Importance of non-pharmaceutical interventions in lowering the viral inoculum to reduce susceptibility to infection by SARS-CoV-2 and potentially disease severity

Matthew A Spinelli, David V Glidden, Efstathios D Gennatas, Michel Bielecki, Chris Beyrer, George Rutherford, Henry Chambers, Eric Goosby, Monica Gandhi

Adherence to non-pharmaceutical interventions to prevent the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been highly variable across settings, particularly in the USA. In this Personal View, we review data supporting the importance of the viral inoculum (the dose of viral particles from an infected source over time) in increasing the probability of infection in respiratory, gastrointestinal, and sexually transmitted viral infections in humans. We also review the available evidence linking the relationship of the viral inoculum to disease severity. Non-pharmaceutical interventions might reduce the susceptibility to SARS-CoV-2 infection by reducing the viral inoculum when there is exposure to an infectious source. Data from physical sciences research suggest that masks protect the wearer by filtering virus from external sources, and others by reducing expulsion of virus by the wearer. Social distancing, handwashing, and improved ventilation also reduce the exposure amount of viral particles from an infectious source. Maintaining and increasing non-pharmaceutical interventions can help to quell SARS-CoV-2 as we enter the second year of the pandemic. Finally, we argue that even as safe and effective vaccines are being rolled out, non-pharmaceutical interventions will continue to play an essential role in suppressing SARS-CoV-2 transmission until equitable and widespread vaccine administration has been completed.

Introduction

Given the heterogeneity in both disease severity and incidence of COVID-19 worldwide, some experts have suggested that adherence to non-pharmaceutical interventions (eg, social distancing and mask-wearing) is an important and intervenable contributor to these observed differences.[1] Non-pharmaceutical interventions have been severely underused as part of the COVID-19 response in the USA, especially with later infection surges of COVID-19. An average of 49% of Americans reported wearing facial masks daily during the months of June to August, 2020, compared with 95% observed adherence in Hong Kong and 100% reported adherence in Vietnam in the same period[1] An emerging hypothesis is that the viral inoculum, and the interventions that might decrease it, could not only limit infections but also lead to less severe COVID-19 disease if these interventions fail to prevent infection.[2–6] In this Personal View, we review data supporting the importance of the viral inoculum for susceptibility to respiratory, gastrointestinal, and sexually transmitted viral infections, and the available evidence linking the inoculum to disease severity. We also argue that, even as safe and effective vaccines are being rolled out, non-pharmaceutical interventions will continue to play an essential and ongoing role in suppressing the transmission of SARS-CoV-2 and any further mutations.

Inoculum, host susceptibility, and outcomes for human pathogens

The importance of pathogen inoculum (ie, the number of organisms to which a host is exposed as a function of the concentration, duration, and viral load of the source's infectious material) on the resulting probability of infection has been well described in humans for several viral pathogens, such as influenza viruses,[7–10] respiratory syncytial virus,[11–13] adenovirus,[14] enterovirus,[15] poliovirus,[16] rhinovirus,[17,18] and rotavirus,[19] and also for several bacteria and parasites, particularly in the context of food safety.[20,21] An important example of the relationship between source viral load and infectivity is HIV, for which a study among HIV serodiscordant couples in Rakai, Uganda showed that the transmission rate was monotonically related to a higher viral load set point of the partner with HIV, with no transmissions observed with a viral load of less than 1500 copies per mL.[22] For respiratory viruses, the relationship between infection and the viral inoculum has been documented through controlled human infection studies, which typically involve increasing the dose of challenge viruses in a stepwise way, by use of intranasal mucosal atomisation devices until mild or moderate illness occurs.[7–10,12] For instance, in an influenza A virus subtype H1N1 human challenge model, no infections occurred until participants received 10^5 or higher 50% tissue culture infectious dose ($TCID_{50}$), with a higher proportion of participants showing viral shedding with each ten-fold increase in $TCID_{50}$.[23] Respiratory viruses typically follow this pattern of higher inoculum leading to a higher probability of infection in human challenge experiments, including influenza viruses, rhinovirus, and respiratory syncytial virus,[7–10,12] although there are exceptions, given the complexity of the pathogen-host interaction.[24–26] In addition, the dose needed to infect 50% of the human population (human infectious dose, HID_{50}) varies between pathogens and their subtypes or strains, which has been documented for influenza viruses, rhinovirus, and others.[21]

