UNCOVER

Usher Network for COVID-19 Evidence Reviews

Review: What is the infectious dose of SARS-CoV-2?

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THE UNIVERSITY of EDINBURGH | usher institute

Request

DataInnovation.AI has created an app which uses technology based on fluid mechanics (e.g. smoke studies showing air flow). Their app produces a heat map showing where viral particles are likely to accumulate in buildings.

They explained that we know how to make buildings fire safe, but we haven't done anything to make them safe in the way of infectious diseases and other pollutants. As lockdowns are alleviated, we want to find out how we can make buildings safe in this way. Currently, COVID-19 safety is a global priority.

In order to improve their app, they need to know how many particles of virus it takes to induce infection from an infected individual to a susceptible infectee.

Summary of question

How many particles of SARS-CoV-2 constitute an infective dose?

Overarching question

What is the infectious dose of SARS-CoV-2?

Summary and future recommendations

Overall, this review found evidence indicating the total number of virions to establish a COVID-19 infection in susceptible individuals is likely to be in the order of hundreds, taken from modelling and microbiological perspectives. However, the overall quality of evidence was low.

There are not enough primary studies to make a review on this topic yet and there are fundamental quality issues with the existing primary studies. Importantly, none of the included studies were able to address all the sub-questions, which represent factors that much be accounted for when estimating the infectious dose of SARS-CoV-2. We recommend that future studies on the infectious dose of SARS-CoV-2 incorporate all these factors and consider the impact of their associated limitations.

We believe that a modelling study which takes into account all of the sub-questions would be most helpful for DataInnovation.AI. Although one modelling study was found which estimated the infectious dose through the number of virions required for infection, this study had significant limitations and did not address all of the sub-questions. We would still invite you to conduct further quality assessments using the available GRADE tool (Joore et al. 2021) as our own quality assessment was empirically based.

Methods

Searches

From our initial scoping search, we realised that there are many factors besides the number of particles, which must also be considered to establish the infectious dose. We identified one review (Karimzadeh et al. 2020) which had directly considered infectious dose. However, the quality of evidence was low.

We proceeded to conduct systematic preliminary searches, predominately interested in reviews and summaries on infectious dose. We searched PubMED, MedRxivr, PreVIEW and WHO COVID-19 database (LG & DK) on 16 April 2021. Search results are shown in the PRISMA diagram.

We also conducted forward citation searches for modelling studies, after data extraction and quality assessment was complete.

Inclusion and Exclusion criteria

We included studies reporting any data on the following sub-questions:

1. What is the evidence on infectious dose of COVID-19 and similar respiratory viruses?
2. What is the evidence for the mode of transmission for SARS-CoV-2?
3. What is the evidence for the route of infection for SARS-CoV-2?
4. What is the evidence for setting type where SARS-CoV-2 infection occurs?
5. What is the evidence for how individual factors affect SARS-CoV-2 infection?
6. What is the evidence for the viability of SARS-CoV-2?

Studies identified from reference lists of the modelling studies were included if they reported data on sub-question 1.

Screening procedure

Title and abstract and full text screening were conducted by following reviewers (LG, MN, DK, JF, YD & PK).

Data extraction and quality assessment

Data extraction and quality assessment for each article was conducted by following reviewers (LG, MN, DK, JF, YD, PK, EP & ET). We extracted any data deemed relevant to any of the sub-questions. Critical appraisal tools were employed, according to study design: reviews were evaluated using Joanna Briggs Institute checklists (Joanna Briggs Institute. 2017). The modelling study by Basu (2021) corresponded most closely to our primary research question and the interest of DataInnovation.AI. since it estimated the infectious dose of SARS-CoV-2 in humans using the measurement number of virions. We evaluated this modelling study (Basu, 2021) using the GRADE system (Joore et al. 2021). Three other modelling studies (Epperly et al. 2020; Lelieveld et al. 2020; Hussein et al. 2021) had data on how infectious dose was used to model transmission in different indoor environments. However, since they were not developed solely to estimate the infectious dose of SARS-CoV-2 in humans, they were not appraised due to time constraints. We adapted an existing tool for the quality appraisal of laboratory experimental studies (Public Health Agency of Canada. 2014) (MN & DK). Details of this adapted tool are provided in Appendix 1.

Data synthesis

Owing to data heterogeneity, results on our primary research question were synthesised narratively.

Results

Here we present results on the evidence on infectious dose of COVID-19 and similar respiratory viruses. Data on the other sub-questions were discussed in our discussion section.

A total of 609 articles were identified. After data extraction and quality assessment, a total of 9 articles were retained for narrative synthesis. Of these, 5 articles addressed our primary question (what is the evidence on infectious dose of COVID-19 and similar

respiratory viruses?): 4 were modelling studies and 1 study was a review. Citations of the modelling studies identified 4 primary studies for analysis, which also addressed our primary question. These were all laboratory or experimental studies.

