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LETTER TO THE EDITOR

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Might the many positive COVID19 subjects in Italy have been caused by resident bat-derived zoonotic β-coronaviruses instead of the Wuhan (China) outbreak?

To the Editor,

In a recent article by Lai et al,[1] published on the *Journal of Medical Virology*, the authors attempted a mathematical reconstruction of the evolutionary dynamics of the new coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) outbreak occurred in Wuhan (China), by analyzing 52 SARS-CoV2 genomes provided at GISAID on 4 February 2020. This evaluation is fundamental to make authorities aware about spreading characteristics of SARS-CoV2 in the Italian population and to earn insightful clues about the presumptive hypothesis that the current viral spreading in Italy surely comes from a Wuhan-borne genotype and/or a Chinese outbreak. The same authors concluded that the SARS-CoV2 in Italy might be present at least since September and October 2019, much before the claimed Wuhan outbreak.[1] According to the World Health Organization evaluation, SARS-CoV2 outbreaks in Europe occurred much before in Germany and France respect to Italy. Therefore, it might be presumed that a significant proportion of Italians were infected by SARS-CoV2 in times greatly preceding the Government dispositions upon the cases enumeration. The daily differences between cases in the highest emergence period, that is, 1 to 9 March 2020, plotted a linear rather than an exponential trend. As x increases (1 day each), y values (cases number) increases by the same amount (1.2 or +20%) (Shapiro-Wilk's exp test $P = .442857$). This possibly suggests that rhinopharyngeal swabs are catching homogeneous clusters of cases from dating back homogeneously, normally distributed preinfected population, depending also on an established maximal number of analyzed swabs for the day.

Questions may be raised, therefore, if, taking into account the genomic distance (or similarity) and the RNA-virus mutation rate, the "Italian" SARS-CoV2 might be the evolutionary balanced genotype (or strain) from a resident zoonotic spillover.

The authors reported their conclusions by taking into account coalescent analysis and a birth-death method to estimate the SARS-CoV2 ancestor and establish unequivocally the origin of pandemics.[1] Despite the trivial observation that a common reader of these issues may raise that is, that genetic divergence cannot be confused with a phylogenetic difference, RNA viruses mutation rate and their maintenance in the population structure or the balancing selection must be debated with great caution.[2] It is well known that, particularly for viral pathogens, their ecological (genetic) and evolutionary dynamics may occur in the same timescale where potential cross interactions represent a fundamental hallmark. As regards RNA viruses, such as coronaviruses, the nucleotide mutation rate is at least a million times higher respect to a vertebrate, frequently recombine their genomes, between animals and humans, and are therefore highly pushed by evolutionary selection to adapt and survive in the animal-human coexisting population.[3] This should suggest that the cross-talk between evolution and epidemiology is closely intertwined, causing that the maintenance of an onward transmission might be crucially associated with a continuous viral adaptation.

Interestingly, the possible comparison of different isolates with the Italian strain (Gene Bank MT008023.1) of only 322 base pair coding the highly conserved M (E1) protein, allows retrieving very high similarity in the early timescale, due to the highly conserved sequence.

Probably, the most stringent topic about SARS-CoV2 should regard virulence, an issue that is strictly dependent on the population health condition and urbanization, more than on the viral mutation speed. Furthermore, the complete sequence diversity of SARS and the Middle East respiratory syndrome (MERS) viruses with further human β-coronaviruses at the S protein, is raising people the anguished question of why the current pandemic alarmed warning did not occur also for SARS, as a completely new emerging virus. One possible answer was forwarded taking into account that the SARS-CoV virus in 2003 outbreak came simply from domestic animals.

The issue of human coronaviruses virulence was recently addressed for SARS-CoV and MERS-CoV and some reports have outlined the role of coronavirus E protein in triggering an inflammatory response, cytokine storm, and/or inhibition of the innate immunity with dampening Th1 interferon-γ signaling.[4] In this context, alarming claims may appear completely justified, despite the fact that for SARS pandemics was not announced an alarming concern as doing currently. In this respect, authorities should inform the social collectivity about the correct patients' stratification, how many hospitalized because of COVID19 and how many deaths because of COVID19.

