Exhaled Breath Aerosol Shedding by Highly Transmissible Versus Prior SARS-CoV-2 Variants

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Running title: Evolution of SARS-CoV-2 Aerosol Shedding

Key points: Highly transmissible variants (Alpha, Delta, and Omicron) demonstrate greater viral aerosol shedding phenotypes compared with prior variants, consistent with a dominant role for airborne transmission of COVID-19. Fully vaccinated and boosted individuals infected with SARS-CoV-2 can shed infectious viral aerosols.

Abstract

Background

Aerosol inhalation is recognized as the dominant mode of SARS-CoV-2 transmission. Three highly transmissible lineages evolved during the pandemic. One hypothesis to explain increased transmissibility is that natural selection favors variants with higher rates of viral aerosol shedding. However, the extent of aerosol shedding of successive SARS-CoV-2 variants is unknown. We aimed to measure the infectivity and rate of SARS-CoV-2 shedding into exhaled breath aerosol (EBA) by individuals during the Delta and Omicron waves and compared those rates with those of prior SARS-CoV-2 variants from our previously published work.

Methods

COVID-19 cases (n=93, 32 vaccinated and 20 boosted) were recruited to give samples, including 30-minute breath samples into a Gesundheit-II exhaled breath aerosol sampler. Samples were quantified for viral RNA using RT-PCR and cultured for virus.

Results

Alpha (n=4), Delta (n=3), and Omicron (n=29) cases shed significantly more viral RNA copies into exhaled breath aerosols than cases infected with ancestral strains and variants not associated with increased transmissibility (n=57). All Delta and Omicron cases were fully vaccinated and most Omicron cases were boosted. We cultured virus from the EBA of one boosted and three fully vaccinated cases.

Conclusions

Alpha, Delta, and Omicron independently evolved high viral aerosol shedding phenotypes, demonstrating convergent evolution. Vaccinated and boosted cases can shed infectious SARS-CoV-2 via EBA. These findings support a dominant role of infectious aerosols in transmission of

SARS-CoV-2. Monitoring aerosol shedding from new variants and emerging pathogens can be an important component of future threat assessments and guide interventions to prevent transmission.

Keywords: SARS-CoV-2; exhaled breath aerosol; convergent evolution; airborne transmission; COVID-19.

Background

The transmissibility of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to increase as new variants emerge[1–3]. Three variants of concern (VOCs), Alpha (B.1.1.7), Delta (B.1.617.2), and Omicron (B.1.1.529), successively became dominant during 2021[4]. Each was identified as having increased transmissibility relative to earlier variants or ancestral strains[1–3].

Multiple lines of evidence point to a dominant role for aerosol inhalation (i.e., airborne transmission) as the primary mode of SARS-CoV-2 transmission[5]. We therefore hypothesize that VOCs associated with increased transmissibility have been selected based on increased fitness for transmission via aerosols. We previously reported that individuals infected with the Alpha variant shed viral RNA copies into *fine* aerosols (≤5 μm in diameter) at an 18-fold greater rate than did individuals infected with ancestral strains and variants not associated with increased transmissibility[6]. It is unknown whether continued evolution of more transmissible variants and subvariants is associated with continued increases in aerosol shedding. Our objectives here were to describe the infectivity and rate of SARS-CoV-2 RNA shedding into exhaled breath aerosol (EBA) by ambulatory cases during the Delta and Omicron waves and to compare those rates with those of prior SARS-CoV-2 variants from previously published work[6]. We also

compared viral aerosol shedding rates among Omicron subvariants and examined associations of viral aerosol shedding with upper respiratory viral load, vaccination, serology, demographic, and clinical predictors.

Methods

We recruited participants with PCR confirmed COVID-19 from the University of Maryland, College Park and surrounding community[6] from June 6, 2020 through March 11, 2022. The University of Maryland Institutional Review Board and the Human Research Protection Office of the Department of the Navy approved this study. All participants provided informed consent.

We previously reported results for participants enrolled from June 6, 2020 through April 30, 2021 [6] and included them here for comparisons with cases enrolled during subsequent waves. Basic demographic data were obtained from a baseline questionnaire. Participants were sampled one to thirteen days post-symptom onset. Each day of sample collection, participants completed online questionnaires to update their symptoms (Supplementary Methods).

During viral shedding assessment visits, participants provided saliva, mid-turbinate swabs (MTS), phone swabs (as a measure of fomite contamination), venous blood samples, and exhaled breath aerosol (EBA) samples collected with a Gesundheit-II (G-II) human exhaled bioaerosol collector[7] following a loud speaking and singing protocol with spontaneous coughing and sneezing[6]. Some participants completed two shedding assessment visits, one to three days apart.

Viral RNA was detected and quantified as previously described[6]. RNA copy numbers were reported per mL for saliva and per sample for all other sample types (except blood). The limit of detection (LOD, 95% probability of detection) was 62 copies/mL for saliva and 75

copies/sample for other samples. Aliquots were sent to the University of Maryland School of Medicine for virus culture. Plasma samples were assayed for antibodies to SARS-CoV-2. IgG antibodies were titered using the SARS-CoV-2 receptor binding domain (RBD) and nucleocapsid (N) proteins (ACRO Biosystems) as targets. Genome sequencing of MTS samples was performed using a MinION sequencing system (Oxford Nanopore Technologies, ONT). See Supplementary Methods for detailed sample processing and laboratory analyses.