We report results, according to study type (modelling studies, microbiology studies forward citated from modelling studies, the review), separately.

Modelling studies

All modelling studies included an indoor environment to model their results upon. The studies modelled infectious dose from data on SARS-CoV-2 (Basu 2021, Hussein et al. 2021, Lelieveld et al. 2020) and Influenza A (Epperly et al. 2020).

Only one optimal modelling study was identified, which directly addressed the original question from DataInnovation.AI (How many particles constitute an infectious dose?). The quality of evidence for this study was low (Appendix 2).

Basu (2021) quantified the number of virions that can initiate an infection in susceptible individuals and the author concluded that 330 virions may be considered a conservative upper estimate of the COVID-19 infective dose in humans. This study estimated that 11 virions will deposit in the nasopharynx of a susceptible individual after a 5-minute exposure to average RNA load in the carrier's sputum. This estimation was based on nasopharyngeal trends derived from the reconstruction of anatomically realistic nasal geometries and computed transport trends therein; virion transmission data was based on distribution of droplet sizes ejected each minute during normal speaking as calculated from previous studies (Xie et al. 2007); and studying the droplet sizes pre-dominantly contributing to virion transmission in the nasopharynx. At the March 2020 Skagit Valley Corale superspreading event, a single COVID-19 carrier was believed to infect 52 other individuals in a 61-member choir group within an exposure time of 2.5 hours (Miller et al. 2021). The author, thus, calculated the number of virions depositing at a susceptible individual's nasopharynx over a duration of 2.5 hours to be around 330 (calculated as (11/5) ×2.5 ×60≈ 330 virions).

The three other modelling studies (Epperly et al. 2020; Lelieveld et al. 2020; Hussein et al. 2021) had data on how infectious dose was used to model transmission and effects of transmission in different indoor environments, which we felt could provide additional useful evidence for DataInnovation.AI. based on the purpose of their app. These modelling studies estimated inhaled dose rate of particles from aerosol transmission (Hussein et al. 2021), infection risk through aerosolised viruses in different environments (ventilation rates) and individual factors (mask-wearing) (Lelieveld et al. 2020), and to describe symptom severity for time periods in different environments (ventilation rates) (Epperly et al. 2020).

Hussein et al. used an indoor aerosol model combined with a respiratory inhaled deposited dose model to study the transmission of COVID -19 through aerosol from an index case to a susceptible case and assessed the potential inhaled dose rate of particles. Assumptions made included a source strength of 10 viruses in emitted expiratory particles of an infected individual which are uniformly distributed on the particles, with an equal number for all particle sizes (range of 0.1–1000 μm). In a well-ventilated room (ventilation rate – 3h-1) the respiratory tract deposited dose rate was only 30–90 viruses/hour whereas it was 140–350 and 100–260 inhaled viruses/hour for males and females, respectively in a closed room with poor ventilation (ventilation rate – 0.5 h-1). Taking the half –life of SARS-CoV-2 into consideration, these values were further reduced by a factor of factor of 1.2–2.2 and 1.1–

1.4 for the tightly closed and the well-ventilated room conditions, respectively. Limitations of the study include using a single virus emission rate for calculations which is contentious as it depends on multiple factors such as factors such as severity of illness (more viral load at symptom onset); type and duration of activity (e.g. breathing, speaking, shouting, loud singing and coughing).

Another modelling study, by Lelieveld et al (2020) estimated the indoor infection risk from aerosolized viruses in four different environments. The study made assumptions on viral load and infection dose as highly infectious viral load of 5 × 10^8 RNA Copies/cm3; super infectious viral load of 5 × 10^9 (10^9–10^10 copies/cm3); infective dose (D50) 316 RNA copies (100–1000 RNA copies); and virus lifetime in aerosol of 1.7 days (0.6–2.6 days). Different settings included office (40m2x4m, 4 ppl, exposure duration 16 hrs); classroom (60m2x3m, 25 ppl, exposure duration 12 hrs); choir practice (100m2x4m, 25 ppl, exposure duration 3 hrs); reception (100m2x4m, 100 ppl, exposure duration 3 hrs) and five scenarios included scenario A with Ventilation Rate (VR) 0.35 hr−1; Scenario B VR 2 hr−1; Scenario C Masks, 70% Efficiency; Scenario D Masks, 95% Efficiency; and Scenario E High-Vol VR 9 hr−1. The index subject was assumed to be either super infectious (10× higher viral load) or a super emitter (10× higher aerosol emission rate). Results showed that in an office environment, ventilation with outside air reduced the risk of transmission by a factor of 2, the use of a common mask by a factor of 7 and finally the use of a high-quality type D mask reduces transmission by a factor of 40. Similarly, reductions were also observed in a classroom depending on the use of masks (and type of mask), the age of the children and their health conditions. Moreover, choir practice and reception tests had similar results. The use of masks and ventilation systems can significantly reduce the infection risk for up to 70%. The major limitation of the study is basing the viral load and infectious dose on limited available literature which makes the estimates unreliable.

