The airborne and gastrointestinal coronavirus SARS-COV-2 pathways

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Running title: airborne coronavirus

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Keywords: coronavirus, droplets, infectivity, surgical mask, cultural behavior

Abstract

Since there is not a clear consensus about the possibility for COVID-2 to be an airborne disease, exists a controversy regarding the need to use surgical masks to prevent its spread. Here, using the Kepler conjecture for ideal packaging, the number of virions of different sizes that can be accommodated inside droplets was calculated and are proportional to the 3rd potency of the droplet/virion diameter. The differences between particles of 5 um and 100 μm are around four orders of magnitude, explaining why the airborne spread is much more difficult but still possible. There is no solid evidence yet that the airborne coronaviruses may reach enough concentration to infect, but this may be the case under certain circumstances. The WHO partially recognizes now this fact in a warning to health workers (from my point of view too late, as it was the declaration of a pandemic). Another issue is whether the virus stays infective in aerosols generated from patients. This has not been directly proved yet except with artificial aerosols, but there are no reasons why the virus cannot remain in the air and be infective if the viral charge and time of exposure are enough. We must also consider whether the virus can infect the

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intestine; there are some signs in this sense. Finally, and most importantly, we need to reduce interactions by using surgical masks to flatten the curve, leave the quarantine and avoid a rebound. For cultural reasons, a social distance of 2 meters (2M) is extremely hard to manage. Surgical masks do reduce the interactions in conditions of proximity and, therefore, help to "flatten the curve". The WHO and CDC "laissez-faire" on this matter do not help and we are running out of time. Anticipated actions, such as the use of surgical masks for the general population, are critical.

Keywords: ACE2, airborne; coronavirus; COV-2; COVID-19; food chain; intestinal infection; Kepler conjecture; rebound epidemic; packaging; particle size; SARS; surgical masks.

Introduction

The COVID-19 pandemic is among us, and it is taking many human lives. I will not describe here the virus nor the disease; many reviews on this subject may be consulted [1-4]. While looking for a vaccine or a pharmacological approach, we are missing an important issue that may save many lives: the use of surgical masks by the general population. This is a remarkably simple and effective solution to reduce Ro and be able to reduce the transmission of the disease. There are many arguments against the use of masks, none of which have a solid basis, from my point of view.

Is airborne SARS COV-2 plausible? The theoretical basis for a continuous distribution

This has been a matter of discussion for many viruses during decades. I think this occurred because the reasoning has been made in the wrong direction. There is not such a thing as either a droplet transmission or an airborne disease. There is not a sharp cutoff. There always exists a gradual transition from droplet to airborne as a function of the size distribution of the droplet and aerosol particles.

I have heard many physicians and journalists saying these days that the virus cannot be transported by air because it is too big and will fall rapidly to the floor, withing two meters (~ 6 feet), which is the social distance recommended (abbreviated here 2M). Clearly, the physics involved was not understood at all.

Respiratory viruses have sizes around 100 nm, as the flu virus, and the coronaviruses SARS COV-2 are not the exception. In a recent paper Kim et al. [5], using electron microscopy on infected Vero cells, reported sizes between 70-90 nm. However, Yun et al. [6], for COV-1, found average sizes between 150-200 nm, and some virions over 400 nm. Although coronaviruses are big compared to the influenza virus, a particle of that size will never reach the floor, not even in 100 years! On the other hand, virus particles will rapidly settle on surfaces if they are inside of droplets with a size over 5 μm. The viruses settle because they are inside of big droplets!

