Viral emissions into the air and environment after SARS-CoV-2 human challenge: a phase 1, open label, first-in-human study

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Summary

Background Effectively implementing strategies to curb SARS-CoV-2 transmission requires understanding who is contagious and when. Although viral load on upper respiratory swabs has commonly been used to infer contagiousness, measuring viral emissions might be more accurate to indicate the chance of onward transmission and identify likely routes. We aimed to correlate viral emissions, viral load in the upper respiratory tract, and symptoms, longitudinally, in participants who were experimentally infected with SARS-CoV-2.

Methods In this phase 1, open label, first-in-human SARS-CoV-2 experimental infection study at quarantine unit at the Royal Free London NHS Foundation Trust, London, UK, healthy adults aged 18–30 years who were unvaccinated for SARS-CoV-2, not previously known to have been infected with SARS-CoV-2, and seronegative at screening were recruited. Participants were inoculated with 10 50% tissue culture infectious dose of pre-alpha wild-type SARS-CoV-2 (Asp614Gly) by intranasal drops and remained in individual negative pressure rooms for a minimum of 14 days. Nose and throat swabs were collected daily. Emissions were collected daily from the air (using a Coriolis µ air sampler and directly into facemasks) and the surrounding environment (via surface and hand swabs). All samples were collected by researchers, and tested by using PCR, plaque assay, or lateral flow antigen test. Symptom scores were collected using self-reported symptom diaries three times daily. The study is registered with ClinicalTrials.gov, NCT04865237.

Findings Between March 6 and July 8, 2021, 36 participants (ten female and 26 male) were recruited and 18 (53%) of 34 participants became infected, resulting in protracted high viral loads in the nose and throat following a short incubation period, with mild-to-moderate symptoms. Two participants were excluded from the per-protocol analysis owing to seroconversion between screening and inoculation, identified post hoc. Viral RNA was detected in 63 (25%) of 252 Coriolis air samples from 16 participants, 109 (43%) of 252 mask samples from 17 participants, 67 (27%) of 252 hand swabs from 16 participants, and 371 (29%) of 1260 surface swabs from 18 participants. Viable SARS-CoV-2 was collected from breath captured in 16 masks and from 13 surfaces, including four small frequently touched surfaces and nine larger surfaces where airborne virus could deposit. Viral emissions correlated more strongly with viral load in nasal swabs than throat swabs. Two individuals emitted 86% of airborne virus, and the majority of airborne virus collected was released on 3 days. Individuals who reported the highest total symptom scores were not those who emitted most virus. Very few emissions occurred before the first reported symptom (7%) and hardly any before the first positive lateral flow antigen test (2%).

Interpretation After controlled experimental inoculation, the timing, extent, and routes of viral emissions was heterogeneous. We observed that a minority of participants were high airborne virus emitters, giving support to the notion of superspreading individuals or events. Our data implicates the nose as the most important source of emissions. Frequent self-testing coupled with isolation upon awareness of first symptoms could reduce onward transmissions.

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Introduction

Currently available vaccines for COVID-19 are highly effective at reducing disease severity, but have limited ability to prevent transmission, particularly of antigenically diverged variants such as omicron. A key strategy for COVID-19 control and future pandemic preparedness is to reduce transmission. To date, reducing transmission has involved non-pharmaceutical interventions such as mask wearing or social distancing; however, considerable effort is now being put into novel vaccines and antiviral agents that aim to block transmission.[1] Understanding when infected people are contagious and how to detect, or

Research in context

Evidence before this study

Understanding when SARS-CoV-2 infected people are contagious to others and how to detect periods of contagiousness is vital for public health strategies to curb transmission. Measuring virus emitted by infected persons could be an indicator of contagiousness and help to identify probable routes. We searched PubMed for articles published between database inception and Feb 20, 2023, using terms "SARS-CoV-2, transmission, infectious or contagious, daily or longitudinal". No language restrictions were applied to this search. Viral load in upper respiratory tract (URT) swabs has been commonly used to infer contagiousness. In most studies, participants were recruited after symptomatic testing, thereby missing virus shedding in the presymptomatic (incubation) period. Three longitudinal cohort studies involving prospective surveillance (in the community, a university, and elite athletes) performed serial URT sampling but did not measure virus emissions into the air and environment. One study found intense air and surface contamination in households with infected children, although no secondary transmission was detected. Routes of SARS-CoV-2 transmission are also not well studied, due to challenges in performing air and environmental sampling in the field. We searched PubMed for "SARS-CoV-2, transmission, daily or longitudinal, airborne or aerosol, environment, fomite, hands" for articles published from database inception to Feb 20, 2022. No language restrictions were applied to this search. Viral RNA has been extensively found in the air and on environmental surfaces in community and hospital settings. However, few studies have systematically or longitudinally correlated air and environmental contamination with viral load in URT swabs, due to challenges in sampling in uncontrolled settings. Only one study that housed patients with COVID-19 individually in a controlled chamber performed paired URT and environmental sampling for a period of 3 days, but participants were recruited some days into the infection, after a positive test. Very few studies have managed to isolate infectious virus from the air and environmental surfaces.

Added value of this study

Real-world studies that sample the air and environment around participants infected with SARS-CoV-2 are unable to control for variations in viral and environmental factors, and usually recruit participants some days into their illness, potentially missing crucial early timepoints when contagiousness was high. SARS-CoV-2 human infection challenge involves the intentional inoculation of healthy participants with a standardised dose of the same virus, providing a highly controlled model in a small number of participants, whereby the exact timepoint of infection is known and sampling can be performed early, densely, and through the whole course of infection. We detected substantial amounts of virus in emissions from infected participants, despite the course of infection being mild. However, the extent, timing, and route of contamination was very heterogeneous, with two of the 18 infected participants emitting a large proportion (approximately 90%) of airborne virus on just 1 or 2 days. The extent of viral emissions was not influenced by how symptomatic a person was, although most viral emissions occurred during the symptomatic period. Viral emissions correlated more strongly with viral load in the nose than in the throat, implicating the nasal mucosa as the most important source of emissions. We detected virus on small frequently touched surfaces and larger surfaces less likely to be contaminated by touch, as well as participants' hands, indicating surface contamination might occur via touch or from depositing airborne droplets. Finally, we observed that lateral flow tests can effectively detect the contagious period when virus is actively being emitted.