The influence of the inoculum on disease severity is more challenging to study in humans, given that some

Division of HIV, Infectious Diseases, and Global Medicine, Department of Medicine
(M A Spinelli MD,
Prof G Rutherford MD,
Prof H Chambers MD,
Prof E Goosby MD,
Prof M Gandhi MD) **and
Department of Epidemiology and Biostatistics**
(Prof D V Glidden PhD,
E D Gennatas PhD), **University of California, San Francisco, CA, USA; Swiss Armed Forces, Medical Services, Ittigen, Switzerland** (M Bielecki MD); **Travel Clinic, Institute for Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland** (M Bielecki); **Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA**
(Prof C Beyrer MD)

Correspondence to:
Prof Monica Gandhi, Division of HIV, Infectious Diseases, and Global Medicine, Department of Medicine, University of California, San Francisco 94110, CA, USA
monica.gandhi@ucsf.edu

viruses only cause mild clinical manifestations in immunocompetent human hosts. Viruses that cause severe disease symptoms are too dangerous to study experimentally in humans, and disease severity is more challenging to measure than is infection itself. However, in one controlled experiment with respiratory syncytial virus, five of the seven human participants who were successfully infected with undiluted, higher dose virus inoculum (10^5 plaque-forming units) administered intranasally, and had an infection confirmed by viral culture, developed symptomatic respiratory syncytial virus disease, whereas none of the 16 participants infected with the diluted, lower dose virus inoculum ($10^{2.7}$ plaque-forming units) developed symptomatic disease.[11] In a human challenge experiment with influenza A virus subtype H3N2, 16 (55%) of 29 volunteers inoculated intranasally with 10^6 or higher $TCID_{50}$ showed viral shedding, but those inoculated with the highest dose self-reported the highest symptom severity score.[9] Other previous experiments that studied the relationship between inoculum and symptom severity did not stratify results by confirmed infections, making the data more difficult to interpret.[10,17,23]

The association between dose and disease severity for some viruses is more easily shown in animal models. The relationship between inoculum and mortality in BALB/c mice has been shown for a mouse adapted-H5N1 strain of influenza A virus, although 100% mortality occurred at relatively low doses. For coronaviruses, there are few human data for dose and response, although there is some evidence of a dose-response relationship from mouse models of severe acute respiratory syndrome coronavirus (SARS-CoV), which is structurally similar to SARS-CoV-2,[27] as well as from murine hepatitis virus, which has been studied as a model of human coronavirus.[28] Intranasally administered, higher dose inocula of SARS-CoV in a BALB/c mouse model showed a dose-dependent association with higher mortality, weight loss, and higher viral titres in the respiratory tract.[29] In an murine hepatitis virus-1 mouse model, escalating doses of virus were associated with increasing mortality rates (p=0·01) in 20 mice with positive viral cultures.[30] Findings in animal models might not, however, translate the complexity of pathogen-host interactions in humans.

Viral inoculum, host susceptibility, and outcomes for SARS-CoV-2

Given the severity of illness associated with SARS-CoV-2 infection, human challenge studies are controversial.[31] However, in an animal model of SARS-CoV-2 infection, Syrian hamsters were successfully infected with two different doses of SARS-CoV-2, intranasally and intraocularly, and the higher dose was associated with greater weight loss and more severe lung abnormalities on chest imaging.[32] In another experiment, when a surgical mask partition was placed between the cages of infected and uninfected Syrian hamsters, only six (25%) of 24 hamsters protected by the surgical mask partition were infected, compared with ten (67%) of the 15 control animals without mask partitions (p=0·018).[33] Several mouse model studies of SARS-CoV-2 examined dose-response effects on disease severity,[34–36] although the relationship between dose and mortality was difficult to interpret due to the absence of confirmed infection at lower doses in one of these,[34] and there was difficulty in distinguishing between increased incubation period and increased severity in another.[35] However, in one mouse model of infection with an adapted SARS-CoV-2, a ten-fold increase in inoculation dose resulted in a 60% mortality, compared with a 20% mortality among BALB/c mice, all with pulmonary infections confirmed via viral culture (p=0·09).[36]