Data cleaning and statistical analyses were completed using R version 4.2.0 and RStudio. Mann–Whitney U Test was used for pairwise comparisons and the Kruskal-Wallis test was used for global comparisons. We used linear mixed-effect models with censored responses[8] to estimate the effect of predictors on EBA viral load, accounting for censored observations below the limit of detection and nested random effects of subjects and samples nested within subjects (Supplementary Methods). We performed sensitivity analyses to determine the impact of cases studied more than five days post-symptom-onset on correlation and regression analyses.

Results

From June 2020 through March 2022, we measured viral load in the exhaled breath of 93 individuals (age range: 6 to 66 years; Table 1). Participants were mildly symptomatic (97%) or asymptomatic (3%) at the time of sampling. Participants enrolled from June 2020 through April 2021[6] were infected with Alpha (n=4) and ancestral/other variants (n=57) prior to widespread vaccination. Participants enrolled from September 2021 through March 2022 had an active Delta (n=3) or Omicron (n=29) infection, were fully vaccinated, and had detectable IgG against SARS-CoV-2 spike protein RBD. Among the later group, 20 (63%) were boosted, and 5 (16%) had detectable IgG against SARS-CoV-2 nucleocapsid (N) protein (Table 1; Supplementary Table 1-2).

Among Delta and Omicron cases, we detected SARS-CoV-2 RNA in saliva, MTS, aerosol, and phone swabs and recovered infectious virus from all sample types except phone swabs (Figure 1; Supplementary Table 3). The majority (21/32; 66%) of Delta and Omicron cases shed detectable viral RNA concentrations in exhaled breath aerosol (EBA). Viral RNA loads in *coarse* (>5 μm) and *fine* (≤5 μm) aerosol fractions ranged from non-detect to $1.8x10^5$ and $1.8x10^7$ RNA copies per 30-minute EBA sample, respectively. The viral RNA load in the *fine* fraction was on average five times greater than in the *coarse* fraction and accounted for most of the total exhaled viral RNA load.

SARS-CoV-2 aerosol shedding during Delta variant infections

We detected viral RNA and cultured virus from EBA provided by two (66.7%) Delta cases. From one, fully vaccinated with NVX-CoV2373, we cultured SARS-CoV-2 from an EBA *fine* fraction that contained $3.0x10^4$ RNA copies. From the other, fully vaccinated with BNT162b2, we cultured virus from an EBA *coarse* fraction that contained $3.6x10^2$ RNA copies. None of the Delta cases were boosted.

SARS-CoV-2 aerosol shedding during Omicron (BA.1, BA.1.1, and BA.2) infections

Among Omicron cases, we detected viral RNA in the EBA of 19 (66%) and two (both BA.1.1) yielded positive virus cultures from their *fine* EBA. One was fully vaccinated (not boosted) with BNT162b2 and emitted the highest number of viral RNA copies in a *fine* EBA sample ($1.8x10^7$) observed over the course of the pandemic. The other individual, fully vaccinated and boosted with BNT162b2, shed $2.9x10^3$ viral RNA copies into *fine* EBA.

Fine EBA viral RNA loads from Omicron cases were, on average, similar to those from Alpha and Delta cases (Figure 2; Supplementary Figure 1). We did not observe a significant

difference in viral aerosol shedding between Omicron BA.1, BA.1.1, and BA.2 ($p>0.05$; Supplementary Figure 2).

Omicron MTS viral RNA load was a weak positive correlate of *fine* EBA viral RNA load (rho = 0.36, p = 0.015), in contrast to ancestral strains and other variants where MTS load was moderately positively correlated with EBA load (rho = 0.59, $p < 0.0001$; Figure 3; Supplementary Figure 3). Omicron viral RNA loads in saliva, however, trended toward a stronger, albeit still moderate, correlation with EBA load (rho = 0.58, $p < 0.0001$) compared with earlier strains and variants (rho = 0.41, $p < 0.0001$). A similar pattern was observed for *coarse* aerosols (Supplementary Figure 4a-4b).

Having received a vaccine booster was associated with shedding more viral RNA in *coarse* EBA ($p=0.0056$; Supplementary Figure 5). However, boosters were not associated with *fine* ($p = 0.97$) or total EBA viral RNA load ($p = 0.81$; Supplementary Figure 5).

Five Omicron cases (one BA.1, one BA.1.1, and three BA.2) were sero-positive for anti-nucleocapsid (anti-N) IgG at enrolment, one to six days post-symptom onset. Four of the five had received a booster >8 days prior to symptom onset. Two reported prior infection(s); two denied prior infection and one did not respond to questions about prior infection. We detected viral RNA in MTS samples from all five. Their MTS, however, contained significantly fewer RNA copies than Omicron infections in the absence of anti-N IgG ($p=0.00045$; Supplementary Figure 6). These five were the only Omicron cases that yielded culture-negative MTS samples (Figure 1). We detected viral RNA in saliva from only one of the five, the non-boosted case, and that sample was culture-negative. None of the five shed detectable levels of SARS-CoV-2 RNA in EBA.

