Clinical Infectious Diseases

REVIEW ARTICLE

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Dose, Infection, and Disease Outcomes for Coronavirus Disease 2019 (COVID-19): A Review

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The relationship between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) dose, infection, and coronavirus disease 2019 (COVID-19) outcomes remains poorly understood. This review summarizes the existing literature regarding this issue, identifies gaps in current knowledge, and suggests opportunities for future research. In humans, host characteristics, including age, sex, comorbidities, smoking, and pregnancy, are associated with severe COVID-19. Similarly, in animals, host factors are strong determinants of disease severity, although most animal infection models manifest clinically with mild to moderate respiratory disease. The influence of variants of concern as it relates to infectious dose, consequence of overall pathogenicity, and disease outcome in dose–response remains unknown. Epidemiologic data suggest a dose–response relationship for infection contrasting with limited and inconsistent surrogate-based evidence between dose and disease severity. Recommendations include the design of future infection studies in animal models to investigate inoculating dose on outcomes and the use of better proxies for dose in human epidemiology studies.

Keywords. SARS-CoV-2; COVID-19; infectious dose; disease severity; inoculum.

The infection process and subsequent disease outcomes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are complex and multifactorial. Recent studies in animals and humans address the impact of different exposure routes, the effect of dose on infection and disease outcomes, and the potential for greater transmissibility and more severe disease from emerging viral variants. However, critical gaps remain in elucidating the relationships between exposure, dose, infection, and severity of disease. We review the existing literature regarding SARS-CoV-2 dose, infection, and disease outcomes; identify knowledge gaps; and suggest opportunities for future research. We focus on evidence for a relationship between infectious dose and coronavirus disease 2019 (COVID-19) severity.

METHODS

Original studies, narrative and systematic reviews, and meta-analyses in peer-reviewed journals were identified using PubMed/MEDLINE. Keywords included "infection," "SARS-CoV-2," "COVID-19," "animal," "human," "severity," "comorbidity," "infectious dose," "dose," "inoculum," and "exposure." Where available, earlier singular studies were excluded in favor of later reviews. We collected and synthesized existing studies on SARS-CoV-2 infectious dose and COVID-19 severity in animal and human studies. Preprints were considered in the absence of peer-reviewed evidence. Theoretical modeling studies were not considered.

SARS-COV-2 INFECTIOUS DOSE AND VIRAL LOAD

Viral transmission and infection are complex, probabilistic processes. The major mode of SARS-CoV-2 transmission is inhalation of respiratory particles containing infectious virions [1]; contact transmission occurs less frequently [2]. Viral viability in respiratory particles is influenced by host physiology

Received 21 July 2021; editorial decision 8 October 2021; published online 15 October 2021.

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Clinical Infectious Diseases® 2022;75(1):e1195–201

https://doi.org/10.1093/cid/ciab903

and biology and environmental conditions [3]. Exposure is a function of viable virus concentration and contact time (Figure 1A).

SARS-CoV-2 infection is mediated primarily through the angiotensin-converting enzyme 2 (ACE2) receptor, found in a variety of tissues including the respiratory tract [4]. An "infectious dose" or inoculum, expressed as minimal infectious dose (the smallest quantity that leads to infection) or median infectious dose (ID_{50}; the dose causing infection in 50% of those exposed), represents the amount of virus received by an uninfected person resulting in cell invasion, active viral replication, and production of infectious virus as well as shedding of detectable viral RNA (Figure 1B) [5]. Infectious dose is a function of ongoing virus viability, particle size and concentration, and breathing rate. Once inhaled and deposited in the respiratory tract, productive infection depends on the dose overcoming multiple factors, including mucus, ACE2 receptor distribution and expression, and innate antiviral immunity in target tissues [6, 7].