Implications of all the available evidence

Viral load in the URT does not perfectly correlate with emissions, indicating a bottleneck between the respiratory mucosa and virus released into the environment that impacts contagiousness. Measuring viral emissions can add to our understanding of contagiousness. Our data implicates the nose as an import portal for virus emissions, which has important implications for public health messaging, such as to cover the nose when using a facemask, and suggests the nasal mucosa as a key target for interventions that aim to block transmission, such as antiviral nasal sprays. Recovering viable virus from surfaces and correlation with viral load on hands suggests a role for hand hygiene and indoor surface cleaning in addition to vital measures to interrupt SARS-CoV-2 airborne transmission. Recognition of early symptoms and self-testing using lateral flow antigen tests could aid in identifying contagiousness in mild community cases.

predict, contagiousness, is vital for the implementation and validation of these strategies. Tractable experimental systems to measure indicators of transmission will be required to test such mitigations.[2]

Transmission of SARS-CoV-2 is a complex process influenced by viral, host, and environmental factors. There are multiple non-mutually exclusive modes of transmission—via direct contact, fomites (contaminated objects), droplets, or aerosols.[3] For transmission to occur, virus needs be expelled through the nose or mouth, or both, then survive for a period of time on fomites or in air before contact with susceptible hosts. Although viral load in upper respiratory tract (URT) swabs has been commonly used to infer contagiousness, measuring viral emissions might be a better surrogate.[4–7] Indeed, recent data have shown the more transmissible omicron variant is more readily detected in airborne particles despite lower viral titres in nasal swabs than previous variants, and another study directly linked emissions collected in masks to household transmission.[8,9]

Previous field studies that sampled the air and environment around SARS-CoV-2 infected participants have mostly recruited participants some days into their illness, potentially missing crucial timepoints when contagiousness was high.[10–12] In such studies, viral factors (such as dose, strain, and site of infection) and environmental factors (such as air flow, temperature, and humidity) are either unknown or uncontrolled. By contrast, SARS-CoV-2 human infection challenge provides a highly controlled model, albeit in a small number of participants, with opportunities to sample early, densely, and through the whole course of infection, including the presymptomatic period.

Previously, we reported on the safety, tolerability, and viral kinetics during the world's first SARS-CoV-2 human challenge in healthy seronegative young adults.[13] Here, we focus on virus emissions from participants who became infected in that study. Longitudinal, daily air and environmental sampling was performed with the aim to inform on the timing of contagiousness and routes of SARS-CoV-2 transmission.

Methods

Study design and participants

Healthy adults aged 18–30 years who were unvaccinated for SARS-CoV-2, not previously known to have been infected with SARS-CoV-2, and seronegative at screening, were recruited to this single-centre phase 1, open label, first-in-human experimental infection study, at a quarantine unit at the Royal Free London NHS Foundation Trust, London, UK. Participants were excluded if they tested positive for anti-SARS-CoV-2 S protein antibodies on the basis of risk factors assessed by clinical history, physical examination, and screening assessments. The QCOVID tool was used to provide a personalised estimated absolute risk of hospitalisation and death, and participants above a predefined risk threshold (equivalent to that for an individual aged 30 years with no risk factors, calculated as a 1:250 000 risk of death or 1:4902 risk of hospitalisation) were excluded. Echocardiography and chest x-ray were performed before inoculation and participants were excluded if their results were outside normal parameters. The full inclusion and exclusion criteria are in the protocol. The protocol was previously published in the supplementary material of our earlier study on safety and tolerability of human challenge.[13] Additionally, per-protocol analysis was performed after exclusion of participants who fulfilled enrolment criteria at screening but were later found to have neutralising and spike-binding antibodies on admission to the quarantine unit.

Participants were recruited via approved advertising (including social media), hVIVO volunteer database, referral, and organic search (eg, via Google or other search engines).

This study was approved by the UK Health Research Authority Ad Hoc Specialist Ethics Committee (reference: 20/UK/0002). Written informed consent was obtained from participants before screening and enrolment. The safety and kinetics of URT infection in SARS-CoV-2 human infection challenge have been described previously.[13]

Procedures

Participants were housed in single occupancy, negative pressure rooms. Participants were inoculated with 10 50% tissue culture infectious dose ($TCID_{50}$) of pre-alpha wild-type SARS-CoV-2 (Asp614Gly virus isolated in 2020) by nasal drops. Participants remained in quarantine for a minimum of 14 days post-inoculation until discharge criteria (two consecutive URT swabs with PCR cycle threshold >33·5 and negative culture) were met.

Nose (mid-turbinate) and throat swabs were collected daily. Each participant wore a sampling mask for 60 min every morning. Room air sampling was performed using a Coriolis µ air sampler (Bertin Technologies, France), approximately 1 m from the participant's head. Hand and environmental (ie, overbed table, bed frame, bedside table, television remote control, bathroom door, toilet flush, and sink tap handles) surface swabs were collected daily (figure 1). All samples were subjected to virological analyses (ie, RT-qPCR for the E gene, plaque assay, and lateral flow antigen test [LFT; BT1309, Innova medical group, USA] for nose and throat samples; RT-qPCR for the E gene, and virus culture for mask, air, and environmental samples). To understand the influence of sampling efficiency or total particle emissions on this observation, all samples were also quantified for human housekeeping gene 18S rRNA by PCR. All samples were collected and tested by researchers (JZ, NG, MM, and FPS). Further assay details are found in the appendix (pp 3–4). Symptom scores were collected using self-reported symptom diaries three times daily.

