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Infectious Dose, Immunity and Transmission of Respiratory Pathogens, with Special Reference to Sars-CoV-2

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Mini Review

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Abstract

Particles of respiratory fluid emitted by an infected host convey respiratory pathogens to the next host. These particles vary in size from aerosols (<5μm) to larger droplets. Pathogens such as *Mycobacterium tuberculosis* depend on aerosols to reach the required target of infection within alveoli. Many respiratory viral pathogens may be implanted along the length of the respiratory tract. The probability of infection depends on the balance of infectious dose versus local defenses at sites of cells bearing relevant viral receptors. In the presence of partial immunity, the infectious dose threshold is likely to be higher. Such doses may only be achieved with exposure to larger respiratory particles like droplets, which by virtue of size convey a higher pathogen load. While droplet respiratory protection may provide good protection in the presence of some immunity, in non-immune populations greater protection against < 5 μm particles is required.

Keywords: Infectious Dose; Immunity; Respiratory Particles; Droplets; Droplet Nuclei; Aerosol; Aerosol Transmission; SARS-CoV-2; Respiratory Transmission

Abbreviations: VZV: Varicella Zoster Virus; AFB: Acid-Fast Bacilli; PPE: Personal Protective Equipment.

Introduction

The transmission of respiratory pathogens has been described since the 1930s as occurring from a source to a human host through respiratory particles travelling through the air or through touch and contact [1]. The routes of transmission are not unique to each pathogen. Instead the varied routes between hosts are shared, but used differentially by pathogens. For each newly identified pathogen, such as SARS-CoV-2, there is a need to determine which routes are both possible and probable. The determinants of transmission should be considered with respect to the ability to establish infection in the next host. This depends on the balance between infectious dose and host defenses at amenable sites of pathogen deposition. The balance between infectious dose and host defence determines which size of respiratory particle is likely to establish infection. Respiratory transmission can be considered with respect to emissions from an infectious host (departures), conveyance through the air (transit) and deposition in the next host (arrivals).

Departures

Beyond touch and fomite transmission, particles of respiratory fluid emitted by one host serve as the means to convey pathogens to the next host. Respiratory particles may be generated in different ways from different parts of the respiratory tract [2-5]. Particles are generated by the closing

and opening of terminal respiratory airways, vibration of the vocal cords, coughing, sneezing and any movement of air sufficient to generate the force required to shear particles from the respiratory fluid lining airway epithelium. The size and number of particles emitted varies between site of generation, manoeuvre and individual [3,6-8]. Breathing, talking, singing, coughing and sneezing generate particles of both aerosol (<5 μm) and droplet size (>5 μm). Additionally, certain procedures increase aerosol production [9,10].

By virtue of size larger respiratory particles are more likely to carry a higher pathogen load. A 100 μm diameter particle will have a volume of ~5 x 10^5 μm^3 and a 5 μm diameter particle a volume of ~65 μm^3. In the case of SARS-CoV-2 there is data on the concentration of viral copies in different tissue fluids [11,12]. Based on these, presumptive calculations on viral copies in a single respiratory particle are shown in Table 1.

The calculations are for a single respiratory particle, but emission will vary between individuals and be continual over time. The calculations presume an equal distribution of viral copies by volume. If there were an unequal distribution, for example, if virions are aggregated in pairs, with a starting concentration of 10^6/ml, there might be two virions in one aerosol particle out of 30,000.

	Number of viral copies in one respiratory particle			
Starting concentration of virus by PCR copies	**Droplet Diameter: 100 μm Volume: $5x10^5$ μm^3**	**Droplet nucleus Diameter: 50 →5 μm Starting vol: $6.5x10^4$ μm^3**	**Aerosol Diameter: 5 μm Volume: 65 μm^3**	**Wang W, et al. [11] Peng L, et al. [12]**
10^{12}/ ml or $10^{12}/10^{12}$ μm^3 or 1 / μm^3	$5x10^5$	$6.5x10^4$	65	Sputum
10^8/ ml	50	~6	1 in 150 aerosol particles	Saliva, nose/throat swabs
10^6/ ml	1 in every 2 droplets	~ 1 in every 16 droplet nuclei	1 in 15,000 aerosol particles	
10^4/ ml	I in every 200 droplets	~ 1 in every 1600 droplet nuclei	1 in 1.5 million aerosol particles	Stool Urine Blood

Table 1: Presumptive calculations on the number of SARS-CoV-2 viral copies per respiratory particle based on starting concentrations for fluids from the work of Wang W, et al. [11] and Peng L, et al. [12].

