Rapid Review on SARS-CoV-2 Aerosol Transmission: update 2

March 2021

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Introduction

What is the emerging cluster/outbreak, experimental and biological evidence implicating aerosol transmission of SARS-CoV-2?

The scientific evidence and understanding of SARS-CoV-2 modes of transmission has rapidly evolved over the past year. Expelled respiratory particles containing infectious pathogens can occur in a continuum of sizes, and smaller respiratory particles (often termed aerosols) can remain suspended in air and disperse further distances than large respiratory droplets. Other

pathogens that are known to primarily transmit through large droplets (e.g., Influenza, SARS-CoV-1, streptococcus pneumonia, and *Legionella*) can also spread by aerosols in some settings and conditions [1, 2, 3, 4, 5, 6]. As such, a range of evidence has been produced to describe the characteristics and relative importance of aerosols in SARS-CoV-2 transmission in different settings and conditions.

This evidence brief summarizes the scientific literature providing evidence on SARS-CoV-2 aerosol transmission published up to March 12, 2021 and is organized into the follow evidence sections:

- SARS-CoV-2 cluster or outbreak investigations that have implicated aerosol transmission;
- Experiments on indirect transmission of SARS-CoV-2 virus using animal models;
- Experimental evidence on SARS-CoV-2 virus stability and viability in aerosols;
- Biological monitoring studies on SARS-CoV-2 RNA in exhaled breath; and
- Biological monitoring studies on SARS-CoV-2 RNA and viability in environmental air samples in patient care and in community (i.e.,. non-hospital/patient care) settings.

The evidence section on fluid dynamic simulations and *in-silico* analyses has been omitted from this update given the increasing amount of empirical evidence supporting the potential for SARS-CoV-2 aerosol transmission.

What's new

This update identified 46 new studies, pre-published and published, between November 6, 2020 (the last update date) and March 12, 2021 on SARS-CoV-2 aerosol transmission potential. These studies are summarized below and identified as new throughout the evidence tables.

- The update includes twelve new reports of SARS-CoV-2 outbreaks/cluster investigations in real-world settings (Table 1). These aerosol implicated transmission events were reported to have occurred inside a post-travel quarantine hotel, nursing home, hospital hematology unit, passenger bus, a restaurant, fitness facilities, department store, and apartments arranged in a vertical line in an apartment building [7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. In these reports, most reported mask use was infrequent or improper at the time of assumed aerosol transmission, and index cases were reported to be at pre-symptomatic or early symptomatic stages of infection.
- Two new animal model-based experiments reported on multiple modes of SARS-CoV-2 transmission, including aerosols, between infected and susceptible hamster pairs [17, 18]. One study suggested aerosol exposure led to earlier virus replication, shedding in

respiratory tissue, and more acute disease manifestation in hamsters, when compared to fomite and intranasal exposures [17]. The other study found aerosols from naïve infected hamsters can infect previously infected hamsters, which suggests recovery from a primary SARS-CoV-2 infection does not eliminate subsequent infection risk by aerosols [18].

- A newly included experimental study investigating the viability and decay rates of SARS-CoV-2 virus in aerosols reported the infectiousness of virus in aerosols to be highly dependent on environmental conditions in the following order of influence: simulated sunlight exposure levels (midday summer, spring midday, indoor/night tested) >temperature (10-40 °C tested)> humidity (20-70% tested) [19]. A 90% reduction in infectious virus was estimated to take 4.8 minutes (at 40^0C, 20% relative humidity) with midday summer sunlight exposure, with similar reductions were estimated to take more than 2 hours (at 40^0C, 20% relative humidity) under no sunlight exposure conditions (i.e., indoors or at night). Sunlight had the largest influence on decay rate, however higher decay was also noted at 30°C with high (70%) humidity or at 40°C regardless of humidity.
- One of the new biological monitoring study on exhaled breath samples from five patients found viral RNA to only be detected in exhaled air samples of COVID-19 patients with positive oropharyngeal, nasopharyngeal, and/or salivary swabs at the time of sampling [20].
- Twenty-eight new biological monitoring studies that investigated SARS-CoV-2 virus contamination in environmental air samples in patient care (includes hospitals and long-term care studies) and in community (i.e., non- patient care) settings are included in this update. The increasing number of studies that confirm the presence of SARS-CoV-2 in environmental air provide additional evidence to support aerosol transmission potential in community settings.
    - Two Canadian studies suggested viral RNA contamination in environmental air samples from a hospital (~ 3 meters from COVID-19 patients) and on no touch surfaces from long-term care home settings to indicate SARS-CoV-2 can spread through aerosols [21, 22].
    - A new study confirmed the presence of viable virus in air samples collected from a car driven by a mildly symptomatic individual [23]. This highlights SARS-CoV-2 virus can be expelled in to the surrounding air, even by a mild case over a short period of time [23].
    - Another study compares environmental air samples collected from inpatient hospital rooms and quarantine households with active cases [24]. The household environmental air samples were estimated to be approximately eight times (OR

8.75 [95% CI 1.21-636.43; p=0.058]) more likely to be contaminated with viral RNA, than hospital air samples. Based on these study findings the investigators suggest differences in air exchanges and ventilation to be a key difference that is more important than disease acuity, with respect to environmental air contamination.

Key points

Highlights from the current literature include:

- In total, 84 studies on the potential for aerosol transmission have now been identified in the published and pre-published literature. Multiple outbreak and cluster investigations suggest aerosol transmission of SARS-CoV-2 may have occurred in some settings. Emerging experimental evidence in separated animals indicates infection can spread from aerosol exposure, and infectious virus can remain stable and viable within suspended aerosols. Biological monitoring studies confirm the presence of viral RNA in exhaled breath and environmental air samples. Clinical evidence informed systematic review and meta-analysis estimates of viable respiratory virus emission of SARS-CoV-2.
- Twenty-six investigations of twenty different COVID-19 outbreaks/clusters in different real-world settings (e.g., nursing home, hospital hematology unit, post-travel quarantine hotel/facility, meat processing plants, indoor choir practice, restaurant, cruise ship, passenger bus, fitness facilities, high-rise apartment building and shopping mall), have implicated aerosol transmission among cases (Table 1). The outbreak investigations suggest aerosol transmission is amplified and/or more likely to occur in some settings and under some conditions such as poorly ventilated or crowded indoor spaces, presence of early symptomatic or pre-symptomatic cases symptomatizes or when individuals are engaged in physically exertive activities (e.g., singing, fitness classes).
- A systematic review and meta-analysis of clinical estimates found the likelihood of viable virus in respiratory aerosols expelled by an individual at peak viral load to be 61.1% (95% CI: 51.8-70.4%), and the likelihood estimates to be substantially lower at ≤ 0.69% (95% CI: 0.43-0.95%) for an individual with a mean viral load Peak viral load was estimated to happen between 1 day before symptom onset to 5 days post symptom onset (Table 7). This is consistent with the findings across outbreaks where the index case was usually pre-symptomatic or very early in their symptomatic illness when transmission occurred.
- Animal studies provide evidence on infection by aerosols, and infection transmission even when infected and susceptible animals are separated by cage setup or barriers. This

indirect infection transmission is at least partially attributed to aerosols and air flow by the study investigators (Table 2).

- Three studies report on aerosol virus stability and viability, as well as the influence of environmental factors (e.g., temperature, humidity and sunlight exposure) on virus persistence in aerosols (Table 3). Experimental evidence has demonstrated prolonged viability of SARS-CoV-2 virus within laboratory aerosols for up to several hours (range 2 to 16 hours).
- Biomonitoring studies measure viral RNA in exhaled breath samples of infected individuals (Table 4) and environmental air from patient care and community settings (Table 5 and Table 6). The increasing number of studies that confirm the presence of SARS-CoV-2 in environmental air provide additional evidence to support aerosol transmission in community settings. The few studies that confirmed virus viability in cell culture reported collecting environmental air samples near (<2 meters) infected individuals (Table 5 and Table 6).

Overview of the evidence

The available body of evidence on the potential transmission of SARS-CoV-2 by aerosols in the published and pre-published literature is rapidly evolving. This review includes studies (n=84) accessed up to March 12, 2021 and deemed relevant by a single reviewer. The overall quality of the evidence reviewed is broadly described below for each section of presented evidence based on study design, quantity, and consistency of the presented data. Briefly, the hierarchy of evidence and general quality ratings consider well-conducted randomized controlled trials to be high quality due to their low risk of bias. Other experimental designs may be considered moderate quality, but may also be downgraded due to power or conduct issues. Experiments using animal models are considered low quality evidence. Observational studies are generally considered to be at high risk of bias and thus low quality, however some large, well-conducted, prospective cohort studies may be assessed to be of moderate or low risk of bias, and thus may be considered moderate to high quality.

There are 26 outbreak/cluster investigation studies focusing on 20 different human outbreaks that suggest aerosol transmission of SARS-CoV-2 (Table 1). These are retrospective observational studies that are at risk of numerous biases. The retrospective nature of these investigations and the lack of genotyping data on the majority of identified cases mean that inferences about aerosol transmission are limited to epidemiological linkages. Multiple cluster investigations include both a description of the investigation as well as *in silico* simulations that explore the potential for aerosol transmission in real world settings.

Animal models provide experimental evidence on infection by aerosol exposure, by assessing indirect transmission from infected to susceptible animals by either passive or directed air flow, when separated using a variety of cage and barrier setups (Table 2). These studies did not provide sufficient details about the symptoms and behaviors of the infected animals (e.g., sneezing and respiratory fluid transfer by sniffing/licking barriers) or the experimental setup to rule out infection transmission by contact with respiratory and oral fluids in most experiments. Overall, animal models of transmission offer the lowest quality of evidence for aerosol transmission.

Three experimental studies provide evidence confirming the stability and viability of infectious virus in artificially generated and suspended aerosols (Table 3). Although these studies provide robust evidence that supports the longevity of infectious virus in laboratory environments for as much as 16 hours, the extension of these findings to real-world settings by biological monitoring studies remains largely unestablished.

The majority of identified biological monitoring studies looked for SARS-CoV-2 viral RNA in exhaled breath (Table 4) and environmental air samples in different settings (Table 5 and Table 6). These provide moderate quality evidence that viral RNA can either travel or linger in air at some distance from an infected individual in some settings and conditions. There is limited reporting of information on virus concentration in positive samples, sampling distance from infected individuals, and whether or not there was virus viability documented by cell culture. The lack of these details limit the generalizability of biological monitoring evidence to all indoor settings and all infected individuals, and hinder the identification of settings and conditions that lead to increased concentration of viable virus from exhaled breath and aerosolized particles in the air. Furthermore, it is likely the large range of sample collection settings (e.g., patient care vs. community settings) and techniques (e.g., exhaled breath vs. exhaled breath condensate) may have influenced the sensitivity of viral RNA quantification and viable virus detection tests, applied in biological monitoring samples. Additional research is needed to confirm the infectiousness and viability of SARS-CoV-2 within air samples to know where and when the risk of aerosol transmission of SARS-CoV-2 becomes more likely.

This review summarizes the evidence on aerosol transmission of SARS-CoV-2 and characterizes the settings and/or conditions that have been studied. Additional evidence is needed to address knowledge gaps on aerosol transmission of SARS-CoV-2:

- Quantifying the infectious dose of SARS-CoV-2
- The case attributes and environmental conditions under which viable virus is likely to be present in exhaled breath, remain suspended and be circulated in environmental air
- Genomic data from cluster and outbreak investigations suggesting indirect SARS-CoV-2 infection transmission in humans

- Confirmation of the relatedness of cases by genotyping data, and better quality data supporting aerosol transmission in the implicated outbreaks/clusters
- Formal review of fluid dynamic literature from experts in this field that best outline the environmental conditions and behavioral activities that increase (or decrease) respiratory aerosol release and infection transmission, as well as quantify aerosol transmission risk estimates

Cluster and outbreak investigations

This section provides a summary of twenty six studies describing twenty different COVID-19 cluster/outbreaks in real-world settings that epidemiologically support infection transmission by aerosols (Table 1). In a number of studies the same COVID-19 cluster/outbreaks were investigated by separate groups of investigators who all concluded aerosols or the long-range indirect transmission of virus to have played a role in infection spread among cases. These outbreaks have been organized according to the setting in which infection transmission was assumed to have occurred by the study investigators. The settings included a hematology unit at a hospital, a travel quarantine hotel, passenger buses, fitness classes and facilities, a meat processing plant, dine-in restaurants, a choir practice, a cruise ship, department stores, and different high-rise apartment buildings. The evidence includes epidemiological investigations, computational fluid dynamic analyses/simulations, video surveillance footage, or spatial analysis of cases during the transmission event.