Epperly and colleagues (2020) extrapolated the viral infectious dose from an Influenza A study as 10^5 to describe “Mild Illness” / “COVID-19 Mild Case” by considering a mean viral concentration of 40 TCID50 units/L as the reference environment that contained one or more sick persons. Using infection fatality rates, R0, and total fatalities of Influenza A and COVID- 19, the authors approximated that the Influenza A to COVID infect scale factor was about 4. The authors showed that outdoor spaces had 176 times more air exchanges compared to indoor spaces like hospital rooms or offices. Outdoor environment was found to be at low risk even without surgical facemask use. Air in indoor places was likely to stagnate in areas like cubicles, furniture, and other semi-enclosed spaces. A non-infected visitor in an indoor environment within 2 to 4.8 meters of two COVID-19 infected persons not wearing any PPE or masks would possibly develop minor illness after 5 minutes and a mild Illness after 52 minutes of exposure in a 6 air exchanges per hour (ACPH) environment. In the same setting, when in contact with a pre-symptomatic COVID-19 patient, a non-infected individual would develop a minor Illness after 1 hour, and possibly develop mild illness after 11 hours. In the indoor environment with two sick patients and 24 air exchanges per hour, minor illness is likely to occur in a normal individual after 20 minutes and mild illness possibly after 3 hours 28 minutes. The authors estimated these exposure times (time for viral load exposure levels) and resulting infection potential in various indoor and outdoor settings for both, Influenza A and COVID-19 by establishing a reference scenario and extrapolating it into several example scenarios that have varied exposure time duration, ventilation amount, with/without surgical mask use, activity/respiration levels, and infected subject shedding levels. Approximate and inaccurate known challenge dose escalation

results for COVID-19 and influenza A versus COVID-19 infectivity adjustment; and having a single reference environment to measure the SARS-CoV-2 aerosol density in a known environment were a few limitations of this study.

Laboratory experimental studies (identified from modelling studies)

Evidence on infectious dose of SARS-CoV-2 was limited and the overall quality of evidence was low in respect to the laboratory animal studies and 1 human microbiological study. Evidence on infectious dose of COVID-19 and similar respiratory viruses was found in four of the primary studies; for SARS-CoV-2 (Johnston et al. 2021, Popa et al. 2020) and Wild-type Influenza A (Memoli et al. 2015). Subjects used were humans and African green monkeys. Studies were conducted in laboratory settings and a hospital room. The measurements used for quantifying infectivity were standard 50% endpoint dilution assay (TCID50 assay) (Memoli et al. 2015), plaque assay (pfu) (Johnston et al. 2021) and the number of virions (Popa et al. 2020).

SARS-CoV-2 Healthy, SARS-CoV-2 serologically naïve African green monkeys (AGM, n = 3), rhesus macaques (RM, n = 4), and cynomolgus macaques (CM, n = 4) were exposed to SARS-CoV-2 on Study Day 1 (i.e. challenge day) by the aerosol route. The mean virus inhaled dose was 3.84x104 plaque forming units (pfu), with AGM receiving an average of 3.80x104 pfu, RM receiving an average of 2.87x104 pfu, and CM receiving an average of 4.86x104 pfu, clinical. Clinical disease findings were noted as early as Study Day 2 and as late as Study Day 18 following the exposure of the animals. Virus was detected for most animals by Study Day 3 in both swab types, with peak levels typically detected on Study Day 3. Peak levels in NP (nasopharyngeal) swabs were between 2.16–4.83 Log10 pfu/mL, and peak levels in OP (oropharyngeal) swabs were between 2.71–4.45 Log10 pfu/mL (Johnston et al. 2021).

MERS ID50 was estimated to be <1 TCID 50 in a transgenic model. 2 out of 2 mice developed clinical infection with 10 TCID50 dose, 3 out of 3 mice with 5 TCID50 dose, 3 out of 4 mice with 2.5 TCID50 dose and 3 out of 3 mice with 1.25 TCID50 dose (Tao et al. 2016).

Wild-type Influenza A An optimal dose of 10^7 tissue culture infectious dose 50 was reached that used mild to moderate influenza disease (MMID) in 69% of individuals who were administered wild-type A (H1N1) pdm09 virus intranasally. The 10^5 and 10^6 TCID50 doses also induced MMID (20% and 47%, respectively). This was not statistically significant (P = .99 and P = .054, respectively). Clinical symptoms of influenza occurred at all doses but were most prevalent at 10^6 and 10^7 TCID (these symptoms lasted about 8 days) (Memoli et al. 2015).

