nature medicine

ARTICLES

https://doi.org/10.1038/s41591-022-01816-0

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Infectious viral load in unvaccinated and vaccinated individuals infected with ancestral, Delta or Omicron SARS-CoV-2

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Infectious viral load (VL) expelled as droplets and aerosols by infected individuals partly determines transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). RNA VL measured by qRT–PCR is only a weak proxy for infectiousness. Studies on the kinetics of infectious VL are important to understand the mechanisms behind the different transmissibility of SARS-CoV-2 variants and the effect of vaccination on transmission, which allows guidance of public health measures. In this study, we quantified infectious VL in individuals infected with SARS-CoV-2 during the first five symptomatic days by in vitro culturability assay in unvaccinated or vaccinated individuals infected with pre-variant of concern (pre-VOC) SARS-CoV-2, Delta or Omicron BA.1. Unvaccinated individuals infected with pre-VOC SARS-CoV-2 had lower infectious VL than Delta-infected unvaccinated individuals. Full vaccination (defined as >2 weeks after receipt of the second dose during the primary vaccination series) significantly reduced infectious VL for Delta breakthrough cases compared to unvaccinated individuals. For Omicron BA.1 breakthrough cases, reduced infectious VL was observed only in boosted but not in fully vaccinated individuals compared to unvaccinated individuals. In addition, infectious VL was lower in fully vaccinated Omicron BA.1-infected individuals compared to fully vaccinated Delta-infected individuals, suggesting that mechanisms other than increased infectious VL contribute to the high infectiousness of SARS-CoV-2 Omicron BA.1. Our findings indicate that vaccines may lower transmission risk and, therefore, have a public health benefit beyond the individual protection from severe disease.

As of 6 March 2022, the Coronavirus Disease 2019 (COVID-19) pandemic has caused more than 443 million cases and just over 5.9 million deaths globally[1]. SARS-CoV-2, the causative agent of COVID-19, primarily infects the cells of the upper respiratory tract (URT) where VL increases during the course of infection[2].

The two key measurements of VL are RNA levels, often expressed in cycle threshold (Ct) values, and infectious virus that is assessed by virus isolation in cell culture. Although the transmission process is complex, higher VL can serve as a proxy for greater risk of transmission. In several epidemiological studies, higher VL measured by viral RNA was associated with increased secondary transmission in household settings[3,4]. Infectious SARS-CoV-2 is shed in the URT, starting, on average, from 2 days before symptom onset. In most studies, infectious virus was not detected in respiratory samples collected from non-hospitalized immunocompetent individuals later than 8 days post onset of symptoms (DPOS)[5–7]. Moreover, viral RNA detection did not correlate with infectiousness in an animal model[8]. Instead, isolation success in cell culture—that is, the ability to replicate the virus in cell culture—was found to correlate with the ability to shed and transmit fully competent viral particles[9]. Virus isolation success from respiratory tract samples can give information only about the presence or absence of infectious virus but is not able to quantify the infectious viral titer in samples of the URT[10].

Since the start of the pandemic, SARS-CoV-2 has constantly evolved, leading to the emergence of new variants. Although most variants vanished quickly, others, such as D614G and the VOCs Alpha, Beta, Gamma, Delta and Omicron, harbor an apparent selection advantage and outcompeted other variants locally or even globally. These VOCs exhibit various mutations[11] that lead to immune evasion and/or higher transmissibility, to which increased viral shedding (among other factors, such as environmental stability) may significantly contribute[12,13]. For Alpha, an approximately ten-fold-higher RNA VL was described compared to pre-VOC viral strains, which was correlated with increased isolation success[14,15]. Similarly, Delta also showed 10–15-fold-higher RNA levels compared to pre-VOC strains in some studies[15,16]. In contrast, a study using longitudinal samples did not find a difference among peak RNA VL of pre-VOC, Alpha and Delta[17]. However, little is known about the quantity of shed infectious viral particles for VOCs, including Omicron.

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There is extensive evidence that vaccines against SARS-CoV-2, which target the original strain, reduce infection case numbers and disease severity. However, the effect of vaccination on infectious viral shedding and transmission from vaccinated individuals remains controversial. All currently approved vaccines are administered intramuscularly; thus, the titer of neutralizing antibodies on the mucosal surfaces lining the URT might be limited, and any sterilizing mucosal immunity might be transient[18]. Epidemiological studies of the secondary attack rate in households of vaccinated versus unvaccinated index cases report contradictory results on the potential effect of vaccination[19–21]. Multiple factors can influence the secondary attack rate in these studies, including patient behavior, age, comorbidities, the infecting variant, time since vaccination and the vaccine used. Therefore, differentiating the effect of vaccination on VL from other factors in purely epidemiological studies is difficult. To our knowledge, no study has directly quantified infectious VL of different VOCs in URT samples of vaccinated and unvaccinated patients with COVID-19.

The dynamics of infectious viral shedding in vaccinated and unvaccinated individuals infected with relevant VOCs require detailed investigation. Understanding of viral shedding in patients would help shape public health decisions to limit community transmission[22]. Here we compare RNA and infectious VL among pre-VOC strains, Delta and Omicron BA.1 in unvaccinated individuals, as well as in fully vaccinated (two doses) or boosted (three doses) individuals infected with Delta and Omicron BA.1, using respiratory samples from mildly symptomatic patients of different age and sex, sampled in the first 5 DPOS.

Results

In this study, we analyzed the VL characteristics in the URT of unvaccinated pre-VOC-infected individuals, as well as fully vaccinated, boosted and unvaccinated Delta-infected or Omicron BA.1-infected individuals, up to 5 DPOS. We included a total of 565 samples in our cohort, of which 118 originated from individuals infected with pre-VOC SARS-CoV-2; 293 originated from individuals infected with Delta; and 154 originated from individuals infected with Omicron BA.1. Of individuals infected with Delta, 166 were fully vaccinated before infection, and 127 were unvaccinated. Among individuals infected with Omicron BA.1, 91 were fully vaccinated before infection, 30 were boosted and 33 were unvaccinated. None of the individuals infected with pre-VOC SARS-CoV-2 was vaccinated, as vaccines were unavailable at the time of infection. All infected individuals had mild symptoms at the time of sampling, but the further course of the disease is unknown. Individuals with asymptomatic infection at the time of sampling were excluded from the study. All infected individuals except five (two with Delta breakthrough infections and three with Omicron BA.1 breakthrough infections) were immunocompetent. Samples of pre-VOC-infected individuals were collected between 7 April and 9 September 2020, before detected circulation of any VOCs; samples of Delta-infected individuals were collected from 26 June until 13 December 2021; and samples of Omicron BA.1-infected individuals were collected from 11 December 2021 until 19 February 2022. Each infected individual provided only one sample at a single time point. All vaccinated individuals included in this study were diagnosed positive at least 14 days after dose 2 or dose 3, which complies with the vaccination breakthrough definition of the Centers for Disease Control and Prevention (CDC)[23]. In total, 274 of 287 patients were vaccinated with mRNA vaccines (Comirnaty or Spikevax); one was vaccinated with a non-replicating viral vector vaccine (CoviVac); one was vaccinated with inactivated virus vaccine CoronaVac; one was vaccinated with viral vector vaccine AZD1222; and, for ten patients, the type of vaccine was not reported by the patient. The median time in days between the second dose and breakthrough infection was 69 days (interquartile range (IQR), 38–122), 160 days (IQR, 137–183) and 154 days (IQR, 86–194) for Delta infections titrated on Vero E6 or Vero E6-TMPRSS cells and Omicron BA.1 infections, respectively. All groups of patients (pre-VOC, Delta-unvaccinated, Delta-vaccinated (two doses), Omicron BA.1-unvaccinated and Omicron BA.1-vaccinated (two or three doses)) had a similar age and sex distribution (Table 1).

We quantified genome copies and infectious viral titers in SARS-CoV-2-positive nasopharyngeal swabs (NPSs) using qRT–PCR and focus-forming assays (FFAs). Only specimens with Ct values below 27 for the E-gene qRT–PCR diagnostic target (Cobas, Roche), as determined by the clinical laboratory at the University Hospital of Geneva (HUG) at the time of diagnosis, were included in our study, as it was shown previously that infectious virus cannot be reliably isolated from samples with higher Ct values[9,24]. In our hands, no infectious virus was detected in 46 pre-VOC and Delta samples with Ct values ≥27. We also compared overall percentages of samples with a Ct ≥27 for time periods with almost exclusive circulation of pre-VOC, Delta and Omicron BA.1 by analyzing the overall diagnostic dataset from our outpatient testing center and separating patients by vaccination status and DPOS. Among pre-VOC samples, 19.4% had a Ct ≥27, whereas, in the Delta-infected unvaccinated and vaccinated groups, as well as in the Omicron BA.1-infected unvaccinated and vaccinated groups, 21.4%, 17.6%, 21.4% and 20.7% of samples fell into this category, respectively. No major difference was observed between the proportion of Ct value ≥27 when divided by DPOS (Supplementary Table).