Interestingly, multiple COVID-19 cluster/outbreaks were described to have occurred in settings similar to one another, which suggests these settings may be more favorable to aerosol transmission. Common attributes were closed spaces with minimal ventilation (i.e. few or no windows, insufficient ventilation and air circulation), presence of pre-symptomatic, early symptomatic index case(s), and the spread of virions beyond 2 meters enhanced by artificial air flow, ventilation systems, air ducts, drainage pipes or inefficient ventilation. Additionally, in some situations the infector and infectees were engaged in activities that typically increase exhalation rates (e.g., physical exercise, singing), or were in crowded spaces during the transmission events.

It is worth noting other than for one of the summarized studies, none reported on the genetic sequences of virus isolated from outbreak/cluster cases, and many studies do not describe mask use during the exposure events. These are important gaps within the existing evidence because genotyping data would confirm the relatedness of cases while mask use data would provide insights regarding the effectiveness of this prevention measure. The lack of this information within the summarized outbreak/cluster investigations is a key limitation of this evidence.

Animal experiments on aerosol exposure and transmission of SARS-CoV-2

This section summarizes the six identified animal model studies that provided experimental evidence supporting aerosol transmission of SARS-CoV-2 (Table 2). The animal models used in the studies were non-human primates, ferrets and hamsters, all of which have been established as suitable animal models for the study of SARS-CoV-2 transmission in humans [25, 26].

Two experiments involved the controlled exposure of non-human primates and hamsters to artificially generated infectious aerosols at different virus concentrations to determine if infection can occur and to follow the clinical course of the infected host animals [17, 27]. These studies provide evidence that SARS-CoV-2 infection can occur from exposure to infectious aerosols in a controlled setting. One of the studies in hamsters, that compared multiple modes of transmission (i.e., aerosol, fomite and intranasal) reported virus replication and shedding in the animals to be linked to the type of exposure. The authors observed earlier viral replication, higher viral loads, acute manifestation of respiratory symptoms, and different viral shedding patterns in the respiratory tissue of animals infected through aerosols compared to animals infected by intranasal and fomites mode of exposure [17].

Five experiments investigated SARS-CoV-2 transmission between infected and susceptible host animals (i.e., transmission pairs) that were physically separated by different barriers and cage setups [17, 18, 28, 29, 30]. In most experiments, transmission pairs were separated by barriers that prevented direct contact but allowed for air flow. This led to 25-100% of the susceptible animals becoming infected after some duration of exposure. These experiments provide evidence supporting aerosol transmission among host animals in experimental settings.

Additional evidence from two experiments demonstrate how air flow and ventilation can impact indirect transmission of infection [17, 29]. In one of these experiments transmission pairs of ferrets were housed in physically separated cages but shared air through connected air ducts, which led to 50% of the susceptible animals becoming infected [29]. In another experiment the influence of air flow direction on likelihood of infection transmission was investigated, and results show infection only occurred in the susceptible hamster housed downwind from the infected hamster [17].

The small sample sizes, animals not being separated by large distances, the lack of details on animal behavior during the experiment (e.g., coughing or sneezing symptoms in infected animals), and at times the lack of detail regarding the permeability of separation barriers to respiratory and oral fluids, fecal and food particle movement, limit this evidence from completely supporting aerosol transmission.

SARS-CoV-2 viability in aerosols

Three studies measured the half-life of viral particles suspended in artificial aerosols within experimental settings, Table 3 [19, 31, 32]. These studies and confirmed the viability of virus in artificial aerosols by plaque assays and cell culture [19, 31, 32] These studies found SARS-CoV-2 virus titers remained stable in artificially created aerosols up to timeframes that ranged from 2 to 16 hours [19, 31, 32]. The stability and infectiousness of virus in artificial aerosols appear to be dependent on environmental factors such as temperature, humidity and simulated sunlight levels, as previously suggested in some computer generated analysis [19]. One experimental study demonstrated simulated sunlight had the largest impact on decay rate, high temperatures (only 10-40°C were tested) had a moderate impact and a significant interaction with sunlight whereas relative humidity had the least influence on decay overall and only at high (70%) relative humidity [19].

SARS-CoV-2 RNA in exhaled breath

Six studies investigated the presence of viral RNA in exhaled air samples or exhaled breath condensate samples of SARS-CoV-2 from human cases, Table 4 [20, 33, 34, 35, 36, 37].

Two different exhaled breath sampling techniques were identified across the studies: some studies collected exhaled breath condensate while others collected exhaled air samples from infected individuals. Exhaled breath condensate techniques collect 1-2 ml of condensate by the cooling and condensation of exhaled air during quiet breathing. Some investigators have suggested the exhaled breath condensate technique is better suited for identifying biomarkers expelled from the lower respiratory tract, and results in reduced false negative results by RT-PCR [34, 38].

Five of the included studies reported at least one exhaled air sample positive for viral RNA. Among the identified studies that confirmed the presence of viral RNA in some exhaled samples, four applied the exhaled breath condensate technique while two were based on exhaled air sampling. Sample positivity ranged from 16%-93.5% in exhaled breath condensate sampling studies to 40% in the single exhaled breath sampling study with positive results. One study reported no positive samples, for both exhaled breath condensate and exhaled air collection techniques [37]. The variability in viral RNA positivity across exhaled breath samples may be linked to the infectious course of sampled individuals. However, the lack of clinical information on exposure and symptom onset dates, symptoms, as well as viral loads and viral replication in respiratory tissue, at the time of sample collection limit inferences between viral exhalation and infection course. Based on evidence that peak viral load occurs between one1 day before and up to five5 days post symptom onset, hospitalized or symptomatic individuals several days into their infection may not be an appropriate sample for measuring viral RNA within exhaled breath [27, 39]. Hospitalized individuals may no longer be infectious at the time of sample collection, which

should not be interpreted as a lack of evidence supporting infection emission by exhaled breath during the early infection period.

SARS-CoV-2 RNA in environmental air

Forty-nine biological monitoring studies that investigated SARS-CoV-2 in air samples collected from patient care settings, such as airborne infection isolation rooms (AIIR), ICU, emergency wards, hospital wards, nursing homes, diagnostic areas (e.g.,. CT scan) and outpatient clinics, were identified (Table 5). All of these studies report the presence of confirmed case(s) in the proximity of sample collection. The majority of studies (n=29/40) reported the isolation of viral RNA from at least one collected air sample by RT-PCR. Three studies successfully cultured virus particles isolated from a very small number of positive air samples in cell culture, thus providing evidence to support virus viability in air [40, 41, 42]. All of these air samples with viable virus were collected close (<2 meters) to confirmed cases. Furthermore, air samples taken at 1-4.8 m from confirmed cases consistently identified SARS-COV-2 RNA, with higher concentrations of viral RNA when the collection apparatus was positioned closer to the case [21, 40, 41, 42, 43, 44, 45, 46, 47, 48].

A single study in a patient care setting demonstrated opening the windows in the COVID-19 patient's room to be beneficial and reduced the viral concentration in the air sample from 10^5/ml to less than 10^4 [45]. Another study compares environmental air samples collected from inpatient hospital rooms and quarantine households with active cases [24]. The household environmental air samples were estimated to be approximately eight times (OR 8.75 [95% CI 1.21-636.43; p=0.058]) more likely to be contaminated with viral RNA than hospital air samples. The investigators suggested variability in air exchanges and ventilation between patient care and community settings to be the main difference that influenced air contamination between the two settings, as such room ventilation was suggested to be a more important factor than disease acuity with respect to environmental air contamination.

Nine biological monitoring studies of SARS-CoV-2 in air samples from community settings (i.e., a car driven by an infected case, shopping mall, concert hall, hotel quarantine rooms and households, mink farm, public buses and subway trains) were identified (Table 6). The majority of these studies (n=5/9) confirm the presence of viral RNA by RT-PCR and in one study, air samples from a car with a COVID-19 case isolated virus in cell culture [23]. The study in the car highlights that significant quantities of viable SARS-CoV-2 can be expelled from even mild cases over short periods of time [23]. A study on a mink farm with an ongoing outbreak highlights high levels of virus within the farm, which is a risk for the workers, however there was low risk outside of the farm to the surrounding community [49]. The identification of positive air samples from quarantine households and hotel rooms with active cases was variable and requires further investigation, as some studies identified positive samples in while others did not [24, 35, 50, 51]. Two environmental sampling studies of public venues, including a concert hall, a shopping mall, and

public buses and subway trains also confirm the presence of viral RNA in air samples [45, 52]. Interestingly, these studies do not confirm the presence of cases in the proximity of air sample collection but indicated high levels of community transmission in sampled settings [45, 52].

A range of air sampling methods were used across the included biological monitoring studies. Some studies used different air sampler models while others used fluid filled petri dishes, gelatin filters, agar plates and novel COVID-19 traps to sample environmental air. The variability in sampling methodologies may have contributed to the observed differences in viral RNA detection and confirmation of virus viability and infectiousness in environmental air samples. Only a small number of studies confirmed the viability of virus in cell culture and all samples were very close (<2 meters) to infected individuals.

The authors who reported no virus contamination in air samples collected from patient care settings often suggested effective disinfection, high efficiency air ventilation and filtration systems fitted to AIIR as possible reasons for negative results. This rationale is further supported by one biological monitoring study which was unable to detect viral RNA in collected samples when the air sampler inlet was covered with a HEPA filter [40].

Among the identified studies, viral RNA concentration within contaminated samples, isolated aerosol particle sizes and fractions, sampling distance from COVID-19 cases, air sample volume, and any attempts to culture isolated virus particles were not consistently reported. Moreover, the majority of studies did not provide clinical information, such as symptom onset data or presence of respiratory symptoms, on the cases present during air sample collection, nor ventilation and air flow in the sampled setting. These data gaps make it difficult to determine the conditions upon which infectious virus in environmental air samples becomes more frequent.

SARS-CoV-2 viral loads in respiratory particles

A systematic review and meta-analysis informed a model to estimate the relationship between viable SARS-CoV-2 virus, case viral loads, and virus laden droplet and aerosol emission, Table 7 [39]. This review found that peak viral load occurred between one day before to five days post symptom onset [39]. The model estimated the likelihood of viable virus in respiratory aerosols expelled by an individual at peak viral load was ≤61.1% (95% CI: 51.8-70.4%), and the likelihood estimate was substantially lower, at ≤ 0.69% (95% CI: 0.43-0.95%), for an individual with a mean viral load.

Methods

A daily scan of the literature (published and pre-published) is conducted by the Emerging Science Group, PHAC. The scan has compiled COVID-19 literature since the beginning of the outbreak and is updated daily. Searches to retrieve relevant COVID-19 literature are conducted in Pubmed, Scopus, BioRxiv, MedRxiv, ArXiv, SSRN, Research Square and cross-referenced with the literature on the WHO COVID literature list, and COVID-19 information centers run by Lancet, BMJ, Elsevier and Wiley. The daily summary and full scan results are maintained in a Refworks database and an excel list that can be searched. Targeted keyword searching is conducted within the COVID-19 database to identify relevant citations using search terms: aerosol, airborne, droplet.

Each potentially relevant citation was examined for relevance, the full text of potentially relevant research was examined to confirm relevance and a synopsis of the study was extracted into the review. This review contains research published up to March 12, 2020.