In addition to aerosols and droplets, there are also 'droplet nuclei', which are larger droplets that have evaporated down to the size of an aerosol particle [1,7]. If a 100 μm particle evaporates down to 50 μm it is still a droplet. Calculations imply that droplets of < 50 μm can evaporate down to aerosol size, and especially droplets of < 20 μm [13]. If a 50 μm droplet (volume $6.5x10^4$ μm^3) evaporates down to 5 μm, it will still contain ~65 virions per particle. If a 20 μm droplet (volume $4.2x10^3$ μm^3) evaporates down to 5 μm, it will still contain ~4 virions per particle. Droplet nuclei actually form very rapidly on being emitted by the source host, and < 5 μm particles measured in the surrounding air will be a combination of droplet nuclei and aerosols.

Transit

Once emitted the trajectory and suspension in the air of respiratory particles depends on size [14]. Evaporation, relative humidity, ambient temperature, surfactant and surface tension affect the size of particles. Particles >20 μm in diameter do not remain suspended in the air for many seconds, and fall to the ground within 1-2 meters. Sneezes and coughs generate cone shaped jets, which may extend dispersion to ~8 meters [10,15]. Particles <20 μm may remain suspended for many minutes. Aerosol sized particles of < 5 μm, can remain suspended in the air and travel greater distances. Droplet nuclei which have reduced to < 5 μm will have the same aerodynamic properties as aerosols.

As respiratory particles disperse they are diluted by distance. Over time there is also potential for loss of viability in laden pathogens. Viability may be affected by ambient ultraviolet radiation, temperature and desiccation. The process of evaporation may concentrate solutes and be osmotically damaging. These parameters are harder to demonstrate for pathogens suspended in the air than when placed on a surface [16-18]. Thus, the greatest concentration of viable pathogen will be close to the source. But an important additional consideration is the person-to-person variation in detectable emissions of pathogens. This is well known

for influenza, with some individuals emitting far less than others [5,19]. For SARS-CoV-2 Cheng et al [20] undertook environmental sampling on one patient in Hong Kong. An air sampler was positioned 10 cm in front of the patient's chin and 1000 L of air at a rate of 180 L/min was collected. While the patient's nasopharyngeal swab and saliva were PCR positive to 10^6 copies/ml, all air samples were negative after normal breathing, deep breathing, speaking and coughing continuously. In a study of 35 COVID-19 infected subjects SARS-CoV-2 was detected in exhaled breath in 5 (14%) subjects [21].

Arrivals

On inhalation air is drawn in through nose and mouth from a radius of about 10 cm, with an average tidal volume of 500 mls [10]. The greatest risk of inhaling pathogen laden air, without any respiratory protection, is in close proximity to the source. With dispersion and dilution of pathogen laden respiratory particles over distance, an individual inhaling air will inhale fewer emitted respiratory particles from a source at 2 meters than 1 meter and at 4 meters than 2 meters.

