**SUPPLEMENTARY MATERIALS**

**Evolution of SARS-CoV-2 Shedding in Exhaled Breath Aerosols**

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# SUPPLEMENTARY METHODS

## Study overview

COVID-19 cases and their close contacts from the University of Maryland and the surrounding community were recruited as part of an ongoing research project “StopCOVID@UMD” aiming to study the transmission of SARS-CoV-2[1]. This study was approved by the University of Maryland Institutional Review Board and the Human Research Protection Office of the Department of the Navy. Electronic informed consent was obtained and questionnaire data were collected and stored using REDCap[2].

## Symptom reporting

On each day of sample collection, participants completed an online questionnaire to update the status of their symptoms and record any medication used. As previously described[1], participants were asked to self-report 16 symptoms on a scale from zero to three. Separate composite symptom scores were then calculated for systemic, gastrointestinal, lower respiratory, and upper respiratory symptoms.

## Sample processing and laboratory analyses

Samples were processed and laboratory analyses were carried out as previously described [1]. Briefly, nucleic acids were extracted from all aerosol, phone swab, mid-turbinate swab (MTS), and saliva samples using the MagMax Pathogen RNA/DNA Kit (Applied Biosystems) on a KingFisher Duo Prime (Thermo Scientific), following the manufacturers’ protocols specific to sample type. MS2 phage was spiked in each extraction to control for extraction and PCR failure. SARS-CoV-2 RNA was detected and quantified using the TaqPath COVID-19 Combo Kit (Applied Biosystems), a multiplex real-time RT-PCR assay that targets SARS-CoV-2 N gene, ORF1ab, and MS2 spike-in. Each quantification plate contained positive and negative extraction controls, a no template control, and all reactions were carried out in duplicate. Ct values of the N gene assay were standardized against serial dilutions of RNA, which were purified from SARS-CoV-2 culture and quantified against inactivated SARS-CoV-2 (BEI Resources NR-52286). Standard curves were established based on N gene Ct values from at least 7 dilutions and had R2 values > 0.99. RNA copy numbers were reported per mL for saliva and per sample for all other sample types. The limit of detection (LOD, 95% probability of detection) was 62 copies/mL for saliva and 75 copies/sample for all other sample types. One aliquot of aerosol, phone swab, MTS, and saliva samples from the first day of case visits (when the MTS and saliva usually showed lower Ct values) were cultured with TMPRSS2-expressing VeroE6 cells and A549-ACE2 cells in a biosafety level 3 laboratory, as described in detail in our previous publication[1]. Plasma samples were also assayed for antibodies to SARS-CoV-2 as previously described[1]. IgG antibodies were titered using the SARS-CoV-2 receptor binding domain (RBD) and nucleocapsid (N) proteins (ACRO Biosystems) as the targets. Genome sequencing of MTS samples was performed using a MinION sequencing system (Oxford Nanopore Technologies, ONT) following the 1200-bp tiled amplicon (“Midnight”) protocol[3]. Fastq reads were uploaded to the EPI2ME platform (ONT) for sequence assembly as well as clade and lineage analyses.

## Statistical analyses

Data cleaning and analyses were completed using R version 4.2.0 and RStudio[4]. Descriptive analyses were done for all participants and by time period of enrollment (from June 6, 2020 to April 30, 2021, and from September 14, 2021 to March 11, 2022). Boosted participants were defined as having received one vaccine booster dose no less than 8 days prior to study enrollment[5].

The Mann–Whitney *U* Test was used for pairwise comparisons of EBA viral RNA loads and number of coughs per 30-minute session for Alpha, Delta, Omicron, and other variants, for pairwise comparisons of composite symptom scores and individual symptoms for Alpha, Delta, Omicron BA.1, Omicron BA.2, and other variants, and to compare EBA viral RNA load by booster and MTS viral RNA load by anti-nucleocapsid IgG status for Omicron cases. The Kruskal-Wallis test was used to compare EBA viral RNA loads among three subvariants of Omicron (BA.1, BA.1.1, and BA.2) and for global comparison among variants in terms of EBA viral RNA load, number of coughs per 30-minute session, composite symptom scores, and individual symptoms. Spearman correlation coefficients (rho) and locally weighted smoothing (LOESS) curve with a 95% confidence interval were used to depict the correlation between EBA and MTS as well as EBA and saliva in terms of viral RNA copy numbers.