Viability Evidence on viability was found in one primary study (Lednicky et al. 2020). Subjects were 2 COVID-19 human patients in a hospital room setting. The measurement used for quantifying viability was a TCID50 assay. Estimates of viable viral concentrations ranged from 6 to 74 TCID50 units/L of air. Viable SARS-CoV-2 was isolated from air samples collected 2 to 4.8 m away from the patients in a hospital room in the absence of an aerosol-generating procedure, indicating to the finding that viable SARS-CoV-2 can be present in aerosols generated by a COVID-19 patient and can serve as a source for transmission of the virus in this setting.

Review

The existing reviews are limited in answering the question of infectious dose, and their quality is low. Karimzadeh (2021) estimated that the infective dose for SARS-CoV-2 is probably lower than for influenza virus (1000 TCID50) as it is more contagious with a slightly higher R0. Experimental animal studies included utilized ferrets, mice, Cynomolgus macaques, Rhesus macaques, African green monkey, hamsters and bats and the routes of infection as intranasal, intragastric, intraocular, intrathecal, intracerebral, intraperitoneal and aerosol. Not all studies measured infectious dose as TCID50, so a virus titre of 0.7 PFU was estimated as theoretically equivalent to 1 TCID50. Results reported by animal below represent the infectious dose required to cause infection with initial clinical presentation (N.B. not the doses required for severity of illness).

The review took note of the findings of the model developed by Basu (2020). In animal studies the minimum dose of SARS-CoV-2 that infected immunocompromised hamsters were also 100 particles, whereas healthy ferrets and transgenic mice were infected at slightly higher dose of 500 particle by nasal and 630 particles by aerosol route. The authors suggested that the higher value of 100 particle might be used as a potential surrogate for estimating the minimum infective dose of SARS-CoV-2 in humans.

Ferrets Intranasal inoculation of 10^5.5 TCID50 (221 359 PFU) of SARS-CoV-2 virus presented raised body temperature and decreased activity in ferrets, while pulmonary histopathological features and viral RNA replication was found at higher doses (50 000– 5 000 000 PFU).

Mice Transgenic mice showed viral RNA, interstitial pneumonia and pulmonary infiltration after at least 25 min exposure to the virus after aerosol inoculation of SARS-CoV-2 isolates at a dose of 630 PFU .21, while begg albino laboratory bred (BALB/c) mice demonstrated viral replication and interstitial pneumonia at a dose of 16000 PFU by the intranasal route.

A study on both young and aged hACE2 mice after infection at a dose of 400 000 PFU (≈5.71 × 105 TCID50) by intranasal route showed mild weight loss (10%) and more severe histopathological features of interstitial pneumonia in aged mice. Infection by the intragastric route at a dose of 4 000 000 PFU (≈5.71 × 106 TCID50) showed pulmonary infection in one of three mice.

Macaques and African green monkey's Inoculation at a dose of 2 600 000 TCID50 (1 820 000 PFU) of SARS-CoV-2 by the intranasal, intratracheal, oral and ocular routes, resulted in various range of clinical signs including weight loss, piloerection, decreased appetite, pallor and dehydration in macaques. A dose of 3 000 000 PFU (≈4.28 × 106 TCID50) resulted in development of respiratory signs of infection along with efficient viral replication in AGMs.

Hamsters Hamsters that were intranasally inoculated at a dose of 56 000 PFU showed weight loss and viral shedding.

Discussion

This rapid review integrates evidence from modelling and microbiological perspectives on the infectious dose of COVID-19. Additionally, evidence from modelling studies was able to offer evidence on the infectious dose in relation to the indoor environment (Epperly et al. 2020; Lelieveld et al. 2020; Hussein et al. 2021). Although both distinct and similar study types used different methods, it is noteworthy that they arrived at similar conclusions (order of hundreds) for quantifying infectious dose in humans. However, the dearth of evidence on SARS-CoV-2 only and overall poor quality of evidence make any

conclusions uncertain. Human challenge studies for SARS-CoV-2 are currently limited, therefore, conclusions drawn are based on animal studies, modelling studies and human studies done with SARS-CoV-2 other respiratory viruses.

Limitations by study type

Modelling studies Only one modelling study was optimal in the sense that it addressed the question of how many virions constitute an infectious dose directly (Basu. 2021). Conclusions drawn from modelling studies are inherently limited by the assumptions which their calculations are based upon (e.g. conditions of the reference indoor environment). Also, neither the optimal study (Basu. 2021) nor the other modelling studies took into account all of the factors which our sub-questions represent, which must be addressed in order to establish the infectious dose.

Basu (2021) addressed the number of virions that were likely to constitute an infectious dose without considering viability or infectivity of virions. The nasopharynx was assumed to be the primary site of infection, subsequently, generalisability is limited as there are multiple possible infection sites and each site can have a distinct influence on both transmission of the virus and the outcome of infection (i.e. severity) (Karimzadeh et al. 2020; Synowiec et al. 2021). Additionally, no distinction was made between aerosols and droplets, which are known to differ in their effects on the infectious dose (Greenhalgh et al. 2021) and pertinent factors such as ventilation rates and indoor airflow were omitted. This study has undergone peer-review but was conducted by a single author.