To validate our FFA, we compared it to the ability to successfully isolate virus in cell culture. Virus isolation success has been used as a correlate of infectious viral shedding for SARS-CoV-2 (refs. [6,25–27]) but lacks the ability to differentiate between high and low VL samples. We were able to quantify viral titers using the FFA in 91.9%, 91.7%, 83.8%, 95% and 85.7% of culture-positive samples in the pre-VOC, Delta-unvaccinated, Delta-fully vaccinated (two doses), Omicron BA.1-unvaccinated and Omicron BA.1-fully vaccinated (two doses) groups, respectively, indicating a high sensitivity (Extended Data Fig. 1a). Overall, the Cohen's kappa agreement, which measures the level of agreement between two methods, was 0.69, 0.41, 0.51, 0.66 and 0.47 for the five groups, showing a moderate to substantial agreement (Extended Data Fig. 1b).

Low correlation between genome copies and infectious VL. First, we investigated whether RNA genome copies are a good proxy for infectious virus shedding. We observed only a very low correlation ($R^2 = 0.1476$, $P = 0.0001$) between viral genome copies and infectious virus particles for pre-VOC samples (Fig. 1a). Likewise, low to moderate correlations between RNA genome copies and infectious viral titers were observed for the samples from unvaccinated and vaccinated Delta patients ($R^2 = 0.3114$, $P < 0.0001$ and $R^2 = 0.4021$, $P < 0.0001$, respectively) (Fig. 1b,c), as well as unvaccinated and vaccinated Omicron BA.1 patients ($R^2 = 0.3638$, $P = 0.0002$ and $R^2 = 0.3055$, $P < 0.0001$, respectively) (Fig. 1d,e).

Next, we tested if infectious VLs are associated with patient age and sex. We did not observe any correlation between the age and infectious VL for all four groups (Extended Data Fig. 2). Similarly, no significant differences of infectious VLs between male and female patients were detected for pre-VOC, Delta (fully vaccinated or unvaccinated) or Omicron BA.1 (fully vaccinated) samples (Extended Data Fig. 3).

Delta-infected unvaccinated individuals have higher infectious VL. Next, we compared genome copies and infectious VLs in pre-VOC and Delta samples from unvaccinated patients during the first 5 DPOS. Overall, pre-VOC samples had significantly more genome copies (2.98-fold, 0.4744 $\log_{10}$, $P = 0.001$, t-value = 3.3512, df = 184.48, Cohen's d = 0.44) than Delta samples, but infectious viral titers were significantly higher in Delta-infected

Table 1 | Patient characteristics of the specimens used in this study

	Unvaccinated SARS-CoV-2 pre-VOC	Unvaccinated SARS-CoV-2 Delta VOC	Vaccinated SARS-CoV-2 Delta VOC	Vaccinated SARS-CoV-2 Delta VOC	Unvaccinated SARS-CoV-2 Omicron BA.1 VOC	Vaccinated SARS-CoV-2 Omicron BA.1 VOC
Number	118	127	104	62	33	121 (among these, 30 are boosted)
Sampling dates	7 April–9 September 2020	26 June–29 August 2021	8 July–4 December 2021	8 October–13 December 2021	16 December–19 February 2022	11 December–19 February 2022
Cell line used for titration	Vero E6	Vero E6	Vero E6	Vero E6-TMPRSS	Vero E6-TMPRSS	Vero E6-TMPRSS
Age (years)						
Median (range)	36 (17–82)	37 (16–83)	41 (16–83)	41.5 (20–70)	32 (17–68)	36 (14–71)
<25	22 (18.6%)	19 (14.9 %)	12 (11.5%)	3 (4.8%)	5 (15.2 %)	14 (11.6 %)
25–35	37 (31.4%)	38 (29.9%)	30 (28.8%)	14 (22.6 %)	14 (42.4 %)	37 (30.6%)
35–50	30 (25.4%)	41 (32.3%)	37 (35.6%)	25 (40.3 %)	6 18.2%)	43 (35.5%)
50–65	23 (19.5%)	25 (19.7%)	21 (20.2 %)	18 (29 %)	4 (12.1 %)	26 (21.5 %)
>65	6 (5.1%)	4 (3.1%)	4 (3.8%)	2 (3.2 %)	4 (12.1 %)	1 (0.8 %)
Sex						
Female	50 (42.4%)	65 (51.2%)	53 (51 %)	32 (51.6 %)	17 (51.5 %)	63 (52 %)
Male	68 (57.6%)	62 (48.8%)	51 (49 %)	30 (48.4 %)	16 (48.5 %)	58 (48 %)
RT–PCR result, Ct (E-gene target, Cobas 6800, Roche)	13.9–26·6	13.8–26.3	16.3–26.1	15.9–26.1	16.6–26.7	14.6–26.7
DPOS median (minimum, maximum)	2 (0,5)	3 (0,5)	2 (0,5)	2 (0,5)	2 (0,5)	2(0,5)
Number of samples selected per each DPOS						
DPOS 0	15	17	15	11	2	12
DPOS 1	22	29	23	11	13	30
DPOS 2	25	17	15	10	9	31
DPOS 3	21	24	18	11	6	23
DPOS 4	21	27	20	9	2	19
DPOS 5	14	13	13	10	1	6
Interval vaccination to infection, days, median (IQR)	na	na	69 (IQR, 38–122)	160 (IQR, 137–183)	na	154 (IQR, 86–194)
Vaccine						
BNT162b2 (Comirnaty)	na	na	38	28	na	43
mRNA-1273 (Spikevax)	na	na	61	32	na	72[a]
CoviVac	na	na	1	-	na	-
AZD1222	na	na	-	1	na	-
CoronaVac	na	na	-	1	na	-
Vaccine unknown	na	na	4	-	na	6

na, not applicable. [a]Four individuals were boosted with Comirnaty.

individuals (2.2-fold, 0.343 $\log_{10}$, $P=0.0373$, t-value = 2.0967, df = 238.82, Cohen's d = 0.27) (Fig. 2a). We found that genome copies for pre-VOC samples were higher at 1 DPOS and 2 DPOS but similar to Delta samples at 0 DPOS, 3 DPOS, 4 DPOS and 5 DPOS (Fig. 2b). Conversely, infectious virus shedding was higher for Delta at 3–5 DPOS but similar at 0–2 DPOS (Fig. 2c). In addition, we observed that genome copies remained largely stable until 5 DPOS, with only a minimal lower number at day 5, whereas infectious VL was significantly lower for pre-VOC (linear model, day 0 versus day 5, slope significantly < 0, $P=0.00036$) but not for Delta (linear model, day 3 versus day 5, slope not significantly < 0, $P=0.07741$) (Fig. 2b,c).

The association of the infectious shedding levels with patient age and sex is highly debated[14]. In this study, we did not detect a correlation between patient age or sex and infectious VL. However, there is increasing evidence of more severe outcomes of COVID-19 disease in older male patients[25,27]. Thus, to eliminate possible confounders, 83 patients infected with Delta were matched with pre-VOC infected patients in regard to sex, age and DPOS (Extended Data Fig. 4a). Similarly, significantly higher infectious VLs (3.44-fold, 0.5361 $\log_{10}$, $P=0.001$, t-value = 3.5261, df = 41, Cohen's d = 0.54) were detected in Delta samples compared to matched pre-VOC samples (Extended Data Fig. 4b).