Three Omicron cases (one BA.1, two BA.1.1) were children aged 6-12 years. None of their *fine* EBA samples contained detectable SARS-CoV-2 RNA; one *coarse* EBA sample contained a trace amount. MTS samples from all of the children were culture-positive. All saliva and EBA samples were culture-negative.

Predictors of viral aerosol shedding from Omicron (BA.1, BA.1.1, and BA.2) infections

Among the 29 Omicron cases, higher saliva viral RNA load, systemic symptom score, and number of coughs per 30-minute sampling session were significant predictors for higher *fine* EBA viral RNA load in a model adjusted for age, sex, and subvariant BA.2 compared with BA.1 and BA.1.1 (Figure 4 a-b). Only higher saliva viral RNA load and systemic symptom score were significant predictors for higher *coarse* EBA viral RNA load in an adjusted model (Supplementary Figure 7 a-b). The BA.2 subvariant was not associated with significantly greater shedding into either *fine* or *coarse* EBA compared with BA.1 and BA.1.1.

Evolution of SARS-CoV-2 aerosol shedding

Over the course of the pandemic (Figure 4 c-d), three highly transmissible SARS-CoV-2 variants (Alpha, Delta, or Omicron), as well as higher systemic symptom score, saliva viral RNA load, age, and number of coughs per 30-minute sampling session were significant predictors for higher *fine* EBA viral RNA load in an age and sex adjusted model. Highly transmissible VOCs were associated with increased *coarse* aerosol shedding in unadjusted analyses but were not significant predictors in adjusted models. Higher systemic symptom score, MTS viral RNA load, and age were significant predictors for higher *coarse* EBA viral RNA load in an adjusted model controlling for age and sex (Supplementary Figure 7 c-d). Day post symptom-onset was not a significant predictor of viral RNA load in EBA and a sensitivity analysis including only cases studied $\leq$ 5 days post onset was consistent with these results (Supplementary Tables 4a-4b).

Delta and Omicron cases coughed more frequently than Alpha, ancestral strains, and other variant cases (Supplementary Figures 8a-8b). The highest cough count was from a BA.1.1 case who coughed 69 times during the 30-minute sampling session. Two participants (one infected with Omicron BA.2 and one with ancestral strain, B.1.509) sneezed during the sampling sessions, each sneezing once. Omicron cases generally reported more upper and lower respiratory symptoms compared with those infected with ancestral strains and other variants (Supplementary Figures 8a-9b).

Discussion

This study, using a well-characterized breath aerosol collector[6,9,10], demonstrated that both fully vaccinated and boosted COVID-19 cases can shed infectious SARS-CoV-2 aerosols. We also observed that Alpha, Delta, and Omicron infections were associated with significantly greater viral aerosol shedding than infection with ancestral strains and variants not associated with increased transmissibility (Figures 2 and 4). These data indicate that a characteristic of highly transmissible variants is a high rate of viral shedding into aerosols. These three highly transmissible variants represent three distinct SARS-CoV-2 clades that independently evolved high viral aerosol shedding phenotypes. This evidence for convergent evolution of increased viral aerosol shedding is consistent with a dominant role for airborne transmission (inhalation of viral aerosols regardless of distance that the aerosol traversed) in the spread of COVID-19[5].

We did not observe statistically significant differences in the geometric mean rates of viral RNA shedding into EBA among the three highly transmissible variants (Figure 4c,d, Supplementary Figure 1). The highest viral EBA shedders had Omicron infections; the highest had 1.8×10^7 RNA copies in a *fine* EBA sample, three orders of magnitude higher than the maximum for Delta and previously reported Alpha variant infections[6], and only 2.4-fold less

than the maximum we previously observed for influenza[11]. This suggests that variants associated with more extreme viral EBA outliers (supershedders) may drive increased transmissibility through superspreading. Thus, superspreading as a biological factor, not just a result of social behavior[12], may be a driving force behind dominance of new variants when they differ minimally regarding immune escape.

The *fine* aerosol fraction (≤5 μm) consistently contained greater numbers of viral particles based on RNA copy number compared with the *coarse* aerosol fraction (>5 μm), and dominated the total aerosol load in all of the SARS-CoV-2 infections studied throughout the pandemic. This pattern mirrored results from earlier studies of influenza[11,13–15]. These observations are consistent with data showing that bubble film burst due to airway closure and reopening is the dominant mechanism of respiratory aerosol generation[16–18] and that bubble films concentrate microorganisms relative to their concentration in bulk fluids by orders of magnitude[19–21]. When considered together with the relatively more efficient concentration and aerosolization of enveloped compared with naked protein capsid viruses[22], it is perhaps not surprising that respiratory viral pandemics of the last >100 years have been caused by enveloped viruses.