By consensus, because there is a gradient of particle sizes expelled when coughing, sneezing or breathing, and not a sharp line, particles over 5 μm are considered able to reach the floor rapidly by gravity (~62 min for 5 μm, 15 min for 10 μm, 4 min for 20 μm,

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10 sec for 100 µm, etc.). Particles below 5 µm essentially do not settle and will remain airborne [7]. This has been known for influenza and other viruses for years. It is obvious that a coronavirus, with a size of 70-90 nm, or even 400 nm, cannot reach the floor and will remain in the air unless it forms aggregates (it might be possible[6]), or it is located inside droplets over 5 µm. We can imagine the picture thinking about what happens when someone smokes a cigarette. The smoke particles have an average size of around 0.1-0.5 µm, depending on the method used to measure [8], very similar to the COV viruses. Do they reach the floor? Never! Of course, density is important, but in this case, it will not make any difference compared to particles over 5 µm. The coronaviruses reach the floor because they are expelled inside the microdroplets that are formed sniffing, coughing, talking or even breathing. Some of the smaller droplets will evaporate very rapidly, within milliseconds, and will form gel-like particles named *droplet nuclei* [9]. Whether or not the distribution of droplets can reach a significant number of nuclei for COV-2 is unknown, but this possibility exists and should not be disregarded. In that case, we would have dry, individual viruses of around 70-400 nm, able to reach very deep regions in the lungs and able to travel far from the patient. The main question is not if we will have virions in droplets smaller than 5 µm. We will have them to a certain degree, always. The main point is how long the small particles or nuclei of COV-2 can remain infective and which concentration can they reach in the air. We know from the recent study at NIH, NIAID [10, 11], that COV-2 particles artificially formed remain infective up to 3 hours. Thus, there is no reason to think that we would not have particles below the arbitrary barrier of 5 µm and infective, although it is true that direct evidence of the infectivity of the RNA found by PCR is yet missing. However, the burden of the proof should be inverted in this case, since many lives are at risk, and assume that the ARN particles collected were infective at some earlier time.

We know from studies with the flu virus, that these microdroplet particles can be between 0.1 µ (even smaller) and over 2 mm. The particles over 5 µm will reach the floor [7, 12, 13]. At least for the flu virus, there are no significant differences in the droplet distribution size with or without a virus [14]. Why should there be any difference between flu and coronavirus microdroplets? There is not a physical or biological barrier that will stop coronaviruses to be expelled in particles <5 µm. Inferring the absence of aerosol-containing viruses because long-range infections are not frequently observed is incorrect for influenza viruses [7], and also for coronaviruses. Many studies with PCR have proven the aerosolization of viruses, including COV-2 [15], although the infectivity of the COV-2 aerosolized particles as not yet been determined.

It should be important to note that there is not a single evidence to assume that above 5 µm we will have coronavirus inside the droplets and below 5 µm we will not have any virion. There is not a physical cutoff there. The 5 µm cutoff is just a convention, a consensus. Although the virus charge load will be reduced as the size of the particles decreases, the particles will have viruses as soon as the size is equal or greater than a virus particle (unless someone can prove the contrary). Of course, below the minimal virion size 70-90 nm, we do not have any chance to find a virus particle in a microdroplet. That we know for sure. At most, we will have a “naked” virus or droplet nucleus of a minimum of 70-90 nm (or even 400 nm). The possibility of droplet nuclei, gel-like viruses, should not be disregarded when a dry cough or respiration in a dehydrated mucosa is present, or with environments with low relative humidity, were the nanodroplets will dehydrate very

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rapidly. Precisely the dry cough could be a survival strategy for the virus to produce more droplet nucleus than other viruses, reaching deeper areas of the lungs. Therefore, in the absence of any evidence on the contrary and due to the evidence from other viruses, we can assume that the coronavirus will be present in microdroplets above 90 nm and not only above 5 μm, which is an arbitrary barrier.

The important question here is not whether the virus is airborne or not, something that is very likely to occur in a given ratio. The important question is for how long the airborne virus will remain intact, and if they can reach enough concentration, during enough time, to be able to have a productive interaction with a target mucosa. This is the key issue. One important issue that emerges from the big size of the COVs is that the number of infective virions in a given droplet will be less than for other viruses. In this sense, the concentration of virions in droplets below 5 μm could be significantly lower than for other airborne viruses, and therefore, be less infective only because the viral load will be lower.

