayan pangolins) well before it jumped into humans, and the second steps occurred in humans following the zoonotic transfer. Here there is an important caveat: with the scarcity of scientific data, we can only postulate two hypotheses. Notably, is it likely that a bat

CoV infected an intermediary animal (potentially a Malayan pangolin) where it recombined with a non-bat coronavirus? (hypothesis 1). We know that coronavirus (like SARS-CoV-2) are single-stranded, positive-sense RNA (ribonucleic acid) virus with a remarkable complexity and genetic diversity, that is linked to the high frequency of viral recombination. This phenomenon of recombination is associated to the gene encoding the S (spike) protein present on the surface or envelope of the virus. If hypothesis 1 is true, and the recombination really happened, SARS-CoV-2 might have jumped into humans only after gaining new (genetic) tools that allowed it to be efficiently adapted to this new host. Being an hypothesis, it needs be tested (and falsified) by collecting more viruses in pangolins, sequencing them and try to piece together a more accurate story of SARS-CoV-2 emergence. More practically, we will have to find an epidemiological confirmation using phylogenetic analyses of the viruses in the wild and animal reservoir (eg pangolins, bats, or some other intermediate animal) that look very similar to SARS-CoV-2 and that are poised to spill-over into humans again.

There is also an alternative, hypothesis 2, which supports the development over time of additional mutations (e.g .a polybasic cleavage site and a nearby o-linked glycan addition site) of SARS-CoV-2, either in the intermediary animal or while amplifying in humans. It is well known that coronavirus typically acquires two mutations a month during the process of spread. This hypothesis fits well to the available data. This is the story: SARS-CoV-2 first infected humans and probably replicated and spread less well. But during multiple replication cycles, as for other viruses (this is the

field of genomic epidemiology) it made mistakes, mutated very quickly and accumulated these changes during the process of transmission from one infected individual to another. Some mutants might gain the tool needed to spread efficiently among humans.

What these two hypotheses might offer in terms of contribution to the study and control of further spread and spill-over of SARS-CoV-2, is how can they be operationalised if they prove to be correct. If hypothesis 1 is deemed to be the correct one, it will lead to extensive epidemiological surveillance programs aimed at identifying viruses with genetic prerequisites for human infection, especially at hot spots with ecological conditions facilitating the spill-over, and informing the most appropriate prevention and control strategies for the management of potential threats to public health. Under the One Health umbrella, many global collaborative projects thrived in recent years, such as the PREEMPT and PREDICT programs to build viral surveillance platforms for identifying and monitoring zoonotic pathogens.

In the scenario hypothesis 2 is correct, the focus of intervention will be the monitoring of humans for new infections, to identify spill-over viruses before they gain high efficiency in person-person transmission.

It is clearly implied from both scenarios (hypothesis 1 and hypothesis 2) that the virus was not designed or modified in a lab.

But the reasoning used in epidemiology converges towards the establishing of a reliable pangolin (or other intermediate host)

Covid-19 epidemiological link, with the gold standard (or the smoking gun): the clear demonstration that the very first human cases in China became ill after being exposed by contact with this species, and that the viral genetic material from humans and animals that might have been present at the Wuhan seafood market, indicate highly similar viruses. This leads us to the third piece of the story: the likely location where the spill-over took place.

Place where the zoonotic spill-over may have occurred

The human epidemiological data link a high percentage of first- and second-generation cases of SARS-Cov-2 infections to Wuhan's Huanan South China Seafood market. A first confirmation of this place emerges from the results of an environmental microbiological investigation carried out in the Huanan market, with 33 swabs taken from surfaces of the stands and equipment in the areas used for the sale of wild animals, all of which were positive for SARS-CoV 2. However, upon further investigation, it was noted that a third of the first 41 human cases had no connection to the market, including the first patient notified on December 1st 2019. Moreover, that latter patient was never linked to any future cases of Covid-19. This data point to the conclusion that the first human cases of SARS-CoV-2 infection must have happened before December 2019, thus did not originate at the Huanan Seafood Wholesale Market.

In summary, based on the above evidence, two hypotheses can be made:

Hypothesis 1: SARS-Cov 2 was introduced into the human population by an animal source in the Huanan market;

Hypothesis 2: humans introduced SARS-Cov 2 to the market. This implies that SARS-Cov was present in animal reservoirs and passed back on to humans during the exposure that might have occurred outside the market, then there was subsequent amplification of the virus in animals and humans. These adjustments (see hypothesis 2 above related to the two-steps process of the natural selection) would then have allowed SARS-Cov-2 to produce the number of cases large enough to be detected by the surveillance system.