Linear mixed-effect models with censored responses[6,7] (R package “lmec”[8]) were used to calculate the geometric means and standard deviations of viral RNA copy numbers for all sample types, and to estimate the effect of predictors on *fine* EBA viral load, accounting for censored observations below the limit of detection and nested random effects of subjects and samples nested within subjects. Linear mixed-effect models (R package “lme4”[9]) were used for the effect of predictors on *coarse* EBA viral load because the models accounting for censored responses were unstable due to a large proportion of censored responses in some strata; a value of 1 was assigned to those with a viral load that was censored below the limit of detection (75 copies) in this analysis. All the adjusted models were selected based on the Akaike information criterion (AIC) while keeping age and sex for models over the course of the pandemic and keeping Omicron subvariants, age, and sex for models among participants with Omicron infections. Interactions between booster status and age, sex, as well as Omicron subvariants were considered in the adjusted models among the Omicron infections; only those that were included in the final models were presented.

# SUPPLEMENTARY TABLES

## Supplementary Table 1. Vaccine and booster types received by Delta and Omicron cases, September 14, 2021 – March 11, 2022.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | Booster type | | | |
| BNT162b2 | mRNA-1273 | NVX-CoV2373 | Not boosted |
| Vaccine Type | BNT162b2 | 9 | 5 | 0 | 10 |
| mRNA-1273 | 1 | 3 | 0 | 0 |
| Ad26.COV2.S | 1 | 1 | 0 | 1 |
| NVX-CoV2373 | 0 | 0 | 0 | 1 |

## Supplementary Table 2. Demographics for SARS-CoV-2 cases, excluding samples collected more than five days post symptom onset

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **Enrolled June, 2020 - April 2021** | **Enrolled September, 2021 - March, 2022** | **All participants** |
| **Number of participants** | | 48 | 30 | 78 |
| **Number of exhaled breath samples** | | 67 | 45 | 112 |
| **Variant, N(%)** | **Other** | 45 (94) | 0 (0) | 45 (58) |
| **Alpha** | 3 (6) | 0 (0) | 3 (4) |
| **Delta** | 0 (0) | 3 (10) | 3 (4) |
| **Omicron BA.1** | 0 (0) | 8 (27) | 8 (10) |
| **Omicron BA.1.1** | 0 (0) | 13 (43) | 13 (17) |
| **Omicron BA.2** | 0 (0) | 6 (20) | 6 (8) |
| **Female, N (%)** | | 19 (40) | 12 (40) | 31 (40) |
| **Age, mean ± SD** | | 23.5 ± 9.3 | 26.5 ± 14.9 | 24.7 ± 11.8 |
| **Age group, N(%)** | **<18** | 1 (2) | 3 (10) | 4 (5) |
| **18-45** | 45 (94) | 23 (77) | 68 (87) |
| **>45** | 2 (4) | 4 (13) | 6 (8) |
| **Race/Ethnicity, N(%)** | **White** | 36 (75) | 18 (60) | 54 (69) |
| **Black/African American** | 6 (12) | 4 (13) | 10 (13) |
| **Hispanic** | 6 (12) | 5 (17) | 11 (14) |
| **BMI, mean ± SD** | | 24.8 ± 4.3 | 24.7 ± 5.6 | 24.8 ± 4.8 |
| **Chronic respiratory illness, N (%)** | | 11 (23) | 6 (20) | 17 (22) |
| **Vaccination status, N(%)** | **Boosted** | 0 (0) | 18 (60) | 18 (23) |
| **Fully vaccinated, not boosted** | 0 (0) | 12 (40) | 12 (15) |
| **Partially vaccinated** | 2 (4) | 0 (0) | 2 (3) |
| **Unvaccinated** | 46 (96) | 0 (0) | 46 (59) |
| **Anti-spike RBD antibody (IgG), N (%)** | | 4 (8) | 30 (100) | 34 (44) |
| **Anti-nucleocapsid antibody (IgG), N (%)** | | 0 (0) | 4 (13) | 4 (5) |
| **Ever symptomatic, N (%)** | | 45 (94) | 30 (100) | 75 (96) |
| **Symptomatic participants** | **Days post symptom onset, mean± SD (range)** | 3 ± 1 (0-5) | 3 ± 1 (1-5) | 3 ± 1 (0-5) |
| **Coughs per 30 min, mean± SD (range)** | 1 ± 3 (0-17) | 7 ± 13 (0-64) | 4 ± 9 (0-64) |
| **Median upper respiratory symptoms (IQR)** | 3 (1 - 4) | 4 (2 - 7) | 3 (1 - 4) |
| **Median lower respiratory symptoms (IQR)** | 0.5 (0 - 2) | 1 (1 - 2) | 1 (0 - 2) |
| **Median systemic symptoms (IQR)** | 2 (0 - 4) | 2 (1 - 7) | 2 (0 - 6) |
| **Median gastrointestinal symptoms (IQR)** | 0 (0 - 1) | 1 (0 - 2) | 0 (0 - 1) |
| **Temperature (C), mean ± SD** | 37.2 ± 0.2 | 37 ± 0.3 | 37.1 ± 0.3 |
| **Oxygen saturation (SpO2), mean ± SD** | 97.9 ± 0.9 | 97.8 ± 0.8 | 97.9 ± 0.9 |