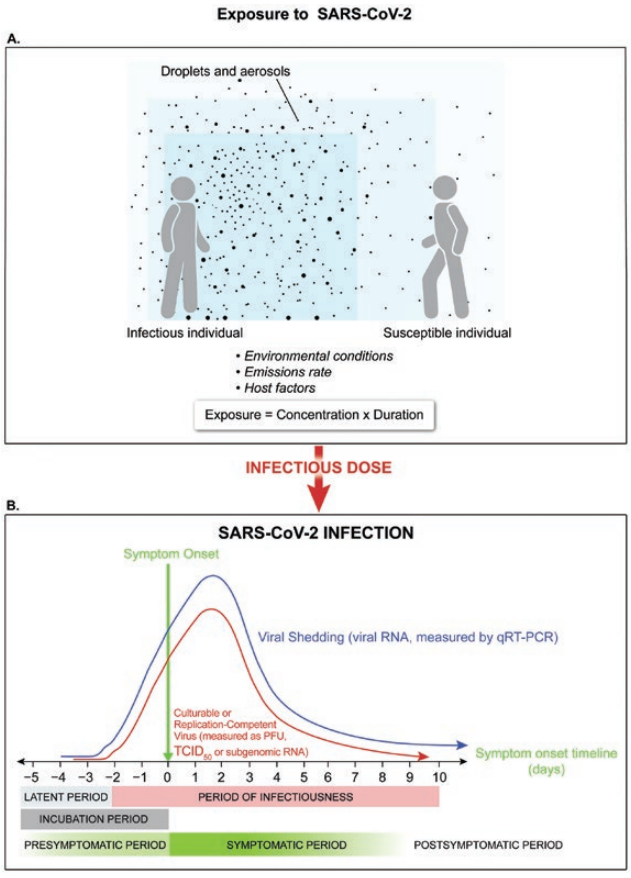

Figure 1. SARS-CoV-2 transmission, exposure, and infection. *A*, Viral transmission and infection are complex, probabilistic processes. Concentration of infectious respiratory particles, exposure duration, and environmental, viral, and host conditions are critical for an infectious dose leading to SARS-CoV-2 infection. *B*, Once an individual is infected with SARS-CoV-2, shedding of virus RNA and viable virus ensues. Viral transmission relies heavily on the viral kinetics around symptom onset. The detection of SARS-CoV-2 RNA exceeds the detection of culturable or replication-competent virus. Abbreviations: qRT-PCR, quantitative reverse transcriptase–polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; $TCID_{50}$, median tissue culture infectious dose.

Viral RNA load, or "viral load," which refers to the amount of SARS-CoV-2 nucleic acid in a sample, is measured by quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) and expressed as either cycle threshold (Ct) or RNA copies per volume. Viral load does not quantify infectious virus, which requires culture-based methods, nor does it correspond to infectious dose. Infectious virus capable of initiating infection, replicating, and producing progeny virus is expressed as plaque-forming units (PFU) or median tissue culture infectious dose ($TCID_{50}$). There is some evidence linking higher viral loads with increased infectiousness [8–10], but the association is not linear. Furthermore, diagnostic assays typically do not discriminate between genomic and subgenomic RNA, which are only produced during productive viral replication. Thus, qRT-PCR metrics alone cannot be used to quantify or infer infectious virus or dose. There is some evidence of an association between viral load and COVID-19 severity [11–13], but this association can be highly variable depending on viral kinetics during infection course, timing of qRT-PCR testing, and presence of symptoms [14].

The incubation period for SARS-CoV-2 (from receipt of an infectious dose to symptom onset) is 2–14 days (median: 4–6 days) [15]. Peak shedding of viable virus leading to infectiousness occurs 1–3 days before symptom onset to 5 days after [5, 16]. Infectious virus is usually not shed beyond 8–10 days after symptom onset; viral RNA can be detected in clinical samples for days, weeks, or even months [17, 18]. Asymptomatic individuals can shed virus and may therefore have a role in transmission, but this is less understood as compared with symptomatic individuals [19]. Throughout SARS-CoV-2 infection, pathophysiological phenomena are complex and variable, resulting in a broad spectrum of symptoms and varying clinical course and outcomes.

COVID-19 SEVERITY

COVID-19 severity has been classified as asymptomatic, mild, moderate, severe, and critical [20, 21]. Proxies used to assess severity include mortality, intensive care unit (ICU) admission, hospitalization, and use of mechanical ventilation.

Several host factors are associated with disease outcomes. Older age (≥65 years), male sex, certain comorbidities (hypertension, diabetes mellitus, chronic obstructive pulmonary disease, obesity, cardiovascular disease, cerebrovascular disease, chronic kidney disease), immunocompromised status (eg, cancer), and smoking are associated with more severe morbidity, ICU admission, invasive mechanical ventilation, disease progression, and increased mortality [22–27]. Pregnancy is associated with an increased likelihood of hospitalization, ICU admission, and mechanical ventilation,

but not mortality [28, 29]. In children, severe COVID-19 cases are less frequent than in adults but are not negligible [30]. Multisystem inflammatory syndrome in children and adults is a rare, life-threatening condition, for which risk factors remain largely unknown [31, 32]. Neither lack of comorbidities nor younger age guarantee mild or favorable disease outcomes, suggesting that unknown host factors may be significant determinants of COVID-19 severity. Evidence on post-acute sequelae of COVID-19 continues to accrue [33, 34].