Fully vaccinated individuals have lower infectious VL in Delta-infected individuals. To determine vaccination's association with virus shedding, we compared genome copies and infectious VLs in unvaccinated ($n=127$) and vaccinated ($n=104$) patients infected

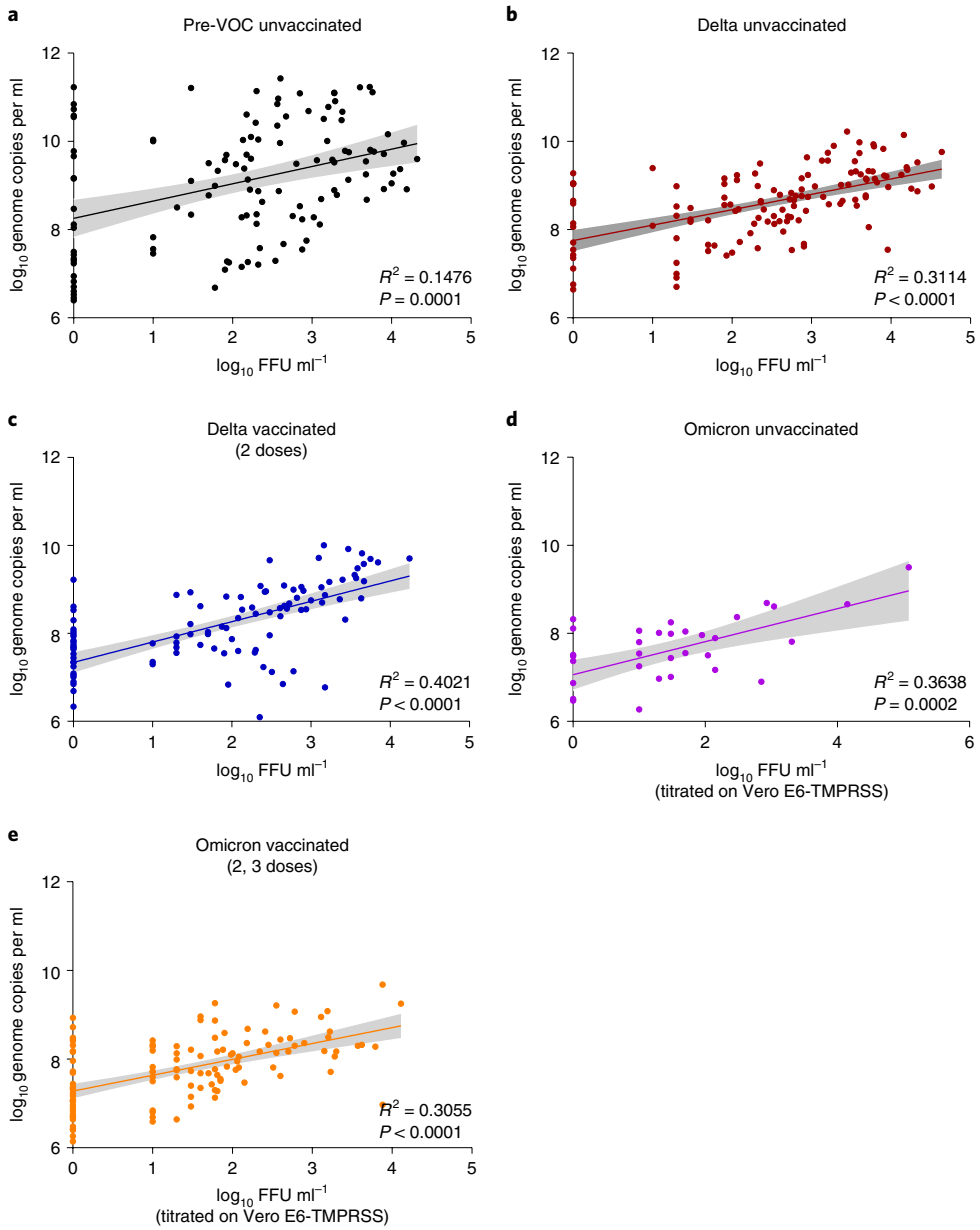


Fig. 1 | Relationship between RNA VLs and infectious viral titers. Linear regression analysis of infectious viral titers in FFU ml^{-1} and the corresponding RNA VLs in NPSs from the unvaccinated patients infected with pre-VOC (**a**), unvaccinated patients infected with Delta VOC (**b**), fully vaccinated patients infected with Delta titrated in Vero E6 cells (**c**), unvaccinated patients infected with Omicron BA.1 VOC (**d**) and fully vaccinated or boosted patients infected with Omicron BA.1 titrated in Vero E6-TMPRSS cells (**e**). Error bars represent 95% confidence bands of the best-fit line. Two-tailed *F*-test was used to determine statistical significance, and no adjustments were made for multiple comparisons.

with Delta for 5 DPOS. Overall, RNA genome copies were significantly lower in vaccinated versus unvaccinated patients (2.8-fold, 0.44 $\log_{10}$, $P = 0.0002$, t-value = 3.7942, df = 197.07, Cohen's d = 0.51). The decrease in infectious VL was even more pronounced in vaccinated patients (4.78-fold, 0.68 $\log_{10}$, $P < 0.0001$, t-value = 3.9903, df = 214.85, Cohen's d = 0.53) (Fig. 3a). The kinetics of RNA genome copies were largely similar between vaccinated and unvaccinated patients until 3 DPOS, with a faster decline for vaccinated patients starting at 4 DPOS (Fig. 3b). In contrast, infectious VLs were substantially lower in vaccinated patients at all DPOS, with the biggest effect at 3–5 DPOS (Fig. 3c). Still, at 5 DPOS, infectious virus was detectable in seven of 13 (53.8%) vaccinated patients and 11 of 13 (84.6%) unvaccinated patients. Additionally, 79 Delta-infected unvaccinated individuals were matched with Delta vaccine breakthrough patients in regard to age, sex and DPOS (Extended Data Fig. 4a). Infectious VLs were elevated in unvaccinated patients in comparison to vaccine breakthroughs (8.12-fold, 0.91 $\log_{10}$, $P = 0.001$, t-value = 3.5789, df = 35, Cohen's d = 0.60) (Extended Data Fig. 4c), confirming a significant reduction of infectious VLs among vaccinated patients. We further analyzed whether infectious VLs correlate with the time interval since administration of the last vaccine dose. A high heterogeneity between patient samples resulted in no significant correlation between the time after vaccination and infectious viral shedding (Extended Data Fig. 5a).

Booster vaccination leads to lower infectious VL in Omicron-infected individuals. Upon the emergence of Omicron BA.1, we analyzed the infectious viral shedding in unvaccinated, fully