The Kepler conjecture defines the optimal packaging for virions

If we assume that the virions are spherical, their volume will be $V = 4/3\ \pi\ r^3$. In a compact arrangement, the packing density will be $\pi/3\sqrt{2}. \approx 0.74048$ (the Kepler conjecture) [16]. The maximal number of virions of radius *r* that can be packaged in a droplet of radius *R* will be, $N = 0.74\ \frac{4}{3}\ \pi\ R^3 / \frac{4}{3}\ \pi\ r^3$. Rearranging this formula, we can use diameters D for the droplet and d for the virion

$$\boldsymbol{N \approx 0.74\ (D/d)^3}$$

Aerosolized particles (< 5 μm), as soon as the leave the body, will be dehydrated almost instantaneously and transformed into droplet nuclei [17]; therefore, we can assume that the aerosolized particles containing viruses, will be reduced in volume until they reach the maximal packaging volume, containing N particles. In other words, droplet nuclei will always have an *N* number of virions; the optimal packaging will determine a limit for the particle shrinkage. It is assumed here that the contribution to the final volume of gel-forming compounds will be minimal compared to the contribution of virions.

For instance, the volume of a sphere of 5 μm will be $4/3\ \pi\ 2.5^3 = 65.45\ \mu m^3$. And the maximal packing volume for spheres will be 65.45 x 0.74 = 48.43 μm^3. A virion of 400 nm has $4/3\ \pi\ 0.2^3 = 0.034\ \mu m^3$. Then, 1445 particles of 400 nm will be packed in that microdroplet. However, if we have a virion of 80 nm, $N = 0.74\ (5/0.08)^3 = 180{,}664$ particles. This is 125-fold over the 1445 virions in a 400 nm particle. Thus, the concentration of virions in a 5 μm droplet decreases two orders of magnitude if the virion is 400 nm instead of 80 nm. For particles of an average of 160 nm, we will have $N = 0.74\ (5/0.16)^3 = 22{,}583$ particles in a 5 μm droplet.

Figure 1 illustrates the theoretical number of virions N vs virion diameter *d* (nm) in droplets of 5 μm and 100 μm. These results agree with earlier experimental results showing that the ability of a particle to carry virion correlates with volume and not with the number of particles; and that the capacity increases with the particle size. The relationship was described by a power law [18]. Thus, our hypothesis of optimal packing

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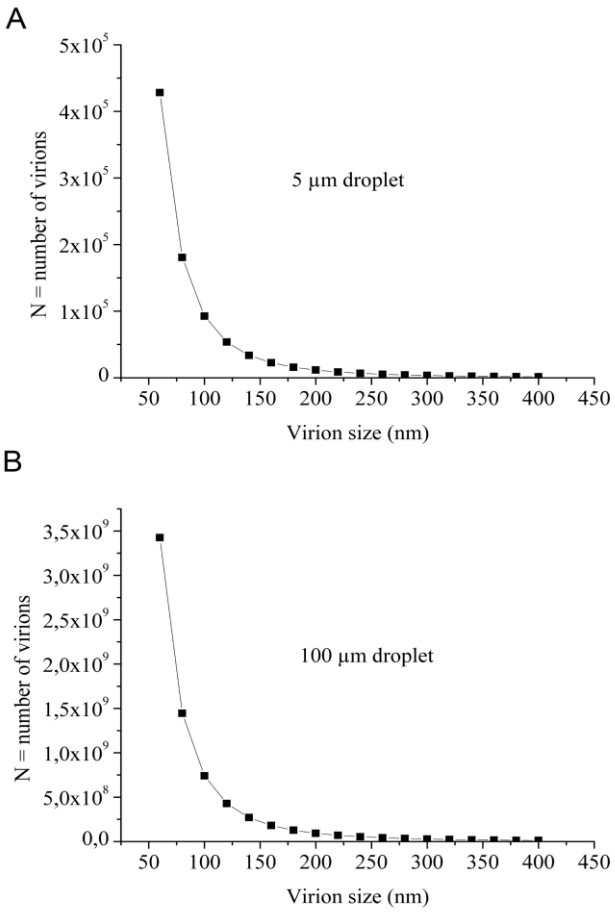