Laboratory/microbiology studies The infectious dose of SARS-CoV-2 based on animal studies is approximately higher than that for SARS-CoV-1 but lower than that of MERS and Influenza. Based on animal studies in our review we found that the infectious dose for SARS-CoV-2 infection via aerosol was in the range of few hundreds to 104 whereas that for other routes of infection such as intranasal, intraocular, intrathecal and intratracheal the values were in a higher range. A caveat to keep in mind is that the infectious dose also varies between species.

Laboratory/microbiology studies investigate infectivity (and viability) of virus under controlled, laboratory conditions, however, these results may not be generalisable to the real world with conditions being very different from non-clinical, community contexts. An issue with collecting air samples is that viral particles may easily be lost. Also, there is no universally accepted critical appraisal tool for these studies.

There was significant heterogeneity amongst animal studies through different endpoint/outcome measures (e.g. mortality, viral load) and differences in inoculation routes (aerosol/nasal/intragastric) affecting the response of the animals to the infection. None of the animal studies reported the same clinical presentations and pathology after infection with SARS-CoV-2, and outcomes were highly variable as have been found in humans. Susceptibility of the animals can also largely vary dependent on various species, ACE2 expression, age and comorbidities. These studies also had small sample sizes and animal comparability to humans is contentious. Study animals such as Rhesus macaques, African green monkeys share nearly 93% DNA with humans (National Institutes of Health. 2007) while specific DNA sequence differences linked to diseases in humans often have counterparts in the mouse genome (National Institutes of Health. 2014), making them ideal candidates for this investigation. However, even with the most ideal laboratory conditions

and using nearly similar genomic animals or transgenic mice, a degree of risk lies in that even with a well-designed strategy the accurate expression profile of the human transgene and its functioning in a mouse context cannot be ensured resulting in variable results (Scheer et al. 2013).

Review The review included (Karimzadeh et al. 2020) did not disclose search strategies nor quality assessments for included studies, limiting transparency and overall reproducibility. The included literature on infective dose in humans was limited and varying endpoints were used for the measurement of infection in animals.

Limitations by sub-heading

Mode of transmission, route of transmission, environmental factors (setting type), individual factors and viability all have influence how infectious dose translates into disease. These factors coexist in a dynamic relationship, each influencing one another and the overall outcome of disease (e.g. symptom severity). None of the studies included took into account all of these factors. These are the areas that any future modelling study should be addressing in order to effectively answer the review question.

Mode of transmission There is uncertainty surrounding the dominant mode of transmission for SARS-CoV-2, however, aerosol transmission has now been established. A lack of direct evidence of SARS-CoV-2 in some air samples has been used to cast doubt on airborne transmission while overlooking the quality and strength of the overall evidence base, however, this is a scientific error (Greenhalgh et al. 2021). A database by Gwenan Knight and colleagues at the London School of Hygiene & Tropical Medicine (LSHTM) show that over 94% of COVID-19 superspreading events occurred in limited ventilation areas, implying that aerosolized transmission is a strong contributor to COVID-19 infections (Epperly et al. 2020). In animals, the infective dose is generally lower with aerosol transmission than other routes. If transmission by aerosol is important, infectious dose in humans could be lower than currently believed (Karimzadeh et al. 2020). Furthermore, there may be risks of more severe respiratory complications by aerosols reaching the lower tract. Karimzadeh (2020) observed it seemed to raise the risk of more severe respiratory complications in animals.

Route of infection SARS-CoV-2 is primarily transmitted via the ACE-2 receptors in human airway epithelial cells. These receptors are found mainly in the upper airway tract (Basu, 2021). This, along with the fact that the lower airway is more protected by mucous, suggests that the upper tract is the main infection site of the virus. Modelled calculation studies on the nasopharynx found that upper tract infections are often due to droplet transmission, whereas lower tract infections are often attributed to aerosol transmission (Basu, 2021). In animal studies in Karimzadeh (2021), the infective dose was generally lower with aerosol transmission than other routes. Aerosol transmission allowed the virus to penetrate into the lower respiratory tract of animals causing severe symptoms (Karimzadeh et al. 2020). Moving forward, infective dose assessment in human studies requires intranasal administration of the virus via drops or aerosols.