## Supplementary Table 3. SARS-CoV-2 in respiratory and phone swab samples from Delta and Omicron cases, September 14, 2021 – March 11, 2022

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample type | Participants with ≥1 PCR positive sample, n/N (%)a | Participants with ≥1 culture positive sample,  n/N (%)b | PCR Positive Samples,  n/N (%)c | RNA copies per sample | |
| GM (95% CI)d | Maximume |
| Saliva | 27/32 (84) | 8/31 (26) | 42/50 (84) | 6.6x104  (9.5x103, 4.6x105) | 8.8x108 |
| Mid-turbinate Swab | 32/32 (100) | 26/31 (84) | 50/50 (100) | 3.6x107  (1.3x107, 9.9x107) | 4.0x109 |
| Phone Swab (fomite) | 14/30 (47) | 0/26 (0) | 20/47 (43) | 16  (2.1, 130) | 4.5x105 |
| Coarse EBAf | 16/32 (50) | 1/31 (3) | 23/50 (46) | 29  (7.1, 120) | 1.8x105 |
| Fine EBA | 20/32 (62) | 3/31 (10) | 28/50 (56) | 150  (35, 600) | 1.8x107 |
| Total EBA (fine + coarse) | 21/32 (66) | 4/31 (13) | 51/100 (51) | 200  (52, 790) | 1.8x107 |

1. Number (n) of participants (N) with at least one sample ≥ the limit of detection (LOD, 62 copies/mL for saliva and 75 copies/sample for other sample types)
2. Number (n) of participants with samples subjected to culture (N) with at least one sample giving a positive culture for SARS-CoV-2
3. Number (n) of samples (N) ≥ LOD, with at least 1 replicate with confirmed amplification after inspection and quality control
4. GM = geometric mean. The GMs were computed by controlling for random effects of subject and sample nested within subjects and for censoring by the limit of detection using a linear mixed-effects model for censored responses (R package “lmec”[8])
5. The largest quantity of RNA copies detected based on the mean of replicate qRT-PCR aliquots
6. EBA = Exhaled breath aerosol. Each EBA sample was collected using a Gesundheit-II machine for 30 minutes

## Supplementary Table 4a. Predictors for SARS-CoV-2 RNA loads in exhaled breath aerosol, including all samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Coarse**  **N=93, n=150** | | **Fine**  **N=93, n=150** | |
|  | **Unadjusted** | **Adjusted** | **Unadjusted** | **Fine Adjusted** |
| **Alpha vs. Other** | 6.8 (1.9, 24) | - | 25 (5.1, 120) | 9.3 (2.7, 32) |
| **Delta vs. Other** | 3.8 (1.1, 14) | - | 17 (2.9, 110) | 4 (1.01, 16) |
| **Omicron vs. Other** | 2.5 (1.4, 4.3) | - | 5.1 (2.5, 10) | 3.5 (2, 6.2) |
| **age** | 1.5 (1.2, 1.8) | 1.4 (1.1, 1.7) | 1.9 (1.4, 2.5) | 1.3 (1.1, 1.7) |
| **Male vs. Female** | 1.3 (0.76, 2.3) | 1.4 (0.86, 2.3) | 1.4 (0.64, 3) | 1.2 (0.7, 2) |
| **Day post-symptom onset** | 0.89 (0.8, 0.97) | - | 0.89 (0.78, 1.02) | - |
| **Log mid-turbinate swab** | 1.9 (1.4, 2.6) | 1.6 (1.2, 2.1) | 2.4 (1.6, 3.5) | - |
| **Log saliva** | 1.6 (1.2, 2) | - | 1.8 (1.3, 2.5) | 1.5 (1.2, 2) |
| **Number of coughs** | 1.01 (0.98, 1.03) | - | 1.1 (1.04, 1.1) | 1.1 (1.03, 1.1) |
| **Upper respiratory symptoms** | 1.2 (0.95, 1.6) | - | 1.5 (1.1, 2.1) | - |
| **Lower respiratory symptoms** | 1.3 (0.94, 1.7) | - | 1.8 (1.2, 2.7) | - |
| **Gastrointestinal symptoms** | 1.3 (1.1, 1.5) | - | 1.4 (1.1, 1.8) | - |
| **Systemic symptoms** | 2.2 (1.6, 2.9) | 1.8 (1.4, 2.5) | 2.4 (1.6, 3.5) | 1.7 (1.3, 2.4) |