Some epidemiological data[1–5,37] suggestive of the viral inoculum effect with SARS-CoV-2 are also worthy of notice. A natural experiment of sorts occurred in the Swiss Alps between March 25, 2020, and April 14, 2020, in two spatially separated homogenous cohorts of soldiers of similar age (median age 21) and without substantial comorbidities.[4] After a COVID-19 outbreak occurred in one of the cohorts, physical distancing and surgical mask-wearing was implemented in both cohorts. An outbreak of COVID-19 in the previously unaffected cohort occurred after the implementation of this policy, with 13 (15%) of 88 asymptomatic soldiers later confirmed to have COVID-19 through mass testing (66 [43%] of 154 were not tested) and none of the 154 recruits developing symptoms. In the cohort impacted before mask-wearing and social distancing were implemented, 102 (47%) of the 215 soldiers who tested positive were symptomatic (132 [37%] of 354 were not tested).[4]

Another study that enrolled participants with PCR-confirmed SARS-CoV-2 infection and their close contacts during the spring 2020 outbreak of SARS-CoV-2 in Catalonia, Spain did a post-hoc analysis of transmission dynamics in a cluster randomised trial of post-exposure prophylaxis with hydroxychloroquine.[38] An outbreak field team visited cases and contacts in homes or nursing homes from March 17, 2020 to April 28, 2020, and measured SARS-CoV-2 viral loads from nasopharyngeal swabs at day 1 and 14. This study found a dose-response relationship between viral load of the index case and the probability of symptomatic disease among contacts, including when adjusting for symptom status of the index case. The viral load of the index case was proportionally related to transmissibility and inversely related to duration of the incubation period the infected contact went through, with higher index viral loads in the cases associated with shorter incubation periods among contacts.[38] The authors suggest that the viral load of cases is an important driver of transmission. Finally, epidemiological data show a higher basic reproduction number (R_0) of SARS-CoV-2 compared with Middle East respiratory syndrome coronavirus or severe acute respiratory syndrome virus[39] (although the relationship

of R_0 with HID_{50} is unknown[40]), and the period of asymptomatic transmission with SARS-CoV-2 probably also plays an important role.

Non-pharmaceutical interventions to reduce viral inoculum

Masks

Surgical masks worn by infected individuals reduce transmission by blocking the release of virions into the air, as has been shown for coronaviruses or influenza viruses.[41] Evidence regarding the ability of cloth face coverings to reduce also the size of the inward viral inoculum was already available for other respiratory viruses,[42–46] and has been accumulating for droplets and aerosols that simulate SARS-CoV-2.[47,48] Increasing evidence from physical sciences research on how cloth masks might protect the wearer (filtration for personal protection), as well as the long-standing evidence on how masks protect others (so-called source control) led to a change in guidance from the US Centers for Disease Control and Prevention, whose original public health recommendation for the public to wear face coverings, from April 3, 2020, provided the reason that masks protect others.[49] However, a scientific brief by the same agency, updated on November 20, 2020, revised the guidance to indicate that masks protect both the user and others, which might also help to increase mask-wearing compliance in the USA.[50]

Rather than criticising the efficacy of cloth masks, investments should be made for the production of high-quality surgical or other types of face coverings to increase their availability outside of health-care settings.[51] Standardisation of recommendations for surgical masks (which use electrostatic-based filtration) and, if not available, high-quality cloth masks (at least two-ply and high-thread count),[52] will reduce confusion.[53] Availability and uniform provision of consistently produced and effective facial coverings could also reduce some mask-wearing hesitancy because a greater perceived efficacy might increase compliance in populations with less adherence to mask-wearing.[54]

In a study in Denmark in April 3, 2020 to June 2, 2020, individuals were randomly assigned to a group where surgical masks were recommended and provided, versus a standard-of-care group. The trial pointed towards a potential association between mask-wearing and reduced SARS-CoV-2 transmission during a low-incidence period, with the point estimate suggesting only a modest benefit.[55] However, several design limitations of the trial probably hindered its ability to show the benefits of mask-wearing for the prevention of COVID-19—underpowering; randomisation at the individual, rather than community, level; flawed outcome measures; and self-reported adherence to mask-wearing in a setting where mask-wearing was not the community norm[56–58]—which suggests that the accumulating epidemiological and physical sciences evidence for the efficacy of masks might be more compelling than this study showed.[59–62] A

modelling study done in the USA has found a correlation between universal mask-wearing and a reduced need for lockdowns and associated economic losses.[63]