Setting type Another factor to take into account is the infrastructure of a room. SARS-CoV-2 survives better on smooth, non-porous surfaces in the damp in low temperatures. (Goodwin et al. 2021). The time taken for aerosols to accumulate in a room is shortened by

inadequate ventilation (Lelieveld et al. 2020). In a modelling study it was found that in comparison to a tightly closed room (low ventilation rate $\lambda = 0.5\ h^{-1}$ and low friction velocity $u^* = 0.01$ m/s), a well-ventilated room (high ventilation rate $\lambda = 3\ h^{-1}$ and low friction velocity $u^* = 0.1$ m/s) reduced the viral dose inhaled from 100-350 viruses/ hour to only 30-90 viruses/hour (Hussein et al. 2021). The study, however, is limited by the sparse literature available on infectious dose and assumed single emission dose. There was also no consideration for the variation in the magnitude of exhaled aerosol particles with different activities such as talking, shouting, coughing or singing.

The type of work environment may also influence infection likelihood. In a typical office setting, a mask-wearing individual doing light work near a pre-symptomatic COVID-19 individual may develop a cold-like illness in 11 hours (Epperly et al. 2020). In contrast, if they are in contact with a symptomatic individual, contact for 8 hours will likely result in infection. This can be extended to 32 hours with better ventilation. In a hair-styling appointment in which nobody is wearing a mask and individuals are in close-contact, infection could occur in 1 hour in contact with an asymptomatic individual. Proper ventilation may extend the time to 4 hours. In a scenario in the hairdressers in which everyone is wearing a mask and there is a symptomatic individual, even in good ventilation, there may be infection in 19 minutes. In hospital, an individual doing light work near a COVID-19 patient, both masked, may develop minor illness in 50 minutes and develop flu like symptoms after 8 hours. It is generally agreed that regular ventilation with outdoor air and the use of face masks can greatly reduce transmission via both aerosols and droplets (Epperly et al. 2020).

Individual factors There are numerous individual factors which can significantly influence how infectious dose translates into disease between infectious individuals and susceptible individuals. For instance, whether somebody becomes infected will depend on their immune response (e.g. vaccinated or not) while the likelihood of an individual infecting others will depend on whether they are asymptomatic or symptomatic, the severity of their symptoms (Cevik et al. 2020). Asymptomatic individuals and those with fewer symptoms may sometimes have similar viral loads as symptomatic individuals (Lelieveld et al. 2020), however, transmission will also depend on physiological differences (e.g. sneezing/speaking loudly/coughing ref indoor/Ignazio). Behavioural factors must also be taken into account, such as social distancing and mask-wearing behaviour.

Peak viral loads were observed in days 7 to 10 of SARS-CoV-2 infection. This is thought to be higher in symptomatic individuals compared to asymptomatic. Asymptomatic individuals also appear to have a faster viral clearance rate and a shorter duration of viral shedding (Cevik et al. 2020). Furthermore, regarding viral shedding, older age and male sex are suggested to be indications for prolonged shedding and delayed viral clearance. This is significant as these individuals may be infective for a longer period of time, contributing to a higher indoor viral dose. Evidence from virus culture studies and large contact tracing studies suggest that COVID-19 patients with mild-to-moderate illness are highly unlikely to be infectious beyond 10 days from symptom onset (Walsch et al. 2020b).

Viability Two of the modelling studies (Hussein et al. 2021 and Lelieveld et al. 2020) did not address viability of virus in relation to infectious dose which is a critical factor for determining infective potential. Viability refers to how stable a viral particle is outside of a host cell (e.g. in air as it travels from infector to infectee) whereas infectivity (or

replicability) refers to its ability to multiply within a host cell. The viability or stability of virions within a dose in the atmosphere is subject to numerous environmental factors such as humidity, temperature, and time (Goodwin et al. 2021).

The correlation between infectious dose and viral load could not be established in this review. Some studies have found higher viral load in mildly symptomatic or asymptomatic stages of disease, suggesting a decline in viral load through disease progression (Karimzadeh et al. 2020), while Walsh (2020a) found no statistical significance between viral load in asymptomatic and symptomatic individuals.

We found evidence of a positive relationship between lower cycle count threshold, likelihood of positive viral culture and date of symptom onset. (Jefferson et al. 2020), implying more people are infectious at onset of illness. However, patients are unlikely to be infectious for the entire duration of viral RNA detection as viral RNA presence may not represent transmissible or replication-competent virus (Walsch et al. 2020a).

Other limitations

Other respiratory viruses The inclusion of other respiratory viruses remains contentious regarding comparability to COVID-19. Although SARS-CoV-2 and influenza are both enveloped, single-stranded RNA viruses, encapsulated by nucleoprotein, having enough similarity for comparison of aerosolized transmission characteristics in modelling studies (Epperly et al. 2020), SARS-CoV-2 has been found to be much more contagious than influenza with a lower number recorded for its infectious dose (Basu 2021).

