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Editorial

May SARS-CoV-2 Diffusion Be Favored by Alkaline Aerosols and Ammonia Emissions?

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Abstract: Ammonia is a common factor linking air in bat caves and air pollution in the proximity of agricultural fields treated with livestock farming sewage and slaughterhouses, where important clusters of COVID-19 have recently been reported all over the world. Such a commonality has a further connection with the known behavior of some viruses of the coronavirus family, such as the murine hepatitis virus, whose spike glycoprotein (S) can be triggered to a membrane-binding conformation at pH 8.0. Within the airborne route of virus transmission, with particular relevance for crowded and enclosed environments, these observations have prompted a hypothesis that may represent a contributing cause to interpret the geographical variability of the virus diffusion and the surging rise of COVID-19 cases in slaughterhouses all over the world. The hypothesis is that, in these environments, the SARS-CoV-2 S protein may find on a fraction of the airborne particles an alkaline pH, favorable to trigger the conformational changes, needed to induce the fusion of the viral envelope with the plasma membrane of the target cells.

Keywords: SARS-Cov-2; aerosol; ammonia; pH; livestock farming sewage; slaughterhouses

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; initially temporarily called 2019 novel coronavirus or 2019-nCoV), made its early appearance in China at the end of 2019. In particular, SARS-CoV-2 originated from bats and, in December 2019, it was transmitted to humans through an unidentified route in Wuhan, Hubei province, China [1]. Wuhan became the center of an outbreak of severe pneumonia of unknown etiology, raising the attention of the scientific community internationally and, on 7 January 2020, researchers isolated a novel coronavirus from patients, responsible for the disease. Immediately after, cases were reported also in Thailand, Japan, Korea, the USA, Vietnam, and Singapore [2] and, over the course of days, SARS-CoV-2 spread almost everywhere in the world [3]. The World Health Organization (WHO), on 30 January 2020, declared the disease determined by SARS-CoV-2 as a Public Health Emergency of International Concern [4] and, on 11 February 2020, the WHO announced that the respiratory disease had been officially called COVID-19; the same agency, given the severity of the infection and the level of spread, on 11 March 2020 declared that the outbreak of COVID-19 could be considered a pandemic [5].

Almost all countries worldwide are currently being affected by this disease, reporting a total of 28,329,790 confirmed cases, 911,877 confirmed deaths, in over 216 countries, areas or territories (Last update: 13 September 2020, 02:00 CEST) [6].

A striking peculiarity of the pandemic is the marked geographical variability of the virus diffusion and of the gravity of its clinical manifestations. In the first instance, such variability reflects the different monitoring and prevention strategies implemented by governments and within each country, by the administrative districts all over the world. Nonetheless, a geographical peculiarity still remains, and emblematic is the case of Italy and of one of its regions—Lombardy—that has been suffering the highest number of positive cases, intensive care recoveries and deaths [7]. Several studies have investigated this issue and have correlated the SARS-CoV-2 morbidity with meteo-climatic conditions [8–13], the median population age of each country and the combined effect of age and gender together to the differences in term of population density and size [9,14] and the pollution levels within the hit regions [9,12,15–17]. In particular, based on such a corpus of data and speculations, both a climatic and a pollution hypothesis have grown [9,12]. The first one relies on the lower virus survival rate in the condition of low relative humidity and high ambient temperatures. Iqbal et al. [11] investigated COVID-19 diffusion all over the world and found that, irrespective of socio-economic conditions, SARS-CoV-2 infection proceeds faster in most of the countries located in relatively lower temperature regions than in those in the warmer climatic regions. Coherently, Huang et al. [10] estimated an optimum temperature range for the virus survival of 5–15 °C and pointed out the role of humidity, reporting that about 73.8% of the confirmed cases over 185 countries were concentrated in regions with absolute humidity in the range of 3–10 g m^{-3}, peaking at 5 g m^{-3}. The influence of climatic conditions on COVID-19 incidence is complex and associated with other potential interfering co-factors. In this context, it can be cited the hypothesis that cold weather and low solar radiation exposures determine a low rate of vitamin D activation, which may play an important role in terms of immune response and anti-inflammatory activity. Indeed, D'Avolio et al. [18] showed that the 25-hydroxyvitamin D (25(OH)D) concentrations in plasma were significantly lower in PCR-positive for SARS-CoV-2 patients than in negative patients. Likewise, Ilie et al. [19] reported that the mean levels of vitamin D in various countries is negatively correlated with the number of COVID-19 cases.