## Supplementary Table 4b. Predictors for SARS-CoV-2 RNA loads in exhaled breath aerosol, excluding samples collected more than five days post symptom onset.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Coarse**  **N=78, n=112** | | **Fine**  **N=78, n=112** | |
|  | **Unadjusted** | **Adjusted** | **Unadjusted** | **Adjusted** |
| **Alpha vs. Other** | 15 (2.9, 82) | 5.9 (1.4, 26) | 28 (3.3, 230) | 8.3 (1.6, 42) |
| **Delta vs. Other** | 5.3 (0.99, 28) | 2.4 (0.52, 11) | 13 (1.5, 110) | 2.7 (0.51, 14) |
| **Omicron vs. Other** | 2.7 (1.3, 5.5) | 2 (1.04, 3.7) | 6.8 (2.8, 17) | 4 (2, 8) |
| **age** | 1.6 (1.2, 2.1) | 1.2 (0.94, 1.6) | 2.2 (1.6, 3.2) | 1.5 (1.1, 2) |
| **Male vs. Female** | 1.5 (0.7, 3) | 1.1 (0.62, 2.1) | 1.6 (0.6, 4.2) | 1.4 (0.71, 2.7) |
| **Day post-symptom onset** | 0.96 (0.77, 1.2) | - | 1.04 (0.78, 1.4) | - |
| **Log mid-turbinate swab** | 1.8 (1.3, 2.6) | - | 2.2 (1.4, 3.4) | - |
| **Log saliva** | 1.6 (1.3, 2.2) | 1.5 (1.1, 1.9) | 1.9 (1.3, 2.7) | 1.7 (1.2, 2.2) |
| **Number of coughs** | 1.05 (1.01, 1.1) | 1.03 (0.998, 1.1) | 1.1 (1.1, 1.1) | 1.1 (1.03, 1.1) |
| **Upper respiratory symptoms** | 1.2 (0.85, 1.7) | - | 1.7 (1.1, 2.7) | - |
| **Lower respiratory symptoms** | 1.4 (0.97, 2) | - | 2.2 (1.4, 3.6) | - |
| **Gastrointestinal symptoms** | 1.3 (1.1, 1.6) | - | 1.6 (1.3, 2.1) | - |
| **Systemic symptoms** | 3.4 (2, 5.8) | 2.4 (1.5, 3.8) | 4.3 (2.1, 8.9) | 2.6 (1.5, 4.6) |

# SUPPLEMENTARY FIGURES

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## Supplementary Figure 1. Viral RNA copies (log 10 scale) in exhaled breath aerosol (EBA) samples for SARS-CoV-2 variants over time, excluding samples collected more than five days post symptom onset.

**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.

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## Supplementary Figure 2. Viral RNA copies (log 10 scale) in fine and coarse exhaled breath aerosol (EBA) samples for SARS-CoV-2 Omicron subvariants over time, December 2021 – March 2022.

**a, c, e,** Scatter plots depict the change of viral RNA copies on the log 10 scale over time. 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 Omicron subvariants. The Kruskal-Wallis p-value indicates the global comparison among the three subvariants. None of the pairwise comparison between two subvariants is significant at the level of 0.05. 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).

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## Supplementary 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, excluding samples collected more than five days post symptom onset.

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.

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## Supplementary Figure 4a. Correlation between SARS-CoV-2 RNA copies in coarse exhaled breath aerosol (>5 µm in diameter) and mid-turbinate swab (MTS) samples as well as saliva, June 6, 2020 – March 11, 2022.

The locally weighted smoothing (LOESS) curves demonstrate the correlation of the RNA copies on the log 10 scale between *coarse* EBA and MTS (**a**, **b**) as well as *coarse* EBA and saliva (**c**, **d**). 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) is the 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.

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## Supplementary Figure 4b. Correlation between SARS-CoV-2 RNA copies in coarse exhaled breath aerosol (>5 µm in diameter) and mid-turbinate swab (MTS) samples as well as saliva, June 6, 2020 – March 11, 2022, excluding samples collected more than five days post symptom onset.