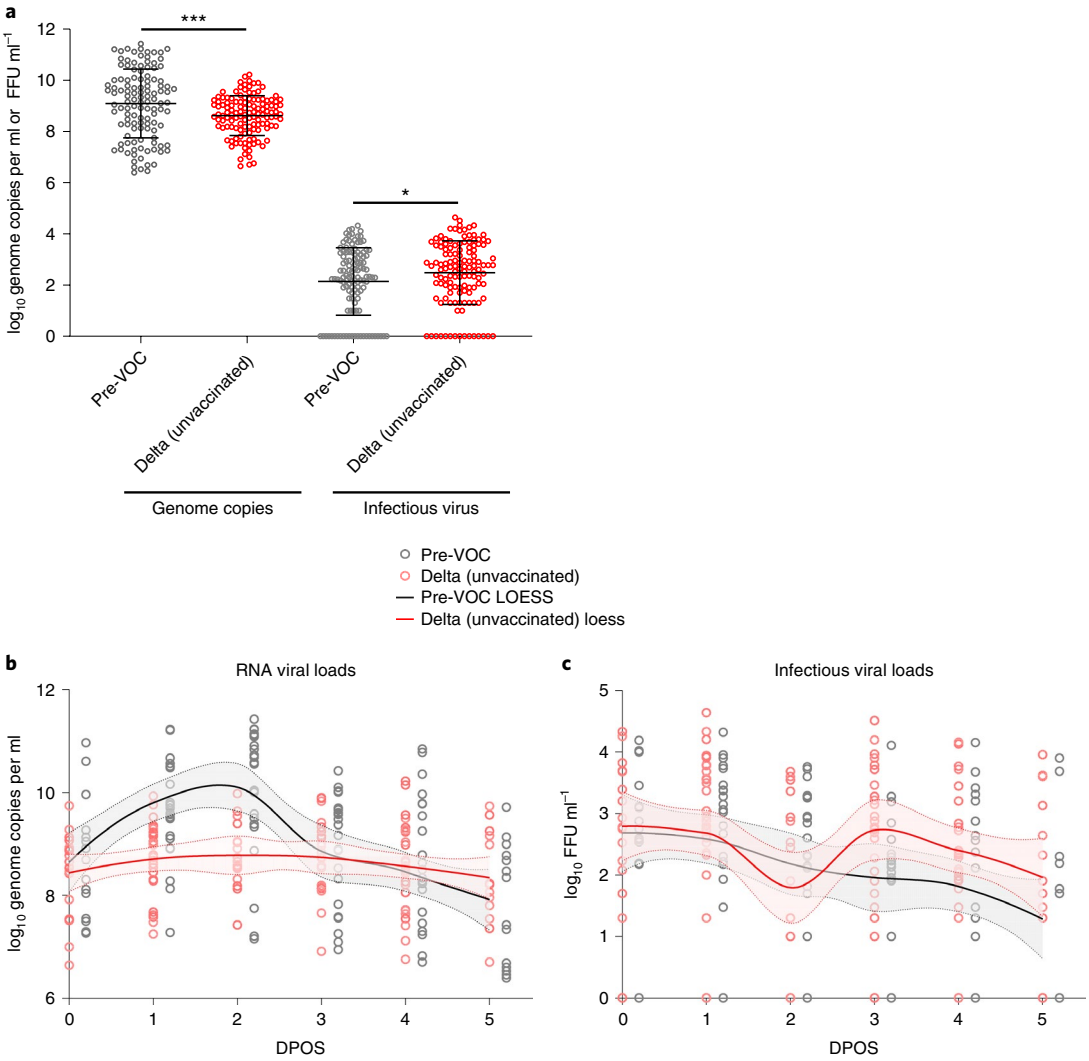


Fig. 2 | RNA VL and infectious viral titers for unvaccinated individuals infected with pre-VOC SARS-CoV-2 versus Delta. a, Genome copies (left) and infectious virus (right) for pre-VOC and Delta unvaccinated patients. Infectious titers (FFU > 0) were detected in 94 pre-VOC and 112 Delta unvaccinated patients; no titers (FFU = 0) were detected in 24 pre-VOC and 15 Delta unvaccinated patients. Error bars indicate mean ± s.d. Two-tailed *t*-test was used to determined differences of means. *$P = 0.0373$ and ***$P = 0.001$. Genome copies (**b**) and infectious VLs (**c**) measured for pre-VOC-infected and Delta VOC-infected patients at different DPOS. The solid lines represent the fitted curve calculated using the LOESS method. Error bars represent 95% confidence bands of the best-fit line. LOESS, locally estimated scatterplot smoothing.

vaccinated and boosted individuals infected with this variant. We compared RNA and infectious VLs in NPS samples of 91 Omicron BA.1-infected patients and 62 Delta-infected patients who received two doses of vaccine more than 2 weeks before diagnosis. Because Omicron BA.1 can be titrated only on Vero E6-TMPRSS cells, we also titrated another set of samples from vaccinated Delta-infected patients on this cell line to assure comparability between infectious VLs. Omicron BA.1 breakthrough infections in fully vaccinated patients resulted in similar genome copies compared to Delta but significantly lower infectious VLs (14-fold, 1.146 $\log_{10}$, $P < 0.0001$, t-value = 5.3336, df = 120.2, Cohen's d = 0.90) (Fig. 4a). A significant reduction of infectious VLs was also observed for Omicron BA.1 samples when matching patients for age, sex and DPOS (29.9-fold, 1.476 $\log_{10}$, $P = 0.00028$, t-value = 4.1887, df = 26, Cohen's d = 0.81) (Extended Data Fig. 4d). Similarly to Delta-infected fully vaccinated individuals, the RNA VLs only slightly decreased over 5 DPOS, whereas infectious VLs declined toward 5 DPOS (Fig. 4b,c). Next, we evaluated whether the vaccination status—that is, unvaccinated, fully vaccinated or boosted—has an influence on RNA or infectious VLs for Omicron-infected individuals. We found no reduction of RNA or infectious VL in fully vaccinated individuals compared to unvaccinated individuals. However, a significantly lower infectious VL, but not RNA VL, was observed for boosted individuals (5.3-fold, 0.728 $\log_{10}$, adjusted $P = 0.001325$, t-value = 3.635, df = 71.237, Cohen's d = 0.64) (Fig. 4d) compared to fully vaccinated subjects. Similarly to Delta-infected fully vaccinated patients, no significant correlation was found between days after vaccination and infectious VL in fully vaccinated Omicron BA.1-infected patients (Extended Data Fig. 5b).

Discussion

In this study, we analyzed virus shedding in COVID-19 patients infected with pre-VOC, Delta and Omicron BA.1 variants and evaluated the effect of vaccination on VL in the URT during the first 5 DPOS. To our knowledge, this is the first study to quantify infectious VLs in individuals infected with different SARS-CoV-2 variants and in vaccination breakthrough cases. We demonstrated

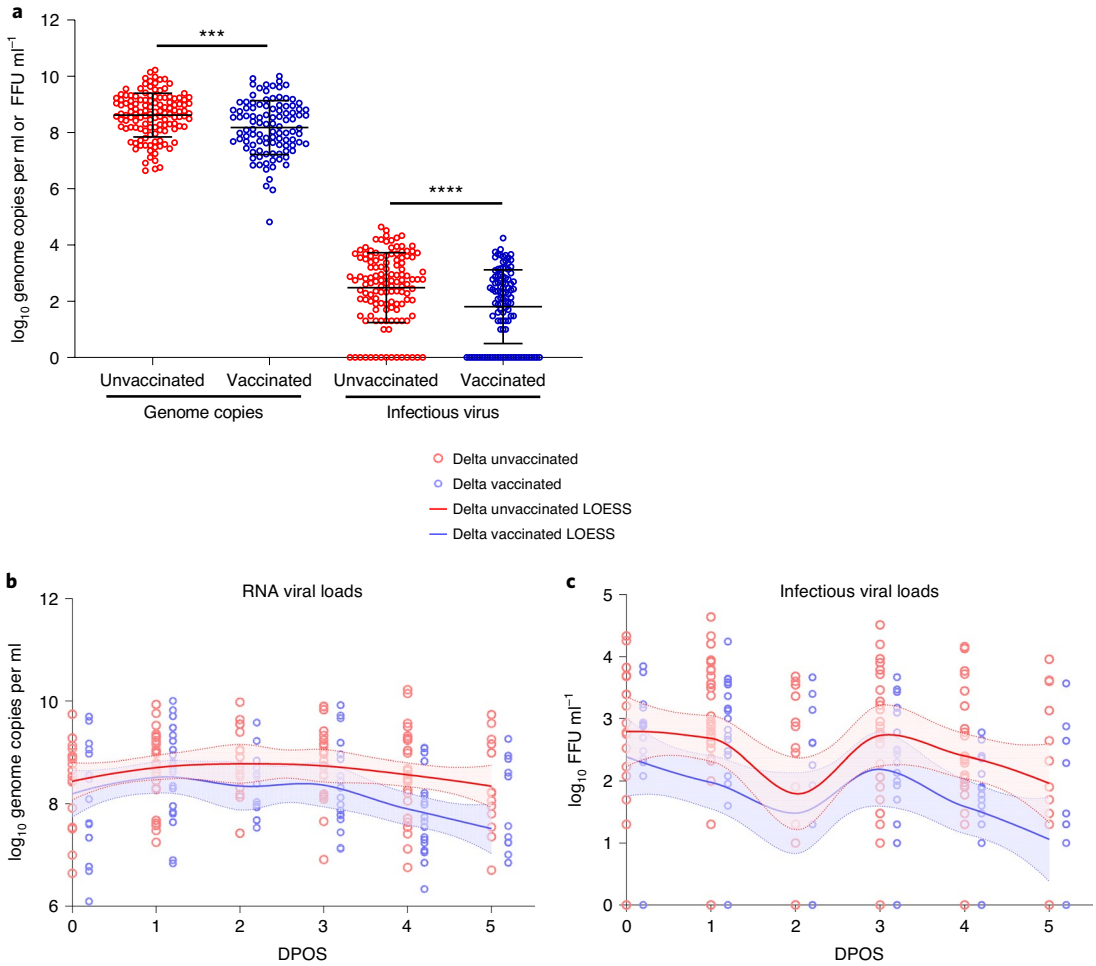


Fig. 3 | RNA VL and infectious viral titers for unvaccinated versus vaccinated individuals infected with Delta. a, Genome copies (left) and infectious virus (right) for fully vaccinated and unvaccinated Delta-infected patients. Infectious titers (FFU > 0) were detected in 112 unvaccinated and 75 fully vaccinated Delta-infected patients; no titers (FFU = 0) were detected in 15 unvaccinated and 29 fully vaccinated Delta-infected patients. Error bars indicate mean ± s.d. Two-tailed *t*-test was used to determined differences of means. ***$P = 0.0002$ and ****$P < 0.0001$. Genome copies (**b**) and infectious VLs (**c**) measured for vaccinated and unvaccinated Delta-infected patients at different DPOS. The solid lines represent the fitted curve calculated using the LOESS method. Error bars represent 95% confidence bands of the best-fit line. LOESS, locally estimated scatterplot smoothing.

a higher infectious VL in unvaccinated Delta-infected individuals compared to pre-VOC-infected individuals and showed a significant reduction of infectious VLs in fully vaccinated Delta-infected individuals. However, only booster vaccination significantly reduced infectious VL in Omicron BA.1-infected individuals. Furthermore, we found a lower infectious VL in Omicron BA.1 breakthrough cases than in Delta breakthrough cases.