Distancing and ventilation

SARS-CoV-2 has generally been shown to have higher RNA concentrations, or a higher viral inoculum, at closer distances to an infected source or closer to COVID-19 patient care areas, as well as downstream (versus upstream) of the air flow from an infected source, although these higher concentrations might not correlate to confirmed culturable virus.[64–66] An air sampling study within a US hospital in the rooms of patients with COVID-19 patients showed higher RNA concentrations with personal air samplers compared with bedroom or hallway air samplers.[64] In another study, in a hospital in Wuhan, China, two (18%) of 11 air samples collected near patients with a COVID-19 infection in the general ward had detectable RNA, compared with none of five samples collected 2·5 m away from patients (sampler positioned upstream of the room's airflow). In the intensive care unit of the same hospital, samples were collected downstream of the room's airflow. Overall, eight (44%) of 18 samples collected 2·5 m away from the patient were positive, while only one (13%) of eight samples collected 4·0 m were positive. Other studies reporting RNA in air samples from COVID-19 care areas did not, unfortunately, measure the distance between sample location and patient.[67,68]

Ventilation to reduce exposure to viral particles has been well described for respiratory viruses. Encouraging human interactions to happen primarily in outdoor spaces and providing engineering and structural changes to increase ventilation in indoor spaces are important non-pharmaceutical interventions.[69,70] Hand-washing could decrease the viral inoculum by reducing the number of viral particles on the hands, which has been shown to be an efficient transmission route, for instance, of rhinovirus (although not identified as a transmission route of SARS-CoV-2).[71] Finally, it is important to note that the efficacy of non-pharmaceutical interventions will increase when multiple strategies are combined, with no single strategy likely to confer an efficacy of 100% in preventing SARS-CoV-2 transmission. Moreover, one strategy can help compensate for another. We propose the concept of the non-pharmaceutical interventions triangle (appendix), in which an individual intervention (masks, distancing, or ventilation) can be reduced as another intensifies (with host susceptibility to SARS-CoV-2 a central figure in the non-pharmaceutical interventions triangle).

There are several possible study designs that could add additional evidence to the relationship between mask-wearing and susceptibility to SARS-CoV-2. Natural experiments, such as the Swiss military study,[4] or case-contact studies, such as the one done in Catalonia,[38] can be examined in other settings. Econometric methods

such as differences-in-differences analyses could be used to study COVID-19 incidence, hospitalisations, and mortality before and after institution of mask-wearing mandates across the world (importantly, controlling for case and testing rates), although adherence to these mandates will also need to be taken into account. Human challenge studies could be attempted with seasonal coronaviruses that do not provoke severe disease. Non-pharmaceutical interventions, such as mask-wearing or physical distancing, could be incorporated into seasonal coronavirus or influenza virus human challenge studies to study their efficacy more rigorously. Furthermore, individuals with SARS-CoV-2 infection could be enrolled into viral culture studies concomitantly with mask-wearing, physical distancing, or both, which could help to quantify the degree of expulsion of viable virus with different non-pharmaceutical interventions.

Non-pharmaceutical interventions and vaccine effectiveness

The effectiveness of a SARS-CoV-2 vaccine could potentially be affected by the population-level burden of COVID-19 disease. The influence of the population-level disease burden on vaccine effectiveness has been well described.[72] Indirect vaccine efficacy (population vaccine efficacy) occurs when a vaccine prevents disease in those who are not vaccinated via sufficient population (herd) immunity. Continuation of non-pharmaceutical interventions will be particularly important for susceptible groups who do not mount a strong immune response to a coronavirus vaccine, and for those who decline a vaccine.[73] Uncontrolled spread of SARS-CoV-2 in much of the USA could limit the initial efficacy of a SARS-CoV-2 vaccine.