Based on the two following studies, we can abandon hypothesis 1, which appears quite feeble. The current data definitely fits better with hypothesis 2 (origin out of the market)

Study 1: ***compare the virus itself in different patients***

In this Chinese study, based on the phylogenetic network, the researchers gathered many sequences of SARS-CoV-2 and compared those sequences to build a family tree. The results indicated that the first infections were from Wuhan, but probably not at the Huanan Seafood Wholesale Market, but imported from other places. Instead, the virus passed to humans earlier. The crowded market boosted the rapid circulation of SARS-CoV-2, which infected a large group of people in the market and spread it to the whole city in early December 2019. In this analysis two additional pieces of information are most significant: — the earliest sequenced case could not be linked to the Huanan Market; — the sequences from patients

connected to the market could not account for the entire diversity of all SARS-CoV-2 sequences collected afterwards. In other words, the comparison of sequences indicate that the viruses aren't "children" of the viruses acquired at the market.

It is worth noting that the conducting of comprehensive phylogenetic analysis of SARS-CoV2 virus isolated in different patients will shed a light on the study of discrete mutations that are occurring between patients. This study can provide strong benefits for the therapy, as the efficacy of different inhibitory drugs might be evaluated vis a vis the kind of mutation in the viral genome.

Study 2: ***compare the virus by extending the phylogenetic network analyses***

The essential concept here is that anywhere that a virus has been, will have its signature written within its genetic history. Researchers from Cambridge, UK, and Germany clearly suggest a secondary source of infection, person-to-person transmission or possibly from infected animals to another market in Wuhan. By extending the phylogenetic network analyses from 160 (previous work) to 1,001 (the paper has not yet been peer-reviewed) complete virus genomes to determine their age and origin, they have suspected that the SARS-CoV-2 may have been spreading quietly in host animals and humans for years, and did not come from the animal market in Hubei province. Then it mutated (a coronavirus typically acquires two mutations a month) and gradually shifted into a highly adaptive and final 'human-efficient' form months ago (but remained inside the reservoir (a bat or other animal) or even human for several

months, without infecting other individuals. They calculated that the initial outbreak occurred in a window between September 13 and December 7. The first Covid-19 outbreak in Wuhan could be a recent event resulted from the last few mutations that completed the leap from harmless strain to deadly pathogen. Despite this work may provide some important clues to future investigations, according to other scientists the conclusions should be treated with caution."

These studies tell us that the investigation of an outbreak and the identification of the zoonotic origin, require a lot of time since a large number of wild and domestic animal would need to be screened. The fact today is that although no animal coronaviruses similar enough to be the direct progenitor of SARS-CoV-2 has been identified, the diversity of coronaviruses in bats and other species is largely under-sampled, and this points to the strengthening of epidemiological surveillance of wildlife, likely reservoir of deadly pathogens.

On this track, Chinese health authorities from the beginning have embarked on three lines of research, which include: investigations into cases with onset of symptoms in Wuhan for the whole month of December 2019; environmental sampling in the Huanan market and in the other markets in the province; and the collection of detailed records on the sources and types of wild species sold in the Huanan market and on the destinations of the animals after the market closure. But a definite proof can only come from analysing more bats, possibly other potential host animals, and preserved tissue samples in Chinese hospitals stored between September and

December 2019, when the first human cases of Covid-19 were notified.

How the scientific evidence gets rid of the man-made theory on SARS-CoV-2

Conspiracy theories on the laboratory origin of SARS-CoV-2 made rounds for quite some time and caused governments to spread disinformation cheaply and easily. Paradigmatic of the mounting controversy is the recent statement of Luc Montagnier, the Nobel Prize-winning scientist and discoverer of HIV (Human Immunodeficiency Virus), who claimed that Covid-19 pandemic has been engineered, since the SARS-CoV-2 contains some genes of HIV-1, the AIDS virus, and is the result of an attempt to manufacture a vaccine against the AIDS virus. He said '*someone added sequences, it is not natural, it's the work of molecular biologists who did a meticulous work*." Countless people commented on this news and the majority of the global scientific communities replied defining his theories 'whimsical'. Montagnier, in support cites a scientific paper, published in March from the Indian Institute of Technology, where the authors claimed that proteins in the coronavirus shared an "uncanny similarity" with those of HIV. Notably the paper claims that, compared to other coronaviruses, SARS-CoV-2 has four insertions in the S glycoprotein gene that the virus deploys to enter the target cells, and that 'these inserts were either identical or similar to the motifs in the some highly variable regions in the envelope glycoprotein or in the Gag protein of some unique HIV-1 strains'. Their study implies that SARS-CoV-2 is the results of gaining gene fragments from the HIV-1

genome. The manuscript was then publicly demolished before being withdrawn by its authors, in an absolutely transparent way.