The magnitude and timing of infectiousness of patients with COVID-19 is critical information necessary to make informed public health decisions on the duration of isolation of patients and on the need to quarantine contacts. Infectiousness is strongly influenced by VL in the URT of infected patients[4]. However, VL is often measured as RNA copy numbers and not actual infectious virus. In this study, we could show that RNA copy numbers in NPS samples poorly correlated with infectious virus shedding. This is in line with several other studies that found that RNA is a poor infectiousness indicator, especially in the presence of infection-induced neutralizing antibodies[9,26]. Nevertheless, in our study, correlation between RNA and infectious VL was equally low between fully vaccinated and unvaccinated Delta-infected patients, indicating that factors other than mucosal neutralizing antibodies may be important for the reduction in infectious VL. In addition, in an animal model, it was demonstrated that infectious virus, but not RNA, is a good proxy for transmission[8].

Virus isolation in cell culture is widely used as a proxy for infectiousness[6,9,28]. Several studies have shown that isolation success significantly drops when RNA VLs are below 6 $\log_{10}$ copies per milliliter in NPS or when samples were collected after 8 DPOS[6]. Of note, with only a qualitative result, isolation success cannot distinguish between high and low infectious VLs in a patient sample, a key determinant of the potential size of the transmitted inoculum. Differences in infectious VL can affect transmission probability; therefore, we used an FFA that can reliably quantify infectious viral particles from NPSs. FFAs have long been a standard to quantify viral shedding in animal infection models for respiratory viruses, such as influenza, and have recently been used to quantify infectious VL in a SARS-CoV-2 human challenge trial, showing that they are considered as one of the best available proxies for infectiousness[29–31]. However, although we can assume that higher infectious VL leads to higher transmission risk, we currently do not know how many focus-forming units per milliliter (FFU ml^{-1}) are required for a patient to actually transmit the virus.

Within 5 DPOS, we found higher RNA VLs but lower infectious VLs in swabs of unvaccinated patients with pre-VOC infections

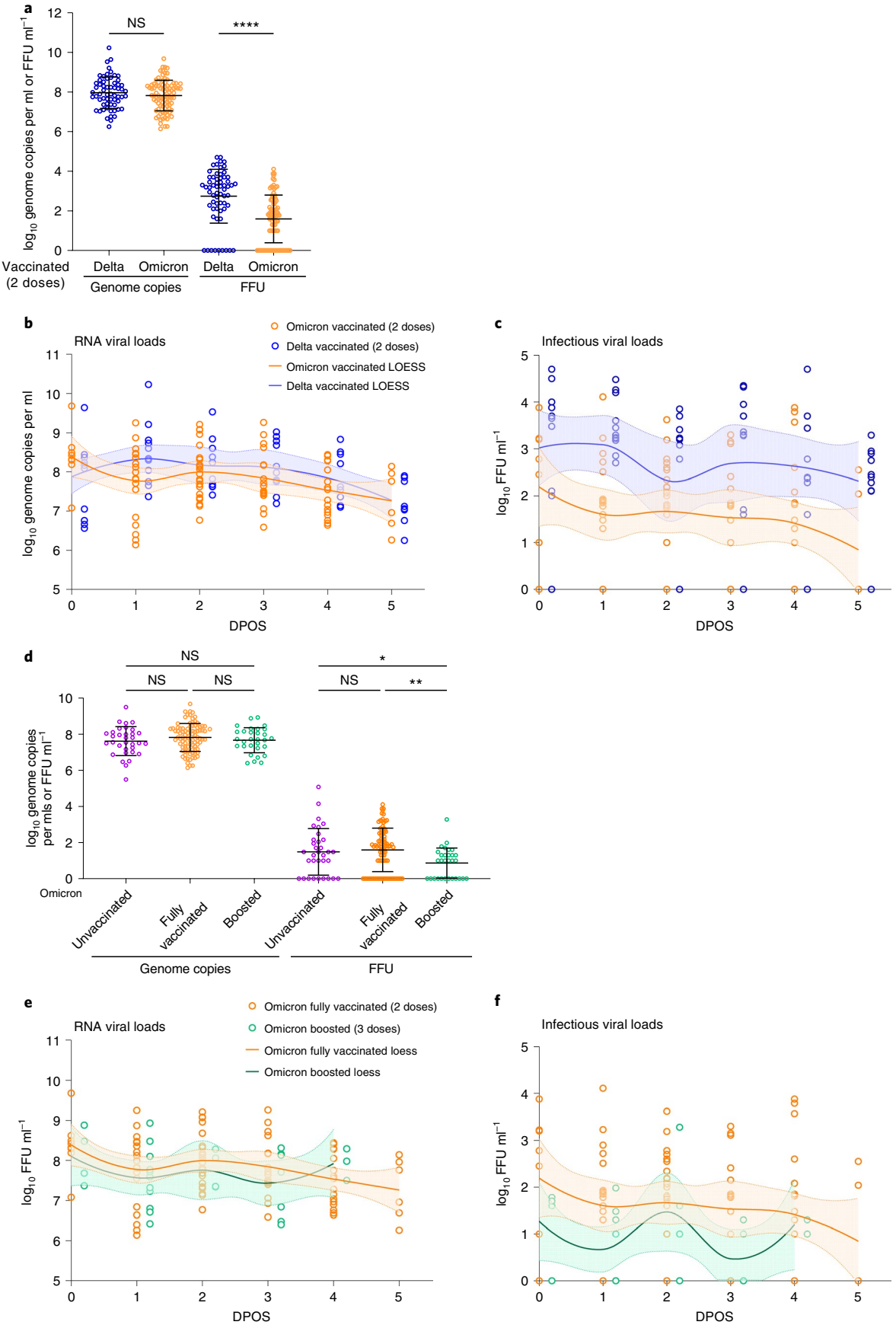
compared to Delta. These results disagree with other studies that analyzed only nucleic acid detection and found 3–10-fold-higher RNA copy number in Delta-infected patients compared to pre-VOC-infected patients[15,32]. However, these studies did not control for DPOS, age or sex. Other studies found either no difference in RNA VL between Delta and pre-VOC swabs[33] or more than 1,000-fold-higher VL for Delta[34], documenting the difficulty of comparing RNA VLs of virus variants during different phases of the pandemic, especially without additional information, such as DPOS. Conversely, in agreement with our results, a higher virus isolation success rate was observed for Delta compared to pre-VOC SARS-CoV-2 or Alpha[35].

Vaccines have been shown to tremendously reduce symptomatic SARS-CoV-2 infections. However, vaccination's effect on breakthrough case infectiousness is unclear. We show that infectious VL and RNA VL is reduced in fully vaccinated Delta patients during the first 5 DPOS. In this time period, approximately 50% of transmissions occur for pre-VOC strains[5], indicating that reduced VL could considerably decrease the secondary attack rate. Other studies showed no difference in RNA VL between the vaccinated and unvaccinated early after symptom onset[36,37] but found a lower virus isolation rate[36]. Conversely, another study detected up to ten-fold-reduced RNA VL in vaccinated patients but only for 60 days after full vaccination[38]. Similarly, two more studies reported decreased RNA VL for vaccine breakthrough infection with pre-VOC and Alpha SARS-CoV-2 (ref. [39]) but no effect around 6 months after vaccination when Delta dominated[40]. Of note, we were still able to detect infectious viral particles in 53.8% of fully vaccinated Delta-infected individuals at 5 DPOS, indicating that shortening of the isolation period to 5 days, as recommended by the CDC, should be carefully evaluated[41]. Whether lower infectious VL translates into lower secondary attack rates remains controversial and depends on other influencing factors, such as environmental stability of virus particles. Several studies found a correlation between VL and secondary attack rate, with VL of the index case being the leading transmission correlate[3,4]. In agreement with these findings, epidemiological studies showed reduced transmission from vaccinated index cases, but the effect size depends on the prevalent variant, the vaccine used and the time since vaccination[19]. In contrast, another study found that the index case vaccination status did not influence the secondary attack rate[21]. Although VL is a key element of transmission, the process of human-to-human transmission is complex, and other factors, such as varying recommended protection measures, overall incidence, perceived risks and the context of contacts (household versus community transmission), can influence outcomes in the studies reported.