The second hypothesis linked to air pollution has been prompted, observing that the cardiovascular effects due to atmospheric Particulate Matter (PM) involve the release of cytokines as mediators of inflammation, the destabilization and rupture of the coronary plaques, the interaction with the coagulation factors and, ultimately, thrombogenesis [20,21]. Similar features are being retrieved in severe cases of COVID-19 as well, namely, the occurrence of cytokine storm [22] and of coagulation cascade leading to thrombotic phenomena [23]. In other words, the paradigm proposed is that the chronic exposure to atmospheric pollutants, by challenging cardiovascular and respiratory health, impairs the host defense ability against virus infections.

Along these lines, Wu et al. [24] showed that an increase of 1 μg m^{-3} in long-term exposure to PM2.5 is associated with an 8% increase in the COVID-19 death rate. Other authors as well have addressed that chronic inflammation and impaired defense ability of people living in pollution hot-spots may be the determinant that links atmospheric pollution to COVID-19 [15,25].

A further interpretation is that, as in the Po valley, the high level of air pollution and the favorable climatic conditions increase the persistence of the virus airborne particles, so that, aside from the direct route of contagion (from individual to individual), an indirect route (airborne viral infectivity) becomes relevant [9,16,17].

On the indirect mechanism of virus transmission, with particular relevance for indoor environments, Morawska and Cao [26] pointed that, while the virus can be emitted through the expiration of large droplets, as their liquid content evaporates, their sizes decrease to the extent that their airborne transport becomes more important than gravitational settling. Consequently, they may be conveyed for several meters from their emission. Most importantly, the authors stressed the

relevance of this route of infection, observing that SARS-CoV-1 did spread in the air. This mechanism may be all the more relevant, considering that such smaller virus-bearing particles may, in some cases, derive not only from the droplet evaporation process, but also directly from exhaled air. Indeed, Houspie et al. [27], with an experimental setting avoiding saliva contamination, detected the presence of Rhinovirus and of Influenza B virus in the exhaled breath condensate of volunteers with upper airway infection, although with a lower detection rate (7%) than for nasal swabs (46.8%).

More recently, Ma et al. [28] showed that SARS-CoV-2 is released directly into the air via breathing by COVID-19 patients and estimated a SARS-CoV-2 emission into the air at a rate of 10^3-10^5 RNA copies/min. As of now, evidence on the indirect route of SARS-Cov-2 transmission has accumulated [29–35] to the point that it can no more be neglected [36] and is being taken under consideration by WHO [37]. On this route of transmission, based on some relevant features of SARS-Cov-2, of the coronavirus family and of atmospheric PM, this paper proposes a hypothesis on the interaction of SARS-Cov-2 and airborne particles.

2. Discussion

2.1. Relevant Issues on the Acidity of Atmospheric Aerosol

It is known that the acidity of atmospheric aerosols changes according to their emission sources. Ding et al. [38] estimated particle pH as a function of particle size, in Beijing. Submicrometer particles clearly exhibited acid pH, whereas coarse particles had pH values over 7.0, approaching 8.0. The authors linked these high pH values to the abundant presence of Ca^{2+} ions in such size fraction, denoting their crustal origin. These findings are congruent with the studies of Ren et al. [39] and of Cao et al. [40] who showed that the acidity of the atmospheric precipitation in China is mitigated in areas where the buffering ability of PM of crustal origin is higher, thanks to the presence of alkaline soils, even if the emissions of acid gas (NO_x, SO_2) are higher than in other regions.

Regarding Italy, size-resolved particle acidity data are not available to our knowledge. Nonetheless, Matta et al. [41] determined the size-resolved aerosol composition in an urban area of the Po valley and found cation-to-anion ratios of 1.2–1.55, for particle sizes above 1 μm, due to the presence of carbonates and other soil-derived alkaline compounds, not revealed by the chemical analysis carried out, supposedly denoting pH values above 7.0. Masiol et al. [42] estimated that 24-h $PM_{2.5}$ samples collected in some cities of the Po valley are mainly acidic (1.5–4.5). In particular, they reported that sulfate and fossil fuel combustion contribute to lower pH, whereas nitrate and biomass burning drive pH toward higher values. They also showed that water accounted for 7–10% of the average $PM_{2.5}$ mass across the sampling sites, following the seasonal trend of relative humidity (RH), with an increase of the $PM_{2.5}$ mass as high as a 3.75-fold factor, under high RH.

As to the pH of airborne particles, an extremely important issue is represented by the massive ammonia emissions all over the world [43]. As evidenced by the European Space Agency (ESA) [44], atmospheric ammonia emissions have largely increased during the last years and models foresee a doubling of emissions by 2050 [45].