The recent news of the high efficacy of the Moderna[74] and Pfizer/BioNTech[75] mRNA vaccines for SARS-CoV-2, as well as of the AstraZeneca[76], Novavax[77], Johnson and Johnson[78], and Sputnik V[79] vaccines, are hopeful and exciting. However, the endpoints for the trials of all of these vaccines were preventing symptomatic COVID-19 disease (in which each of the mRNA vaccines showed more than 94% efficacy versus a placebo). Because asymptomatic infection could not be ruled out in patients receiving the vaccine, continued adherence to non-pharmaceutical interventions (even by the vaccinated) will need to be maintained until the pandemic is controlled and widespread vaccination is achieved. During this period, lower priority groups, such as the young, healthy, and people not working in essential services might have delays in being offered vaccines. Non-pharmaceutical interventions will, therefore, remain essential for the near future. While building the infrastructure to stockpile and administer a vaccine at a mass scale, investments should simultaneously be made in the scientific study, production, and promotion of non-pharmaceutical interventions, such as standardised masks, to prevent continued transmission of SARS-CoV-2.

Conclusion

We reviewed the influence of the viral inoculum on disease susceptibility for several human pathogens and the preliminary data available for SARS-CoV-2. We make a plea for continued or enhanced adherence to non-pharmaceutical interventions in combatting SARS-CoV-2 transmission as we await equitable distribution of a safe and effective vaccine. Non-pharmaceutical interventions, including social distancing, mask-wearing, and improved ventilation, especially if associated with higher compliance in settings with unmitigated SARS-CoV-2 transmission, might make an important and positive difference in disease severity and transmissibility worldwide as we approach the second year of the COVID-19 pandemic.

References
1 Leffler CT, Ing E, Lykins JD, Hogan MC, McKeown CA, Grzybowski A. Association of country-wide coronavirus mortality with demographics, testing, lockdowns, and public wearing of masks. *Am J Trop Med Hyg* 2020; **103:** 2400–11.
2 Gandhi M, Rutherford GW. Facial masking for Covid-19—potential for "variolation" as we await a vaccine. *N Engl J Med* 2020; **383:** e101.
3 Gandhi M, Beyrer C, Goosby E. Masks do more than protect others during COVID-19: reducing the inoculum of SARS-CoV-2 to protect the wearer. *J Gen Intern Med* 2020; **35:** 3063–66.
4 Bielecki M, Züst R, Siegrist D, et al. Social distancing alters the clinical course of COVID-19 in young adults: a comparative cohort study. *Clin Infect Dis* 2020; published online June 29. https://doi.org/10.1093/cid/ciaa889.
5 Guallar MP, Meiriño R, Donat-Vargas C, Corral O, Jouvé N, Soriano V. Inoculum at the time of SARS-CoV-2 exposure and risk of disease severity. *Int J Infect Dis* 2020; **97:** 290–92.
6 Little P, Read RC, Amlôt R, et al. Reducing risks from coronavirus transmission in the home-the role of viral load. *BMJ* 2020; **369:** m1728.
7 Murphy BR, Clements ML, Madore HP, et al. Dose response of cold-adapted, reassortant influenza A/California/10/78 virus (H1N1) in adult volunteers. *J Infect Dis* 1984; **149:** 816.
8 Murphy BR, Clements ML, Tierney EL, Black RE, Stienberg J, Chanock RM. Dose response of influenza A/Washington/897/80 (H3N2) avian-human reassortant virus in adult volunteers. *J Infect Dis* 1985; **152:** 225–29.
9 Han A, Czajkowski LM, Donaldson A, et al. A dose-finding study of a wild-type influenza A (H3N2) virus in a healthy volunteer human challenge model. *Clin Infect Dis* 2019; **69:** 2082–90.
10 Watson JM, Francis JN, Mesens S, et al. Characterisation of a wild-type influenza (A/H1N1) virus strain as an experimental challenge agent in humans. *Virol J* 2015; **12:** 13.
11 Mills J 5th, Van Kirk JE, Wright PF, Chanock RM. Experimental respiratory syncytial virus infection of adults. Possible mechanisms of resistance to infection and illness. *J Immunol* 1971; **107:** 123–30.
12 Lee FE, Walsh EE, Falsey AR, Betts RF, Treanor JJ. Experimental infection of humans with A2 respiratory syncytial virus. *Antiviral Res* 2004; **63:** 191–96.
13 DeVincenzo JP, Wilkinson T, Vaishnaw A, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010; **182:** 1305–14.
14 Couch RB, Cate TR, Douglas RG Jr, Gerone PJ, Knight V. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol Rev* 1966; **30:** 517–29.