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Evidence Tables

Table 1: SARS-CoV-2 cluster or outbreak investigations that have implicated aerosol transmission (n=26)

Study	Method	Key outcomes
Hospital hematology setting		
Saidel-Odes (2021) [53] Cluster Investigation Israel Sep – Oct 2020	Investigation of a COVID-19 outbreak linked to an index patient who was immunocompromised with multiple myeloma and underwent stem cell	Seven healthcare staff in the transplant unit housing the index patient were diagnosed with the infection; attack rate of 19% (n=7/37). The infected staff included 2 doctors, 4 nurses, and 1 housekeeping worker. Two of the positive

new	transplant. Patient was treated in an AIIR following COVID-19 diagnosis, and healthcare staff donned appropriate PPE (i.e., N95, face shield, gown and gloves) prior to entering the patient's isolation room. All staff in the unit wore gowns, gloves and masks.	staff did not report any direct contact with the index patient. The outbreak investigation and univariate analysis found infected healthcare workers were more likely to have reported spending time in the unit's corridor or nurses' station ([RR], R = 7.2; 95% CI, 1.22–42.49; *P* = .018), but not in the index patient's room. The investigators suggest aerosol transmission to be the only plausible explanation for this outbreak.
Hotel quarantine and airplane setting		
Eichler (2021) [14] Cluster Investigation New Zealand Sep 2020 **new**	Investigation of a COVID-19 outbreak that occurred during repatriation, at a mandatory isolation and quarantine facility (hotel) and among household contacts in the community. Cases who were returning other countries were required to complete a mandatory. All persons went to a Mandatory quarantine and isolation at a facility for 14 days after landing at their destination.	Based on the application of video surveillance data and viral genomic analysis of cluster cases, the investigators suggest a multiple transmission chains. The suspected sites of transmission were international and domestic flights, the quarantine facility, and households. While aerosol transmission may have been the mode in each transmission event, but an in-depth investigation concluded aerosol transmission was the primary mode of infection transmission at the hotel quarantine facility The case exposures/transmissions in the outbreak are assumed to be as follows: 2 index cases infected around the time of repatriation travel from India to Christchurch, NZ. 1 exposed on the flight to Christchurch, 2 exposed in hallway at the quarantine facility (by aerosols see below), 1 exposed on the flight from Christchurch to Auckland (transmission from the 2 aerosol exposed cases who tested negative on day 12 of quarantine),

		with additional cases among household contacts of the last three cases. Based on computer footage the investigators concluded aerosol transmission to have taken place at the doorway of two different hotel rooms that housed individuals in quarantine by suspended aerosols. Transmission was assumed to have occurred during a 50-second window between closing the door to the room of one case and opening the door to the room of other cases. Note: A review of the hotel's ventilation system found the rooms in question had a net positive pressure compared with the corridor so air flow into the corridor when the door was open was likely
Nursing home setting		
de Man (2020) [15] LTE Cluster Investigation Netherlands Jun –Jul 2020 **new**	Investigation of a COVID-19 outbreak in a single ward of a 7 ward Dutch nursing home.	A total of 17/21 residents in the ward and 13/34 healthcare workers from the ward, and an additional 4 laboratory workers were confirmed cases linked to the cluster; attack rate among residents was 81% and among healthcare workers was 50%. All 106 healthcare workers and 95 residents of other wards remained negative for COVID-19. Healthcare workers wore facemasks during patient care and worked in designated wards to limit contacts. The outbreak was limited to a single ward (out of 7 wards in the facility) with a ventilation system that recirculated unfiltered air. The ventilation system was new and monitored CO2 concentrations to determine when to refresh the air with outside air. Viral RNA

		was detected in the ventilation system (air conditioners and ventilation cabinet dust filters). The investigators state transmission was likely the result of aerosol transmission in an inadequately ventilated setting/ward given the simultaneous detection of a large number of cases limited to one ward, and occurred during a time of low community transmission.
Transportation bus setting		
Luo (2020) [7] Cluster Investigation China Jan 2020 **new**	Investigation of a COVID-19 outbreak linked to multiple bus trips by the index case, in Hunan China.	A total of 12 cases were identified following the investigation of 243 individuals who were epidemiologically linked to multiple bus trips taken by the index case, attack rate of 7.0%. The seated distance of the infected individuals during bus rides ranged from 1-4.5 meters from the index case. The 2.5 hour coach ride resulted in 8 cases and not all cases were clustered close to each other during the trip. Infection was also identified in an individual who sat (approximately) on the same seat as the index case, after the index case had existed the bus. 2 cases were identified from the 1 hour minibus trip. 2 cases were tertiary cases to one of those infected on the bus. None of the identified cases reported wearing face masks during bus rides. The investigators suggest aerosols traveling beyond 2 meters and air flow to have influenced infection transmission in

		the crowded and closed environment of these transportation buses.
Shen (2020) [54] Cluster Investigation China Jan 2020	A COVID-19 outbreak among 128 people driven to a worship event in Eastern China on two separate buses. Round trip was 100 minutes on the bus. Attack rates were measured for Bus 1 vs. Bus 2 that had the index case. Air conditioning systems of both buses were on recirculation mode. Spatial analysis of passenger seating was estimated.	None of the passengers on Bus 1 were infected, 24 of the 68 passengers on Bus 2 developed COVID-19. Passengers riding Bus 2 with the index case had an attack rate of 34.3% (95% CI, 24.1%-46.3%), compared to passengers on bus 1. Although sitting near bus windows and doors appeared to have had a protective effect on infection transmission, the authors conclude, the lack of a significant increase in infection risk between individuals sitting in high risk zones (i.e. closer to the index case) and low risk zones, and elevated attack rates among bus passengers riding with the index case, to be partially explained by aerosol transmission of infection.
Meat processing plant setting		
Guenther (2020) [55] *Preprint* Cluster Investigation Germany Spring 2020*	Investigation of a super-spreader event among meat processing plant workers that included: possible routes of transmission, spatial relationship between workers, climate/ventilation conditions, sharing of living quarters and transportation, and genetic typing of oropharyngeal swab samples.	The analysis of index cases (flatmates) and 18 co-worker cases suggest working the early morning shift (140 early shift workers) to be the common source of infection. Statistically significant infection rates were observed for workers working within an 8-meter radius of the suspect index case. Authors conclude indoor confined settings, demanding physical work, and the facility's environmental conditions (i.e. air being constantly re-circulated and cooled to 10°C, with low air exchange

		rates) all created conditions for aerosol transmission. Note: quantitative risk estimates were not provided.
Restaurant setting		
Kwon (2020) [8] Cluster Investigation Korea Jun 2020 **new**	Investigation of a cluster among 3 patrons at a restaurant. The investigation considered epidemiological data, television images, air flow patterns and cell phone location information.	The investigators concluded infection transmission occurred from an infected customer to two individuals at the restaurant venue, attack rate of 15.4% (2/13). Source and time of exposure was assumed to be one day prior to symptom onset in the index case. The assumed mode of transmission was aerosols that travelled greater than 6.5 meters via air conditioner air flow current inside the restaurant venue. The exposure time between the infector and the infected is reported to be approximately 5 minutes. Mask use is reported to have been improper among staff and patrons.
Lu (2020) [56] Cluster Investigation China Jan-Feb 2020	Investigation of a COVID-19 cluster among restaurant patrons. The investigation included a spatial analysis of restaurant table arrangement and where cases were seated.	An outbreak among 91 individuals at a restaurant, 83 had dined at 15 tables, and the remaining 8 individuals were staff. A single asymptomatic case led to 9 COVID-19 infections among diners from three families. None of the families had met previously and did not have any close contact during lunch. No additional cases were identified during the 14 days quarantine of the remaining diners. Spatial analysis of case tables during lunch (i.e., exposure event reveal) found the affected tables had been arranged in

		line with airflow from an air conditioning unit. Authors suggest infection transmission could not be explained by droplets alone, and aerosols travelling with air flow may have contributed to infection transmission.
Li (2020) [57] *In silico* study China Feb 2020 Note: Same outbreak described by Lu (2020).	An investigation and analysis of a COVID-19 cluster among 3 families who ate at the same restaurant. The analysis included: epidemiological data, spatial analysis of restaurant table arrangement, video surveillance data, and computer fluid dynamic and tracer gas simulations of event's fine droplet spread.	10 people from three different families seated at different tables were found to have been infected with SARS-CoV-2 following a Chinese New Year's Eve (January 24, 2020) lunch. None of the waiters or patrons at the remaining tables became infected. Ventilation rate was estimated to be 0.9L/s (0.75-1.04 L/s) per person. No close contact or fomite contact was observed among cases, aside from back-to-back sitting by some patrons. Using computer simulations the authors demonstrate infection distribution to be consistent with the spread pattern of exhaled virus aerosols. These results highlight transmission occurring in a crowded and poorly ventilated indoor situation.
Choir practice setting		
Charlotte (2020) [16] Cluster Investigation France Mar 2020 **new**	The investigation of a COVID-19 outbreak linked to a choir practice, in Whir au Val, France.	Twenty-seven participants (25 singers, 1 conductor and 1 accompanist) attended the indoor choir practice that took place in a non ventilated space of 45 m^2. No attendees reported being symptomatic in the 14 days prior to the practice date. 19/27 attendees developed COVID-19 infections at 1 to 12 days post practice

		date (median of 5.1 days). The secondary attack rate was 70% among all attendees. The investigators assume infection transmission occurred by aerosols from presymptomatic or asymptomatic individual(s) who attended the choir practice as the index case was not definitively identified.
Hamner (2020) [58] Cluster Investigation US Mar 2020 Note: Same outbreak described by Miller (2020).	The investigation of a COVID-19 outbreak linked to a choir practice, in Skagit County, Washington. The practice lasted for 2.5h. During practice people were singing and seated 6-10 inches apart, socializing with communal snacks, and stacking chairs. None of the attendees reported physical contact.	Among the 61 choir members attending the practice, at least one singer was known to be a symptomatic COVID-19 case. The epidemiological investigation reported 53 cases (33 confirmed, 20 probable cases). Secondary attack rates were 53.3% among confirmed cases and 86.7% among all cases. The odds of infection were 125.7 (95% CI: 31.7-498.9) times greater among members who attended the March 10 practice (assumed exposure event). The investigators introduce the potential for aerosol emission and COVID-19 transmission during singing in the COVID-19 literature.
Miller (2020) [59] *In silico* study US Mar 2020 Note: Same outbreak described by Hamner (2020).	Monte Carlo simulations and mathematical modeling were used to estimate aerosol emission rates in the outbreak linked to a choir practice, in Skagit County. The applied model assumes infection transmission during the outbreak was dominated by inhalation of respiratory aerosols in a	*In silico* analysis supported aerosol transmission from respiratory aerosols based on assumption that high emission rates occurred given the high attack rate (53-87%), which was higher than would be expected if the transmission was due to fomites or large respiratory droplets. The model estimates the mean respiratory aerosol emission rate for a single infected case at the exposure event

	well mixed indoor environment (i.e. the aerosols were evenly distributed in the air). The viral load emitted was expressed as quanta emission rate (quanta per hour^{-1}) where a quantum was defined as the dose of aerosol droplet nuclei required to cause infection in 63% of susceptible persons.	to be 970 [IQR 680-1190] quanta per hour^{-1}.
Multiple outbreaks		
Buonanno (2020) [60] *In silico* study China and US (sites of applied outbreaks) Feb-Mar 2020 Note: A different analysis of restaurant and choir practice outbreaks described above	This is an emission and exposure model that used a step-wise approach to quantify individual infection risk among susceptible subjects exposed to an asymptomatic/ mildly symptomatic case in choir practice and dine-in restaurant. Also used Monte Carlo method; individual infection risks were calculated as a function of quanta emission characteristics.	The model illustrated individual infection risk increased based on ventilation rates, activities and amount of virus exhaled. For instance, sedentary activities for 1 hour may have an infection risk of 2.1%, which can increase to 27% with higher emission rates. Based on risk assessment approach and available data, quanta emission rates were estimated to be 61 quanta/hour for the restaurant and 341 quanta/hour for the Skagit Valley choir practice. In both of the examples, varying the ventilation would not have achieved an individual risk <0.1. The authors concluded aerosol transmission represents the main route of transmission for both outbreaks.
Kriegel (2020) [61] *In silico* study	An extension of the Wells-Riley equation was used to	In nine out of the twelve outbreaks the observed attack rates were in range with