Figure 1: Number of virions N vs virion diameter *d*(nm) in droplets of 5 and 100 µm. For a 5 µm droplet, the virion number will be in the order of 10^5 from 60-100 nm; for a 100 µm particle the number will be about 10^9. Thus, the difference in particle number is around 4 orders of magnitude between a particle of 5 µm (aerosol) and a particle of 100 µm (droplet). This may explain why an airborne infection is less likely to occur. However, crowed areas and with poor ventilation could make the difference, since virions could reach enough concentration and the probability of infection will increase with time and with the time of exposure. For this reason, health personnel are with the higher risk. A: number of virions in a 5 µm droplet. B: number of virions in a 100 µm droplet. Thus, the efficient packaging makes the difference.

following a relationship with a potency of three (x^3) was already verified experimentally for MS2 (a bacteriophage of 27-34 nm), TGEV (transmissible gastrointestinal virus; a spherical 100-150 nm coronavirus of pigs), SIV (swine influenza virus) and AIV (avian influenza virus) viruses; the three animal viruses share genetical and physical similarities with human viruses. The slope values of *N* vs particle diameter *D* were a little over 3: 3.7 for infectious and 3.2 for total MS2 viruses. What is important here is that the N value does not find a theoretical limitation with the volume of the particle. The only consequence of having smaller particle sizes will be a reduction in the air concentration that will follow the relationship $C = N/m^3 = 0.74(D/d)^3/m^3$.

It should be noted that in a 100 µm droplet, we may have up to 3×10^9 virions of 60 nm compared to 4×10^5 in a 5 µm droplet. Four orders of magnitude less! This may

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explain why the virus is much more infective when the droplets are over 5 μm, or even 100 μm, or more. With a big size in the virions, normally the amount in the air may not be enough to reach an infectivity threshold. The virions will be probably degraded before they have a chance to infect. However, in a crowded place or a room without appropriate ventilation, things could be different.

Which is the evolutionary advantage for the virus to have a big size if the infectivity is lower? It is unknown. One possibility is that the big particles have more stability against oxidation and UV radiation, because of a shield effect. This might explain why COV-2 viruses survive a long time on surfaces; this possibility is worth being explored.

I think this is the reason why there exists such a strong controversy with the airborne nature of COV viruses, recently commented by Dyani Lewis [19]. On the other hand, it is unknown under which circumstances a cell will form virions of 60 nm or 400 nm. This will make a strong difference in airborne infectivity.

Noteworthy, one droplet of 5 μm has enough room to host up to 4 x 10^5 virions; this is a concentration of ~0.8 x 10^8 /ml. Therefore, knowing that a concentration over 10^5/ml is considered infective (less than 100,000 RNA copies/ml are not considered a risk [20]), in just one microdroplet of 5 μm we have enough room for near 1000 infective doses. Thus, it is plausible and theoretically possible to reach infective concentrations of SAR-COV-2 even with small droplets below 5 μm, which are by definition aerosolized. And we already know that the COV-2 viruses can remain infective in aerosols of 5 μm at least for 3 hours [11].

Experimental evidence for COV-2 as an airborne disease

With the SARS-COV-2 coronaviruses, so far, we have evidence of the possible existence of aerosolized particles in a preprint from physicians from Wuhan, Hong Kong, and Shanghai, among other places; not yet peer-reviewed [15]. By using PCR, the authors found evidence for airborne coronaviruses in toilets from patients, in the rooms where the medical staff change the protective equipment and outside hospitals, in crowded places. Thus, we can reasonably assume that the virus is in aerosols because there is not any scientific reason why it should not be present in particles below 5 μm.