One critical aspect in assessing severity is understanding that clinical course evolves over time, warranting the differentiation of presymptomatic from asymptomatic individuals, the latter estimated to represent 16–25% of SARS-CoV-2 infections [35–40].

SARS-COV-2 VARIANTS

Certain viral characteristics are associated with the likelihood of infection and disease severity. Ongoing transmission of SARS-CoV-2 has led to the emergence of variants of concern (VOCs) with the potential for increased transmissibility, host immune evasion, or more severe outcomes [41, 42]. Human studies of the SARS-CoV-2 D614G mutant, globally dominant in 2020, consistently found no association with clinical outcomes compared with wild-type SARS-CoV-2 [43, 44]. More recently, 4 major circulating variants (Alpha, Beta, Gamma, Delta) have emerged in various locations and spread to other countries. Epidemiological data suggest that some of these VOCs may result in worse clinical outcomes.

Alpha variant (lineage B.1.1.7) has been associated with greater risk of hospitalization (40–63%) and death (28–71%) compared with non-VOCs [45–49]. One hospital-based study found no association between the Alpha variant and severe and fatal COVID-19 [50]. Studies have found that Gamma (lineage P.1) infections are more likely to result in hospitalization or death compared with non-Gamma infections [51, 52]. The Delta variant (lineage B.1.617.2) has emerged as a significant driver of waves of infection worldwide, and initial observations suggest that it may be associated with an increased risk of hospitalization as compared with other variants [53, 54]. A hamster study has shown that Delta is more pathogenic than a D614G-bearing B.1.1 isolate, independent of dose [55].

DATA FROM ANIMAL STUDIES

No single wild-type animal model replicates the full range of human disease from asymptomatic or mild symptoms to severe or fatal health outcomes. While viral RNA has been found in the lung, brain, liver, kidney, spleen, and gastrointestinal tract in nonhuman primates (NHPs), hamsters, and ferrets, there are no studies in any animal model that fully investigate the long-term effects of infection [56].

Nonhuman primates have emerged as reasonable higher-order species for COVID-19 pathogenesis, vaccine, and therapeutic studies. The COVID-19 rhesus macaque model uses a large mucosal dose, resulting in mild upper respiratory infection that resolves in 14 to 21 days [57–63], and models the milder form of the disease in humans. Hamsters and transgenic/transformed human ACE2 (hACE2) mice, in contrast, are the predominant rodent models in use [64–69] and progress to severe pathologies from experimental mucosal infection. Ferrets, which develop mild symptoms only in the upper respiratory system, have limited utility [70–72].

Most COVID-19 animal infection models require high doses—10^4 to 10^6 $TCID_{50}$ or PFU—delivered to the upper mucosa to produce clinical disease. There are no reports in the literature of attempts to reliably determine a minimum infectious dose in the macaque COVID-19 model using any NHP species. Rodent studies (hamsters) show a correlation between viral load and dose; the ID_{50} for hamsters has been determined at approximately 5 $TCID_{50}$ [73].

Route of exposure is a key factor in animal models of COVID-19 and can influence severity, kinetics, and sites of infection. Early studies in NHPs showed preferential infection when rhesus macaques received 10^6 $TCID_{50}$ by conjunctival and intratracheal but not intragastric routes [74]. Although the conjunctival route resulted in infection, disease was milder compared with the intratracheal route. This contrasts with other NHP studies that did not find severity differences attributable to the route of exposure [62, 75]. Route of exposure influences clinical outcomes in rodent models as well. Aerosol exposure in hamsters accelerated and worsened clinical signs even if dosed at more than 1 log lower than mucosal exposure (1.5×10^3 vs 8×10^4 $TCID_{50}$) [76]. More lung inflammation and disease symptoms were observed in hamsters receiving 10^5 PFU by intranasal than oral inoculation; oral and fecal shedding were similar by both routes [77]. This may be related more to viral fitness than infectious dose, as the Alpha variant outcompeted an earlier A-lineage variant in hamsters exposed to either virus by the aerosol route [78].