For more on the **QCOVID tool** see https://www.qcovid.org/

See **Online** for appendix

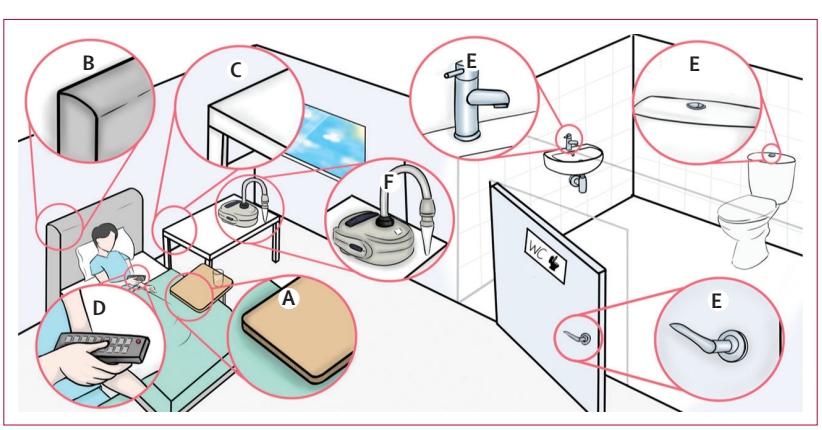


Figure 1: **Schematic of air and environment sampling in participants' rooms**
Environmental surface swabs were collected from overbed table (A), bed frame (B), bedside table (C), television remote control (D), and bathroom handles (door metal handles [inside and outside], flush metal handles, and sink metal handles; E). A Coriolis µ air sampler (Bertin Technologies, France) was placed on the bedside table, about 1 m distance to the participant's head.

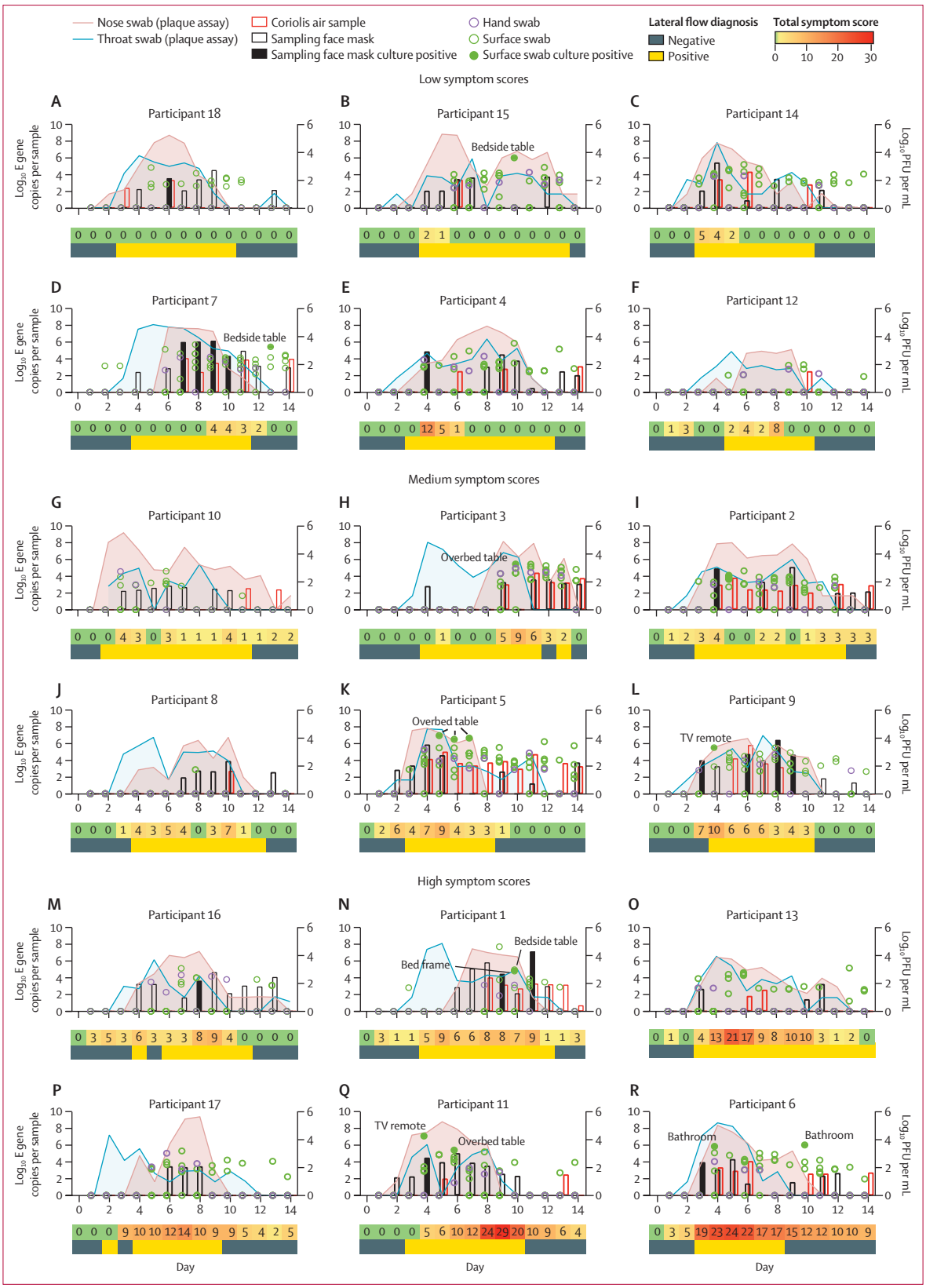


***Figure 2*: Longitudinal viral emissions into the air and environment from SARS-CoV-2 infected participants (n=18)**

Sampling masks, Coriolis air samples, hand swabs, and environmental surface swabs were collected daily, and E gene copies were quantified by RT-qPCR (left y axis). Culture-positive mask and surface swabs are indicated. Nose and throat swabs were collected daily and infectious virus was quantified by plaque assay (right y axis). Total symptom score was calculated using self-reported symptom diaries three times daily. The total symptom scores are displayed in the upper heatmap under each plot, ranging from green (no symptom) to red (highest symptom score). Lateral flow diagnosis from combined nose and throat swabs is shown in the lower heatmap under each plot. Participants are numbered in line with Killingley and colleagues.[13] PFU=plaque forming units.

Outcomes

The primary outcome of this study was to explore air and environmental contamination in the SARS-CoV-2 human challenge model in healthy adults by air sampling, exhaled breath sampling, and surface swabbing. The secondary outcome was to describe the transmission pathways of SARS-CoV-2 and the correlations between viral emissions and host factors.

Statistical analysis

Repeated measures correlation coefficients were computed to assess correlations between viral load in emissions (RNA), viral load in URT (RNA and infectious), and symptom burden with the participant as a random effect. Virus titres were log transformed before the analysis. Correlations were visualised as a heatmap matrix organised by hierarchical cluster analysis, done with the standard hierarchical clustering algorithm included in the base R heatmap function, which uses 1 – correlation as the dissimilarity (distance) metric and the complete method for merging points.