A virus particle can remain infective as soon its structure remains intact. This depends on environmental factors and time. The laboratory of virology at NIAID, NIH published a preprint on March 9, 2020, suggesting that the virus remain infective in aerosols <5 μm for up to 3 h (the time used for the assay) and in different surfaces more than three days (they measured up to 3 days)[10]. The preprint was later published [11]. One important conclusion of that letter is that the virus can remain active in aerosols! The criticism that some people rise here is that perhaps the real environment and conditions in an ICU are different and this is not comparable. It does not matter. The important issue here is that the virus can remain, under certain circumstances, infective in aerosols. Given these results, the WHO released a document warning physicians regarding the possibility of aerosols under certain circumstances in which they may be generated in ICUs. Again, as it was with the declaration of a pandemic, from my point of view, the WHO issued this

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statement too late. Many physicians and health personnel are infected in Italy, Spain and the rest of the world, and many may die. Now they are cautious again, stating that there is not enough evidence for airborne transmission. Does it matter? We know now from the Wuhan preprint that the virus can be in any droplet size in ICU rooms and toilets, and we know that it will remain active in droplets based in the NIH paper. So, what is missing here? The WHO wants direct evidence that the viruses isolated from a hospital room can be infectious. This will be just a matter of time. Meanwhile, many people in charge of health care units are being infected because the warning to use protective equipment was released too late. Even so, the WHO still sustains that the virus will be aerosolized only under procedures capable of producing aerosols and denying the possibility that the virus can exist in particles below 5 μm. On which grounds? They go even further, sustaining that the general public should not use masks because the virus is not airborne. What are we missing here? Perhaps the WHO is not considering the fact that we need the concurrence of two important variables for a virus to be infective: the concentration of viable virus and the time of exposure. If we have a low viral concentration in the air due to a low proportion of particles below 5 μm, below a certain threshold, it is true, the probability of infection will be very low. However, what happens if the room gets too crowded with infected patients and the virus concentration increases? And if the room does not have negative pressure? Or the caudal of extraction is not good enough? And the protective medical equipment is deficient? Well, eventually we will have an accumulation of aerosolized viruses that at a certain point will have a high probability to infect.

The variables that determine the capability to infect in a closed room with or without ventilation has been known for many years. Many simulations and mathematical models explain the influence of the different variables [13, 21, 22]. These studies emphasize the importance of the room size, the ventilation rate, the number of infected people, the time spent inside the room, and other variables. In a closed room, the airborne viruses will increase with time and faster if the number of infected people increases (in an epidemic for instance). The ventilation rate is important but more important is the virus load that each patient contributes. At some point, even if usually the virus cannot infect because of the low load in the air, eventually the threshold will be reached if the time of permanence is long (critical for health staff), the number of infected people is high, and the ventilation is deficient. Something similar may occur even outside hospitals if a place is crowded and does not have enough ventilation. This reflects what happed in Wuhan, where viruses were detected in ICUs, in the patient´s toilets, and outside, in a very crowded store, and an area near a hospital entrance [15]. Also, it should be considered that in environments with high viral load, the lungs could be colonized in multiple points with a high load, making the innate immunity useless, and perhaps determining a very strong and irreversible infection [23, 24]. In this sense, the medical staff are at a big risk and should wear always complete protection.

The concentration of viruses in the air will strongly depend on the ventilation rate of a room. This is a very important variable for the ICUs, or rooms crowded with infected people. How many hospitals have ICUs with negative pressure and with enough air changes per hour? The ventilation rate is critical! In a room with many patients, even if the patients produce few contaminated droplets to infect, as soon as the room gets crowed, the concentration will increase with time. If the time of exposure is long enough, the health personnel may be infected. However, if the room air is renewed rapidly enough,

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even with natural ventilation [25], the threshold value will never be reached. Of course, the use of full protection is mandatory in cases in which the disease can produce aerosolized viruses. This is probably the reason why so many doctors were infected with COV-2 in Italy and Spain. The warning from the WHO was late and ambiguous. Until we have enough evidence, we should protect the health personnel assuming that the virus is highly aerosolized and infective in ICUs or places with many potentially infected people. Again, the burden of proof should be inverted here.