Clarisse et al. [46] identified by infrared satellite observations in 2008 elevated ammonia columns (also >1 mg m^{-2}) in the Po Valley as well as in the Ebro Valley and in the North China Plain. The authors localized 28 hotspots with an ammonia column above 0.5 mg m^{-2}. Some were due to biomass burning and fires, others were above agricultural areas in North America, Europe and Asia. The highest values were observed over agricultural valleys surrounded by mountains, namely, in orographic configurations hindering pollutant dispersion. This is the case of the Po valley in Italy, surrounded by the Alps and the Apennines, where frequent atmospheric stability conditions favor pollutant build-up [47,48].

The storage facilities for animal slurry and digestate storage, also, represent ammonia emission sources. Zilio et al. [49] showed that in Lombardy Region (Italy) such storage facilities emit ammonia at

a rate ranging from about 9 to 30.68 g N-NH_3 m^{-2} d^{-1}. Moreover, biomass burning emissions produce particles with the outer layer composed mainly of alkali salts [50].

Ammonia has a marked effect on particle nucleation, causing, in an experimental setting, a 130-fold particle concentration increases in a matter of seconds, followed by particle coagulation [51]. Raffaelli et al. [52] estimated ammonia emissions in 2020 of about 250,000 tons in the Po Valley, with a potential reduction of 22% to be expected for 2025, if some measures in main sectors such as biomass burning, transportation of goods and passengers, domestic heating, industry and energy, and agriculture, are activated.

Overall, these studies show the presence of mildly alkaline aerosol aside from acidic aerosol. The origin of such a fraction of aerosol can be crustal and under particular meteorological conditions, favoring pollutant stagnation, and for some specific areas, may derive from ammonia emission hotspots.

2.2. Relevant Issues about SARS-CoV-2

SARS-CoV-2 entry into the target cells proceeds through the binding of its transmembrane spike glycoprotein (SARS-CoV-2 S) to the angiotensin-converting enzyme 2 (ACE2) as the entry receptor [53,54]. Within S protein, two relevant units have been recognized: the surface unit S1 and the transmembrane unit S2. S1 has the function of binding to the ACE2 receptor of the host cell through its Receptor-Binding Domain (RBD). S2 allows the fusion of the viral membrane with the cellular membrane [55,56]. The first step of the viral infection is the binding of RBD to ACE2. Wrapp et al. [56] described that RBD undergoes a transient conformational change, through a hinge-like movement that brings up or down the specific amino acid sequence responsible of the binding to the ACE2 receptor. Only the "up" conformation, that is thought to be less stable than the "down" conformation, can bind to ACE2. After that, a two-step activation sequence, both involving a proteolytic cleavage, is required. The first cleavage is carried out by the host–cell furin protease at the multibasic S1/S2 cleavage site. Only after the first cleavage, the second activation step, important for the virus entry into the lung cells, occurs, operated by the host enzyme Type II Transmembrane Serine Protease (TMPRSS2) [57–59]. Differently from SARS-CoV S1/S2 that is monobasic, the multibasic character of SARS-CoV-2 S1/S2 is due to the presence of multiple arginine residues. Its presence enables the activation of the SARS-CoV-2 S by the almost ubiquitous furin-like proteases. This occurrence explains the systemic spread of the viral infection [54,57].

2.3. Hypothesis on the Interaction of SARS-CoV-2 with Airborne Particles

A crucial step for virus entry into cells is the fusion of its envelope with the membrane of the host cells [60]. To this end, the S proteins of the coronavirus envelope undergo a conformational change that is triggered either upon interaction with the specific cell receptor or by an optimal pH range. Such range may be acidic, proceeding through an endocytosis mechanism, as for avian coronavirus infectious bronchitis virus (IBV) [61] and influenza virus [62] or mildly alkaline as for bovine coronavirus [63] and mouse hepatitis virus [64–66]. In particular, Chu et al. [61] observed an optimal pH of 5.0 at 37 °C for IBV-BHK cell fusion, with fusion reaching maximal extent within 60 s, whereas they reported little or no fusion above a pH of 6.0. On the contrary, Zelus et al. [66] showed that the S glycoprotein of the murine coronavirus mouse hepatitis virus (MHV-A59 strain) can be triggered to a membrane-binding conformation at 37 °C either by binding to the receptor at neutral pH or by alkaline pH (pH = 8.0) alone, in an apparently irreversible way.