Germany, China, US, (sites of applied outbreaks) Feb-Mar 2020 Note: Included the following clusters: Meat Processing plant- Guenther (2020), Choir Practice- Hamner (2020), Bus Passengers – Shen (2020), and Restaurant – Lu (2020)	estimate predicted infection risk via aerosols in twelve published and unpublished COVID-19 outbreaks. Predicted infection risks were compared to observed attack rates in each event. To estimate a "credible interval" for model predicted infection risks, the quanta emission rate, the respiratory rate as well as the air volume flows were varied. The analysis assumes long range aerosol transmission in an ideally mixed environment.	the predicted infection risk via aerosols and the corresponding ranges (with the variation of the boundary conditions). Predicted Infection Risk via Aerosols (PIRA)/attack rate (AR) Meat processing plant: 25% (17-35)/ 26% Choir: 97% (88-99)/ 87% Restaurant: 40% (35-56)/ 45% Bus tour: 35% (19-58)/ 34% The attack rates from all these outbreaks are reported to be in-line with estimated infection risk via aerosols.
Cruise ship settings		
Azimi (2021) [62] *In silico* study Cruise ship Jan-Feb 2020 Note: Same outbreak described by Almilaji (2020) and Xu (2020)	Analysis of case data from the Diamond Princess cruise ship outbreak. Applied a framework based on stochastic Markov chain and negative exponential dose-response modeling with empirical data, to inform a modified version of the Reed-Frost epidemic model, to predict case count rates. Assumed infected individuals could be infectious upto 1 day post incubation period,	712 COVID-19 cases were identified in 3711 passengers and crew members, yielding an attack rate of 19%. Key estimates derived from the model included • short-range droplets and aerosols (35%), long-range aerosols (35%), and fomite (30%) as modes of infection transmission • large respiratory droplets (41%) and small respiratory aerosols (59%) as source of infectious virus • Case transmission proportions prior to and after the passenger

	effective incubation periods were estimated to be 5-11/13 days, and considered different modes of transmission. Note: Case data from early 2020 were included in the analysis.	quarantine was 58% (±5% SD) and 42% (±5% SD), respectively. T • Effective reproduction number before and after the quarantine period was 3.8 (±0.9 SD) and 0.1 (±0.2 SD). Based on the modeled estimates the authors concludes smaller respiratory aerosols contributed to a greater proportion to infection transmission aboard the cruise ship, on average, across all time periods (i.e., both before and after passenger quarantine). It was estimated that aerosol transmission was the dominant mode of transmission (>70% of cases) despite the high ventilation rates (9-12 air changes per hour) with no air recirculation in the cruise ship.
Almilaji (2020) [63] Cluster Investigation Cruise ship Jan-Feb 2020 Note: Same outbreak described by Azimi (2020) and Xu (2020)	Analysis of clinical and case count data from Diamond Princess cruise ship outbreak. Post quarantine symptomatic infection onset rates (SIRR) among lab confirmed cases were examined and the design of the cruise ship's air conditioning system was considered. Note: Case data up to February 20, 2020 were included in the analysis, and a median 5 day	Infection rates among passengers in cabins without previously confirmed cases was 1.2%, which was higher than rates among passengers in cabins with previously confirmed cases 0.8%. Based on this difference, the authors suggest airborne transmission of SARS-CoV-2 through the cruise ship's ventilation system may have contributed to the outbreak, and explain the higher infection rates in cabins without previously confirmed cases.

	incubation period was assumed.	
Xu (2020) [64] *Preprint* Cluster Investigation Cruise ship Jan-Feb 2020 Note: Same outbreaks described by Azimi (2020) and Almilaji (2020).	Analysis of COVID-19 case data from the Diamond Princess cruise ship outbreak was analyzed based on individual risk factors, stateroom occupancy and the air conditioning (i.e. HVAC) system of the ship to explore the most plausible modes of transmission. Case data from January 20 to February 18, 2020 were included in this analysis.	Daily infection rates for passenger cases (n=146) were predicted based on close contact vs. non-close contact status, and pre- and post-quarantine data (February 5 was the start of quarantine). The investigators concluded most passenger cases were likely exposed before the passengers were quarantined and the cruise ship's air conditioning system did not play a role in long-range aerosol transmission of COVID-19.
Fitness/sports facility settings		
Groves (2021) [9] Cluster Investigation US Jun 2020 **new**	Investigation of a COVID-19 outbreak associated with multiple exposure events in Hawaii between a stationary cycling class instructor, a kick boxing/personal training instructor and their clients. Note: The average community transmission rate at the time of the outbreak was 2–3 cases per 100,000 persons per day, which suggests alternative exposures were unlikely.	20 cases were linked to two pre-symptomatic fitness instructors, at different fitness classes. The kick boxing/personal training instructor was a secondary case of the stationary cycling class instructor. The index instructor wore a face mask during the stationary bike class but the participants did not. The instructor was > 6 feet away and facing participants during the class. The room windows and doors were closed and floor fans (for cooling) directed air at participants. The transmission events with the second instructor are assumed to have occurred during small group kick boxing and personal training sessions when the

		instructor did not wear a mask and mask use was minimal among participants. Both instructors taught classes prior to symptom onset and the following aggregate attack rates for were calculated: • <1 day pre symptom onset (attack rate 95%) • 1 to <2 days pre symptom onset (attack rate 13%) • ≥2 days pre symptom onset (attack rate 0%) The outbreak investigators suggest infection transmission to have been facilitated by the lack of face mask use, extended close contact, poor room ventilation, and aerosol emission during physical activity and loud speech.
Lendacki (2021) [10] Cluster Investigation US Aug-Sep 2020 **new**	Investigation of a COVID-19 outbreak associated with indoor fitness classes that were conducted at ≤25% capacity (i.e., 10–15 persons) with participants ≥6 ft apart. Note: the building was not originally designed for exercise classes and the buildings ventilation system was not assessed.	55 COVID-19 cases (49 confirmed and 6 probable cases) were identified among 81 individuals who participated in indoor high-intensity exercise classes, attack rate of 68%. Mask use during the exercise sessions was reported to have been infrequent and approximately 75%. Outbreak was attributed to 2 index cases who attended multiple exercise classes when symptomatic and potentially infectious. Both attendees reported mask use ≤60% of the time in class (infrequent mask use). The odds of infrequent mask use compared to consistent mask use during classes was more common among attendees with COVID-19 than attendees

		not diagnosed with COVID-19 (odds ratio [OR] = 3.5; 95% CI = 0.9–15.1). Attendees diagnosed with COVID-19 also reported attending more exercise classes (median of 5 vs. 3 classes) The authors suggest infrequent mask use, increased respiratory exertion during exercise, aerosol transmission, and sub-optimal ventilation may have contributed to infection transmission in this outbreak.
Jang (2020) [65] Cluster Investigation South Korea Feb-Mar 2020	Investigation of a COVID-19 outbreak associated with Zumba classes at 12 different fitness sports facility locations following an instructor workshop in Cheonan, South Korea.	The initial transmission event is assumed to have occurred among instructors at a 4-hour workshop where 8 of the 27 attendees tested positive for SARS-CoV-2. In the following weeks case counts associated with infected instructors grew to 112 cases across multiple fitness facilities. The workshop attack rate was 26.3% (95% CI 20.9%–32.5%) and the secondary attack rate from 8 instructors was 4.10% (95% CI 2.95%–5.67%, 830 close contacts). The investigators state approximately half of identified cases (50.9%) were due to transmission from instructors to fitness class participants; 38 cases (33.9%) were in-family transmission from instructors and students; and 17 cases (15.2%) were from transmission during meetings with coworkers or acquaintances. No secondary cases were observed among Pilates and yoga class students, led by an infected instructor. Authors state intense physical activity, large number of participants in a fitness

		class (i.e. crowded space), and the moist warm atmosphere of the sports facility may have contributed to high rates of infection in the outbreak.
Brlek (2020) [66] Cluster Investigation Slovenia Feb-Mar 2020	Investigation of a COVID-19 cluster linked to a squash court in a fitness facility.	The cluster involved 6 cases assumed to be linked through indirect transmission of infection. Epidemiological investigation indicated the index case developed symptoms during the game of squash, and four confirmed and one suspect case were linked to the same squash hall and potentially the same change rooms. None of the cases shared sports equipment or had contact with the facility staff. No additional cases were identified. Authors suggest the infection transmission within the cluster likely occurred due to aerosolization of virus in the indoor setting including small confined space, inadequate ventilation and strenuous physical activity.
Apartment building settings		
Hwang (2021) [11] Cluster Investigation South Korea Aug 2020 **new**	Investigated the role of aerosol transmission in a COVID-19 outbreak associated with an apartment building in Seoul.	10 COVID-19 cases in 7 households were identified along two vertical lines of apartments in a building, each line of apartments were connected through a single air duct in the bathroom for natural ventilation. Attack rate of 2% among the apartment building residents (n=10/437). None of the tested surfaces, including household ventilation grills and drains were positive for viral RNA.

		Transmission through droplets when occupying the same common space (e.g., an elevator) was assumed to have been unlikely due to the spatial arrangement of case apartments. The outbreak investigators assume vertical air flow through a single air duct in the bathroom (consistent with air flow in a vertical shaft) to have spread the infection to upstairs and downstairs apartment residents in the building.
Lin (2021) [12] Cluster Investigation China Jan – Feb 2020 **new** Note: A different analysis of the cluster described by Kang (2020).	Investigation of a COVID-19 cluster linked to families living in three vertically aligned units of the same apartment building. The index family reported possible travel related exposure in Wuhan, but the two other families with subsequent cases did not. Tracer gas was used to simulate air flow among units.	10 COVID-19 cases were identified in 3 households, phylogenetic analysis confirmed all cases were infected by the same strain. Video surveillance footage did not identify exposures between the index household members and other cases. Investigators conclude infection transmission to have likely occurred through drain pipes connected to the toilet which were connected to sewer pipes and flood drains, a system that was shared by the vertically aligned apartments.
Kang 2020 [67] Cluster Investigation China Jan – Feb 2020 **new** Note: A different analysis of the	Investigate infection transmission among three families living in the same apartment building. The index family reported possible travel related exposure in Wuhan, but the two other families with subsequent cases did not. Investigators used Ethane tracer gas as a surrogate	10 COVID-19 cases among three families who lived in vertically aligned apartments connected by drainage pipes in the master bathrooms. Attack rate of 4% among apartment building residents and staff (n=10/217) No exposure from the building's elevators were identified, and viral RNA was not detected on elevator buttons or air vent surfaces. The surfaces most frequently contaminated with viral RNA

cluster described by Lin (2021).	for gas in the buildings drainage system and computational fluid dynamics to investigate possible sources of infection and transmission among families.	were in the master bathrooms, suggesting these areas to be probable infection transmission sites. Based on the epidemiological and *in-silico* analyses the investigators conclude infection transmission from the index family to the other two families likely occurred through fecal aerosols traveling within vertical drainage stacks of the apartment building. Note: none of the collected air samples from this setting were positive for viral RNA.
Shopping malls/stores		
Jiang (2020) [13] Cluster Investigation China Jan – Feb 2020 **new**	Investigation of 43 SARS-CoV-2 cases linked to a cluster at a department store in Baodi, China. Epidemiological data, video surveillance footage, store layout and ventilation conditions were considered in the investigation.	43 COVID-19 cases linked to the outbreak at a department store: 6 salespersons, 18 customers, and 19 of their close contacts. The close contact cases were determined to have been secondary cases without exposure to the department store. The investigators conclude aerosols to have been a significant mode of transmission among 11 cases of the outbreak. The index case was not identified but it was assumed to have been one of the infected salespersons.
Cai 2020 [68] Cluster Investigation China Jan 2020	Investigation of a SARS-CoV-2 cluster linked to a shopping mall. Clinical, epidemiological and laboratory (RT-PCR) data of cases was analyzed to assess possible modes of infection transmission.	Two shopping mall co-workers were the index cases: this was associated with 7 infections among co-workers on the same floor, 7 mall staff from other floors, 10 mall shoppers, and 2 close case contacts outside of the mall. Shoppers and co-workers from other floors denied close contact with the index cases.

		Based on the available data the authors suggest infection spread could have resulted from spread via fomites or virus aerosolization in a confined public space (e.g. restrooms or elevators).

LTE= letter to the editor

Table 2: Animal experiments on aerosol exposure and indirect transmission of SARS-CoV-2 (n=6)

Study	Method	Key outcomes
Edwards (2020) [27] *In Vivo Study* US Oct 2020*	Eight non-human primates Macaca mulatta (rhesus macaque) and Chlorocebus aethiops (African green monkey)) were infected with aerosols (≈2 µm) containing SARS-CoV-2 (~2.5x10^3 TCID50) using a laboratory inhalation system	Mucosal sampling by nasal swabs showed viral RNA detected as early as +1 day post infectious aerosol exposure with viral titers reaching peak levels at +7 days post infection, and clear decline of RNA by day +14 days post infection, and undetectable by +28 days post infection. These findings confirm SARS-CoV-2 infection by infectious aerosols can occur. Mucosal sampling by nasal swabs showed viral RNA detected as early as +1 day post infectious aerosol exposure. Exhaled breath particle production started 3 days post infection rose to day 7 and decreased to baseline by day 14 in primates. Exhaled breath particle production was temporally consistent with viral replication in nasal swab samples. There was a significant association between exhaled breath particles and viral load in most primates and correlated with viral kinetics.