We previously reported that, for infections studied through April of 2021, high MTS viral RNA load was a strong risk factor for high viral RNA load for both *coarse* and *fine* EBA fractions[6]. With Omicron, however, we see a clear shift toward saliva being a stronger predictor of the viral RNA load in EBA. This was evident for both *coarse* and *fine* EBA viral RNA in our regression models for Omicron infections (Figures 4a,b) and can be clearly seen in our correlation plots. These results are consistent with previous reports that Omicron cases tend to have lower viral loads in their nasopharynx compared with Delta cases[23,24]. Therefore, the

observation that Omicron cases have similar or higher rates of viral RNA shedding suggests that nasopharynx is not the source of exhaled viral aerosols. By contrast, detailed studies of respiratory aerosol generation point to the small airways, larynx, and oropharynx as the major sources of exhaled particles during breathing, talking, and singing, and small airways and larynx as the primary sites of fine particle generation [16,25]. Taken together, these data suggest that selection may be favoring variants that replicate more efficiently at sites where aerosols are generated, and that viral RNA in EBA and saliva may reflect viral load in the posterior pharynx and mucociliary transport of virus from the lower respiratory tract.

Hui *et al*[26] found that Omicron variants replicated to 70-fold higher titers in human bronchial *ex vivo* cultures than wild-type or Delta strains at 24 and 48 hours after infection, suggesting that Omicron infections may produce higher viral loads in conducting airways. Higher viral load and resulting inflammation and irritation of intrathoracic airways could explain the higher cough counts. However, if cough related shear forces were a major mechanism of viral aerosol generation, cough should be a stronger predictor of viral load in coarse than in fine aerosol. That the reverse is true, as we previously observed for influenza [11], indicates that cough is not a primary mechanism of infectious aerosol generation in these viral infections.

Omicron BA.2 appeared to be more transmissible than BA.1 in a study of Danish households[27]. However, the reported increase in transmissibility of BA.2 over BA.1 was limited to unvaccinated primary cases; fully vaccinated and boosted primary cases infected with BA.2 were significantly less likely to transmit BA.2 than BA.1[27]. Antibody escape is not thought to be responsible for the dominance of BA.2 over BA.1[28,29]. One recently observed advantage of BA.2 is an increased competence for replication in human nasal and bronchial tissues[30]. This change did not appear to impact average viral aerosol shedding rates among

vaccinated/boosted individuals with Omicron breakthrough infections; we did not see evidence of a significant difference in viral RNA aerosol shedding between people infected with BA.1, BA.1.1 and BA.2. Given that the dominance of BA.2 seems to have been associated with transmission by unvaccinated individuals, we might expect to see increased aerosol shedding from unvaccinated cases. Our data cannot address that possibility because all Omicron cases in our study were fully vaccinated and some boosted.

Five participants with an Omicron infection were positive for anti-N protein IgG at the time of enrolment. The presence of anti-N IgG may indicate prior infection (reported by two participants) and a broad immune response to infection, including IgA secretion, which is a potent neutralizer of SARS-CoV-2 during early infection[31]. Infection produces a more robust IgA response than intramuscular vaccination[32] and concentrations decline more slowly after infection than those of IgG[33]. These participants had no PCR-detectable levels of virus in EBA, phone swabs and all but one saliva sample, and the viral RNA load in their MTS was significantly lower than that of other Omicron cases. These observations together suggest that acquired immune responses including specific IgA in these participants may have played a role in reducing viral loads overall and limiting shedding in EBA. However, because subsequent Omicron subvariants, particularly BA.2.12.1, BA.4 and BA.5, can escape antibody neutralization elicited by both vaccination and prior Omicron infection[34,35], we might not expect to observe such a reduction in viral aerosol shedding among seropositive individuals infected with future variants.

Our study has several limitations. Although we recruited throughout the pandemic, our sample size is relatively small and enrollment rates were low during the Delta wave. As a result, we are limited in making comparisons such as the correlation between EBA viral RNA load and

culture positivity for specific variants. Although we were able to sample children infected with Omicron, our sample size is too small to make conclusions about viral aerosol shedding from children. The EBA collection procedure is not suitable for children under age 6 years. Lastly, we did not sample participants throughout their entire infection. Because viral loads in aerosol samples were low, we opted for a sensitive but non-quantitative measure of infectiousness. Thus, we are unable to assess the impact of variants and Omicron subvariants on the duration of viral aerosol shedding and infectious virus titers in EBA.

In conclusion, our findings demonstrate that COVID-19 cases can shed infectious SARS-CoV-2 aerosols even when fully vaccinated and boosted. Evolutionary selection appears to have favored SARS-CoV-2 variants associated with higher viral aerosol shedding. The combination of immune evasive properties *and* high viral aerosol shedding were likely responsible for Omicron's rapid spread and replacement of Delta, even as infection- and vaccine-acquired immunity increased. Thus, non-pharmaceutical interventions, especially indoor air hygiene (e.g., ventilation, filtration, and disinfection with germicidal UV) and targeted masking and respirators, will continue to play an important role in limiting SARS-CoV-2 transmission in vaccinated communities to prevent post-acute COVID-19 sequalae[36] and to protect vulnerable populations.

NOTES

Author contributions

D.K.M. conceived the project and obtained funding.