SARS-CoV2 genome shares about 70% similarity with SARS coronavirus[5] but probably, its completely new emergence via the mechanism of jumping species, is not a novelty in the biology of human *Beta-coronaviridae*. Table 1 shows the Waterman-Eggert score and similarity of main zoonotic coronavirus genomes and the SARS-Cov2 Italian genomic sequence (Gene Bank MT_008022.1) of about 322 nucleotides, from a BLAST evaluation on 60 different

TABLE 1 Comparison between the Italian SARS-CoV2 sequence MT008022.1 with zoonotic spillover SARS CoVs genomics

Gene bank ID	Name	waterman-eggert score	simi-larity	Notes
DQ071615.1	Bat Rp3 SARS coronavirus	1177; 176.2 bits; E(1) <8.9×10^{-47}	85.3%	85.3% Identity (85.3% similar) in 320 nt overlap (26 532-26 851:3-322)
JX993988.1	Bat coronavirus Cp/Yunnan2011	1199; 179.1 bits; E(1) <1.2×10^{-47}	86.2%	86.2% Identity (86.2% similar) in 319 nt overlap (26 437-26 755:3-321)
MG772933.1	Bat SARS-like coronavirus isolate bat-SL-CoVZC45	1412; 221.9 bits; E(1) <1.5×10^{-60}	93.2%	93.2% Identity (93.2% similar) in 322 nt overlap (26 610-26 931:1-322)
MG772934.1	Bat SARS-like coronavirus isolate bat-SL-CoVZXC21	1412; 222.4 bits; E(1) <1.1×10^{-60}	93.2%	93.2% Identity (93.2% similar) in 322 nt overlap (26 541-26 862:1-322)
MN996532.1	Bat coronavirus RaTG13	1466; 214.0 bits; E(1) <3.7×10^{-58}	95%	95.0% Identity (95.0% similar) in 322 nt overlap (26 672-26 993:1-322)

Note: Italy SARS-CoV2 isolate = 322 nt. Similarity with Wuhan seafood market pneumonia virus isolates Wuhan HU-1 = 100% (Lalign Software version 2.1.30 (12 May 2010) Readseq version 2.1.30 (12 May 2010).
Abbreviations: nt, nucleotide; SARS-CoV2, severe acute respiratory syndrome coronavirus 2.

genome sequences. The similarity is higher than 85%, a similarity quite similar for previous human SARS outbreaks, for example, the 2004-SARS epidemic virus (Gene Bank NC_004718.3), with a similarity of 84% but not with MERS coronavirus (Gene Bank NC_019843.3), with 56.9% of similarity.

Previous reports have outlined that the genetic difference in the Spike protein sequence, between the most frequent human coronavirus genotypes such as HCoV-NL63, HCoV-229E and HCoV-OC43, and SARS or MERS coronaviruses, is practically absolute (100%), so describing new emerging species in the human coronavirus outbreaks.[6] This suggests that SARS had the same genetic novelty without immune protection in the population as COVID19 to date, though with a much lesser extent in the emergence claim.

The low kinship with other human coronavirus outbreaks, such as MERS, suggests that human coronaviruses rapidly evolve in the local population and rapidly disappear, probably via evolutionary adaptation, via an immune pressure. Briefly speaking and despite the fact that SARS HCoV was a newly emerging virus likewise SARS-CoV2, the COVID19 causing agent, when the outbreak occurred in 2002 to 2003, worldwide alarming and warnings widespread in lesser extent respect to the current SARS-CoV2 claim.[7] Actually, the genomic evolution of the β-coronavirus family in Italy may come from zoonotic spillover and a wide interplay vectors/human hosts.[8] While Liu et al[8] suggested turtles as intermediate vectors of the SARS-CoV2, Li et al,[9] dismiss the hypothesis of pangolin as a possible intermediate, mainly focusing on bats. We do not know if in Italy a wide genetic subfamily of β-coronaviruses coexisted in the country due to zoonotic spillover and population spreading caused by the very frequent human exchanges with also Chinese people from endemic areas. This issue has not been thoroughly investigated so far, because of the urgent need to diagnose infected individuals just following the Wuhan outbreak, but it might have an important consequence also in political decisions.