15 Schiff GM, Stefanović GM, Young EC, Sander DS, Pennekamp JK, Ward RL. Studies of echovirus-12 in volunteers: determination of minimal infectious dose and the effect of previous infection on infectious dose. *J Infect Dis* 1984; **150:** 858–66.

16 Koprowski H. Immunization against poliomyelitis with living attenuated virus. *Am J Trop Med Hyg* 1956; **5:** 440–52.

17 Hendley JO, Edmondson WP Jr, Gwaltney JM Jr. Relation between naturally acquired immunity and infectivity of two rhinoviruses in volunteers. *J Infect Dis* 1972; **125:** 243–48.

18 Bradburne AF, Bynoe ML, Tyrrell DA. Effects of a "new" human respiratory virus in volunteers. *BMJ* 1967; **3:** 767–69.

19 Ward RL, Bernstein DI, Young EC, Sherwood JR, Knowlton DR, Schiff GM. Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. *J Infect Dis* 1986; **154:** 871–80.

20 Wu F, Rodricks JV. Forty years of food safety risk assessment: a history and analysis. *Risk Anal* 2020; **40:** 2218–30.

21 Yezli S, Otter J. Minimum infective dose of the major human respiratory and enteric viruses transmitted through food and the environment. *Food Environ Virol* 2011; **3:** 1–30.

22 Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. *N Engl J Med* 2000; **342:** 921–29.

23 Memoli MJ, Czajkowski L, Reed S, et al. Validation of the wild-type influenza A human challenge model H1N1pdMIST: an A(H1N1) pdm09 dose-finding investigational new drug study. *Clin Infect Dis* 2015; **60:** 693–702.

24 Smith AM, Smith APA. A critical, nonlinear threshold dictates bacterial invasion and initial kinetics during influenza. *Sci Rep* 2016; **6:** 38703.

25 Castanha PMS, Erdos G, Watkins SC, Falo LD Jr, Marques ETA, Barratt-Boyes SM. Reciprocal immune enhancement of dengue and Zika virus infection in human skin. *JCI Insight* 2020; **5:** 133653.

26 Haas CN. Microbial dose response modeling: past, present, and future. *Environ Sci Technol* 2015; **49:** 1245–59.

27 Shang J, Ye G, Shi K, et al. Structural basis of receptor recognition by SARS-CoV-2. *Nature* 2020; **581:** 221–24.

28 Watanabe T, Bartrand TA, Weir MH, Omura T, Haas CN. Development of a dose-response model for SARS coronavirus. *Risk Anal* 2010; **30:** 1129–38.

29 Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathog* 2007; **3:** e5.

30 De Albuquerque N, Baig E, Ma X, et al. Murine hepatitis virus strain 1 produces a clinically relevant model of severe acute respiratory syndrome in A/J mice. *J Virol* 2006; **80:** 10382–94.

31 Douglas AD, Hill AVS. Immunological considerations for SARS-CoV-2 human challenge studies. *Nat Rev Immunol* 2020; **20:** 715–16.

32 Imai M, Iwatsuki-Horimoto K, Hatta M, et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc Natl Acad Sci USA* 2020; **117:** 16587–95.

33 Chan JF, Yuan S, Zhang AJ, et al. Surgical mask partition reduces the risk of non-contact transmission in a golden Syrian hamster model for coronavirus disease 2019 (COVID-19). *Clin Infect Dis* 2020; **71:** 2139–49.

34 Zheng J, Wong LR, Li K, et al. COVID-19 treatments and pathogenesis including anosmia in K18-hACE2 mice. *Nature* 2020; published online Nov 9. https://doi.org/10.1038/s41586-020-2943-z.

35 Winkler ES, Bailey AL, Kafai NM, et al. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat Immunol* 2020; **21:** 1327–35.

36 Leist SR, Dinnon KH 3rd, Schäfer A, et al. A mouse-adapted SARS-CoV-2 induces acute lung injury and mortality in standard laboratory mice. *Cell* 2020; **183:** 1070–85.e12.

37 IHME COVID-19 Forecasting Team. Modeling COVID-19 scenarios for the United States. *Nat Med* 2020; published online Oct 23. https://doi.org/10.1038/s41591-020-1132-9.

38 Marks M, Millat P, Ouchi D, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. *Lancet Infect Dis* 2021; published online Feb 2. https://doi.org/10.1016/s1473-3099(20)30985-3.