D.K.M., F.H., B.A., Y.E., J.L., S.S.T., J.G., I.S.M. conceptualized the project and designed the study.

I.S.M., A.K.S., M.O., and N.F. recruited study volunteers.

K.K.C., A.K.S., and M.O. collected exhaled breath samples.

B.A., Y.E., J.L., K.M.M., M.O., and N.F. collected clinical samples.

S.S.T., J.G., and M.S. processed and analysed samples.

S.W. and M.F. performed virus culture.

K.M. performed antibody tests on sera samples.

F.H. performed data management and curation.

J.L. performed data analyses.

J.L., K.K.C., and T.L.G. drafted the original manuscript.

All authors participated in reviewing and editing the manuscript.

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Data and Code Availability

Deidentified data for the accepted manuscript will be made available on the Open Science Framework repository. Custom code used to analyse the data will be made available on a public github repository with linkage to the Open Science Framework repository.

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for New England Workplace (CPH-NEW) External Advisory Board; and Editor-in-Chief (paid) of Workplace Health & Safety Journal via American Association of Occupational Health Nurses (AAOHN). D.M. reports support for attending meetings and/or travel from The Flu Lab (gift from charitable foundation); receipt of Upper room GUV fixture from Aeromed Inc and Far UV-C GUV fixtures from FarUV Inc. None of the other authors have potential conflicting interests.

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Table

Table 1. Demographics for SARS-CoV-2 cases enrolled June 6, 2020 – March 11, 2022

		Enrolled June 2020 - April 2021[a]	Enrolled September 2021 - March 2022	All participants
Number of participants		61	32	93
Number of exhaled breath samples		100	50	150
Variant, N (%)	**Ancestral strains and other**	57 (93)	0 (0)	57 (61)
	Alpha	4 (7)	0 (0)	4 (4)
	Delta	0 (0)	3 (9)	3 (3)
	Omicron BA.1	0 (0)	8 (25)	8 (9)
	Omicron BA.1.1	0 (0)	14 (44)	14 (15)
	Omicron BA.2	0 (0)	7 (22)	7 (8)
Female, N (%)		23 (38)	13 (41)	36 (39)
Age, mean ± SD		23.6 ± 9	27.2 ± 15.3	24.8 ± 11.6
Age group, N (%)	**<18**	1 (2)	3 (9)	4 (4)
	18-45	57 (93)	24 (75)	81 (87)
	>45	3 (5)	5 (16)	8 (9)
Race/Ethnicity, N(%)	**White**	48 (79)	19 (59)	67 (72)
	Black/African American	7 (12)	5 (16)	12 (13)
	Hispanic	8 (13)	5 (16)	13 (14)
BMI, mean ± SD		25.2 ± 4.5	24.7 ± 5.4	25 ± 4.8
Chronic respiratory illness, N (%)[b]		13 (21)	6 (19)	19 (20)
Vaccination status, N (%)[c]	**Boosted**	0 (0)	20 (63)	20 (22)
	Fully vaccinated, not boosted	0 (0)	12 (37)	12 (13)
	Partially vaccinated	3 (5)	0 (0)	3 (3)
	Not vaccinated	58 (95)	0 (0)	58 (62)
Anti-spike RBD antibody (IgG), N (%)		6 (10) [d]	32 (100)	38 (41)
Anti-nucleocapsid antibody (IgG), N (%)		N/A[e]	5 (16)	5 (5)
Ever symptomatic, N (%)		58 (95)	32 (100)	90 (97)
Symptomatic participants	**Days post symptom onset[f], mean ± SD (range)**	5 ± 3 (0-13)	3 ± 2 (1-7)	4 ± 2 (0-13)
	Coughs per 30 min, mean ± SD (range)	1 ± 4 (0-24)	8 ± 15 (0-69)	4 ± 10 (0-69)
	Median upper respiratory symptoms[g] (IQR)	2 (1 - 3.8)	3.5 (2 - 6)	3 (1 - 4)
	Median lower respiratory symptoms (IQR)	0 (0 - 1.8)	1 (0.2 - 2)	1 (0 - 2)
	Median systemic symptoms (IQR)	1 (0 - 3)	2 (1 - 6)	2 (0 - 4)

	Median gastrointestinal symptoms (IQR)	0 (0 - 1)	1 (0 - 2)	0 (0 - 1)
	Temperature (C), mean ± SD	37.2 ± 0.3	37 ± 0.3	37.1 ± 0.3
	Oxygen saturation (SpO2), mean ± SD	97.8 ± 1	97.9 ± 0.8	97.8 ± 1

BMI = Body mass index; RBD = Receptor Binding Domain; IgG = Immunoglobulin class G; IQR = Interquartile range

a. Previously reported cases (57) and four others lacking blood samples [6].
b. Chronic respiratory illness = volunteers with any chronic obstructive pulmonary disease, asthma, other lung diseases.
c. Boosted = received one vaccine booster dose ≥ 8 days prior to study enrollment; Fully vaccinated, not boosted = received only two doses of BNT162B2, mRNA-1273, or NVX-CoV2373, or one dose of Ad26.COV2 ≥ 14 days prior to study enrollment; Partially vaccinated = received only one dose of BNT162B2 or mRNA-1273.
d. Serologic status data for four participants were missing due to a lack of blood samples.
e. Anti-nucleocapsid antibodies were not measured for these participants.
f. Days since symptom onset at the time of each sample collection visit.
g. Symptoms at the time of each sample collection visit. Sixteen symptoms were rated from 0 to 3 with a maximum possible composite score of 15 for upper respiratory, 9 for lower respiratory, 12 for systemic symptoms and 12 for gastrointestinal symptoms.