The intestinal pathway

One of the most studied receptors for coronaviruses is the angiotensin I converting enzyme 2 (ACE2)[26-30]. Angiotensin was first discovered in Argentina by Luis F. Leloir and colleagues, at the Institute of Bernardo A. Houssay (see a brief description in [31]). As shown in Figure 2, the expression of ACE2 in the small intestine and duodenum is several-fold over the expression in the lungs. The common reasoning to explain why lungs are most affected is that the lungs express ACE2 in particular cells, the alveolar type II cells (AT2), where the ACE2 expression is high [32]. Similar results regarding the relative expression in different organs were obtained by Zou et al. [33] by using single-cell RNAseq data; the authors made a map for the susceptibility of the different human organs.

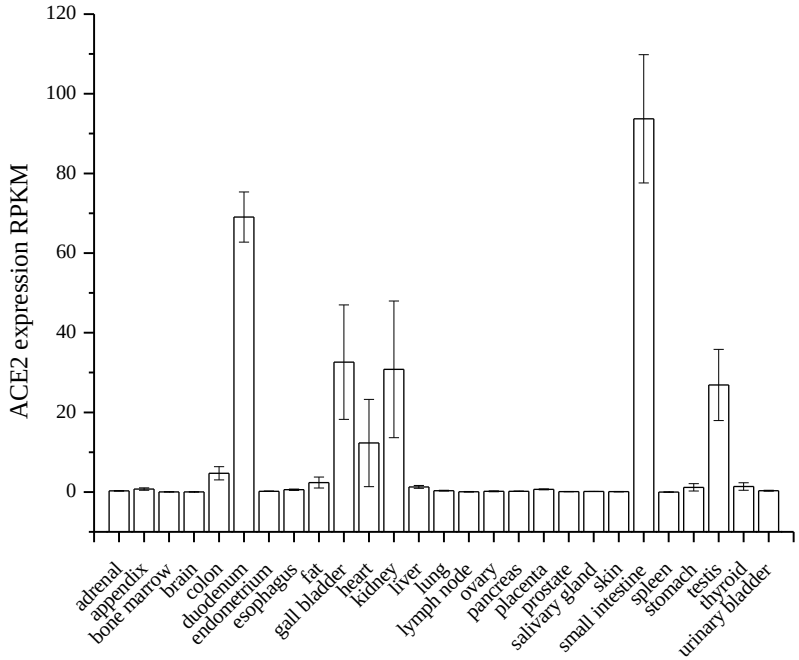

Figure 2: Expression of ACE2 in different tissues. RNA-seq performed on tissue samples from 95 human individuals representing 27 different tissues to determine tissue-specificity of all protein-coding genes; corresponds to the data deposited by Fagerberg et al. [34].

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The ACE2 expression levels in the testis are relatively high, and there is some evidence for possible affected gonadal functions [35]. It should also be noted that kidney and heart tissues, in which the ACE2 expression is also high, are affected in the late stages of the disease [36], with kidney damage to a lesser degree or not even affected [37]. Gall bladder levels are similar to those in the kidney and it might be a susceptible organ.

The diarrhea symptoms that often are seen in patients with coronavirus infections might be related to the high ACE2 expression levels in the gastrointestinal tract [38, 39]. Therefore, the intestinal route of infection cannot be disregarded as a possible source of viruses, that could be transmitted through toilets and lack of proper hand sanitation. And this also raises a concern regarding food distribution if proper hygiene is not maintained. If fact, COV-2 RNA has been found in toilets [15] and stool samples [40]. Although the potential fecal-oral transmission is considered unlikely, the persistence of active viral replication for up to 11 days is a concern [40], and this possibility should not be disregarded without further data regarding the infectivity of this route of transmission. In this regard, Wolfel et al. [41] were not able to replicate the virus from stool samples in which abundant RNA was found. Somehow the gastrointestinal truck inhibits the virus infectivity of stools but not their ability to replicate inside the intestinal cells, as evidenced by the abundant RNA detected in the stools. The infectivity might increase in certain periods or with patients with higher viral loads and, therefore, this route of transmission cannot be disregarded until further studies are done. Noteworthy, these authors did not find detectable viruses in blood or urine.