39 Petersen E, Koopmans M, Go U, et al. Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. *Lancet Infect Dis* 2020; **20:** e238–44.

40 Delamater PL, Street EJ, Leslie TF, Yang YT, Jacobsen KH. Complexity of the basic reproduction number (R_0). *Emerg Infect Dis* 2019; **25:** 1–4.

41 Leung NHL, Chu DKW, Shiu EYC, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med* 2020; **26:** 676–80.

42 Davies A, Thompson KA, Giri K, Kafatos G, Walker J, Bennett A. Testing the efficacy of homemade masks: would they protect in an influenza pandemic? *Disaster Med Public Health Prep* 2013; **7:** 413–18.

43 van der Sande M, Teunis P, Sabel R. Professional and home-made face masks reduce exposure to respiratory infections among the general population. *PLoS One* 2008; **3:** e2618.

44 Rengasamy S, Eimer B, Shaffer RE. Simple respiratory protection—evaluation of the filtration performance of cloth masks and common fabric materials against 20–1000 nm size particles. *Ann Occup Hyg* 2010; **54:** 789–98.

45 Drewnick F, Pikmann J, Fachinger F, Moormann L, Sprang F, Bormann S. Aerosol filtration efficiency of household materials for homemade face masks: influence of material properties, particle size, particle electrical charge, face velocity, and leaks. *Aerosol Sci Technol* 2020; **55:** 63–79.

46 Pan J, Harb C, Leng W, Marr LC. Inward and outward effectiveness of cloth masks, a surgical mask, and a face shield. *medRxiv* 2020; published Nov 20. https://doi.org/10.1101/2020.11.18.20233353.

47 Klompas M, Baker MA, Rhee C. Airborne transmission of SARS-CoV-2: theoretical considerations and available evidence. *JAMA* 2020; **324:** 441–42.

48 Chughtai AA, Seale H, Macintyre CR. Effectiveness of cloth masks for protection against severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis* 2020; **26:** e200948.

49 Centers for Disease Control and Prevention. Regarding the use of cloth face coverings, especially in areas of significant community-based transmission. April 3, 2020. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/cloth-face-cover.html (accessed Nov 9, 2020).

50 Centers for Disease Control and Prevention. Scientific brief: community use of cloth masks to control the spread of SARS-CoV-2. Nov 10, 2020. https://www.cdc.gov/coronavirus/2019-ncov/more/masking-science-sars-cov2.html (accessed Nov 10, 2020).

51 Aydin O, Emon B, Cheng S, Hong L, Chamorro LP, Saif MTA. Performance of fabrics for home-made masks against the spread of COVID-19 through droplets: a quantitative mechanistic study. *Extreme Mech Lett* 2020; **40:** 100924.

52 Agro C, Grundig T, Szeto E. Marketplace tested over 20 different masks. Here's what will best protect you and others during the pandemic. Nov 13, 2020. https://www.cbc.ca/news/canada/marketplace-masks-test-1.5795481 (accessed Nov 23, 2020).

53 Konda A, Prakash A, Moss GA, Schmoldt M, Grant GD, Guha S. Aerosol filtration efficiency of common fabrics used in respiratory cloth masks. *ACS Nano* 2020; **14:** 6339–47.

54 Galasso V, Pons V, Profeta P, Becher M, Brouard S, Foucault M. Gender differences in COVID-19 attitudes and behavior: panel evidence from eight countries. *Proc Natl Acad Sci USA* 2020; **117:** 27285–91.

55 Bundgaard H, Bundgaard JS, Raaschou-Pedersen DET, et al. Effectiveness of adding a mask recommendation to other public health measures to prevent SARS-CoV-2 infection in Danish mask wearers: a randomized controlled trial. *Ann Intern Med* 2020; published online Nov 18. https://doi.org/10.7326/m20-6817.

56 Laine C, Goodman SN, Guallar E. The role of masks in mitigating the SARS-CoV-2 pandemic: another piece of the puzzle. *Ann Intern Med* 2020; published online Nov 18. https://doi.org/10.7326/m20-7448.

57 Frieden TR, Cash-Goldwasser S. Of masks and methods. *Ann Intern Med* 2020; published online Nov 18. https://doi.org/10.7326/m20-6817.