Figure Legends

Figure 1. Viral RNA load and culture results from SARS-CoV-2 Delta and Omicron (BA.1, BA.1.1, and BA.2) cases. Violin plots present the viral RNA copies on the log 10 scale of culture negative and positive samples from SARS-CoV-2 Delta and Omicron cases by sample type from September 14, 2021 to March 11, 2022, with one sample of each type per case. Each point represents a case. **a,** Mid-turbinate swab (MTS), saliva, and phone swabs. **b,** *Coarse* (>5 μm in diameter) and *Fine* (≤5 μm in diameter) exhaled breath aerosol (EBA) from 30-minute sampling events. The *n* at the bottom of the plots indicates the number of cases. Cases with no detectable viral RNA were assigned a copy number value of one.

Figure 2. Viral RNA copies (log 10 scale) in exhaled breath aerosol (EBA) samples for SARS-CoV-2 variants over time. a, c, e, Scatter plots depict the change of viral RNA copies on the log 10 scale from June 6, 2020 to March 11, 2022. Each point represents a sample collected for an individual on a specific date. **b, d, f,** Boxplots present the comparison of viral RNA copies on the log 10 scale by SARS-CoV-2 variants. The Kruskal-Wallis p-value indicates the global comparison among the four variants. The asterisks indicate the pairwise comparison between two variants. Only those with a p-value less than 0.05 are shown (*: p <= 0.05; **: p <= 0.01; ***: p <= 0.001; ****: p <= 0.0001). The *n* indicates the number of samples included in each boxplot. **a**, **b,** Fine EBA (≤5 μm in diameter); **c**, **d,** Coarse EBA (>5 μm in diameter); **e**, **f,** Total EBA (fine and coarse combined). *Ancestral/other* means SARS-CoV-2 ancestral strains and other variants not associated with increased transmissibility. *Omicron* includes BA.1, BA.1.1, and BA.2 subvariants.

Figure 3. Correlation between viral RNA copies in fine (≤5 μm in diameter) exhaled breath aerosol (EBA) and mid-turbinate swab (MTS) samples as well as saliva. The locally weighted smoothing (LOESS) curves and spearman correlation coefficients (rho) demonstrate the correlation of the RNA copies on the log 10 scale between *fine* EBA and MTS (**a** and **b**) as well as *fine* EBA and saliva (**c** and **d**) from June 6, 2020 to March 11, 2022. The shaded areas represent the 95% confidence interval of the smooth curves. Each point represents samples collected from an individual on a specific day. Rho (ρ) means spearman correlation coefficient. **a** and **c** depict the correlations among Pre-Omicron (ancestral/other, Alpha, and Delta) infections. **b** and **d** depict the correlations among Omicron (including BA.1, BA.1.1, BA.2) infections. *Ancestral/other* means SARS-CoV-2 ancestral strains and other variants not associated with increased transmissibility.

Figure 4. Predictors for SARS-CoV-2 RNA loads in *fine* exhaled breath aerosol. a-b, Predictors for viral RNA loads in *fine* exhaled breath aerosol among 29 participants with Omicron (BA.1, BA.1.1, BA.2) infections enrolled from December 16, 2021 to March 11, 2022. **c-d,** Predictors of viral RNA loads in *fine* exhaled breath aerosol over the course of the pandemic from June 6, 2020 to March 11, 2022. Unadjusted models show the effect of one predictor at a time; adjusted models include the multiple predictors shown so that the effect of each predictor is adjusted for the effect of other predictors. Linear mixed-effect models with censored responses analyses accounted for samples below the limit of detection and repeated measures from the same subject. Potential confounding by age and sex were controlled by including them in all adjusted models.

Effect estimates and their 95% confidence intervals are shown as the ratio of RNA copy number of samples: variant to variants other than Alpha/Delta/Omicron, Omicron BA.2 to Omicron BA.1

and BA.1.1, received to not received a booster, anti-nucleocapsid positive to negative, male to female, or as the fold-increase in RNA copy number for a 10-year increase in age, 1-day increase in day post-symptom onset or days since last vaccine/booster, 1-count increase in numbers of coughs, and an interquartile range change in symptom scores, mid-turbinate swab and saliva RNA copy number.