		Viral RNA was undetectable in nasal swab samples of infected primates by day 28 post-infection.
Port (2020) [17] *Preprint* *In Vivo Study* US Dec 2020* **new**	Three groups of female *Mesocricetus auratus* (Syrian hamsters) (n=36) were infected with SARS-Cov-2 virus aerosols and other types of exposures (i.e., fomites and intranasal). Infected animals were compared to unexposed controls (n=12). Exposure by SARS-CoV-2 aerosols ($1.5x10^3$ TCID50) was by a 3 jet collision nebular; particle size ranged from 1-5 um. Viral shedding and replication patterns measured in respiratory tissue and by fecal and oropharyngeal swabs. During separate airborne transmission experiments two transmission pairs (n=4) were co-housed in cages separated by a perforated plastic divider that prevented direct contact. The susceptible animals were placed in the direction or against the air flow from infected animals; four transmission pairs (n=8). Infection	The authors found mode of SARS-CoV-2 infection transmission played a factor in disease severity, viral load, and virus shedding in the animal model. Early virus replication (1 day post infection), peak respiratory shedding of virus at 2 days from exposure, and higher early viral loads in lung and tracheal tissue ($p = <0.0001$), was observed among aerosol exposed animals when compared to other exposure types. Viral titers in lung tissue showed a positive relationship with upper and lower respiratory tract pathology and weight loss, which lead the authors to suggest early respiratory shedding (as observed among animals infected by aerosols) may predict acute disease manifestation. In the indirect contact experiments assessing airflow based transmission no symptoms linked to infection were observed in the susceptible animals but 25% (n=1/4) seroconverted. This seroconversion (i.e., virus exposure) was linked to directional airflow from the infected to host animals. Of note, it was observed fomite transmission was linked to delayed disease manifestation with longer duration of time between exposure and viral replication in respiratory tissue, which led to reduced disease severity.

	detected based on serum seroconversion.	
Zhang (2021) [18] *Preprint* *In Vivo Study* US Jan 2021* **new**	Investigated the risk of SARS-CoV-2 infection transmission from aerosol exposure, and risk of re-infection at re-challenge between naïve infected and previously infected transmission pairs of *Mesocricetus auratus* (Syrian hamsters). In the aerosol transmission experiments infected and donor hamsters (n=6) were housed in transmission cages with wire mesh partitions that prevent indirect and direct contact between animals, but permitted airflow. Live virus levels in respiratory tissue over the post exposure period was measured to confirm re-infection.	Experiments found SARS-CoV-2 was efficiently transmitted from infected naïve hamsters to previously infected hamsters by airborne transmission, among all transmission pairs. Based on viral RNA levels in respiratory tissue, the investigators conclude prior infection to have provided provide some good protective immunity but not complete immunity against re-infection at re-challenge; replicating live virus was present in the re-infected animals.
Sia (2020) [28] *In Vivo* Study Hong Kong* May 2020*	Experimental study that investigated SARS-CoV-2 infection transmission via aerosols in Mesocricetus auratus (Syrian hamsters). Infected and susceptible animals were housed in adjacent wire cages placed 1.8 cm away from one another (3 different pairs)	Efficient indirect transmission of infection to susceptible hamsters occurred in all three pairs within experimental settings. Peak viral load in aerosol exposed hamster was at 3 days post contact.

	were exposed to one another for 8 hours.	
Kutter (2020) [29] *In Vivo Study* Netherlands* Oct 2020*	An experimental set-up where infected and susceptible pairs (i.e. transmission pairs) of individually housed *Mustela putorius furo* (ferret) (n=8) were connected through a hard 15 cm air duct opening with multiple 90^0 turns. Airflow was directed upwards from the donor to indirect recipient animals. Air travelled an average of 118 cm (approx. 3 ft.) through the tube systems. Note: transmission of SARS-CoV-2 and SARS-CoV was investigated.	Indirect transmission of SARS-CoV-2 between two ferrets more than 1 meter away was confirmed in 50% separately housed transmission pairs. Infection in susceptible animals were confirmed through the detection of viral RNA in throat and nose swabs.
Kim (2020) [30] *In Vivo Study* Hong Kong* May 2020*	Experimental study of SARS-CoV-2 transmission via aerosols in Mesocricetus auratus (Syrian hamsters). Infected and susceptible and infected ferrets.	Efficient indirect transmission to have occurred among indirect contact ferrets.

*Estimated based on author affiliations and publication date.

Table 3: Experimental evidence confirming SARS-CoV-2 virus stability in aerosols (n=3)

Study	Method	Key outcomes
Dabisch (2020) [19]	Measured the viability and persistence of SARS-CoV-2 in	The time needed for a 90% decrease in infectious virus at

Simulation experiments US* Aug 2020* **new**	artificially generated aerosols within a drum aerosol chamber across a range of temperatures (10 °C, 20 °C, 30 °C and 40°C), relative humidity (20%, 45% and 70%), and simulated sunlight levels of zero integrated UVB irradiance (i.e., indoor/night/darkness), 0.9 W/m2 UVB irradiance and 1.9 W/m2 integrated UVB irradiance (i.e., summer midday sunlight). Viral concentration was held at mean value of 2.3 ± 0.4 $\log_{10}$ $TCID_{50}$/L-air during the experiment. Infectiousness of virus within aerosols was measure using micro titration assay and Vero cells. Experiment results informed a regression model predicting aerosolized SARS-CoV-2 decay under variable conditions.	40^0C, 20% relative humidity increased from 4.8 min with sunlight representative of noon on a clear summer day outdoors, to more than 2 hours under conditions representative of indoors or at night. Across other temperature and humidity levels the decay per minute in midday sun simulations ranged from. 38.1% ± 8.9% per minute at 40 °C and 20% relative humidity, to 18.9 ± 4.8% per minute at 10 °C and 20% relative humidity. For moderate sunlight representing spring and fall intensity at 40 °C, ,decay rates ranged from18from.0 ± 6.2% per minute at 30 °C and 45% relative humidity, to 11.1 ± 4.6% per minute at 10 °C and 20% relative humidity. In the absence of sunlight <2% decay (mean) per minute rate was estimated, for most tested temperature and humidity levels. Exceptions were in the higher temperature and/or humidity ranges, • Decay rate at 30 °C with 70% relative humidity was 6.3 ± 2.6% per minute. • Decay rate at 40 °C with 20% relative humidity was 3.9 ± 0.4%.

		In the regression analysis sunlight had the largest influence on decay followed by temperature and a sunlight x temperature interaction. Humidity (relative or absolute) had the lowest influence on the decay constant. Thus, more intense sunlight and high temperatures >30°C had faster decay rates.
Fears (2020) [31] Simulation experiments US* Sep 2020*	The long-term persistence of artificially generated viral aerosol suspensions of SARS-CoV-2 was measured at different time intervals. Viral contents were quantified by RT-PCR, and infectiousness of virus was measured by plague assay. Samples were qualitatively assessed by electron microscopy.	Infectious SARS-CoV-2 was detected at 10 minutes, 30 minutes, 2, 4, and 16 hours during the aerosol suspension stability experiment. A minimal reduction in viral genome copies in aerosol samples (as measured by RT-PCR) was noted for the measured time points. A minor but constant fraction of the SARS-CoV-2 virus in aerosols maintained replication-competence at all measured time points, including at 16 hours. Qualitative assessment of virion integrity revealed virions were either ovoid or spherical in shape, and maintained the expected morphologies up to 16 hours in aerosol suspension.
Van Doremalen (2020) [32] *LTE* Simulation experiment	In this experiment SARS-CoV-2 and SARS-CoV-1 virus titer stability and decay was measured from artificially generated aerosols, in Vero 6	SARS-CoV-2 virus remained viable in experimentally generated aerosols up to 3 hours (duration of the experiment), with a

US* Spring 2020*	cell culture. Analysis used a Bayesian regression model.	reduction in infectious titer from $10^{3.5}$ to $10^{2.7}$ $TCID_{50}$ per liter of air In aerosols the half life of SARS-CoV-2 virus was estimated to be 1.1-1.2 with a 95% credible interval of 0.64-2.64.

*Estimated based on author affiliations and publication date.

LTE= letter to the editor

Table 4: Biological monitoring studies on SARS-CoV-2 RNA in exhaled breath (n=6)

Study	Method	Key outcomes
Reporting SARS-CoV-2 in samples		
Ryan (2020) [34] Exhaled breath condensate monitoring study Ireland Apr - May 2020 **new**	Exhaled breath condensate samples were collected from COVID-19 patients using RTUBE condensers. The sample included nasopharyngeal swab positive (n=16) and nasopharyngeal swab negative with clinical diagnosis of COVID (n=15) patients. Additional samples from pre-SARS-CoV-2 were included as controls (n=14). Virus in samples was detected by RT-PCR using different viral gene assays. Note: Clinical diagnoses of COVID-19 were based on clinical expertise and imaging results.	93.5% (29/31) of the collected exhaled breath samples from SARS-CoV-2 patients (clinically confirmed and/or positive nasopharyngeal swab results) were positive by RT-PCR targeting all four genes (E, S, N, ORF1ab). All pre-pandemic control samples were negative. In this study exhaled breath was shown to be a sensitive and non-invasive sample type. Positivity of samples varied by RT-PCR assay target sequence among nasopharyngeal swab negative patients (n=15): • 66% (10/15) positivity for viral envelope (E)/ spike (S) proteins gene assays (used for the nasopharyngeal swab). • 73% (11/15) positivity for viral nucleocapsid (N)/open

		reading frame (ORF1ab) gene assays. The combined results equated to 14/15 clinically diagnosed cases positive on at least one assay.
Zhou (2021) [33] Exhaled breath condensate monitoring study China Feb - Mar 2020 **new** Note: Additional results on viral RNA in air samples are summarized in Table 5.	COVID-19 patients (n=10) about to be discharged from hospital (as per negative throat and nasal swabs) were recruited from multiple hospital sites. Exhaled breath condensate was sampled using using a BioScreen II device. SARS-CoV-2 RNA in breath samples was quantified using RT-PCR.	22.2% of 9 COVID-19 patients about to be discharged from hospital had SARS-CoV-2 in their exhaled breath samples at a concentration of ~10^5 RNA copies/m^3. Both patients were over the age of 70. It was estimated that some COVID-19 patients in the sample were exhaling the virus at a rate of ~1400 RNA copies per minute into the air at discharge.
Ma (2020) [35] *Preprint* Exhaled breath condensate monitoring study China Spring 2020* Note: Additional results on viral RNA in environmental air samples are summarized in Table 5.	Exhaled breath condensate samples were collected from COVID-19 patients (n=30) using a BioScreen device.	The study confirms the emission of SARS-CoV-2 virus RNA into the air from exhaled breath condensate of infected individuals (16.7% n=5/30). The positive samples were detected either <3 days from symptom onset (n=3) or within 7-14 days from symptom onset (n=2). SARS-CoV-2 levels in exhaled breath were estimated to reach 10^5-10^7 copies/m^3 if an average breathing rate of 12 L/min is assumed and is highest during early stages of infection.

Di Carlo (2021) [20] Exhaled air monitoring study Italy Apr - Jun 2020 Note: Additional results on viral RNA in environmental air samples are summarized in Table 5. **new**	Tests the release of SARS-CoV-2 RNA into the air during normal breathing – without coughing, sneezing or talking among infected cases (n=5). These cases were all in hospital patients admitted to hospital for non-COVID symptoms who later developed COVID symptoms during hospital stay. Sampled individuals were housed in airborne infection isolation rooms (AIIR) and exhaled breath samples were collected using Sartorius AirPort sampler. Patient oropharyngeal, nasopharyngeal and salivary swabs samples were also collected at the time of breath samples.	The days since symptom onset to sample collection varied from 7- 56 days in the patient sample. Oropharyngeal and nasopharyngeal swabs of 4 patients were positive at the time of sampling. Viral RNA was detected at 1 cm distance from patients' mouths in two patients (40% n=2/5). Both had positive oropharyngeal, nasopharyngeal and salivary swabs. Salivary swabs were negative in the patients with negative exhaled air samples who were reported to be infected based on consecutive positive swab samples. In one of these patients, wearing a surgical mask effectively blocked viral RNA detection at 1 cm (masked samples were not collected from the other patient with positive exhaled air samples). Air samples at 1 cm from RT-PCR negative patients were negative for viral RNA.
Feng (2020) [36] Exhaled air & exhaled breath condensate monitoring study China Feb - Mar 2020 Note: Additional results on viral RNA in environmental air	Sampled exhaled breath and environmental air of COVID-19 patients using a NIOSH bio-aerosol sampler. Exhaled breath condensate was sampled using a sterile laboratory-made collection system. Air samples were segregated by aerosol size. Samples were collected from COVID-19 patients in	SARS-CoV-2 RNA was not detected in any of the patients' expired breath samples (n=0/9). Viral RNA was isolated in exhaled breath condensate (25%; n=2/8), and bedside environmental air samples (8%; n=1/12). The authors attributed minimal contamination of viral RNA in study samples to reduced respiratory viral

samples are summarized in Table 5.	the later stages of infection in hospital settings.	shedding among patients in later stages of infection.
Reporting NO SARS-CoV-2 in samples		
Ding (2020) [37] *Preprint* Exhaled air and exhaled breath condensate monitoring study Hong Kong Feb 2020 Note: Additional results on viral RNA in environmental air samples are summarized in Table 5.	Exhaled breath condensate samples (n=2) and expired air samples (n=2) were collected from COVID-19 patients housed in airborne infection isolation rooms (AIIR). Multiple devices were used for air sample collection (n=27), which was conducted on different days. Note: sample collection distances from patient(s) are not reported.	All collected exhaled condensate samples and expired air samples were negative for SARS-CoV-2 RNA.