To date, few data exist on VL in vaccine breakthrough infections caused by Omicron, owing to its recent emergence in late November 2021. Reduced neutralization of Omicron by infection-derived and vaccine-derived antibodies was reported in vitro, but the effect was less pronounced for boosted individuals[42,43]. Furthermore, epidemiological studies show an increased risk of (re-)infection with Omicron in vaccinated and recovered individuals[44] with high secondary attack rates among fully vaccinated and boosted individuals[45–47]. Higher RNA VLs as described in some studies were discussed as one potential contributing factor for the emergence of Alpha and Delta, although, for Delta, we could confirm this only for infectious VL in our data. Recent studies have shown that infection with Omicron caused shorter viral RNA shedding and lower peak viral RNA concentrations in comparison to Delta variant[48,49]. In contrast, other studies found a similar RNA VL for Omicron-infected and Delta-infected patients[46,50]. These finding are in line with our study, where only infectious VL, but not RNA VL, was significantly lower in Omicron BA.1 breakthrough cases compared to Delta breakthrough cases. In combination, these results indicate that the observed high transmissibility of Omicron BA.1 is not caused by elevated VLs, and the mechanism behind the higher transmissibility remains to be investigated. First, in vitro data hint toward alternative entry mechanisms as well as early replication peaks in cell culture[51,52], but no clinical data for these exist so far. Our findings indicate that, with lower infectious VL, the higher transmissibility of Omicron BA.1 seems to be unrelated to an increased shedding of infectious viral particles in vaccinated individuals. Furthermore, we could show that, in the case of Omicron BA.1 breakthrough infections, only boosted individuals had lower infectious VL, but not RNA VL, compared to unvaccinated individuals. These findings are partially in agreement with a recent household transmission study from Denmark where both fully vaccinated and boosted primary cases showed reduced onward transmission[46].

Our study has several limitations. We included only samples from symptomatic, but not asymptomatic, infected individuals that were collected ≤5 DPOS with Ct values <27. Therefore, absolute RNA copy numbers are biased toward higher VLs as patients with low VL were not included here. However, patients with low VL have likely little relevance in terms of transmission, and the fraction of patients with Ct values ≥27 was similar for all groups at all DPOS. Furthermore, our focus was on infectious virus shedding, and it has been shown that SARS-CoV-2 culture is unlikely to be successful from samples with higher Ct values[24] and that the vast majority of secondary transmission occurs before 5 DPOS, although this requires assessment in Omicron cases[5]. Other factors, such as poor swab quality, can be a confounding factor leading to low VLs. Also, our results could be affected if the timing between peak VL and the observed onset of symptoms would be considerably different between the variants or between unvaccinated and vaccinated individuals. However, VL trajectories of variants and of vaccinated and unvaccinated individuals run in parallel, indicating that they largely follow similar kinetics. Also, we would like to emphasize that there is currently no agreed cutoff for FFU ml^{-1} above which a patient could reliably be classified as infectious. In addition, comparisons between variants—that is, between pre-VOC and Delta as well as between Delta and Omicron BA.1—might be affected

Fig. 4 | SARS-CoV-2 infectious VLs in vaccine breakthrough infections with Omicron or Delta. a, Genome copies (left) and infectious virus (right) for fully vaccinated patients infected with Delta or Omicron BA.1 VOC. Infectious titers (FFU > 0) were detected in 53 Delta-infected and 66 Omicron BA.1-infected fully vaccinated patients; no titers (FFU = 0) were detected in nine Delta-infected and 25 Omicron BA.1-infected patients. Error bars indicate mean ± s.d. Two-tailed *t*-test was used to determined differences of means. ****$P < 0.0001$; NS, not significant. Genome copies (**b**) and infectious VLs (**c**) measured for fully vaccinated Omicron BA.1-infected and Delta-infected patients at different DPOS. The solid lines represent the fitted curve calculated using the LOESS method. Error bars represent 95% confidence bands of the best-fit line. **d**, Genome copies (left) and infectious virus (right) for unvaccinated, fully vaccinated or boosted Omicron BA.1 breakthrough cases. Infectious VLs for Delta and Omicron BA.1 samples were determined by FFA on Vero E6-TMPRSS cells. Infectious titers (FFU > 0) were detected in 24 unvaccinated, 66 fully vaccinated and 18 boosted Omicron BA.1-infected patients; no titers (FFU = 0) were detected in nine unvaccinated, 25 fully vaccinated and 12 boosted Omicron BA.1-infected patients. Error bars indicate mean ± s.d. Two-tailed t-tests were applied for each pair. P values were adjusted for multiple testing with the Benjamini–Hochberg method. NS, not significant; * adjusted $P = 0.0384$ and ** adjusted $P = 0.001325$. Genome copies (**e**) and infectious VLs (**f**) measured for fully vaccinated (two doses) and boosted (three doses) Omicron BA.1-infected patients at different DPOS. The solid lines represent the fitted curve calculated using the LOESS method. Error bars represent 95% confidence bands of the best-fit line. LOESS, locally estimated scatterplot smoothing.


a
NS
****
log10 genome copies per ml or FFU ml−1
Vaccinated (2 doses)
Delta
Omicron
Delta
Omicron
Genome copies
FFU
b
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Delta vaccinated (2 doses)
Omicron vaccinated LOESS
Delta vaccinated LOESS
log10 genome copies per ml
DPOS
c
Infectious viral loads
log10 FFU ml−1
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*
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log10 genome copies per mls or FFU ml−1
Omicron
Unvaccinated
Fully vaccinated
Boosted
Unvaccinated
Fully vaccinated
Boosted
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DPOS
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Infectious viral loads
log10 FFU ml−1
DPOS

by differences in adaption of variants to the cell lines used in this study. Lastly, we also would like to mention that almost all individuals in this study were vaccinated with mRNA vaccines that induce high titers of neutralizing antibodies in the blood but relatively low mucosal antibodies. Therefore, our results cannot be generalized to other vaccines—that is, those that are used mainly in low- and middle-income countries.

In conclusion, this study provides significant evidence for higher infectiousness of SARS-CoV-2 Delta as well as a significant effect of full vaccination on infectious VL and its speed of clearance. In addition, we show that Omicron BA.1 has lower infectious VLs compared to Delta in fully vaccinated individuals. Last, after Omicron BA.1 infection, lower infectious VL is observed only in boosted individuals. Our findings highlight the beneficial effect of vaccinations beyond the individual protection from severe disease and underscore the importance of booster vaccination. Thereby, we provide guidance for public health measures, such as shortening of the isolation period and vaccination certificates.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-01816-0.

Received: 24 January 2022; Accepted: 6 April 2022;
Published online: 8 April 2022

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Methods

Participants. *Ethical approval.* The study was approved by the Cantonal Ethics Committee at the University Hospital of Geneva (CCER no. 2021-01488). All study participants and/or their legal guardians provided informed consent.

Sample collection and setting. NPSs collected from symptomatic (self-reported) individuals by trained professionals in the outpatient testing center of the Geneva University Hospital (HUG), for SARS-CoV-2 qRT–PCR diagnostics, were included in this study. Samples from asymptomatic individuals were not included. Infection with SARS-CoV-2 was diagnosed by qRT–PCR assay (Cobas 6800, Roche). For this study, samples were included between 7 April 2020 and 19 February 2022.

Only specimens with Ct values below 27 for the E-gene qRT–PCR diagnostic target were included in our analyses. All samples originated from the diagnostic unit of the hospital's virology laboratory and were received for primary diagnosis of SARS-CoV-2. Remaining sample volume was stored at −80 °C, on the same day or within 24 hours. All samples had only one freeze–thaw cycle for the purpose of this study. All specimens from unvaccinated and vaccinated Delta-infected individuals were characterized by full genome sequencing or mutation-specific PCR for their infecting SARS-CoV-2 variant. Initial identification of Omicron BA.1 was done by S-gene target failure of the TaqPath COVID-19 assay (Thermo Fisher Scientific) and confirmed by partial Sanger sequencing of spike[53], followed by next-generation sequencing. No sequence information was obtained for samples collected before the first detection of VOCs in Switzerland—that is, pre-VOC samples. Clinical information of the patients was collected by a standardized questionnaire in our testing center and/or through the Cantonal Health Service. The day of symptom onset was defined as day 0 in this study. Only specimens collected within the first 5 DPOS were selected for this study.

Viral load quantification by qRT–PCR. For initial inclusion of samples into this study, Ct values for the E-gene target of the diagnostic qRT–PCR (Cobas 6800, Roche), determined by the diagnostic laboratory of the HUG at the time of sampling, were used. Afterwards, to minimize variability between measurements, all selected samples were re-extracted after thawing, and RNA VL in each sample was determined by E-gene qRT–PCR using SuperScript III Platinum One-Step qRT–PCR Kit (Invitrogen) in our research laboratory. Quantification of genome copy numbers was performed using an in vitro transcribed RNA standard for the E-gene assay as described previously[28]. Only results obtained from this latter measurement were used for further analysis.

Quantification of SARS-CoV-2 by FFA. Vero E6 and Vero E6-TMPRSS cells were cultured in complete DMEM GlutaMAX I medium supplemented with 10% FBS, 1× non-essential amino acids and 1% antibiotics (penicillin–streptomycin) (all reagents from Gibco). Vero E6-TMPRSS cells were kindly received from the National Institute for Biological Standards and Controls (cat. no. 100978). All infection experiments were performed under Biosafety Level 3 conditions.