Although no transmission studies have been reported in NHPs, rodents (hamsters) and ferrets have been shown to successfully transmit to naive animals by contact, fomites, and aerosol routes [70, 79–82]. Quantitative estimation of dose from infected to naive animals has not been done and is impossible to accomplish; thus, SARS-CoV-2 transmission studies rely on observation as a qualitative measure of dose.

Severe disease outcomes related to age, sex, and comorbidities have been replicated in NHPs [62, 75, 83], hamsters [84, 85], and some transgenic mice models [86–88]. The latter must be carefully interpreted, because transgenic hACE2 species may replicate infection or disease in a different manner than humans. Young (4–6 weeks) transgenic mice expressing hACE2 with a cytokeratin 18 promoter (K18-hACE2 mice) showed greater

and more rapid weight loss in the high-dose (10^3 $TCID_{50}$) versus the low-dose (10^4 $TCID_{50}$) group and uniform lethality in the high-dose group. All mice shed SARS-CoV-2 and developed pulmonary pathology following SARS-CoV-2 inoculation, with limited dose-dependent differences [89]. Another study with K18-hACE2 mice that assessed doses at 10^3, 10^4, and 10^5 PFU showed 0, 50%, and 100% lethality, respectively, suggesting a median lethal dose (LD_{50}) of 10^4 PFU [90].

Some studies in animals with inducible comorbidities (ie, diabetes, obesity, or compromised immunity) replicate serious health outcomes in young animals, as seen in younger humans with similar conditions. For example, among cyclophosphamide-treated hamsters (representing immunosuppression), those exposed intranasally to 10^2, 10^3, and 10^4 PFU showed significant weight loss compared with mock-challenged hamsters; viral shedding continued until cyclophosphamide treatment ended, after which all animals recovered. An intranasal dose of 10^4 PFU was lethal in all exposed recombination activating gene 2 (*RAG2*) knockout hamsters, which are unable to produce functional T or B cells [91].

DATA FROM EPIDEMIOLOGIC STUDIES

Observational epidemiologic studies can provide insight into the relationships between dose and infection and dose and disease outcomes, but many have methodologic limitations. For example, SARS-CoV-2 transmission is reported more frequently in indoor spaces, particularly when poorly ventilated or crowded, compared with outdoor settings [92, 93]. This suggests that infection is less likely in settings with lower virus concentrations, but, in most cases, no quantitative assessment of dose is reported. Instead, proxies have been used, such as the route and duration of exposure, proximity to an infectious source, number of contacts with infected sources, source infectiousness measured by infectious virus concentration, use of respiratory protection, and environment in which the exposure occurs. Proxies can have important limitations, however; for example, many cannot be directly or continuously observed in real time and rely instead on self-report of past behavior.

Studies of healthcare workers (HCWs) provide an opportunity to assess such proxies, given the risk of occupational exposure and the ability to control for confounding factors. While the strength of evidence for the likelihood of SARS-CoV-2 infection among HCWs and the dose received (often measured by directness or intensity of contact) trends toward a positive association, most studies to date are considered to be of low to moderate certainty due to limitations in methodology, such as recall bias, low participant numbers and participation rates, collinearity, and failure to control for confounding variables [94–103].

An ongoing review of SARS-CoV-2 infection rates and risk factors in HCWs consistently found a wide range of SARS-CoV-2 infection incidence (0.4–50%) and seropositivity prevalence (2–32%), thought to be due to differences among studies in locations, exposures, community infection rates, and control measures, among others [94–99]. Risk factors for higher rates of infection include being a nurse or working in hospital non-emergency wards [104], lack of personal protective equipment or adequate handwashing, direct patient contact or care for patients with COVID-19, and presence during intubation [94]. Each of these risk factors support an association between exposure and dose and infection. Race or ethnicity (Black, Asian, ethnic minority, Hispanic) has also been reported as a risk factor for HCW infections [101–103, 105], which may be due to job or community exposures.

Rates of hospitalization and severe disease range from 0–14% and 1–10%, respectively [100]. Mortality rates in HCWs are less than 1% [100]. Seroprevalence in HCWs ranges from 4% in Asia to 13–18% in North America [105, 106]. There is a paucity of evidence to suggest that dose is associated with disease severity among HCWs. Studies among US HCWs have shown that they may have less severe illness despite higher risk of unprotected or repeated exposure, with rates of severe disease significantly lower in HCWs (10%) than in all COVID-19–positive patients (29%); the same is true for mortality rates (0.3% vs 2.3%) [106]. Rather, age, a well-known, host-related risk factor, was associated with higher rates in HCWs over 50 years, with the highest rates in those over 70 years [107].