Likewise, SARS-CoV S and MERS-CoV S, SARS-CoV-2 S can be triggered to fuse at either the plasma membrane or the endosomal membrane, which route is followed depends on the protease present in the specific environment. The endosomal route is indirectly activated by low pH. An acid environment is, in fact, required to activate cathepsin L protease that cleaves the S protein at a specific site and triggers the fusion pathway. The direct plasma membrane fusion route is followed in presence of membrane-bound proteases such as TMPRSS2 and trypsin [67]. As to whether this direct route

may be activated by alkaline conditions, no study has been published to our knowledge. However, considering that, differently from the RBD that is variable within the coronavirus family, the membrane fusion domain represents the less variable portion of the S protein, the hypothesis of an alkaline activation, as observed for MHV [66], is worthy of consideration.

We can reasonably consider that SARS-CoV-2 bearing particles and droplets, in relation to their size, density and shape, undergo the same dynamic processes as airborne ordinary particles, such as coagulation, impaction and gravitational settling [68]. We hypothesize that when a collision occurs with airborne particles, the SARS-CoV-2 S protein finds, on some of them, a pH environment favorable to the conformational changes needed for virus–cell fusion. We propose the hypothesis that such an environment is possibly mildly alkaline. This hypothesis relies on the ground of some observations.

1. Ammonia emissions are particularly relevant in the Po basin, the area most heavily hit by COVID-19 in Italy. In this area, the practice of using sewage sludge form livestock farming for agricultural purposes is widely adopted. In view of the ammonia capability of promoting the formation of secondary inorganic aerosol [69], the use of sewage sludge from cattle farming has been regulated by the Lombardy Region [70]. To this end, the limits of 170 and 340 kgN ha^{-1} $year^{-1}$ (kg of Nitrogen from livestock farming sewage allowed in a year per hectare of agricultural soil) have been fixed, respectively for areas classified as "nitrate-vulnerable" and "not vulnerable". In particular, in 2019 the limit of 170 and 340 kgN ha^{-1} $year^{-1}$ was exceeded in great part of the agricultural areas, respectively in the Bergamo province and in some municipalities in the province of Lodi, both heavily hit by COVID-19. Such provinces include agricultural areas respectively of 70,963 and 55,643 ha [71]. It is, therefore, reasonable to suppose that a fraction of airborne aerosol exhibits an alkaline pH, particularly in the proximity of the agricultural treated areas.
2. The hypothesis formulated may represent a possible key to interpret also the important COVID-19 clusters recently signaled in several slaughterhouses, considered as major COVID-19 hotspots [72]. In these occupational settings as well, the possibly favorable alkaline environment for the virus is due to ammonia. Indeed, ammonia concentrations of 18.4 ± 17.5 ppm have been reported in poultry houses [73]. In such environments, the relevant role exerted by ammonia adsorbed on airborne particles, in consideration of their ability to penetrate deep into the respiratory system, has been pointed out by Donham et al. [73]. Not only is ammonia formed through the bacterial action on animal wastings, but it is also used in meat processing as an antimicrobial agent [74]. Ammonia leaks from the refrigeration system may also occur. Moreover, remarkable amounts of oil deriving from ammonia compressors are entrained into the gas circulation. Therefore, the oil must be periodically purged. On carrying out this operation, once oil is purged, ammonia comes out from the purging valves [75].
3. Bats have been recognized as the most likely natural reservoir of both SARS-CoV and SARS-CoV-2 [76,77]. High ammonia concentrations build up in bat caves, due to bat waste product decomposition [78]. McFarlane et al. reported ammonia peak concentrations as high as 1779 ppm at a bat cave containing several million insectivorous bats [79]. A similar ammonia concentration has been reported by Studier [80,81], who proposed that the presence of an efficient ammonia filtering system, possibly dependent on the mucous of the respiratory tract and on its production rate and composition, is at the base of the bat high ammonia tolerance.

We argue that on airborne particles, in the proximity of animal sewage treated fields and in slaughterhouses as well, without reaching such high ammonia concentrations, lethal to humans, alkalinity and humidity conditions are—to some extent—reproduced.

3. Conclusions

There are two common threads linking environments such as bat caves, slaughterhouses and inhabited areas in proximity to animal sewage-treated fields in the Po valley (Italy). Such environments are SARS-Cov-2 hot-spots and are characterized by ammonia emissions. We hypothesize that this circumstance is causal rather than casual. In a similar way as other viruses of coronavirus family, SARS-Cov-2 S protein might be triggered to a membrane-binding conformation by mildly alkaline pH, in that way favoring COVID-19 diffusion. We propose that the hypothesis formulated may be worthy of consideration, in view of its possible implication on COVID-19 diffusion, with specific relevance for enclosed and crowded environments.

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