FFAs used in this study were adapted from published protocols[54]. NPS samples were serially diluted and applied on a monolayer of Vero E6 cells in duplicates. After 1 hour at 37 °C, the media were removed, and pre-warmed medium mixed with 2.4% Avicel (DuPont) at a 1:1 ratio was overlaid. Plates were incubated at 37 °C for 24 hours and then fixed using 6% paraformaldehyde for 1 hour at room temperature. Cells were permeabilized with 0.1% Triton X-100 and blocked with 1% BSA (Sigma-Aldrich). Plates were incubated with a primary monoclonal antibody targeting SARS-CoV-2 nucleocapsid protein (Geneva Antibody Facility, JS02, diluted to 0.2 µg ml^{-1}) for 1 hour at room temperature and then with peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, 109-036-09, diluted to 1:2,000) for 30 minutes at room temperature. Foci were visualized using True Blue HRP substrate (Avantor) and imaged on an ELISPOT reader (CTL). We defined a cluster of adjacent cells expressing viral antigen as a foci. Foci were counted and expressed as FFU ml^{-1}. FFAs for comparison of infectious VLs in Delta versus Omicron were performed in Vero E6-TMPRSS cells using the same protocol.

Virus isolation. NPSs were applied on Vero E6 cell monolayers in 24-well plates. Next, 100 µl of each sample was added and incubated for 1 hour at 37 °C. After the incubation, the infectious supernatant was discarded, and virus culture medium was added. Then, 50 µl of the medium was collected to determine VL by qRT–PCR as described above at day 0. Then, 3–4 days after inoculation, the medium was replaced, and 6 days after infection, the infectious medium was collected to determine VL. A genome copy number change of at least 1 log of from day 0 to day 6 indicated a successful isolation.

Statistical analysis. Data collection was done using Excel 2019. All statistical analyses were performed using R statistical software version 4.1.1 (Foundation for Statistical 185 Computing) and Prism version 9.3.1 (GraphPad). All FFU and RNA genome copies were $\log_{10}$ transformed, and samples with no detectable FFU were set to 1 FFU ml^{-1} for the purpose of analysis. Cohen's d effect size is interpreted as follows: small = 0.2 - <0.5; medium = 0.5 - <0.8; large = ≥0.8

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data are included as a source data file in this manuscript.

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Acknowledgements

We thank all patients for their willingness to participate in our research. We thank the staff of the laboratory of virology from the University Hospitals of Geneva for their support. We would like to thank M. Essaidi-Laziosi for helpful advice and Y. Cambet, V. Jaquet and A. R. Corvaglia for technical assistance. We also thank E. Boehm for help with editing the manuscript.

Author contributions

O.P., P.V., I.E. and B.M. designed the study. O.P., K.A. and P.S. performed the laboratory experiments. C.G., A.I., F.J.B., L.K. and P.V. contributed to data collection. O.P., N.H., I.E. and B.M. analyzed and interpreted the data. I.E. and B.M. supervised the work. O.P., I.E. and B.M. wrote the manuscript. O.P., K.A., N.H., P.S., C.G., A.I., F.J.B., L.K., P.V., I.E. and B.M. reviewed the manuscript. Funding: This work was supported by Swiss National Science Foundation 196644 (I.E.) and 196383 (I.E.), National Research Program 78 Covid-19 Grant 198412 (I.E. and B.M.), the Fondation Ancrage Bienfaisance du Groupe Pictet (I.E.) and the Fondation Privée des Hôpitaux Universitaires de Genève (I.E.). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-022-01816-0.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-022-01816-0.