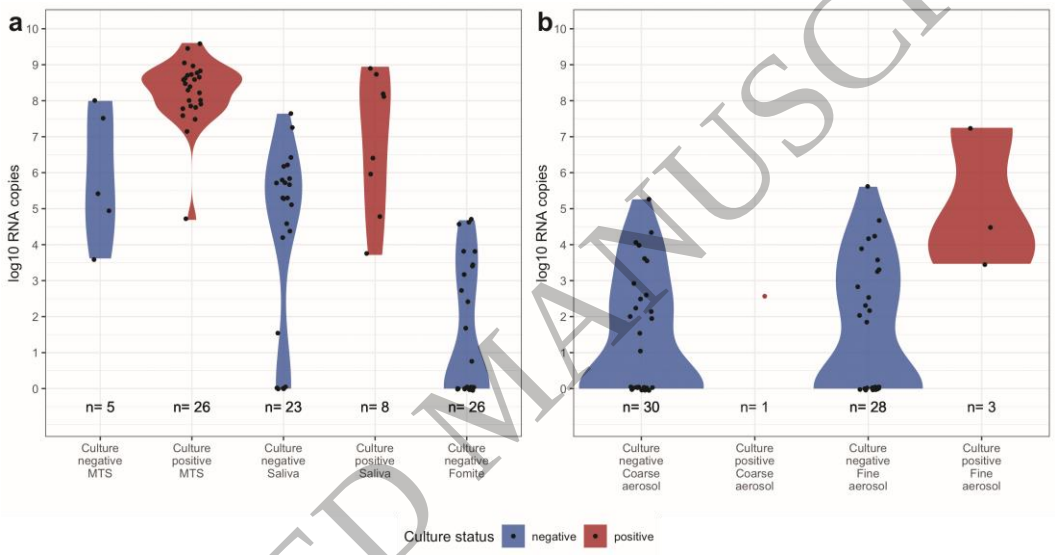

Figure 1
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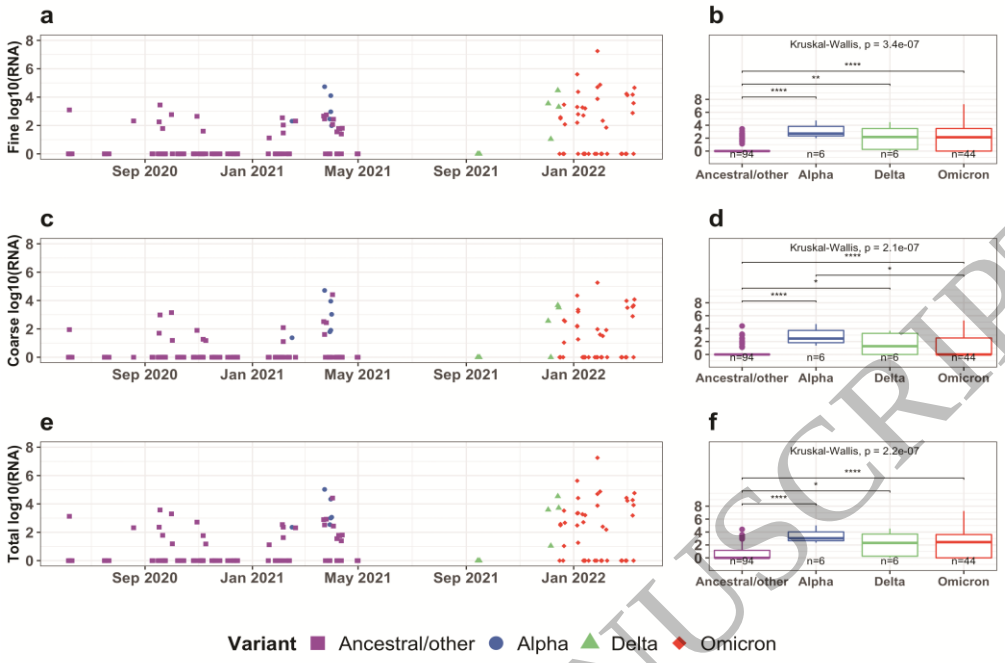

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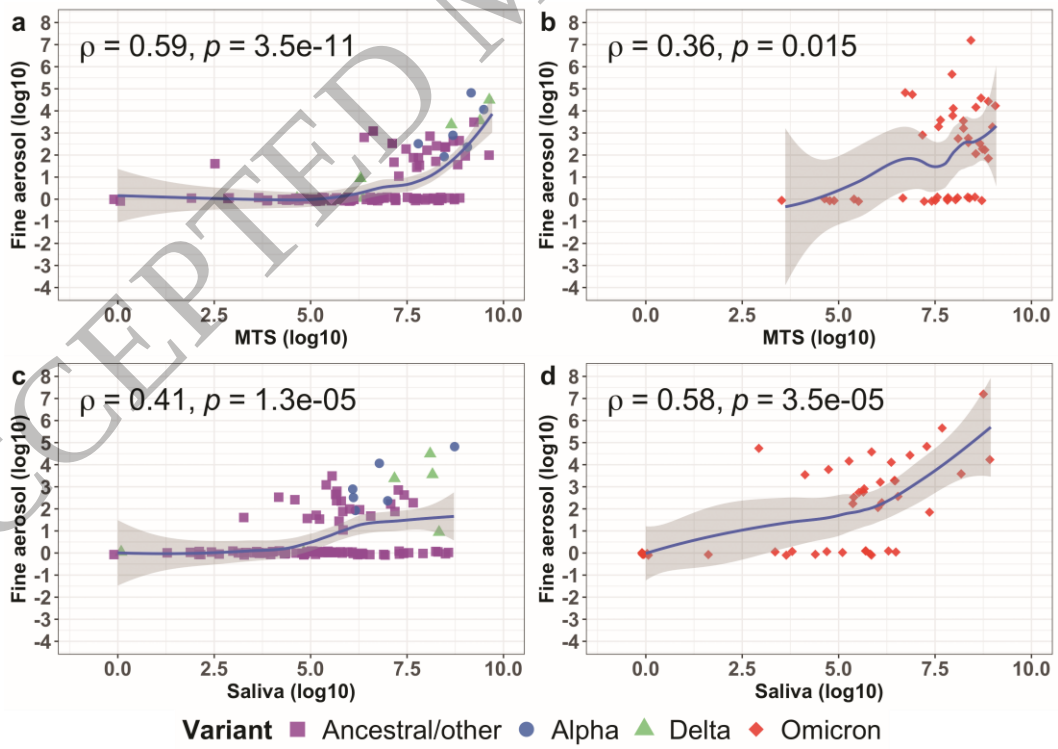