*Estimated based on author affiliations and publication date.

Table 5: Biological monitoring studies investigating SARS-CoV-2 within air in patient care settings (n=40)

Study	Method	Key outcomes
SARS-CoV-2 in cell culture samples		
Lednicky (2020) [40] Biological monitoring study US* Nov 2020*	Air samples were collected in triplicate from hospital rooms of COVID-19 patients in the absence of aerosol generating procedures. Air samples were collected using a VIVAS air sampler at 2 to 4.8 meters away from patients, with and without a HEPA filter on the	Viable (infectious) SARS-CoV-2 was found to be present in aerosols sampled from hospital patient rooms by RT-PCR and cell culture (via cytopathic effects)

	air sampler inlet tube. Virus in isolated air samples was measured using RT-PCR, and infectiousness was measured based on cytopathic effects in cell culture (LLC-MK2 and Vero-E6). The genomes of isolated virus was sequenced.	All air samples collected without a HEPA filter was positive for viral RNA. A single nearly complete virus sequence was isolated from the air samplers that collected environmental air. This genetic sequence matched the virus strain isolated from nasopharyngeal sample of one of the two patients who occupied the room during sampling. The matched person was diagnosed with acute infection at the time of air sampling.
Santarpia (2020) [41] *Preprint* Biological monitoring study US Apr 2020	Patient generated aerosols in hospital air samples were collected using a NIOSH BC251 aerosol sampler at the foot of COVID-19 patient beds. Aerosol sizes and concentration was concurrently measured during sample collection using an Aerodynamic Particle Sizer Spectrometer. Aerosols were distinguished by the proportion of different sizes (>4.1 µm, 1-4 µm, and <1 µm) among samples. Presence of the virus in isolated aerosols (<5µm) was measured using RT-PCR, western blot, and transmission electron microscopy and infectiousness of isolated	RNA was detected in all six patient rooms, and included all aerosol particle size fractions (defined as >4.1 µm, 1-4 µm, and <1 µm). Replicating virus in cell culture was observed in most <1 µm aerosol samples, two of the 1-4 µm size aerosol samples and two of the >4.1µm samples. Western blot and TEM analysis of these samples also showed evidence of viral proteins and intact virions. The authors conclude the infectious nature of the

	viral particles was examined using cell culture (Vero-E6). Note: The study does not specify if the patients were housed in regular wards or AIIR.	aerosols collected in this study suggests that aerosol transmission of COVID-19 is possible.
Santarpia (2020) [42] Biological monitoring study US Mar 2020	Air samples from negative pressure isolation spaces and wards housing COVID-19 cases were collected using a Sartorius Airport MD8 air sampler and tested for SARS-CoV-2 viral RNA by RT-PCR. A subset of positive samples were examined for viral propagation in Vero E6 cells. Several indicators were utilized to determine viral replication including cytopathic effect, immunofluorescent staining, time course PCR of cell culture supernatant, and electron microscopy.	63.2% of in-room air samples were positive by RT-PCR (mean concentration 2.42 copies/L of air). Two samples placed at different proximity to a patient, including a sample from <2 meters away the patient, were positive. Viral concentration was higher in the air sample collected closer to the patient (4.07 vs. 2.48 copies/L of air). 58.3% of air samples collected from hallways were positive (mean concentration of 2.51 copies/L of air). Some viral replication was observed in a single positive sample collected from a hallway.
SARS-CoV-2 RNA in samples		
Munoz-Price (2021) [24] Biological monitoring study US Spring 2021* **new** Note: Additional results on viral RNA in	Air samples (n=16) collected from hospital ICU inpatient rooms occupied by confirmed COVID-19 cases. Air samples were collected using the Sartorius MD8 airscan sampler, positioned at 0.3 -1.8 meters from patients' head. Viral RNA in samples was measured by RT-PCR.	12.5% (n=2/16) of hospital air samples were only positive for viral RNA. These samples where collected at 0.3 meters from confirmed cases. The patients had mild severity of illness and were not on supplemental oxygen at the time of samples collection.

environmental air from household settings are summarized in Table 6.		
Razzini (2020) [69] Biological monitoring study Italy Apr 2020* **new**	Air samples (n=5) collected from hospital areas with COVID-19 cases using a MD8 Airport Portable Air Sampler with gelatin membrane filters. Samples were collected from three zones, contaminated (corridor for patients and ICU), semi-contaminated (undressing room) and clean areas (medical staff dressing and locker areas). Viral RNA in samples was measured by RT-PCR.	All the air samples collected from COVID-19 cases' ICU and corridor were positive for viral RNA; samples from other areas were negative. Viral concentration in (mean Ct) across ICU air samples was 22.6, and 31.1 for corridor air samples.
Ge (2020) [70] Biological monitoring study China Jun 2020* **new**	Air samples (n=33) were collected using the NIOSH bioaerosol sampler BC251. Sampled settings were ICU, hemodialysis clinics, fever clinics, and respiratory wards. Viral RNA in samples was measured by RT-PCR.	All air samples (n=3) from the ICU setting were positive for viral RNA. Viral concentrations in samples ranged from Ct 36.5 - 37.8.
Hu (2020) [71] Biological monitoring study Feb – Mar 2020 China **new**	Air (aerosol) samples were collected from multiple hospital sites using a centrifugal aerosol-to-hydrosol sampler (n=123). Viral RNA in samples was measured by RT-PCR and viability by Vero-E6 cell culture.	Eight air samples (21%) from ICU environments and one same from (16%) from the CT (computerized tomography) room were positive for viral RNA. The range of virus concentrations in the positive air (aerosol) samples was 1.11 × 10^3 to 1.12 × 10^4 RNA copies

		m^{-3}. The virus could not be cultured from positive samples.
Seyyed (2020) [72] Biological monitoring study Iran May 2020* **new**	Air samples (n=10) were collected from COVID-19 patient ICU wards using a sampling pump with a porous midget impeller. Viral RNA in samples was measured by RT-PCR.	60% of air samples were positive for viral RNA. The highest RNA concentrations were measured in samples from between patient beds (3913 copies per ml). These were air samples collected at 1.5 to 2 meters from patient beds.
Moore (2020) [44] Biological monitoring study England Mar –May 2020 **new**	Air samples (n=55) were collected from COVID-19 patients with and without respiratory symptoms, from eight different hospitals. Sampled settings included AIIR, general COVID-19 wards and non-COVID-19 wards. Samples were collected using a Coriolis µ air sampler and/or Sartorius MD8 air sampler. Viral RNA in samples was measured by RT-PCR, and viability by Vero-E6 cell culture.	7% (n=4) of air samples were positive for viral RNA, at low concentrations from <10 to 460 genomic copies/m^3 air. The virus could not be cultured from positive samples. All positive samples were collected at 1 meter distance from patients, and days since symptom onset among the patients ranged from 8-10 days.
Hernández López (2021) [44] Biological monitoring study Jan 2021* Mexico **new**	Air samples (n=15) from emergency, internal medicine and COVID-19 patient rooms and multiple bed patient care rooms in two hospitals were collected using a Millipore sampler. Viral RNA in samples was measured by RT-PCR.	Three air samples (20%) were positive for viral RNA. All positive samples were from the COVID-19 patient room and near the patient.
Tan (2020) [73]	Air samples (n=12) were collected from ICU and isolation wards of	A single air sample from a patient care area, collected

Biological monitoring study China Mar 2020 **new**	COVID-19 cases in hospital, and corridors. Air samples were collected at 1 meter from a patient's head.	when the patient was undergoing intubation, was positive for viral RNA.
Gehrke (2021) 45 *Preprint* Biological monitoring study Germany Oct 2020 - Jan 2021 **new** Note: Additional results on viral RNA in community setting air samples are summarized in Table 6.	Air samples were collected from patient rooms in two COVID-19 isolation units, and an outpatient endoscopy facility using non-powered cold traps. Viral RNA was quantified by RT-PCR.	Hospital setting samples: No viral RNA was isolated from the first isolation unit ventilated permanently by two windows. Viral RNA was detected in samples collected from a non-ventilated corridor next to the isolation room. In the second isolation unit samples, SARS-CoV-2 RNA levels reached concentrations of 10^5/mL in non-ventilated rooms, but when windows were open to increase ventilation SARS-CoV-2 RNA concentrations in samples were reduced to 10^4/mL or less. In the endoscopy facility, 50% of cold traps were positive for viral RNA (n=6/12). 57% of cold traps (n=4/7) from endoscopy operation rooms were positive for SARS-CoV-2, but RNA concentrations (initially at 12 copies/ml) and the number of positive samples were reduced when the ventilation levels in the room was increased.

Zhou (2021) [31] Biological monitoring study China Feb- Mar 2020 **new** Note: Additional results on viral RNA in exhaled breath samples are summarized in Table 4.	Air samples (and exhaled breath samples) of hospitalized COVID-19 patients were collected from multiple hospital sites. Air samples were collected from hospital corridors, waste storage rooms, ICU rooms, toilets, medical preparation rooms, clinical observation rooms, and general wards. Air samples were collected using the Air-nCoV-Watch (ACW) system and viral RNA quantified by RT-PCR.	6.8% of the air samples (n =3/ 44) were positive for viral RNA at digital PCR concentration levels ranging from 9-219 viruses/m^3.
Yarahmadi (2021) [47] Biological monitoring study Iran Feb 2021* **new**	Air samples were collected (n=20) from an ICU ward at, 1) a COVID-19 patient breathing zone (i.e., side table next to patient's head), general area (10 meters from the ICU unit), and breathing zone of health care personnel near the COVID-19 patient's bed, and 1 meter from the patient's bed. Samples were collected using NIOSH and ASHRAE bioaerosol samplers. Viral RNA was detected by RT-PCR.	50% (n=2/4) of samples from the patient breathing zone were positive for viral RNA. The positive samples were from a confirmed case, and negative samples were from a suspected case. 12.5% (n=1/8) of samples from health care personnel breathing zone were positive for viral RNA. 12.5% (n=1/8) of samples from the general area were positive for viral RNA. Authors suggest bioaerosols maybe present due to the re-aerosolization of previously airborne SARS-CoV-2 particles from health care personnel walking between different wards and stations of the ICU.

Ong (2021) [46] Biological monitoring study Singapore Jan 2021*	Air samples were collected from AIIR in a hospital and community isolation facility (naturally ventilated) housing confirmed COVID-19 patients, at 1 meter away from the patient. Air samples were collected using a BioSpot-VIVAS BSS300-P bioaerosol sampler (collecting particles <4.34 µm in size). NIOSH aerosol samplers were used to validate the Biospot results. SARS-CoV-2 RNA was detected by RT-PCR, and cultured using Vero C1008 cell culture.	50% of BioSpot air samples from hospital AIIR (n=6/12) were positive for SARS-CoV-2 RNA (concentrations ranging from 178.9 to 2,738.4 copies/m^3). Positive samples were collected from rooms with at least one symptomatic patient (<7 days post symptom onset). NIOSH aerosol samplers detected SARS-CoV-2 RNA in aerosols <1 µm, 1–4 µm, and >4 µm in diameter. Only 1 of 9 samples from the community isolation facility was positive for SARS-CoV-2 RNA, with a concentration of 978.3 copies/m^3. Virus cultures of all positive samples were negative.
Dumont-Leblond (2020) [22] Biological monitoring study Canada Mar-June 2020 **new**	Sampled air from AIIR rooms from a Quebec hospital housing non-severe COVID-19 patients (n=22). Air samples were collected (with 3 µm gelatine filters and 0.8 µm polycarbonate filters) using a SASS 3100 dry sampler, at 1.5m away from the patient bedside. Viral contamination was measured by RT-PCR and Vero E6 cell culture.	11% (n=11/100) of air samples collected at the bedside of 6 patients were positive for viral RNA. These patients had higher frequency of fever, dyspnoea, and cough compared to others in the sample. Most were males and had a slightly longer duration of hospitalization. Average SARS-CoV-2 viral load in the positive sample rooms was estimated at 4.86E+4 virus genomes per hour. No virus isolated from air samples could be cultured.