Infected participants were ranked in order of the quantity of total viral load in emissions (RNA), total viral load in URT (RNA and infectious), total 18S rRNA, BMI, and baseline lung function (forced expiratory volume in 1 s [FEV_1], forced vital capacity [FVC], and peak expiratory flow [PEF]); Spearman's correlation coefficients were computed to assess correlations between pairs of variables. The Mann-Whitney test was used to compare viral load on different environmental surfaces, and viral load in emissions and viral load in URT between male and female participants. For all tests, a value of $p<0·05$ was considered significant. Coefficient of variation (the ratio of the standard deviation to the mean) was used to assess the stability of 18S rRNA in samples. Area under the curve (AUC) was calculated by the sum of E gene copies (log transformed). The proportion of emissions that occurred before and after participants met symptom-based definition of a suspected case defined by WHO, onset of fever, self-reported onset of early symptoms, and a positive LFT was calculated by the sum of viral RNA (log transformed) detected before or after the criteria divided by AUC.

Statistical analysis was performed using Graphpad Prism 9 and R (version 4.20).

The study is registered with ClinicalTrials.gov, NCT04865237.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between March 6 and July 8, 2021, 36 participants were recruited (ten female and 26 male participants; mean age 21·8 [SD 2·9]) and 18 (53%) of 34 seronegative participants became infected, resulting in protracted high viral loads in the nose and throat (appendix pp 2, 5–6) following a short incubation period, with mild-to-moderate symptoms. Two participants were excluded from the per-protocol analysis owing to seroconversion between screening and inoculation, identified post hoc. No viral contamination was detected in the breath, air, or rooms of uninfected participants. All 18 infected participants emitted virus-laden particles into the air or contaminated the surrounding environment, or both. Viral RNA was detected in 63 (25%) of 252 room air samples from 16 (89%) of 18 participants between 3 and 14 days post inoculation (figure 2; appendix p 6), and in 109 (43%) of 252 masks from 17 (94%) of 18 participants between 2 and 14 days after inoculation (figure 2; appendix p 6). Viral RNA was detected on 67 (26·6%) of 252 hand swabs from 16 (89%) of 18 participants collected between 3 and 13 days after inoculation (figure 2; appendix p 6). In all 18 infected participants' rooms, viral RNA was detected in 371 (29%) of 1260 surface swabs, including from 74 (29·4%) of 252 overbed table swabs, 62 (24·6%) of 252 bed frame swabs, 80 (31·7%) of 252 bedside table swabs, 68 (27·0%) of 252 television remote control swabs, and 87 (34·5%) of 252 bathroom swabs, between 2 and 14 days after inoculation (figure 2; appendix p 6). The extent of viral contamination was similar across the five different surfaces swabbed ($p=0·12$; appendix p 7). Viable virus was recovered from 16 masks and 13 surface swabs (two from overbed table swabs, one from bed frame swab, six from bedside table swabs, two from television remote control swabs, and two from bathroom metal handle swabs), but not from any Coriolis air samples or hands. However, the Coriolis sampler is not designed nor expected to preserve infectivity of SARS-CoV-2.

In a correlation analysis that encompassed all the samples from all participants, we looked for association of viral load in swabs and emitted virus, and symptoms (figure 3). Viral load measures from air and surfaces clustered together (group 1). Hand viral load and masks clustered with nasal viral load. Bathroom handles and television remote controls showed higher correlations with hand swabs. Mask, air, hand, and surface viral loads all correlated more strongly with nasal viral load than with throat viral load. There was little correlation between symptoms scores and emissions. To explore these findings further at the individual level, plots of longitudinal emissions, symptom burden, and infectious viral load in nose and throat swabs were analysed (figure 2). In several participants, emissions coincided with periods of high nasal viral load, more so than throat viral load. This finding was especially apparent for four participants (figure 2D, H, N, P) in whom the onset of nasal shedding was delayed (≥2 days) and emissions to the air and environment were scarce when virus was only present in throat swabs. This observation confirmed findings from the correlation analysis, that nasal viral load more strongly correlated with emitted virus than