While infection and seropositivity rates suggest that HCWs are at greater risk than non-HCWs, these wide ranges illustrate the difficulty in using such measures to understand the nature of risk in the absence of information about community rates and exposures at and away from work. Most HCWs are young and healthy, which probably accounts for their low rates of severe disease and mortality. Higher fatality rates in HCWs over 50 years is consistent with disease outcomes in the general population, suggesting that dose is not associated with disease severity.

DISCUSSION

In this review, we sought to understand the relationships of dose to infection and disease severity by examining evidence from relevant animal models, clinical studies, and epidemiologic data. We found that there is some evidence of a relationship between dose and infection based on animal studies and human epidemiology but minimal data supporting a relationship between dose and disease severity. Instead, host responses and potentially viral genotype primarily determine disease outcomes.

Animal studies are the method by which the relationships between dose, infection, and disease severity will be further elucidated. Existing human clinical studies do not, in general, include or reveal information about the level of exposure or dose received. If the low median infectious dose of 5 $TCID_{50}$ found in 1 hamster study is relevant to humans [73], it would

suggest that human dose–response will be difficult to detect in nonexperimental settings.

Epidemiology data suggest an ill-defined dose–response relationship between SARS-CoV-2 and infection. Challenges include limited control for confounding factors, the potential for recall bias in retrospective studies, the potential for selection bias, low participation rates, inconsistencies across studies, and imprecise estimates of exposure and dose [103]. While human challenge studies and randomized controlled trials would provide the strongest evidence, researchers cannot ethically inoculate or randomize people to different SARS-CoV-2 doses. To quantify dose more fully, we recommend that future epidemiology studies assess and address as many of the proxies for dose as possible in reporting results. Disease severity should also be assessed objectively and consistently while recognizing that hospitalization rates and ICU admission are indicative of healthcare capacity, rather than disease pathogenesis. Future research must account for variables such as simultaneous interventions, changes in testing criteria over time, treatment improvements, and host risk factors for severity.

The complex interplay of host and virus is a much stronger determinant of disease severity than simply the size of the dose. Based on available evidence, once infection takes place, disease outcomes are a function of biological and physiologic response and defense mechanisms, which are host-dependent, and environmental factors, such as access to medical care and healthcare system capacity. Most animal models for SARS-CoV-2 replicate only mild to moderate respiratory disease outcomes; a few show more severe outcomes in aged animals. Studies in small-animal models suggest that host factors are a strong determinant of disease severity. This is also supported by species-specific differential COVID-19 severity across different animal models experimentally infected with the same dose via the same route [108–113]. While disease outcomes in humans range from asymptomatic to mild to severe, including death, the most compelling factors associated with disease severity are certain host factors, such as age, sex, smoking, pregnancy, and some comorbidities. It may be that less severe health outcomes are associated with lower doses, but there are few data to support that hypothesis in the human clinical studies conducted to date.

Few data from clinical or epidemiologic studies conducted to date support the hypothesis that lower dose is associated with less severe health outcomes, being limited by inferring viral dose from inappropriate surrogates, ecological approaches that cannot control for potentially relevant confounding factors, and failure to longitudinally follow subjects for misclassification of asymptomatic infections. Epidemiologic studies could be more robust by the use of better measurement tools, such as genotyping and environmental sampling, and through greater efforts to control for confounding and selection and information biases.

Thus, we conclude that, while there is an association between SARS-CoV-2 dose and infection, data do not support a relationship between dose and COVID-19 severity. Nonpharmaceutical interventions may limit the inoculum dose from an exposure, thereby reducing the risk of infection, but they are unlikely to individually have an impact on COVID-19 severity [114, 115].

Notes

Author contributions. All authors had full access to all the data in the study and take responsibility for the integrity and the accuracy of the data analysis. Concept and design: L. M. B., K. E., and A. K. U. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: L. M. B., A. K. U., K. E., A. L. R., G. J. B., S. V. P., and C. J. R. Critical revision of the manuscript for important intellectual content and administrative, technical, or material support: all authors. Supervision: L. M. B., A. K. U., and M. T. O.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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