Binder (2020) [74] Biological monitoring study US Apr-May 2020 **new**	Environmental swabs and aerosol samples were collected from single patient hospital rooms housing COVID-19 patients (n=20, 16 symptomatic and 4 asymptomatic) within a dedicated COVID-19 ward. Bioaerosols were collected using a NIOSH BC 251 sampler placed at different distanced from the patients' heads (1, 1.4, 2.2, and 3.2 meters). Empty hospital rooms, hallways, staff break room and staff workstations were sampled as controls. SARS-CoV-2 viral contamination was measured by RT-PCR, and Vero EC6 cell culture.	Three samples collected from three of the COVID-19 patient rooms were positive for viral RNA; samples were collected at 1.4-2.2 meters from the patients. These patients were 4-10 days post symptom onset (runny nose, headache, fever, cough, difficulty breathing, fatigue, loss of smell, gastrointestinal symptoms). Viable virus could not be identified in bio aerosols samples via cell culture. Samples from other area were negative for SARS-CoV-2 RNA.
Passos (2021) [75] Biological monitoring study May-Aug 2020 **new**	Air samples were collected from two different hospital environments (n=52) using active and passive aerosol sampling methods. Active sampling used multiple portable/hand-held air sampler models (AIRIDEAL 3P, MD-8 AirPort, HANDI-VOL) and filter types (cellulose, PTFE or quartz microfiber filters). The passive method used petri dishes for the collection of sedimentable particles. Both hospitals housed COVID-19 patients in dedicated ICU wards at the time of sample collection. Aerosol samples were also collected from multiple outdoor public spaces using high-	Air samples from four areas sampled in one hospital (at 100% ICU occupancy at the time of sampling) were positive for viral RNA in suspended particles (filter pore size >0.2 µm and >0.3 µm), as well as 11% of sedimentable particles (n=4/36). Positive suspended air particle samples were detected from inside the COVID-19 ICU ward (concentration of 0.33 genomic units m^{-3} @ distance >2 from patient), in a protective apparel removal room (0.14 genomic units m^{-3}), storage room for patient mobile toilets and soiled patient linens (0.19

	volume air samplers (AGV, Energética). SARS-CoV-2 RNA in samples was detected by RT-PCR.	genomic units m^{-3}). The storage room was open to natural ventilation. SARS-CoV-2 RNA positive sedimentable particles were detected in samples collected from an external corridor adjacent to the COVID-19 dedicated ICU. All outdoor air samples and samples collected at the other hospital (i.e., Hospital 1) l (at 33% occupancy at the time of sampling) were negative for SARS-CoV-2 RNA.
Lane (2021) [48] Biological monitoring study Jan 2021* **new**	Air samples were collected from multiple hospital units, AIIR, ICU nursing stations, family/visitor corridors outside of ICUs, medical unit, and patient room hallways. NIOSH 251 2-stage cyclone samplers collected air samples (limit of detection of 8 viral copies/m^3 of air). Two patient rooms housing COVID-19 patients were used as positive control samples. Viral RNA was detected by RT-PCR.	The two positive control samples collected from two confirmed COVID-19 patient rooms were positive for viral RNA. None of the air samples (n=528) from other sampling area were positive for SARS-CoV-2 RNA.
Liu (2020) [76] Biological monitoring study China Feb-Mar 2020	SARS-CoV-2 RNA concentration and aerosol size distributions in air samples (n=30) from multiple sites within or near a hospital and field hospital. All aerosol samples (n=30) were collected on pre-sterilized gelatin filters (Sartorius). Three size-	SARS-CoV-2 contamination in air samples was low to undetectable. In the field hospital setting, the greatest suspended SARS-CoV-2 RNA in aerosols was identified in a temporary patient toilet room (1 m^2 area)

	segregated aerosol samples were collected using a miniature cascade impactor (all sampled from staff areas). Viral RNA was detected by RT-PCR.	with low ventilation, likely from the patient breathing or aerosolization of virus from feces and urine of infected patients. Samples from the field hospital staff personal rooms demonstrated the greatest virus concentrations. Aerosols from 0.25 to > 2.5 µm were identified. The authors hypothesize this came from healthcare worker PPE surfaces and apparel. Low but detectable viral RNA concentrations were found at a department store entrance and an outdoor site near the hospital suggesting this may have occurred due to high traffic flow and crowding. Note: The specific concentrations of airborne SARS-CoV-2 in each aerosol sample by site are provided in the publication.
Chia (2020) [76] Biological monitoring study Singapore Spring 2020*	Detection of air contamination by SARS-CoV-2 in airborne infection isolation rooms (AIIR) housing COVID-19 patients, in hospital settings. Air samples were collected, and aerosol sizes were measured by NIOSH BC 251 bio-aerosol samplers. Viral RNA was detected by PCR.	66% (n=2/3) of the air samples collected from AIIR environments were SARS-CoV-2 RNA positive. The smallest aerodynamic size fraction that contained detectable levels of SARS-CoV-2 RNA was 1–4 µm. Total SARS-CoV-2 concentrations in air ranged

		from 1.84×10^3 to 3.38×10^3 RNA copies per m^3 air sampled. The authors suggest the presence of SARS-CoV-2 in the sampled air is likely highest during the first week of illness, when respiratory viral load is high.
Jin (2021) [78] Biological monitoring study China Feb-Mar 2020 **new**	Air samples were collected from an ICU housing a single ready-for-discharge COVID-19 patient, 2 days after the patient tested negative for SARS-CoV-2. Air samples were collected from the isolation room and staff PPE dressing room. High-volume air samples were collected using a WA 400 Portable viral aerosol sampler. Viral RNA in samples was detected using RT-PCR.	A single air sample from the ICU ward isolation room was positive (1/7, 14.29%) for viral RNA. The authors state their findings suggest the virus may be shed via aerosol for days, even after a patient has tested negative.
Zhou (2020) [79] Biological monitoring study UK Apr 2020	Three to five air samples were collected from multiple hospital environments using a Coriolis air sampler, viral RNA was measured by RT-PCR, Vero E6 and Caco2 cells cultures were used to culture virus.	38.7% (n=14/31) of the collected air samples were positive for viral RNA, but SARS-CoV-2 virus could not be cultured due to low recovered viral loads. The odds of contamination in public areas was lower than areas immediately occupied by a COVID-19 patient (OR 0.5 95% CI 0.2-0.9).
Orenes-Piñero (2020) [80] Biological monitoring study	Investigators develop and apply "COVID traps" to measure the capacity of SARS-CoV-2 aerosol transmission in hospital patient	In the ICU, none of the "COVID traps" were positive for COVID-19; all COVID-19 patients were intubated. In the ward setting,

Spain Spring 2020*	care settings. "COVID traps" were placed 1 meter away from patients in ICU and ward settings. Viral RNA was detected by RT-PCR.	two "COVID traps" were positive for SARS-CoV-2, both were near a patient requiring the use of respiratory assistance. The authors conclude it was unequivocally the result of virus transmission in air.
Feng (2020) [36] Biological monitoring study China Feb-Mar 2020 Note: Additional results on viral RNA in exhaled breath samples are summarized in Table 4.	Environmental air from the rooms of recovering COVID-19 patients in isolation hospital wards and ICU were sampled using a NIOSH sampler. Air samples (n=12) were collected and aerosol size measured. Samplers were also placed on a tripod 1.2 m in height and 0.2 m away from the bed at the side of the patient's head for 30 minutes.	SARS-CoV-2 RNA was detected in a single air sample from SARS-CoV-2 patients. The maximum viral RNA concentrations detected in the positive air sample by particle size was 1112 copies/m^3 (<1 µm) and 745 copies/m^3 (>4 µm). The authors attribute minimal contamination of viral RNA in study samples to reduced respiratory viral shedding among patients in later stages of infection.
Ding (2020) [37] *Preprint* Biological monitoring study Hong Kong Feb 2020 Note: Additional results on viral RNA in exhaled breath samples are summarized in Table 4.	Air samples (n=46) were collected from airborne infection isolation rooms (AIIR) housing COVID-19 patients, nursing stations, corridor and air-conditioning units at a hospital treating COVID-19 cases. Multiple air samplers were used for sample collection, which was conducted on different days, and RNA was detected by RT-PCR.	A single air sample (n=1/46) from the corridor outside a storage room with a medical waste bin was weakly positive for SARS-CoV-2 RNA. All other tested air samples from patient rooms, washrooms, and air supply inlets were negative. RNA copies for the weakly positive sample was not quantified.

Guo (2020) [81] Biological monitoring study China Feb-Mar 2020	Air samples were collected from hospital ICU (n=40) and general wards housing (n=6) COVID-19 patients, at different distances from patients and the doctors office (n=8). Air samples were collected using a SASS 2300 Wetted Wall Cyclone Sampler.	SARS-CoV-2 virus particles were identified in 35% (n=14/40) of ICU air samples, 12.5% (n=2/16) of general ward air samples. SARS-CoV-2 aerosol was detected in 35.7% (n=5/14) of samples near air outlets, 44.4% (n=8/18) of samples in patients' rooms, and 12.5% (n=1/8) in the doctors' office area. No SARS-CoV-2 virus were identified in patient corridor air samples. Based on site(s) of positive air sample collection authors conclude virus-laden aerosols to concentrate near and downstream from patients, and the maximum transmission distance of virus laden aerosols to be 4 meters.
Nissen (2020) [82] Biological monitoring study - Hospital setting Sweden Spring 2020*	Open liquid containing petri dishes were placed at air entrances to ward rooms and near exhaust filters of a hospital's ventilation system for 24 hrs to collect viable virus. Infectivity was assessed using Vero E6 cell culture.	SARS-CoV-2 RNA was detected in fluid samples placed in the ventilation system, and in 33% of samples (n=1/3) placed near air entrances of wards. Viability of the isolated virus could not be established by cell culture.
Zhang (2020) [83] *Preprint* Biological monitoring study - Hospital setting China	The study sampled outdoor environment aerosols (n=16) at three hospitals receiving COVID-19 patients. Aerosol samples were collected using bioaerosol	SARS-CoV-2 virus was identified within sampled aerosols at 285-1,130 copies/m^3 concentrations, similar to contamination levels observed in ICU units.

Mar-Apr 2020	samplers WA-15. Viral RNA was quantified by RT-PCR.	Viral RNA was identified up to 5 meters away from outpatient buildings (n=1/2), as well as in hospital waste water treatment areas (n=1/3).
No SARS-CoV-2 RNA in samples		
Dumont-Leblond (2021) [22] Biological monitoring study Canada Spring 2020 **new**	Air and no-touch surfaces of 31 rooms from multiple long-term care facilities (n=7) in Quebec were sampled. The air samples were collected 8 to 30 days after the residents' symptom onset using an IOM Multidust sampler attached to a portable pump Gillian Air 5, placed ~2m from SARS-CoV-2 positive residents. The sampled rooms did not have significant aerosol mitigation measures in place. SARS-CoV-2 RNA was quantified by RT-PCR, and cultured by Vero E6 cells. Note: the distance of surfaces from SARS-CoV-2 patients was not provided.	All air samples (n=31) were negative, but viral RNA was recovered from 32% (n= 20/62) no-touch surface samples of door frames and shelving units (concentrations range 13-36,612 genomes/surface). Authors suggested the viral load recovered from the no touch surfaces without direct contact to be indicative of viruses spread through air. Virus could not be cultured from positive samples.
Song (2020) [84] Biological monitoring study China Feb 2020 **new**	Air samples were collected from Airborne infection isolation rooms (AIIR) housing COVID-19 cases. Air samples were collected using the automatic sampling system Derenda PNS 16T-3.1, at approximately 1 meter from patient beds. Viral RNA in samples was measured by RT-PCR.	All of the samples were negative for SARS-CoV-2 RNA.
Faridi (2020) [85]	Air samples (n=10) were collected from COVID-19 patient wards	All of the samples were negative for SARS-CoV-2 RNA.