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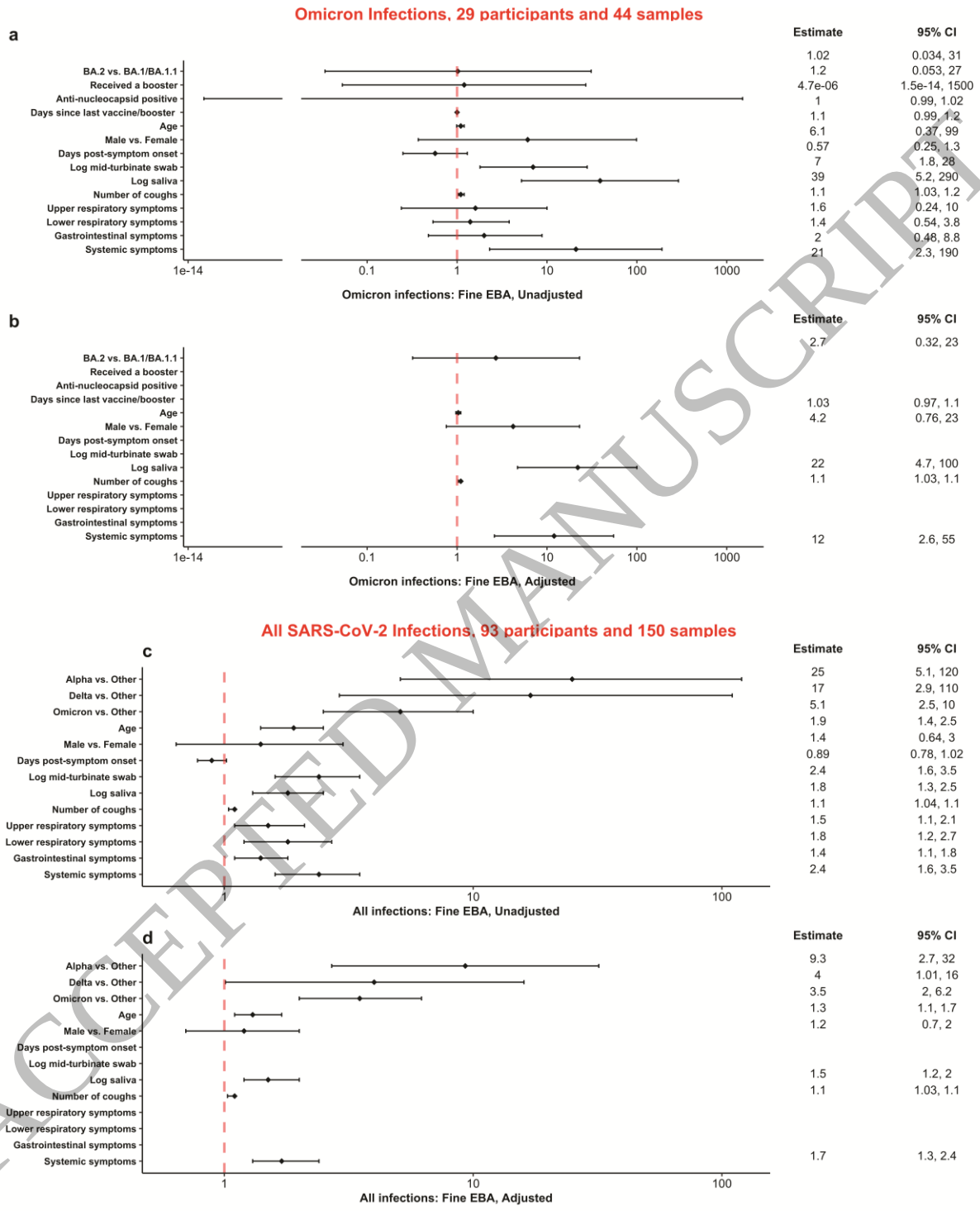

Figure 4
305x356 mm (x DPI)