The locally weighted smoothing (LOESS) curves demonstrate the correlation of the RNA copies on the log 10 scale between *coarse* EBA and MTS (**a**, **b**) as well as *coarse* EBA and saliva (**c**, **d**). 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) is the 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.

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## Supplementary Figure 5. Viral RNA load in exhaled breath aerosol samples by booster status for Omicron cases, December 16, 2021 – March 11, 2022.

**a, c, e,** Scatter plots depict the change of viral RNA copies on the log 10 scale over time. 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 booster status. 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).

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## Supplementary Figure 6. Viral RNA copies (log 10 scale) by status of anti-nucleocapsid IgG for Omicron cases, December 16, 2021 – March 11.

Boxplots present the comparison of viral RNA copies on the log 10 scale by the status of anti-nucleocapsid IgG at baseline for 29 Omicron cases (24 negative, 5 positive). The *n* indicates the number of samples included in each boxplot. **a,** Mid-turbinate swab (MTS); **b,** Saliva; **c,** Fomite (swab of participant’s mobile phone); **d** and **e,** *Fine* (≤5 µm in diameter) and *Coarse* (>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 samples.

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## Supplementary Figure 7. Predictors for SARS-CoV-2 RNA loads in coarse exhaled breath aerosol.

**a-b, P**redictors for viral RNA loads in *coarse* exhaled breath aerosol among 29 participants with Omicron infections enrolled from December 16, 2021 to March 11, 2022. **c-d,** Predictors of viral RNA loads in *coarse* exhaled breath aerosol over the course of the pandemic from June 6, 2020 to March 11, 2022. Effect estimates and their 95% confidence intervals from linear mixed effect models (observations censored by the limit of detection coded as 1) 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.

Diagram

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## Supplementary Figure 8a. Number of coughs per 30-minute sampling and composite symptom scores for SARS-CoV-2 variants over time, June 6, 2020 – March 11, 2022.

**a**, Each point in the scatter plot represents a sample collected for an individual on a specific date. **b,** The boxplots present the comparison of number of coughs per 30-minute sampling session by SARS-CoV-2 variants. **c - f,** The boxplots present the comparison of four composite symptom scores by SARS-CoV-2 variants/subvariants. The Kruskal-Wallis p-value indicates the global comparison among the four (**a-b**) or five (**c - f**) variants/subvariants. The asterisks indicate the pairwise comparison. 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/O* means SARS-CoV-2 ancestral strains and other variants not associated with increased transmissibility.

Diagram, schematic

Description automatically generated

## Supplementary Figure 8b. Number of coughs per 30-minute sampling and composite symptom scores for SARS-CoV-2 variants over time, June 6, 2020 – March 11, 2022, excluding samples collected more than five days post symptom onset.

**a**, Each point in the scatter plot represents a sample collected for an individual on a specific date. **b,** The boxplots present the comparison of number of coughs per 30-minute sampling session by SARS-CoV-2 variants. **c - f,** The boxplots present the comparison of four composite symptom scores by SARS-CoV-2 variants/subvariants. The Kruskal-Wallis p-value indicates the global comparison among the four (**a-b**) or five (**c - f**) variants/subvariants. The asterisks indicate the pairwise comparison. 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/O* means SARS-CoV-2 ancestral strains and other variants not associated with increased transmissibility.

Calendar

Description automatically generated

## Supplementary Figure 9a. Self-reported symptoms for SARS-CoV-2 variants over time, June 6, 2020 – March 11, 2022.

The boxplots present the comparison of 16 self-reported symptoms (on a scale of zero to three) by SARS-CoV-2 variants/subvariants. The Kruskal-Wallis p-value indicates the global comparison among the five variants/subvariants. The asterisks indicate the pairwise comparison. 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/O* means SARS-CoV-2 ancestral strains and other variants not associated with increased transmissibility.

Diagram, calendar

Description automatically generated

## Supplementary Figure 9b. Self-reported symptoms for SARS-CoV-2 variants over time, June 6, 2020 – March 11, 2022, excluding samples collected more than five days post symptom onset.

The boxplots present the comparison of 16 self-reported symptoms (on a scale of zero to three) by SARS-CoV-2 variants/subvariants. The Kruskal-Wallis p-value indicates the global comparison among the five variants/subvariants. The asterisks indicate the pairwise comparison. 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/O* means SARS-CoV-2 ancestral strains and other variants not associated with increased transmissibility.

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