Heterogeneity in measurements for infectious dose There was heterogeneity in how infectious dose was measured across the studies. The review by Karimzadeh (2021) and animal laboratory studies measured infectious dose through dilution of virus studies for cytopathogenic effect (CPE) in 50% of inoculated culture cells (TCID), or by counting plaque-forming units; each plaque in a layer of host cells indicating colonization by a single virus particle (PFU). Lednicky et al. (2020) considered specific infectivity as the ratio of SARS-CoV-2 genome equivalents present for every one able to infect a cell in culture while Basu (2021) calculated the infectious dose in terms of the number of virions that can go on to start an infection.

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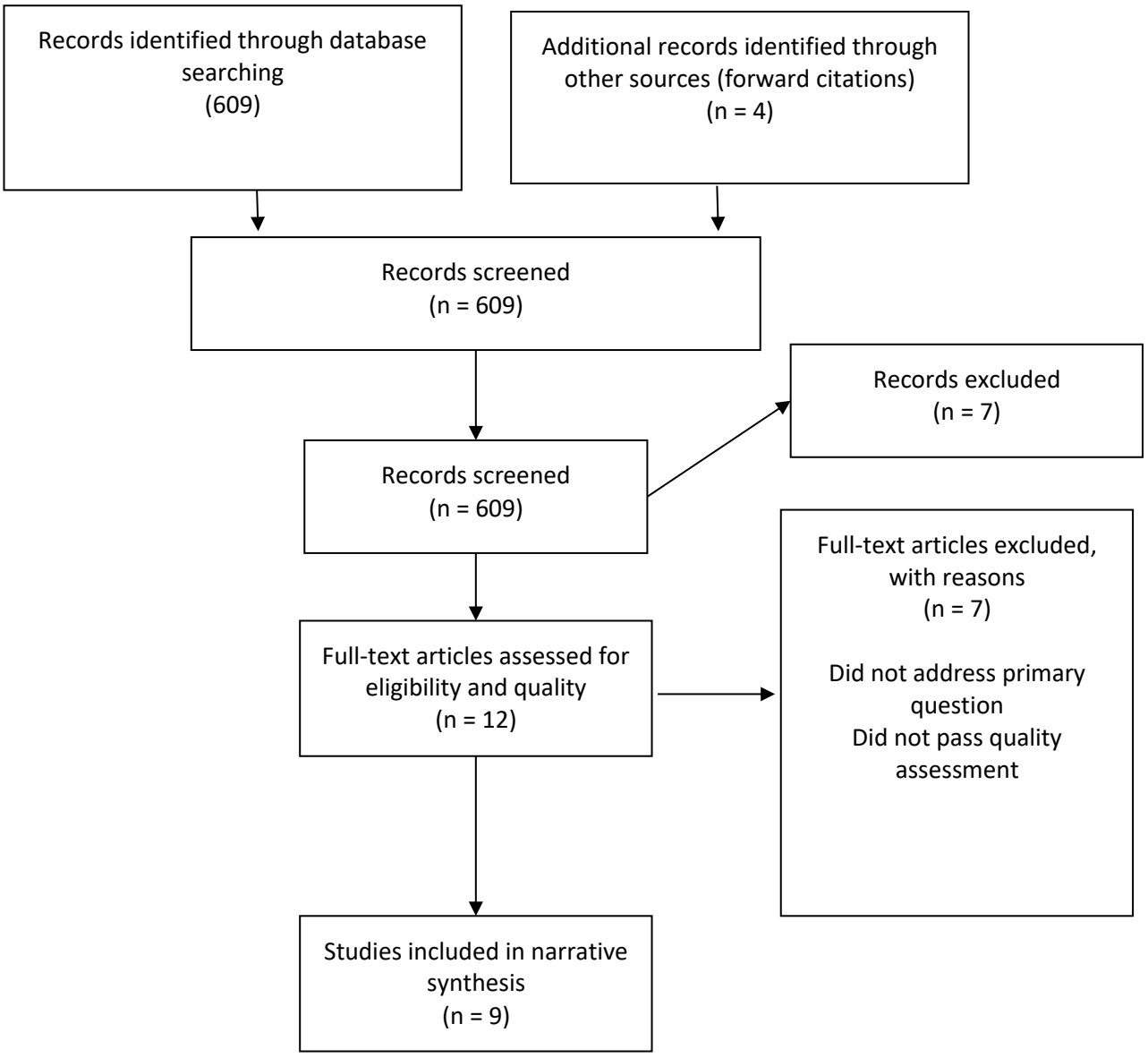
Figure 1. Prisma flow diagram of publications screening and appraisal:



GLOSSARY

ACPH	Air Changes Per Hour; higher the number better the ventilation in a room.
Asymptomatic individuals	Individuals who have COVID-19 (RT-PCR positive) and are capable of shedding virus but show no symptoms throughout the course of the disease.
Pre-symptomatic individuals	Individuals who have COVID-19 (RT-PCR positive) and are capable of shedding virus with no symptoms and develop symptoms anytime between 2-14 days of acquiring infection.
Infectious dose of virus	The quantity of virus needed to produce infection in a susceptible individual.
Infectivity	Ability of a pathogen to reproduce in the host and produce disease; depends on multiple factors such as infectious dose, viability of pathogen, immune status etc.
PFU	Plaque Forming Unit; an assay to measure viruses capable of lysing host cells and forming a plaque in culture media.
TCID 50	Tissue Culture Infective Dose 50%; an assay to measure the amount of viruses needed to kill 50% of infected hosts cells or cause lysis in 50% of tissue culture cells.
Viability	Live pathogen with the potential of multiplication
Virion	Whole viral particle with outer shell and inner nuclear material

APPENDIX

1. Adapted Quality Assessment tool for laboratory experimental studies

Adapted from the INFECTION PREVENTION AND CONTROL GUIDELINES CRITICAL APPRAISAL TOOL KIT, Public Health agency of Canada (2014)

For commentary like a review:

1. Strength of study design
a. Strong - Meta- analysis/ SR/RCT/Lab-study
b. Medium – Cohort, case-control, non-systematic review
c. Weak – Cross sectional, ecologic studies
2. Quality –
a. High – No major threats to internal validity (chance, bias, confounding) which affect the ability to draw a conclusion about estimate of effect
b. Medium – Minor threats to internal validity (chance, bias, confounding) which affect the ability to draw a conclusion about estimate of effect
c. Weak – Major threats to internal validity (chance, bias, confounding) which affect the ability to draw a conclusion about estimate of effect
3. Directness of evidence
a. Strong- Specifically researched the association of interest
b. Weak – Extrapolated from other study that measured the association of study

For laboratory/animal studies:

1. Was RQ well defined? Strong, moderate, weak
2. How was sample selected?
a. Strong-Samples have clearly targeted characteristics
b. Medium -Samples seem to have targeted characteristics
c. Weak – Unclear
3. Control of selection bias
a. High- sample studied for e.g. material/ micro-organism similar for all samples
b. Low- Not similar for all samples
4. Control of information bias-
a. Strong- strategies used to minimize bias in collection and blinding used
b. Medium – no blinding but strategies used to minimize bias in collection
c. Weak – No blinding/ no effort to minimize bias
5. Validity and reliability of collection instruments or methods
a. Strong- Validity and reliability assessed
b. Medium- no effort to assess validity but assumed due to standard methodology or involvement of experts in tools used
c. Weak- No attempt made nor can be assumed
6. Lost to follow up (improper handling of samples and loss of viability)

a. Strong- study takes into account
b. Weak – no mention
7. Ethics
a. Strong- Approval sought
b. Weak – No approval sought
8. Statistical testing
a. Strong – P values and Cis interpreted correctly
b. Medium – Simple tests were used correctly but data warranted more sophisticated tests and control of confounding was limited.
c. Weak: Tests were incorrect for the data or information was not given regarding tests used. Results were not interpreted correctly.
9. Sample size
a. Strong- Significant differences were found, therefore the sample size was sufficient or no significant differences were found but researchers reported the power was sufficient to find such a difference.
b. Moderate: Significant differences were not found, and the researchers reported that the study power was insufficient. Sample size seemed reasonable for the design/research questions, e.g., justified by other studies.
c. Weak: Significant differences were not found, the sample size was small, and the researchers did not report on the adequacy of the power of the study.
10. Generalizability
a. Strong: Characteristics of the study population were very similar to the group to which one wishes to generalize results.
b. Moderate: Characteristics of the study population were somewhat similar to the group to which one wishes to generalize results.
c. Weak: Characteristics of the study population were not at all similar to the group to which one wishes to generalize results.

2. Quality Assessment for Basu, S. (2021)

Title		Computational characterization of inhaled droplet transport to the nasopharynx
Author		Basu S.
Journal		Scientific reports
Citation		Basu, S. Sci Rep 11, 6652 (2021)
Link		https://doi.org/10.1038/s41598-021-85765-7
Risk of bias	***Credibility of the model***	straightforward conceptualisation, structure (single numerical protocol used that has been developed, published validated by the same team), calibration (n/a), no validation, no replication
	Certainty of all its inputs	Majority of inputs are based from single papers and they are estimates
Directness	***Directness of input data with respect to ideal target model's input***	Indirectness of input data since many inputs were estimates
	Directness of model outputs with respect to the decision problem at hand.	The output of the model represents the problem of interest
Precision		No variability of the estimate (i.e. infectious dose) given
Consistency		Consistency wasn't assessed within the model (by utilising different input parameters); Model outcome was compared with animal models, models of other coronaviruses and expert opinions and it was found to be consistent
Risk of publication bias		Other models may have been developed but not published (This is unlikely given how topic the research question is); There might be risk of publication bias in relation to the model input parameters.

Overall quality assessment	Low (due to lack of validation, lack of replication, indirectness, lack of precision, no information on consistency)

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