Respiratory articles <10 μm can transgress the vocal cords, and those <5 μm are capable of reaching the alveoli. If several pathogen laden respiratory particles are inhaled, each particle will likely land in a different place on the respiratory epithelium lining airways or within different alveoli. The local infectious dose is the number of viable organisms in the arriving particle, and this has to contend with local defenses. By virtue of size a single droplet is much more likely to deposit an infectious dose in one spot of the respiratory tract to overcome local defenses compared with a single aerosol sized particle. Numerous aerosol sized particles must be inhaled to match the same number of organisms as a droplet, but each aerosol particle is expected to land in a different spot. Wherever each aerosol particle alights the ratio of one virion to local defenses will favour the latter [22]. This is likely to hold true for whatever quantity of aerosol particles is inhaled. Surface mucus acts as a trap and mucociliary action conveys pathogens away. A variety of secreted molecules add to the innate, natural defence to which is added the contribution of the adaptive, specific immune response. Any pathogen specific antibodies in epithelial and alveolar lining fluid can potentially neutralize incoming organisms and prevent progression of infection. Provided viability is preserved, reaching a successful infectious dose becomes more likely with inhalation of several droplet nuclei than source derived aerosols. Proving that an infection occurs after exposure to droplet nuclei or to aerosol is difficult. The converse to this argument is that aerosol transmission is more likely when very small infectious doses are successful in overcoming host defenses. In this case, transmission is still more likely in close proximity to a source, but is still possible at distances reached by aerosols.

Recognized Aerosol Transmission

The archetypal examples of pathogens transmitted by aerosol sized particles are tuberculosis, measles and varicella zoster virus (VZV) [2]. In the case of tuberculosis, the degree of infectivity relates to acid-fast bacilli (AFB) smear positivity of sputum. Higher mycobacterial loads in the lung will result in higher smear positivity. This correlation with infectivity does hold true, but there is a more subtle variation in the production of so-called cough aerosols. Around each globule of expectorated mucus is dispersed a finer aerosol. Aerosol production varies person to person, and those with a higher degree of aerosolization may be more infectious [5]. This may reflect the physico-chemical properties of mucus, and these properties may vary between infected and non-infected states. Mycobacteria do not usually establish infection in the airway, but do so in the alveoli, where they have the opportunity to replicate in the preferred intra-cellular niche of alveolar and interstitial macrophages [23,24]. Thus, transmission is dependent on aerosols reaching the alveoli. This was demonstrated in classic experiments using guinea-pigs in chambers beyond the ventilation exhausts from side-rooms accommodating tuberculosis patients [25].

The potential site for pathogen implantation is not so restrictive for measles and VZV. Measles virus binds to CD150 (SLAMF1) expressed on alveolar macrophages, dendritic cells and lymphocytes [26]. VZV surface glycoproteins at least bind to sialic acid binding immunoglobulin like lectins (Siglec-4 in particular) [27]. Siglecs are expressed on a variety of cells. It is likely that dendritic cells play a key role in uptake from the respiratory epithelium. In animal models VZV infection can be established by nasal inoculation [28]. Primary infection confers solid immunity to VZV and measles as immune parents of infected children do not get re-infected. The transmission of VZV and measles has to take into account their very high reproductive number (the number of secondary cases arising from index cases) among the non-immune. This ranges from 12-18 for measles [29]. Estimates for VZV include 3.7-5 in Norway and 3-68 in Belgium [30,31]. Aerosol transmission is used to explain the high reproductive numbers, with aerosols able to reach susceptible hosts not in very close range and being implanted throughout the respiratory tract.

Aerosol transmission is possible because of a low infectious dose and the cumulative probability of exposures. Prolonged, close proximity to the source increases the probability of exposure to an infectious dose. With tuberculosis the duration of exposure to an infectious host matters. A cumulative exposure time of 8 hours is regarded as a risk, based on experiments with guinea pigs [25]. This

could be interpreted as the time taken for sufficient single organism bearing particles to onslaught the guinea pigs to achieve the probability of an infection, or the time taken for the likelihood of exposure to a single ~65 organism bearing particle. Experiments in mice suggest that the infectious dose of M.tuberculosis can be as little as one organism, but is consistently successful when the infectious dose is >10 organisms [32]. This illustrates that low infectious dose pathogens are more likely to be transmitted by <5 μm particles, bearing few organisms.