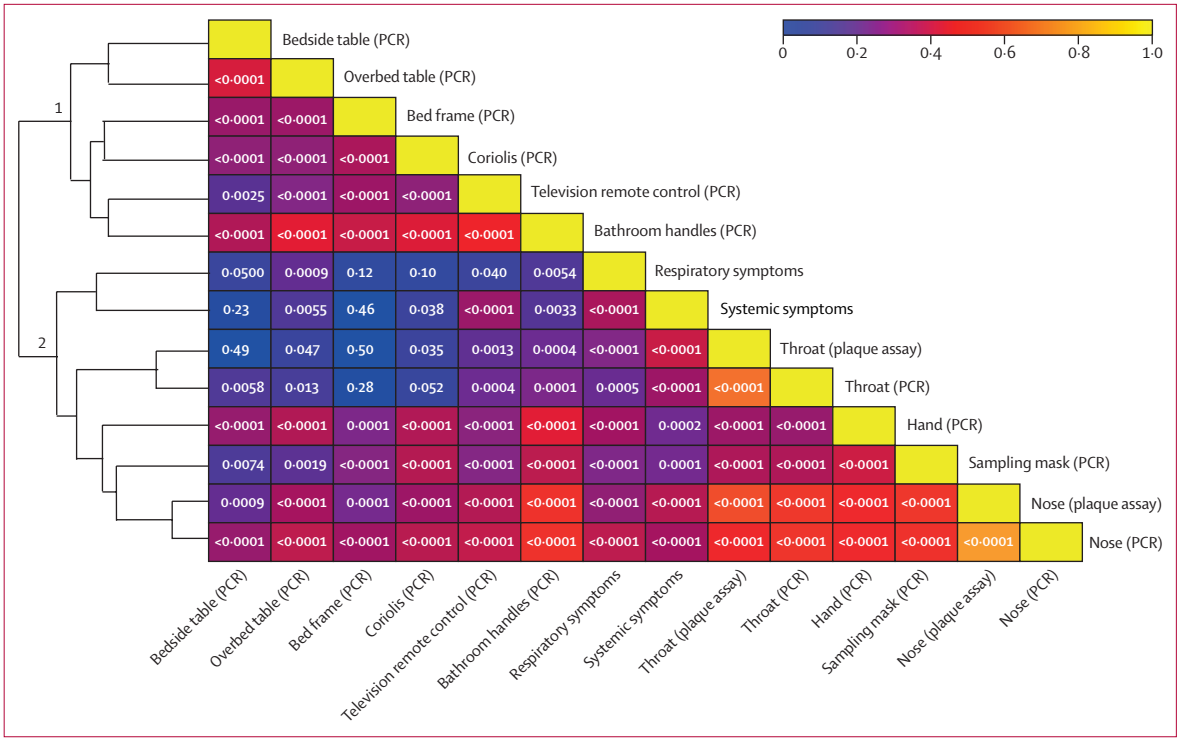


Figure 3: **Correlation between viral load in the upper respiratory tract, viral emissions into the air and environment, and symptoms**
Heatmap matrix between systemic and respiratory symptom scores, viral load in nose and throat swabs (PCR and plaque assay), viral emissions in sampling masks (PCR), Coriolis air samples (PCR), hand swabs (PCR), and environmental surfaces (PCR) are shown.

did viral load in the throat. Again, symptom burden did not determine the extent of viral emissions. Participants who were most symptomatic (figure 2M–R) did not emit more virus than participants who manifested fewer symptoms (figure 2A–F). For example, participant 17 (figure 2P) in the high symptom score group emitted relatively minimal virus, whereas asymptomatic participant 7 (figure 2D) emitted large amounts of virus, including viable virus in mask on several days.

Even for a single individual, there were some days when high quantities of virus were detected in swabs, but little virus was emitted, or days when lower quantities were detected on URT swabs but emissions were higher. Human housekeeping gene 18S rRNA levels in daily air samples from the same individual were relatively consistent (coefficient of variation=13·3%), indicating that the quantity of expelled airborne particles did not vary through infection and the collection of particles into the air sample remained consistent (appendix p 8). By contrast, there was larger variability in 18S rRNA from masks (coefficient of variation=22·2%; appendix p 9), suggesting that this sampling is more variable, perhaps affected by expiratory events (eg, speaking or coughing), individual activity levels, or the alignment of the mask during the 1-h sampling period.[11,14,15] Comparing total virus in Coriolis air samples from each individual, two (11%) of the 18 infected participants (participants 9 and 5) generated 86% of total airborne viral RNA detected (figure 4A) on only 3 days (figure 4B). These bursts of high airborne viral emissions coincided mostly (but not exclusively) with times of high nasal viral load (figure 2K–L) but were not associated with an increased release of 18S rRNA at the time (appendix p 8). Although the two high emitting participants were male, no correlation was found across the cohort between sex (appendix p 10) or BMI and total airborne viral or 18S rRNA emissions (figure 4C). Of note, 18S rRNA emissions and baseline lung function (FEV_1, FVC, and PEF) correlated with total viral air emissions (figure 4C). To further explore indicators of high airborne virus emitters, we ranked participants in order of the quantity of viral air emissions, alongside the other variables tested (figure 4D). Participant 5, who emitted 19% of all airborne virus, had very high nasal viral load, was the highest emitter of 18S rRNA, and was also one of the highest emitters of virus onto surfaces, hands, and masks. By contrast, participant 9, who emitted 66% of airborne virus, did not shed especially high virus in the URT nor were they a high producer of 18S rRNA, although they did have the highest baseline PEF volume of the cohort. Interestingly, discordance was also seen for participant 17 who had extremely high peak nasal infectious viral load and coincident respiratory symptoms (runny nose and sneezing) yet did not emit any virus into the air (figure 2P).

We calculated the proportion of emissions that occurred before participants met the symptom-based definition of a suspected case defined by WHO or before