Implications for the food chain

Given the results from NIH suggesting that the COV-2 can remain infective for days on surfaces, and having present that the gastrointestinal infection route cannot be disregarded yet, then a critical issue is the preservation of the food chain. The use of surgical masks and gloves (often changed) in the entire food chain should be mandatory, including the last steps in the food chain, the shops. This will also contribute to reduce the environmental contamination and to reduce the Ro value, shortening the quarantine period and reducing the possibility of a rebound epidemic.

Implications for the use of surgical masks for the general population

Even if the circumstances by which time or concentration may never reach an infective threshold in real life with airborne coronaviruses, the use of surgical masks for the health personnel and the general population are critical and should be mandatory for several reasons. The most important reason is that surgical masks help to reduce the Ro and the probability of infections. Why will the masks reduce Ro? It is simple because masks help to reduce the interaction between people and contact with contaminated areas or persons. For many cultures in the world, it is extremely hard to keep a social distance of 2M. Also, in many areas, we do not even have water to drink and less to wash hands! Thus, people with contaminated hands can touch their face all the time (children the most!), up to 15 times per hour [42]. Without masks, people will talk with nearby persons with an enormous possibility of infection. Besides, the elderly need to go to the teller machines

to get cash, or to banks or pharmacies, because they do not know how to do it differently. Long and crowded queues are made, perhaps in the presence of contaminated teller machines or cashiers. Thus, the use of surgical masks, even the home-made ones will help to avoid contamination until N95 masks can be acquired. This is critical and the WHO cannot ignore these facts. Even if the coronavirus would have a "magic limit" at 5 μm, which is highly unlikely [43], the surgical masks will still save lives by lowering the Ro. On the other hand, without enough kits to test a critical proportion of the population, the masks are the only way to exit the lockout/quarantine without having a strong rebound. Just imagine a crowded train or subway, with millions commuting without masks. The rebound would be inevitable and deadly.

Conclusions

Taken into account that there is not a physical barrier precluding virions inside droplets of less than 5 μm, we can conclude that the airborne pathway of infection is possible in a crowded environment if the quanta for the COV-2 is high enough or the ventilation of the rooms is deficient.

It is a fact that droplets below 5 μm might remain in the air for hours. There is no reason to believe that particles with less than 5 μm will not have COV-2 viruses. The number of viruses in a particle will decrease with the droplet size up to the limit of the virus size. The size of the virion has an enormous effect on the viral concentration inside particles. Up to four orders of magnitude reach the difference in the number of virions between 5 and 100 μm particles. A different issue is whether virions in particles below 5 μm are concentrated enough or intact enough to be able to infect. Until evidence to the contrary is found, the possibility of airborne COVID-19 should be taken as a fact, to prevent further damage of this pandemic both to health workers and the community. Avoiding high viral concentration (crowded places with infected people, symptomatic or not) and long times of exposure are critical variables. Also, the ventilation of the contaminated areas is another key factor to reduce the possibility of airborne COVID-19.

On the other hand, due to the enormous level of expression of ACE2 in the intestine and with the evidence of RNA samples in toilets and stools, we cannot exclude the possible fecal-oral contamination route. Therefore, the food chain should also be preserved by using appropriate measures (surgical masks, gloves, etc).

Finally, the WHO, the CDC, and the different governments must take action towards the obligatory use of surgical masks for the general population as a way to reduce infectivity. Not just as a soft recommendation but as an obligation, since many lives are at risk. Besides, the use of masks will be an appropriate way to exit the lockdowns/quarantines without the risk of a rebound. Although there is not yet direct evidence that the RNA found in aerosolized particles remain infective, the burden of the proof should be inverted in this case, assuming that these measured RNA-containing particles were infective at some point, since many lives are at risk and it is not casual the high number of deaths that we have among health workers. The mandatory use of surgical masks for the general population should be recommended to governments at once, to save lives and avoid a rebound.

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