Respiratory Viruses

Unlike VZV and measles, immunity to other respiratory viruses does not provide a solid defence with, for example, repeated episodes of influenza, parainfluenza, respiratory syncytial virus and coronavirus of different strains. However, higher infectious doses may be required for subsequent episodes. These may only be conveyed in droplets. In experimental challenge studies the infectious dose for influenza is $>10^3$ TCID50 [33]. It is conceivable that background influenza immunity raises the threshold infectious dose to these levels. Conversely, infection for the very first time may arise from smaller infectious doses conveyed in smaller respiratory particles. Thus, the same viral pathogen may require droplet precautions for most when there is background immunity, but in the absence of immunity protection against droplet nuclei and aerosols may be more important.

SARS-CoV-2

SARS-CoV-2 binds to the ACE-2 receptor, and this requires the co-factor of TMPRSS2, or potentially furin [34]. The ACE2 receptor is expressed from conjunctiva to alveoli. Conjunctival expression may be limited, and local innate defenses seem to make local conjunctival infection less likely. In experimental models, infection with SARS-CoV-2 is clearly established with inoculation of the nose [35,36]. If the first implantation of infection is in the lower respiratory tract this would be a pointer to aerosol transmission. In a high proportion of patients with COVID-19, the lower respiratory tract is involved. Abnormal chest radiology is reported in 65% of children and 54-95% of adults [37,38]. However, this does not necessarily preclude that the first implantation of viral particles is in the upper respiratory tract, followed by progression to the lower tract. In rhesus macaques, inoculation of the nose was followed by lung changes within a few days [35]. In in vitro experimental models, as few as 1-3 virions of SARS-CoV-2 are sufficient to infect individual respiratory epithelial cells [39]. From nose to alveoli, given local defence mechanisms the probability of a small number of virions reaching cellular receptors will depend on much higher numbers landing on the epithelial lining fluid. Therefore, droplet nuclei as well as droplets may be more relevant. Non-specific, innate and specific, adaptive host defenses may both play a part. The proportionate contribution of the innate defenses is greater when the specific, adaptive host response does not provide solid immunity to re-infection.

For SARS-CoV-2, World Health Organization Infection Control guidance states that transmission is through droplets [40]. As droplets follow a ballistic trajectory an effective barrier for respiratory protection is provided by a fluid resistant surgical face mask. Certain aerosol generating procedures may release more pathogen laden respiratory particles into the air that can ingress around the loose sides of a surgical face mask, and in such situations a tight-fitting respirator mask (N95 or FFP2 and above) is advised. Many, but not all, countries around the world are aligned to the WHO guidance on respiratory protection [8].

The argument presented here is that droplet transmission remains the most probable route for many respiratory pathogens, including SARS-CoV-2. However, in a non-immune population there should be greater consideration and protection against droplet nuclei of aerosol size in the absence of aerosol generating procedures. Fluid resistant surgical face masks in close proximity to infected sources, whilst providing excellent droplet barrier protection, do not provide equivalent protection against droplet nuclei and aerosols which are present in exhaled air in the absence of aerosol generating procedures, and thereby could be inhaled through the gaps around a surgical face mask [41]. The movement of non-droplet sized particles has been demonstrated with particles laden with influenza and in studies using manikins [42-45]. Health and Social Care Workers have had higher rates of COVID-19 infections than the general population, despite guidelines advising fluid resistant surgical face masks in caring for patients with COVID-19 [46,47]. Admittedly, teasing out the contribution of respiratory protection from other items of personal protective equipment (PPE) and broader infection control measures is not easy.

Conclusion

The departure of a pathogen from an infected host and transit to the next host share common means and variables. The dose of pathogen arriving at the next host may depend on the pathogen load in the source and survival in transit. Whether that dose is infectious depends on the probability of overcoming host defenses. It is only through understanding transmission that we can attempt to control the spread of infection. The key point made here is that different measures may need to be applied according to host immunity. With non-immune populations and a pathogen associated with

significant morbidity and mortality a precautionary principle should direct us to do more rather than less in preventing transmission. For some pathogens this may mean applying more than just droplet precautions to prevent respiratory transmission. Droplet precautions using surgical face masks does make a difference to the transmission of many respiratory pathogens, but for a novel pathogen against which populations do not have background immunity greater protection against smaller respiratory particles will provide greater protection for health care staff.

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