All studies of the genetics of the coronavirus point to the overwhelming probability that SARS-CoV-2 leapt from animal to human in a non-laboratory setting, as was the case with HIV, Ebola, SARS and MERS.

Now there are three lines of scientific evidence, that by discarding the laboratory-scenario theory and debunking the Indian study, clearly show that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus; I would like to cite an article from Nature, BLAST software, and a Chinese study.

Let us start with the first one.

Nature's article

One answer that refutes the man-made scenario behind the emergence of SARS-CoV-2 comes from a recent paper published on Nature untitled 'The proximal origin of SARS-CoV-2'. The authors review the information on the origin of SARS-CoV-2 from comparative analysis of genomic data based on the distinctive features of the genome and discuss scenarios for the likely origin. Their structural studies and biochemical experiments suggest that SARS-CoV-2 may bind human ACE2 (but also other animal species) with high affinity, but computational analyses indicate that this interaction is not ideal and that the receptor binding domain (RBD) sequence in the S protein is optimised with an efficient solution

different from those shown in SARS-CoV. If someone was seeking to manipulate/engineer a new coronavirus as a pathogen, the authors note, they would have constructed it from the backbone of a virus known to cause illness. But the backbone of SARS-CoV-'differed substantially' from those of already known coronaviruses, and mostly resembled related viruses found in bats and pangolins. The genetic data provided by the authors, some derived from reputable published scientific papers, incontrovertibly show that SARS-CoV-2 is not derived from any previously used virus backbone. They deduce that 2 features of the virus, the mutations in the RBD portion of the S protein and its distinct backbone, rule out laboratory manipulation as a potential origin for SARS-CoV-2, notably the high-affinity binding of the SARS-CoV-2 to human ACE2 is most likely the result of natural selection on a human or human-like ACE2.

BLAST

A recent post by the researcher Philippe Lacoude corroborates the evidence of the natural selection of SARS-CoV-2 and discards the odd hypothesis of Montagnier pointing to SARS-CoV-2 containing HIV genetic code. To debunk this theory, he uses a tool called BLAST (Basic Local Alignment Search Tool (developed by the National Institute of Health (NIH)), which finds regions of similarity between biological sequences of viruses. The BLAST program works in this way: it analyses the complete genomes of viruses, compares nucleotides or protein sequences to sequence databases and calculates the statistical significance of said similarities. For each similarity, it even documents which protein it encodes. By applying

BLAST to SARS-CoV-2, the researcher didn't find any sequence of HIV-1, thus SARS-CoV-2 is not made of the bat coronavirus plus small bits of the HIV virus. However, he points out that there are sequences of HIV-1 that would be "vaguely" similar to some other sequences of SARS-CoV-2, as both viruses have a glycoprotein envelope. Notably the part of the HIV-1 genome and the part of the SARS-CoV-2 genome that encode the two (different) glycoprotein envelopes are circa 90% similar. Based on this analysis, to further discard the likely HIV insertion, is shows that the genetic sequence of SARS-CoV encoding its lipidic envelope, while it looks a bit like that of HIV-1, is also part of many other viruses such as the humans coronavirus HCoV-229E (which gives the common cold) and HCoV-OC43 (also a variant of the common cold), which were discovered well before the HIV.

The Chinese study

A chinese study published on February 2020 clearly demonstrates no evidence that the sequences of the four inserts are HIV-1 specific, or the SARS-CoV-2 obtain these insertions from HIV-1, because three out of four of the HIV inserts naturally existed in three bat CoV viruses, before SARS-CoV-2 was identified. This undoubtedly refutes the possibility that SARS-CoV-2 is generated through obtaining gene fragments from the HIV-1 genome. Instead, it is much more likely that SARS-CoV-2 originated from RaTG13-like CoV.

The molecular clock (temporal) evaluation of spike and nucleocapsid genes of these viruses indicates that the most current frequent ancestor of all genotypes of these viruses is dated back to 1950s,

hence is practically impossible that anyone in a Wuhan laboratory in 2019 could have affected the RNA code of a virus in the mid-1950s.

In conclusion, drawing on the above different kinds of reasonings hinged on the Nature paper, the BLAST analysis results and the Chinese study, we are certain that the SARS-CoV-2 emerged from a natural selection process. But, I would like to add that these conspiracy theories of the laboratory-scenario origin of SARS-CoV-2 built on biased and incorrect scientific analysis, can undermine the integrity of the scientific process and vanish the effort to curb the public health threat of Covid-19.

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Coronavirus; Covid-19, uncertainties

Following

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- Covid 19
- Sars Cov2
- Uncertainty