The crowded debate burst on the epidemiological causes of the SARS-CoV2 outbreak in Italy never addressed the possibility that, also in a European country, the animal-mediated intermediate transmission may have a role in the positive SARS-CoV2 diagnosed individuals so far. Interestingly, the paper by Lai et al,[1] discussed the hypothesis that a COVID19-like coronavirus causative agent, might have existed in Italy quite earlier than the Wuhan outbreak, probably since September or October 2019. The question if we are currently "mapping" a family of SARS-CoV2-corelated genotypes derived from pre-existing human autochthonous β-coronavirus, is particularly concerning. In Italy, the Lombardia region, which accounts for the highest number of SARS-CoV2 positive subjects, has also the highest percentage of Chinese resident people (>23%), according to recent ISTAT data in 2019. Interestingly, Tuscany (Toscana) region, with Prato, accounting on about 18% Chinese individuals, appeared to have a very low number of people purported to have contracted the SARS-CoV2.[10]

Besides China, a spillover of zoonotic pathogens from wildlife was reported also in Italy from bats.[11] In Italy, the association between β-coronavirus species, closely akin to SARS-CoV, and bats

(*Rhinolophus ferrumequinum* L), has been recently reported.[9,12] Lelli et al[13] reported a phylogenetic investigation about the origin of the many different coronavirus genotypes in Italy from wild bats and identified two alpha-coronaviruses from Kuhl's pipistrelle (*Pipistrellus kuhlii*), three clade 2b β-coronaviruses from lesser horseshoe bats (*Rhinolophus hipposideros*), and 10 clade 2c β-coronaviruses from Kuhl's pipistrelle, common noctule (*Nyctalus noctula*), and Savi's pipistrelle (*Hypsugo savii*), mainly in the Italian Regions Lombardia and Emilia-Romagna, where major SARS-CoV2 focuses are present.[13] The group of Moreno et al,[14] described two β-coronaviruses closely associated with MERS-CoV and present in very common bat species, that is, *P. kuhlii* and *H. savii*), widespread in the Northern regions of Italy. This study fills a substantive gap in the knowledge of bat-CoV ecology in Italy and extends the current knowledge of clade 2c β-coronaviruses with new sequences obtained from bats that have not been previously described as hosts of these viruses. According to the authors, the mean evolutionary rate was estimated as 2.15×10^{-6} substitutions/site, corresponding to a value of 7.8×10^{-4} substitutions/year, so retrieving an estimated tMRCA dating back to 10 September.[1] The conclusions reported by the authors showed that the evolutionary rate of the novel SARS-CoV2 is online with the SARS and MERS ones.[1]

Zhang and colleagues[15] have investigated the adaptive evolution of the S (Spike) protein in SARS-CoV and SARS-CoV-like viruses in animals, reaching the conclusion that both the positive selected pressure and the positively selected target site contributes in the adaptive evolution of the S protein from animals to humans, evidence that supports the suggestion to analyze this issue to comprehend the coronavirus spillover in Northern Italy during the current SARS-CoV2 outbreak.[15]

Bats are well-known reservoirs of mammals coronaviruses, including humans. Therefore, a keen survey about the genetic variants and phylogeny of SARS-related coronaviruses in Italy is mandatory to shed light on the very recent SARS-CoV2 in Italy from spillover zoonosis. This should enlight many still debated concerns.

Moreover, the current literature on the field is still scanty about the clinical aspects of COVID19 in Italian patients. So far, evidence was reported only for individuals having held direct contact with Wuhan.[16] Death rates from pulmonary infectious agents account for about 11% to 14%, usually involving people aged 75 to 90 years, therefore the current estimation of about 2.83% to 3.6% of deaths caused by COVID19, may appear limited respect to the big claim burst on the SARS-CoV2 infection. Furthermore, the biggest concern currently forwarded by media regards the SARS-CoV2 impact on the resuscitation units of the many health care structures in Italy. This may appear controversial with the number of patients with confirmed pulmonary pathology undergoing hospitalization. During the 2002-SARS epidemics evidence that transmission may occur during cardiopulmonary resuscitation was reported but did not raise particular concern.[17] Therefore, the Italian authorities engaged to face at the SARS-CoV2 outbreak should inform citizens also about the stratified number of hospitalized people in the resuscitation units, to have a cleared overview of the SARS-CoV2 epidemics in our country.

In 2018, the total number of resuscitation units (intensive care and cardiovascular arterial units) were estimated to be 69 921 + 148 124 (a total of 218 045 hospitalized cases, ie, 597.38 cases per day), which means about 30 cases/day/region and 600 subjects in those units in a time range of about 20 days. Data from the Health Ministry were sensitively lower (351) on 5 March 2020 and showed a very modest upgrowth only very recently (877 on 10 March 2020), which might explain the big concern about health care services.

Much better communication and the elucidation about the actual origin of the SARS-CoV2 outbreak in Italy, by deepening the evolutionary phylogeny of the virus, may give fundamental insights also about the security and safety dispositions held by the institutional authorities and politics.

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