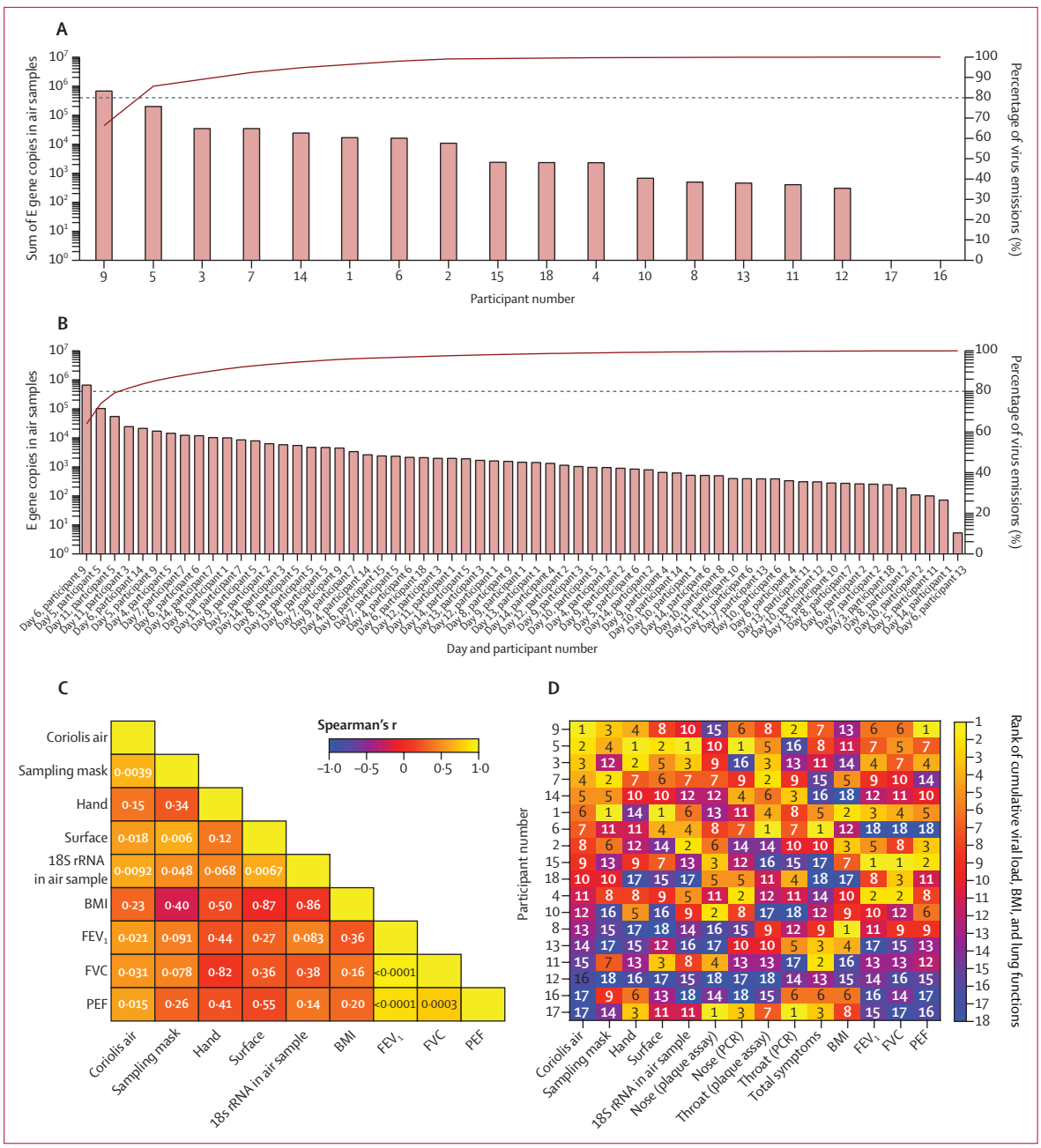


Figure 4: **Heterogeneity in virus emissions after SARS-CoV-2 human challenge**

(A) The sum of E gene copies in air samples (quantified by RT-qPCR) from each infected participant were ranked from highest to lowest (left y axis). (B) E gene copies in each positive air sample were ranked from highest to lowest (left y axis). In A and B, percentage of the cumulative total virus emissions are represented by the red curved line (right y axis) and the dashed line represents the 80% level of cumulative curve. (C) Heatmap matrix between the sum of E gene copies in air samples, sampling masks, hands, surfaces, 18S rRNA in air samples, BMI, FEV_1, FVC, and PEF are shown. Spearman correlation coefficients were calculated to assess correlations between pairs of variables. p values are shown in each cell. (D) Heatmap of the ranks of each variable of cumulative viral loads in nose and throat, air, sampling masks, hands, surfaces, total symptoms, human housekeeping genes are visualised in the heatmap, ranging from yellow (highest rank) to blue (lowest rank), except BMI, which is ranked from the lowest value to the highest value. BMI=body-mass index. FEV_1=forced expiratory volume in 1 s. FVC=forced vital capacity. PEF=peak expiratory flow.

the onset of fever (which is often used as an entry screening tool).[16] We found that AUC for air (mean 31% [SD 48]), mask (42% [44]), hands (32% [43]), and surface (37% [45]) emissions occurred before participants met WHO case criteria and the AUC for air (64% [49]), mask (72% [40]), hands (67% [41]), and surfaces (66% [44]) occurred before onset of fever (figure 5A–B; appendix p 11), consistent with previous descriptions of presymptomatic transmission identified through modelling studies.[17]

Participants were directly questioned three times a day about their symptoms, allowing us to pinpoint the onset of early symptoms with high precision. Before the onset of any reported symptom, viral RNA was detected in air (two [11%] of 18 infected participants), masks (four [22%]), hand swabs (one [6%]), and surface swabs (two [11%]; figure 5C; appendix p 11). However, these viral RNA emissions constituted only 9% (SD 26) of the total AUC for air emissions, 10% (26) for mask emissions, 2% (8) for hand swab emissions, and 8% (25) for surface swab emissions. Overall, 7% (21) of emissions into the air and environment occurred before the first reported symptom; this finding indicates that most contagiousness occurred after the participant first felt unwell.

We previously noted that nine (50%) of the 18 infected participants shed infectious virus in the URT before becoming LFT positive, although the proportion of overall infectiousness that this lag period contributes has been uncertain.[13,18] We detected no virus in Coriolis air samples before the first LFT positive sample and only a small proportion of participants emitted virus into masks (three [16%] of 18), or had virus detectable on hand (one [6%]) and surface swabs (two [11%]; figure 5D–E; appendix p 11). Moreover, the proportion of the AUC of each viral emission type was negligible (2% [SD 3]) before LFA positivity (0% [0] air emissions, 2% [4] mask emissions, 1% [4] hand swab emissions, and 2% [4] surface swab emissions) and after two consecutive LFT negative days (9% [17] air emissions, 4% [8] mask emissions, 3% [7] hand swab emissions, and 8% [11] surface swab emissions), indicating that LFTs can effectively detect the main contagious period.