58 Gandhi M. Perspective on DANMASK study. Nov 18, 2020. Healio Infection Control. https://www.healio.com/news/primary-care/20201118/study-covid19-risk-slightly-lower-for-mask-wearers (accessed Dec 1, 2020).

59 Hendrix MJ, Walde C, Findley K, Trotman R. Absence of apparent transmission of SARS-CoV-2 from two stylists after exposure at a hair salon with a universal face covering policy - Springfield, Missouri, May 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69:** 930–32.

60 Wang X, Ferro EG, Zhou G, Hashimoto D, Bhatt DL. Association between universal masking in a health care system and SARS-CoV-2 positivity among health care workers. *JAMA* 2020; **324:** 703.

61 Lyu W, Wehby GL. Community use of face masks and COVID-19: evidence from a natural experiment of state mandates in the US. *Health Aff (Millwood)* 2020; **39:** 1419–25.

62 Gallaway MS, Rigler J, Robinson S, et al. Trends in COVID-19 incidence after implementation of mitigation measures - Arizona, January 22-August 7, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69:** 1460–63.

63 Hatzius J, Struyven D, Rosenberg I. Face masks and GDP. June 29, 2020. Goldman Sachs Research. https://www.goldmansachs.com/insights/pages/face-masks-and-gdp.html (accessed Nov 9, 2020).

64 Santarpia JL, Rivera DN, Herrera VL, et al. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. *Sci Rep* 2020; **10:** 12732.

65 Zhou J, Otter JA, Price JR, et al. Investigating SARS-CoV-2 surface and air contamination in an acute healthcare setting during the peak of the COVID-19 pandemic in London. *Clin Infect Dis* 2020; published online July 8. https://doi.org/10.1093/cid/ciaa905.

66 Guo ZD, Wang ZY, Zhang SF, et al. Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, Wuhan, China, 2020. *Emerg Infect Dis* 2020; **26:** 1583–91.

67 Liu Y, Ning Z, Chen Y, et al. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature* 2020; **582:** 557–60.

68 Ong SWX, Tan YK, Chia PY, et al. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. *JAMA* 2020; **323:** 1610–12.

69 Morawska L, Tang JW, Bahnfleth W, et al. How can airborne transmission of COVID-19 indoors be minimised? *Environ Int* 2020; **142:** 105832.

70 Center for Disease Control and Prevention. COVID-19 employer information for office buildings. Oct 29, 2020. https://www.cdc.gov/coronavirus/2019-ncov/community/office-buildings.html (accessed Dec 2, 2020).

71 Gwaltney JM Jr, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. *Ann Intern Med* 1978; **88:** 463–67.

72 Coelingh K, Olajide IR, MacDonald P, Yogev R. Efficacy and effectiveness of live attenuated influenza vaccine in school-age children. *Expert Rev Vaccines* 2015; **14:** 1331–46.

73 Bennett A, Pollock L, Jere KC, et al. Direct and possible indirect effects of vaccination on rotavirus hospitalisations among children in Malawi four years after programmatic introduction. *Vaccine* 2018; **36:** 7142–48.

74 Baden LR, El Sahly HM, Essink B. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021; **384:** 403–16.

75 Polack FP, Thomas SJ, Kitchin N. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2020; **383:** 2603–15.

76 Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397:** 99–111.

77 Novavax. Press release: Novavax COVID-19 vaccine demonstrates 89·3% efficacy in UK phase 3 trial. Jan 28, 2021. https://ir.novavax.com/news-releases/news-release-details/novavax-covid-19-vaccine-demonstrates-893-efficacy-uk-phase-3 (accessed Feb 5, 2021).

78 Johnson and Johnson. Johnson and Johnson announces single-shot Janssen COVID-19 vaccine candidate met primary endpoints in interim analysis of its phase 3 ENSEMBLE trial. Jan 29, 2021. https://www.jnj.com/johnson-johnson-announces-single-shot-janssen-covid-19-vaccine-candidate-met-primary-endpoints-in-interim-analysis-of-its-phase-3-ensemble-trial (accessed Feb 5, 2021).

79 Logunov DY, Dolzhikova IV, Shcheblyakov DV, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet* 2021; published online Feb 2. https://doi.org/10.1016/s0140-6736(21)00234-8.