Biological monitoring study Iran Mar 2020 **new**	housing individuals with server and critical symptoms. Air samples were collected into the sterile standard midget impingers using a vacuum pump. Air samplers placed 1.5 to 1.8 m above the floor and approximately 2 to 5 m away from patients' beds. Some patients coughed during the sample collection. Viral RNA in samples was measured by RT-PCR.	
Declementi (2020) [86] Biological monitoring study Italy Sept 2020* **new**	Air samples (n=8) were collected from a COVID-19 non-Intensive Care Unit, following cleaning and disinfection processes. Viral RNA in samples was measured by RT-PCR. Note: The sampler model, the presence of COVID-19 cases during sampling nor the sampling distance from cases are provided.	All of the samples were negative for SARS-CoV-2 RNA.
Anh (2020) [87] Biological monitoring study Korea Mar 2020 **new**	Air samples were collected from the isolation rooms of severe COVID-19 patients requiring mechanical ventilation or high-flow oxygen therapy. Samples were collected using a SKC BioSampler and swab sampler. Viral RNA was measured by RT-PCR.	All of the samples were negative for SARS-CoV-2 RNA.
Masoumbeigi (2020) [88] Biological monitoring study Iran	Air sampling (n=31) was conducted in a hospital with COVID-19 patients; all of the patients had a severe form of cough and sneezing. Air samples	All of the samples were negative for SARS-CoV-2 RNA.

Sept 2020* **new**	were collected from Emergency wards, ICU, CT-SCAN, and laundry unit. Air sampling was performed by all-glass impinger (AGI) at ~0.5-4 m away from patients' beds. Viral RNA was detected by RT-PCR.	
Alsved (2020) [89] Biological monitoring study Sweden* Spring 2020*	SARS-CoV-2 RNA measured from COVID-19 cases (n=2) within 2 days of symptom onset. Air samples were collected 0.8 meters away from the case, as the individual was talking or singing. The measurements were carried out in an experimental airtight chamber with human volunteers.	Air samples collected within 0.8 meters of COVID-19 cases were negative for viral RNA. Viral loads in subject airways at the time of the experiment could not be obtained. Authors state qPCR Ct values of 22–25 to have been reported in clinical reports for the subjects within 24hrs of the experiment.
Cheng (2020) [90] Biological monitoring study China Jan-Apr 2020	Air samples were collected within 10 cm of asymptomatic and symptomatic COVID-19 patients (n=6) with and without surgical masks in AIIR were tested for SARS-CoV-2 contamination. Viral loads in respiratory patient fluid samples were also tested by having patients sneeze and spit into gelatin filters within air samplers. Viral loads were measured using assays (not specified) and RT-PCR.	No virus was detected in air samples from rooms with both surgical masked and non-masked patients. Except for one patient who had a respiratory fluid viral load of 2.54×10^4 copies/ml, all other patients' samples from sneezing were negative for virus RNA. Authors suggest aerosol transmission is not the predominate mode of infection transmission in the sampled settings. Appropriate PPE use, environmental disinfection, and single occupancy within AIIR are provided as reasons for observed results.

Kim (2020) [91] Biological monitoring study South Korea Mar-Apr 2020	Air samples (n=52) were collected 2 meters away from COVID-19 patients (n=8), before admission, and on hospital days 3, 5, and 7 using a MD8 Airport Portable Air Sampler. Some patients were housed in negative pressure rooms (e.g. AIIR). RNA was measured by RT-PCR.	All collected air samples were negative for viral RNA.
Ong (2020) [92] Biological monitoring study Singapore Jan-Feb 2020	Air samples were collected from COVID-19 patients (n=3) in AIIR at a dedicated SARS-CoV-2 outbreak center between day 4 and day 11 from symptom onset using SKC Universal pumps a Sartorius MD8 microbiological sampler. RNA was measured using RT-PCR.	No air samples were positive for SARS-CoV-2 viral RNA.
Ma (2020) [35] *Preprint* Biological monitoring study China Spring 2020* Note: Additional results on viral RNA in exhaled breath samples and community setting air samples are summarized in Table 4 and Table 6.	Air samples (n=26) were collected from hospital settings and unventilated quarantine hotel rooms of cases using a robot. RNA was detected by RT-PCR.	No air samples from hospital settings were positive for SARS-CoV-2 viral RNA.

*Estimated based on author affiliations and publication date.

Table 6: Biological monitoring studies investigating SARS-CoV-2 within air in community settings (n=9)

Study	Method	Key outcomes
SARS-CoV-2 in cell culture samples		
Lednicky (2021) [40] *Preprint* Biological monitoring study US Jan 2021* **new**	Air samples were collected from a car driven by a COVID-19 patient (mildly symptomatic, no cough) using a Sioutas personal cascade impactor sampler at multiple sampling stages of aerosol sizes (PCIS); the sampler was attached to the sun-visor on the passenger side of the car and approximately 3 feet from the subject's face. The air conditioner in the car was on during the 15 minute long drive. SARS-CoV-2 RNA was detected by RT-PCR, virus viability measured by Vero E6 cell culture, as well as genome sequencing.	SARS-CoV-2 viral RNA was detectable at all PCIS aerosol size sample stages, at concentrations ranging from 1.24E+03 to 3.14E+04 genome/ m^3 of air. These findings suggest virions are present in different sized respiratory secretions. SARS-CoV-2 was cultured from the sampling stages collecting particles in the 0.25 to 0.50µm size range.
SARS-CoV-2 RNA in samples		
Munoz-Price (2021) [24] Biological monitoring study US Spring 2021* **new** Note: Additional results on viral RNA in environmental air from patient care settings are summarized in Table 5.	Air samples (n=9) collected from households occupied by confirmed COVID-19 cases. Air samples were collected using the Sartorius MD8 airscan sampler, positioned at 0.3 -1.8 meters from patients' head. Viral RNA in samples was measured by RT-PCR.	55% (n=5/9) of air samples, collected from three different households, were positive for viral RNA. The odds of positive air samples in household settings, when compared to hospital settings, werewas estimated to be 8.75 [95% CI 1.21–63.43; P = .058]. The median number of days from the last positive SARS-CoV-2 test to the day of air sampling was 3 (range, 2.5–5).

		Of the households with positive air samples, one had A/C running and all three had opened windows or doors immediately prior to air sampling. Anecdotally, most households felt warm and humid at the time of sample collection.
Gehrke (2021) [46] *Preprint* Biological monitoring study Germany Oct 2020 - Jan 2021 **new** Note: Additional results on viral RNA in hospital settings air samples are summarized in Table 5.	Air samples were collected from a concert hall and a shopping mall for 6 hours during operation using non-powered cold traps. Viral RNA was quantified by RT-PCR in an area with high community transmission in an effort to identify potential hotspots for transmission.	Indoor COVID-19 hotspots were found in non-ventilated areas and in zones that are predisposed to a buoyancy (chimney) effect. In the concert hall, 1/10 cold traps were positive for viral RNA. In the shopping mall, 35% of traps were positive for viral RNA (n=5/14) located on the ground floor, 2nd and 3rd kiosk area as well as around the escalators. The 4th floor fashion section that usually has 25% capacity was negative. The highest viral concentration (2.7-5.4 x10^3 copies/mL) was found on the ground level between the escalators. However, follow-up measurements where cold traps were placed directly under 2 inflows and 2 outflows on the 3rd floor (to exclude any contamination of the

		central ventilation system) were negative. Samples taken on Friday when a high volume of patrons were twice as high as those taken on Monday.
Moreno (2021) [52] Biological monitoring study Spain May- Jul 2020 **new**	Air samples were collected from subway trains and buses during operation. Ambient air ($PM_{2.5}$) was sampled using Teflon filters with PEM (Personal Environmental Monitor) equipment, A/C filters were also sampled. Viral RNA in samples was measured by RT-PCR.	2/6 subway train air samples were SARS-CoV-2 positive and none of the air conditioner filter samples were positive. 1/6 bus air samples and 4/9 air-conditioning filters were positive. Cleaning and disinfection results indicated this process was successful at decontamination.
de Rooij (2021) [49] *Preprint* Biological monitoring study Netherlands Apr-Jun 2020 **new**	Air samples were collected from mink farms with ongoing COVID-19 outbreaks (3 farms at late stage of outbreak, 1 at early stage of outbreak). Samples were collected from indoor farm environments, outdoors at the farm premises, and nearby residential sites. Air samples were collected using teflon filters, and in parallel with particulate size fraction PM_{10} (<10µm) and inhalable dust (<100µm) collection. Personal air samples from the investigation field staff were also collected. Viral	Based on sample contamination the investigators conclude mink farms to be highly contaminated with viral RNA inside but the dispersion of virus outdoors and to nearby areas to be negligible. Farm in acute stage of outbreak: • SARS-CoV-2 RNA was detected in 62.5% (n=5/8) of the inhalable dust samples and 50% (n=4/8) of PM_{10} samples collected

	contamination in air samples was measured by RT-PCR.	inside the farm. All (n=2) personal air samples were positive for viral RNA. • SARS-CoV-2 RNA was also detected in outdoor samples at 1.5-10 meters from the farm's open entrance, but samples collected at 20m from this entrance were negative. Farms in late stage of outbreak: • Viral RNA was detected in 9.8% (n=4/41) of inhalable dust samples, at ~4x103 copies/m3. SARS-CoV-2 RNA was not detected in the inhalable dust samples collected outside the mink houses (n=9) or from the PM10 samples collected inside the farm (n=9) or near the mink houses (n=9). One of the personal air samples was positive for SARS-CoV-2 RNA. • All of air samples from residential sites were negative for SARS-CoV-2 RNA.
Ma (2020) [35]	Air samples (n=26) were collected from hospital settings and	A single positive air sample (3.8%) was identified in an

Preprint Biological monitoring study China Spring 2020* Note: Additional results on viral RNA in exhaled breath samples and hospital settings air samples are summarized in Table 4 and Table 5.	unventilated quarantine hotel rooms of cases using a robot. RNA was detected by RT-PCR.	unventilated quarantine hotel toilet room.
No SARS-CoV-2 RNA in samples		
Wong (2020) [50] *Preprint* Biological monitoring study Singapore Feb-Mar 2020 **new**	Air samples (n=6) were collected from accommodation rooms, corridors, toilets and elevators used by COVID-19 cases in a community settings (not specified). Viral RNA in samples was measured by RT-PCR.	All air samples were negative for viral RNA.
Döhal (2020) [51] *Preprint* Biological monitoring study Germany Mar 2020 **new**	Environmental air from 21 quarantine households (n=15) were collected by cyclone sampling using a Coriolis 154 Micro Air sampler. Viral RNA in samples was measured by RT-PCR and Vero-E6 cell culture. Note: At least one person in each included household was positive for SARS-CoV-2 at the time of sample collection.	All air samples were negative for viral RNA.

Di Carlo (2020) [93] Biological monitoring study Italy May 2020 **new**	Samples were collected on a city bus in a town which had a high number of COVID-19 cases, during the last week of lockdown and first week of gradual opening (approx. 1100 passengers used the bus during the study period). Air was sampled using microbiological gelatin membrane sample filters of 80 mm. Viral RNA in samples was measured by RT-PCR.	No air samples (or surface samples) were positive for SARS-CoV-2. Public health measures in place included hand sanitized upon entry to the bus, mandatory masks and keeping the bus windows open. Authors state these precautions prevented SARS-CoV-2 circulation and detection in the investigation.

*Estimated based on author affiliations and publication date.

Table 7: SARS-CoV-2 viral load in respiratory particles (n=1)

Study	Method	Key outcomes
Chen (2020) [39] Systematic Review informed *in-silico* analysis Canada* Aug 2020	A systematic review and meta-analysis were conducted (Aug 2020) to developed a dataset and summarize data on SARS-CoV-2 respiratory viral load (rVL). A model was developed to estimate the likelihood of respiratory droplets and aerosols containing viable virus assuming different viral load estimates, and different activities.	The meta-analysis showed there was a large degree of heterogeneity in viral loads across individuals, studies, and stages of infection. This suggests intrinsic virological factors mediate the over dispersion seen in the pandemic. Many cases present minimal transmission risk, whereas highly infectious individuals were estimated to shed 9.84 (95% CI 9.17-10.56,) $\log_{10}$ SARS-CoV-2 virions/ml via droplets and aerosols while breathing, talking and singing. The model estimates coughing increased the contagiousness of symptomatic cases. The likelihood of viable

		virus in respiratory aerosols at peak viral load was estimated to be ≤61.1% (95% CI: 51.8-70.4%) for the most infectious cases, and ≤ 0.69% (95% CI: 0.43-0.95%) for cases with mean viral load.

*Estimated based on author affiliations and publication date.

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