Discussion

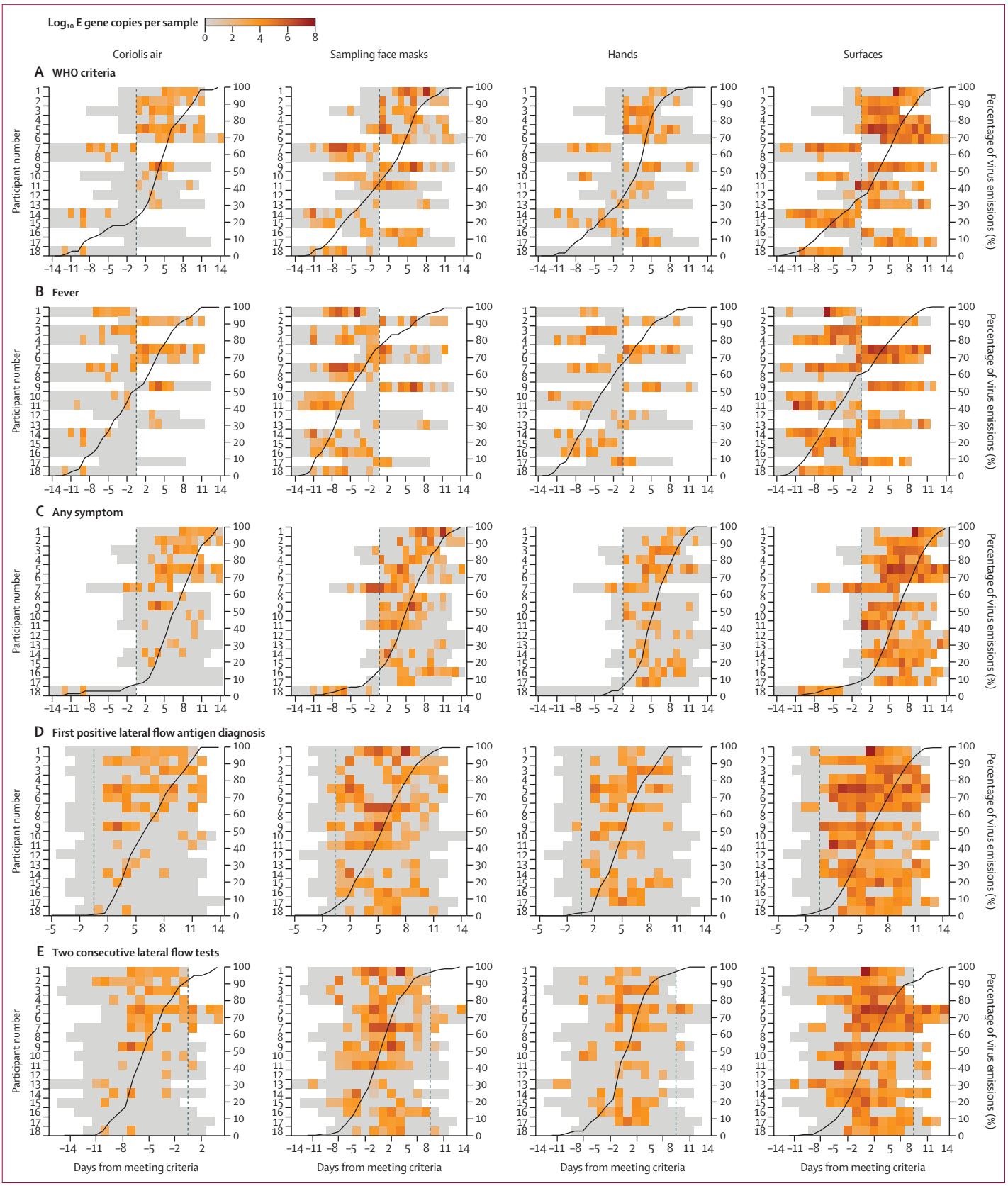
Our high-resolution analysis of virus emissions after uniform inoculation of healthy, seronegative human participants found extensive, but heterogenous, viral contamination of the air and surrounding environment, which might largely arise from the nasal epithelium. Viral emissions mostly occurred after participants developed early symptoms and began to test positive by LFT, indicating that a heightened awareness of early symptoms prompting self-testing could identify a large proportion of infectiousness.

Viral emissions correlated more strongly with viral load in the nose than in the throat, implicating the infected nasal mucosa as an important source of virus for transmission. In-vitro studies have shown that nasal epithelial cells are particularly susceptible to SARS-CoV-2 infection.[19,20] In ferrets, airborne transmitted influenza virus predominantly originates from the nasal respiratory epithelium.[21,22] Our findings highlight the importance of messaging that masks should cover the nose and suggest that transmission-blocking agents should target the nasal epithelium.[2] Although URT viral load has commonly been used as a surrogate for a person's infectiousness, our data are the first to refine this concept, showing that it is the quantity of virus in nose swabs (rather than throat swabs) that correlates best with emissions. However, at an individual level, even nasal viral load is not a perfect predictor of how much virus a person emits into the environment. For example, we identified participants with relatively low nasal viral load who emitted large amounts of virus. This finding suggests that measuring viral emissions is a better surrogate for infectiousness. Indeed, mask sampling of infected individuals in the community has shown a better correlation with transmission within households than viral loads detected in URT swabs.[9] Future human infection challenge studies that include emission monitoring might offer a means for testing the efficacy of agents and vaccines aimed at blocking transmission.

How symptomatic a person is has often been assumed to indicate their contagiousness. Here, we found that symptom scores did not influence the extent of viral emissions. Nonetheless, we found that most virus was emitted during the symptomatic period, with only 10% of emissions occurring before the first reported symptom. However, around one third of emissions occurred before participants met the WHO suspected case criteria that would have triggered symptoms-based testing. Modelling studies estimated that at least 30–50% of community transmission occurred before reported symptoms.[17] However, self-isolation after symptoms would prevent transmission that might otherwise have occurred and lead to an underestimation of the post-symptomatic component in epidemiological studies. No other real-world studies have previously quantified presymptomatic viral emissions; indeed, our previous community-based household transmission study is the only one that performed longitudinal quantification of infectious virus in swabs throughout the presymptomatic period.[23] We found infectious virus on URT swabs in the pre-symptomatic period in 20% of infected contacts. In a real-world setting, early symptoms probably go undocumented, particularly given an individual had to meet a case definition to trigger testing and recommended self-isolation.[24] We found that the WHO suspected case definition and the onset of fever were relatively poor definers of the onset of contagiousness. However, LFTs effectively detected the contagious period. Thus, for healthy individuals with mild infections in the community, awareness of early symptoms prompting daily self-testing

Figure 5: **Virus emissions in relation to timing of symptoms and diagnosis on lateral flow antigen tests**

Proportion of viral RNA detected from air, sampling masks, hands, and on surfaces before WHO SARS-CoV-2 symptom criteria (A), before fever (B), before any symptom (C), before first lateral flow antigen diagnosis (D), and before two consecutive negative lateral flow antigen tests (E), from combined nose and throat swabs. Each row of the heat map represents a participant, which is separated by a dotted line (indicating when criteria are met). The cumulative total virus emissions are represented by the curved line.


Log10 E gene copies per sample
0 2 4 6 8
Coriolis air
Sampling face masks
Hands
Surfaces
A WHO criteria
B Fever
C Any symptom
D First positive lateral flow antigen diagnosis
E Two consecutive lateral flow tests
Participant number
Percentage of virus emissions (%)
Days from meeting criteria

by LFT could be a valuable tool to avert community transmission.

Even within this small cohort and highly controlled study design, we observed heterogeneity in the timing, extent, and routes of viral emissions. We observed short windows of high airborne viral emission, with two (11%) infected participants contributing 86% of airborne virus over three individual timepoints, giving support to the phenomenon of superspreading individuals or events.[25] Across the whole cohort, higher airborne viral emissions correlated with 18S rRNA and baseline lung function, suggesting that participants with larger lung capacities emit more airborne virus. However, the three high emission timepoints were not associated with greater human marker (18S rRNA) and the highest emitter of the cohort did not produce especially high outputs of the human marker. Predicting or identifying people who might be high virus emitters, perhaps even before they are infected, is of interest because they could be prioritised for interventions to block transmission. Our analysis suggests both host (eg, sex, BMI, mechanics of breathing) and viral factors (eg, timing, extent, or anatomical site of virus) might contribute to overdispersion. However, further work is required to understand these findings. It would be interesting to investigate the importance of mechanics of breathing that might lead to differences in emitted particle size distribution, including breathing more through the nose than the mouth, or during exercise or singing. Furthermore, physiological factors such as the composition of respiratory secretions (eg, how viscous or acidic mucous is), the diversity of nasal microbiome, pre-existing mucosal immune tone, and mucosal immune responses triggered in response to infection, could affect virus release into airborne particles.[26]

Airborne transmission of SARS-CoV-2 is well described, but evidence for the contribution of fomites has been relatively lacking.[27,28] Only a few studies have recovered viable SARS-CoV-2 from surfaces.[29] Here we successfully cultured viable virus from several environmental surfaces within the rooms housing infected cases, and in one case, detected infectious virus on a bedside table 1 day after the cessation of infectious virus detection in the URT, possibly indicating the persistence of infectivity on a surface for some time after it was released. Detection of virus on hands correlated with nasal viral load and with frequently touched points, suggesting a causal pathway—hands soiled by virus in the nose then contaminate surfaces. The infectiousness of virus was preserved through this journey—infectious virus was frequently isolated from television remote controls and small bathroom surfaces that are more likely to be contaminated by touch rather than depositing droplets. Conversely, the clustering of surfaces around the bed with air samples suggests that these items, with relatively larger, flat surfaces, might be contaminated by respiratory droplets that settle out of the air. Overall, our data demonstrate the complexity of SARS-CoV-2 transmission pathways, but cannot define the relative contribution of different routes to onward transmission, which would require the use of susceptible participants. Nonetheless, our findings support measures such as hand washing and surface cleaning in close indoor settings where there has been a case of COVID-19, as one of a combination of non-pharmaceutical interventions to reduce transmission risk.

This study has limitations. First, our sample size was small and included only intranasally inoculated healthy young adult participants. Second, the study was carried out in negative pressure hospital rooms. It is possible that intranasal experimental inoculation could have induced less symptomatic disease than natural infection, which might have reduced overall transmissions or masked the effect of more severe symptoms on virus emission. The negative pressure room could have removed virus more quickly after emission and understated contagiousness in our study. Our team has also conducted air and environmental sampling in real-world settings, such as households, public transport, campus, student accommodation, and hospitals.[30–32] The same equipment and protocols were applied in these projects. The levels of emitted virus we found in these real-life situations were not substantially different from those measured in this study. Our ability to detect viral emissions on surfaces might have been enhanced by the controlled nature of the study. Participants underwent a strict quarantine whereby movement of people (except the study team) in and out of the room was restricted, with lower opportunity to dislodge surface virus than would be expected in normal environments. Our inability to culture virus from Coriolis air samples might reflect the inactivation of the virus during collection, rather than the absence of infectious virus in the air. Our inability to culture virus from hands probably reflects the lower viral load collected on these swabs compared to surfaces, which might depend on the swabbing technique or the frequency at which participants were inclined to decontaminate their hands versus their surrounding environmental surfaces. Although we did not find a correlation between respiratory symptom score and viral emissions, we cannot exclude that transient symptoms such as sneezing would not increase viral load in the air momentarily at a time when air sampling was taking place. Emissions are used to imply contagiousness here, but to truly understand transmission risk the use of naive sentinel participants, or susceptible animals (eg, hamsters), would be required. Finally, this study was performed in unvaccinated seronegative individuals with an early strain of SARS CoV-2 (pre-alpha). Currently circulating SARS-CoV-2 omicron variants transmit in a population with varying levels of existing immunity. A recent study suggested that later variants, including omicron, were more readily detected in breath aerosols than first wave virus, suggesting emissions encountered in 2022 might be greater than those we report here.[8] Future

challenge studies with new variants can be compared with the current study as a baseline, to understand changes in contagiousness as SARS-CoV-2 continues to evolve.

Our findings suggest that the human challenge model is useful to understand kinetics and routes of transmission of SARS-CoV-2. As such, it can inform policy and be a vehicle to test mitigations that aim to block transmission. Future studies should aim to address the basis of heterogeneity in timing, extent, and routes of viral emissions to attempt to identify high emitters. Our analyses suggest that LFT could be used to quickly identify those likely to be infectious far more effectively than screening for fever. Finally, our findings suggest roles for hand hygiene and surface cleaning in reducing transmission risk.

Contributors

JZ, AS, NG, BK, MK, AJM, APC, MRB, NMF, CC, and WSB conceived or designed the work. JZ, NG, MM, FPS, BK, AJM, and CC conducted the study or collected data. All authors analysed or interpreted the data. JZ, AS, NG, NMF, and WSB drafted the manuscript. BK, AJM, MRB, and CC critically revised the article. JZ, AS, MRB, NMF, CC, and WSB verified underlying data of the study. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

MK, AJM, and APC are employees of hVIVO Services and hold shares in Open Orphan and Poolbeg Pharma. All other authors declare no competing interests.

Data sharing

Individual participant data that underlie the results reported in this article after de-identification will be made available for individual participant data meta-analysis beginning 12 months and ending 5 years after article publication upon written request. Data will be shared with investigators whose proposed use of the data has been approved by the UK Vaccine Taskforce Human Challenge Steering Committee to achieve the aims in the approved proposal. Additional shareable documents include the statistical analysis plan. Proposals should be directed to Wendy S Barclay (w.barclay@imperial.ac.uk) and Christopher Chiu (c.chiu@imperial.ac.uk) and to gain access, data requestors will need to complete a data request form and sign a data access agreement.

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