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Peer review information *Nature Medicine* thanks the anonymous reviewers for their contribution to the peer review of this work. Alison Farrell was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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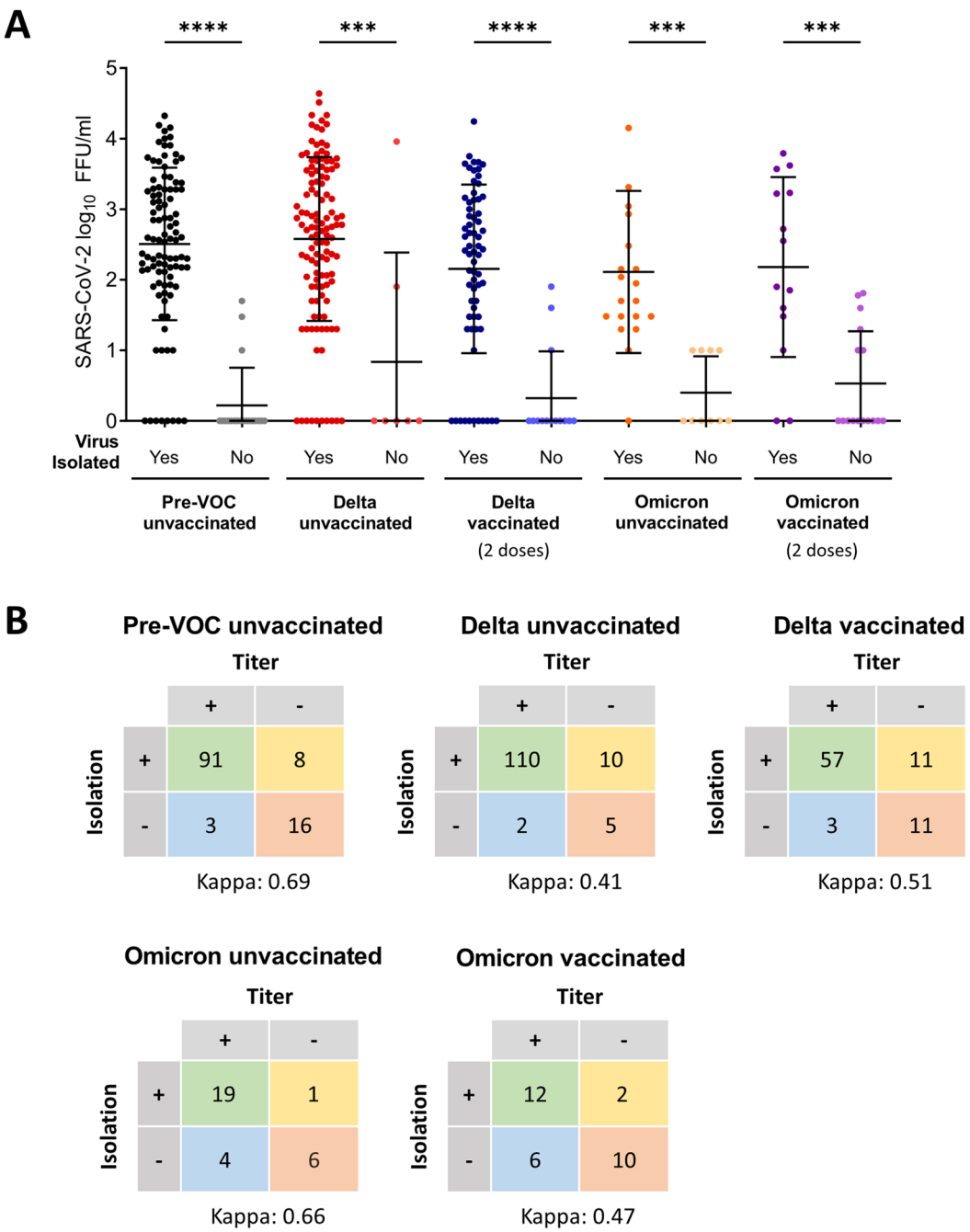
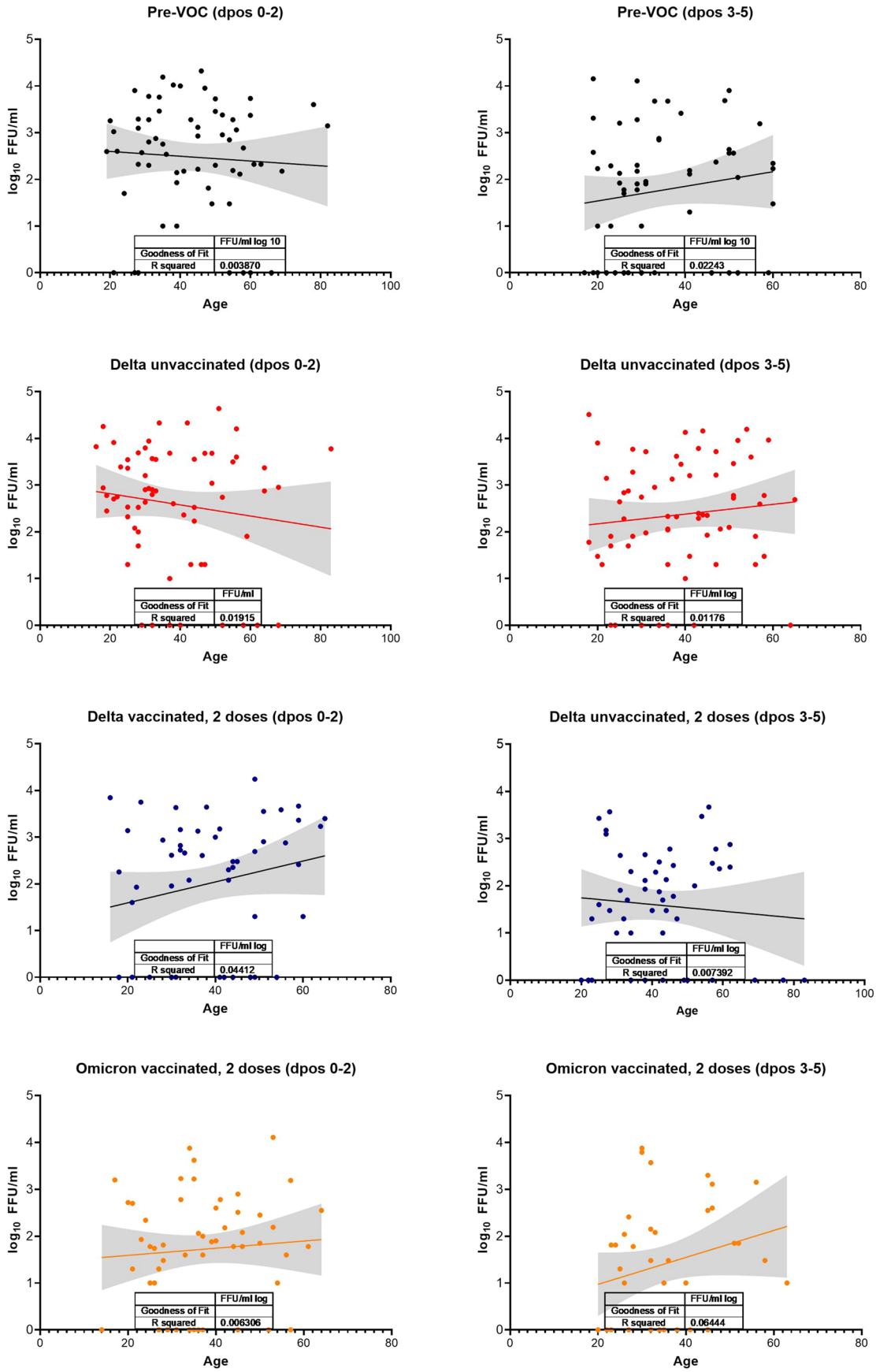
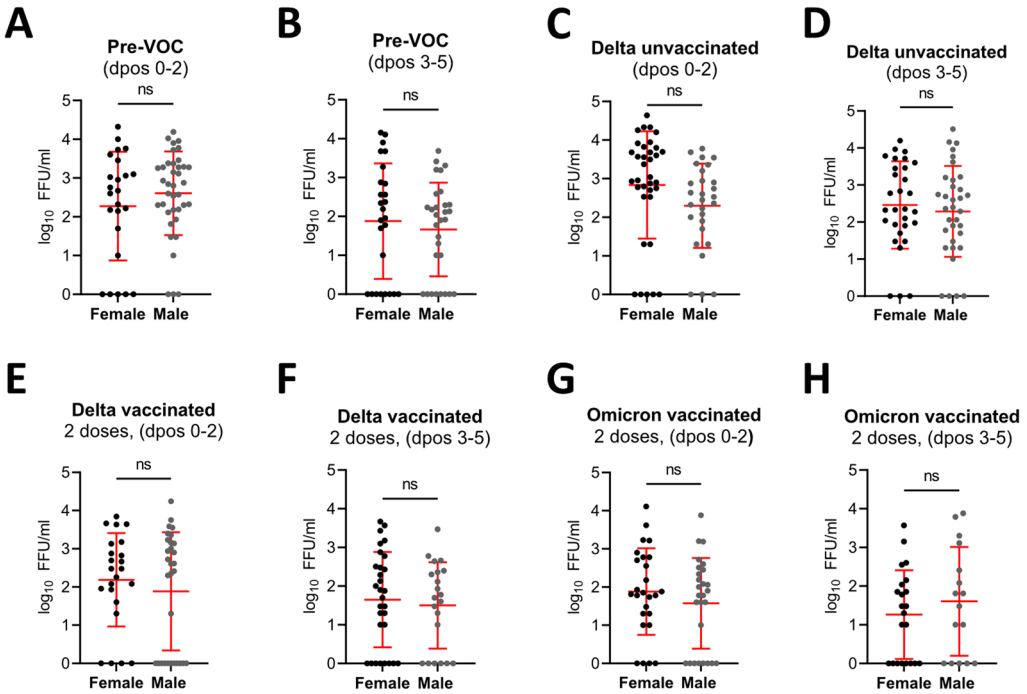


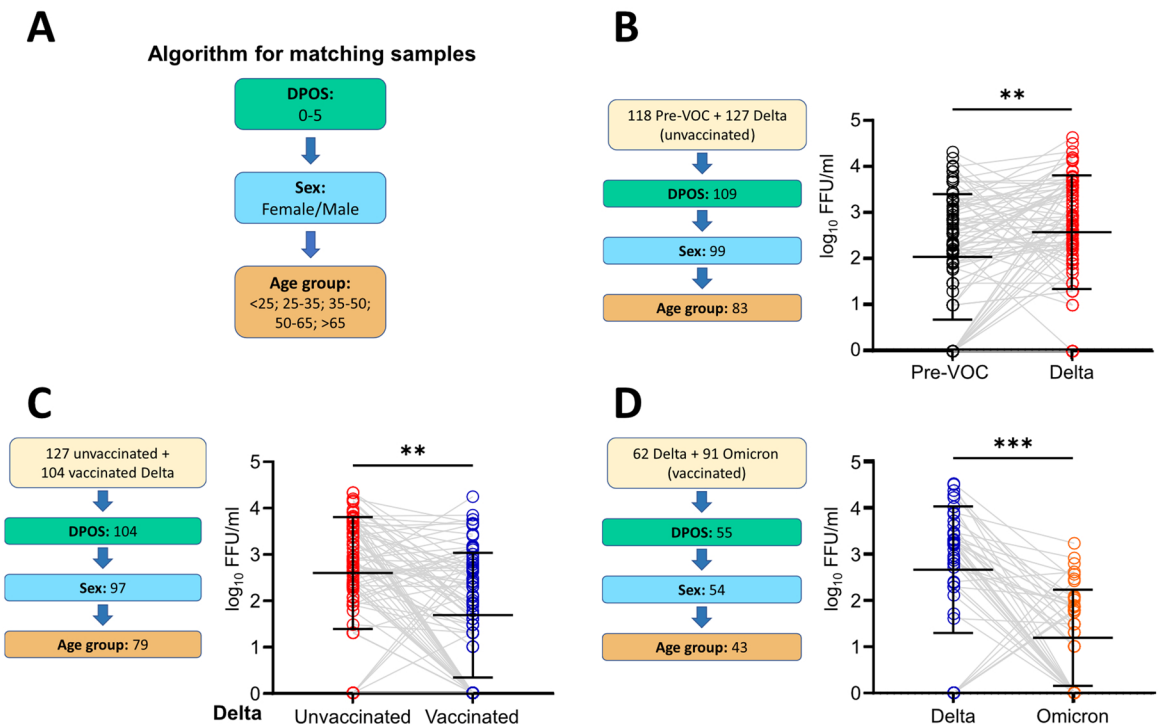
Extended Data Fig. 1 | Quantitative infectious viral loads versus overall virus isolation success. (**a**) Vero E6 (Pre-VOC and Delta) or Vero E6-TMPRSS (Omicron BA.1) cells were inoculated with 10-fold serial dilutions of nasopharyngeal swabs collected from SARS-CoV-2 infected individuals. Plates were fixed 27 h post-infection and following the staining with SARS-CoV-2 specific antibodies, the number of focus forming units (FFU)/mL was calculated for each sample. Error bars indicate mean ± SD. p-values were calculated using one-way ANOVA. ***: $p < 0.0003$; ****$p < 0.0001$. (**b**) The total number of positive and negative samples defined by titration and virus isolation for each patient group. Patients with detectable foci-forming units were assigned to the (+) group while patients without detectable focus-forming units were assigned to the (-) group). Cohens kappa agreement is shown.



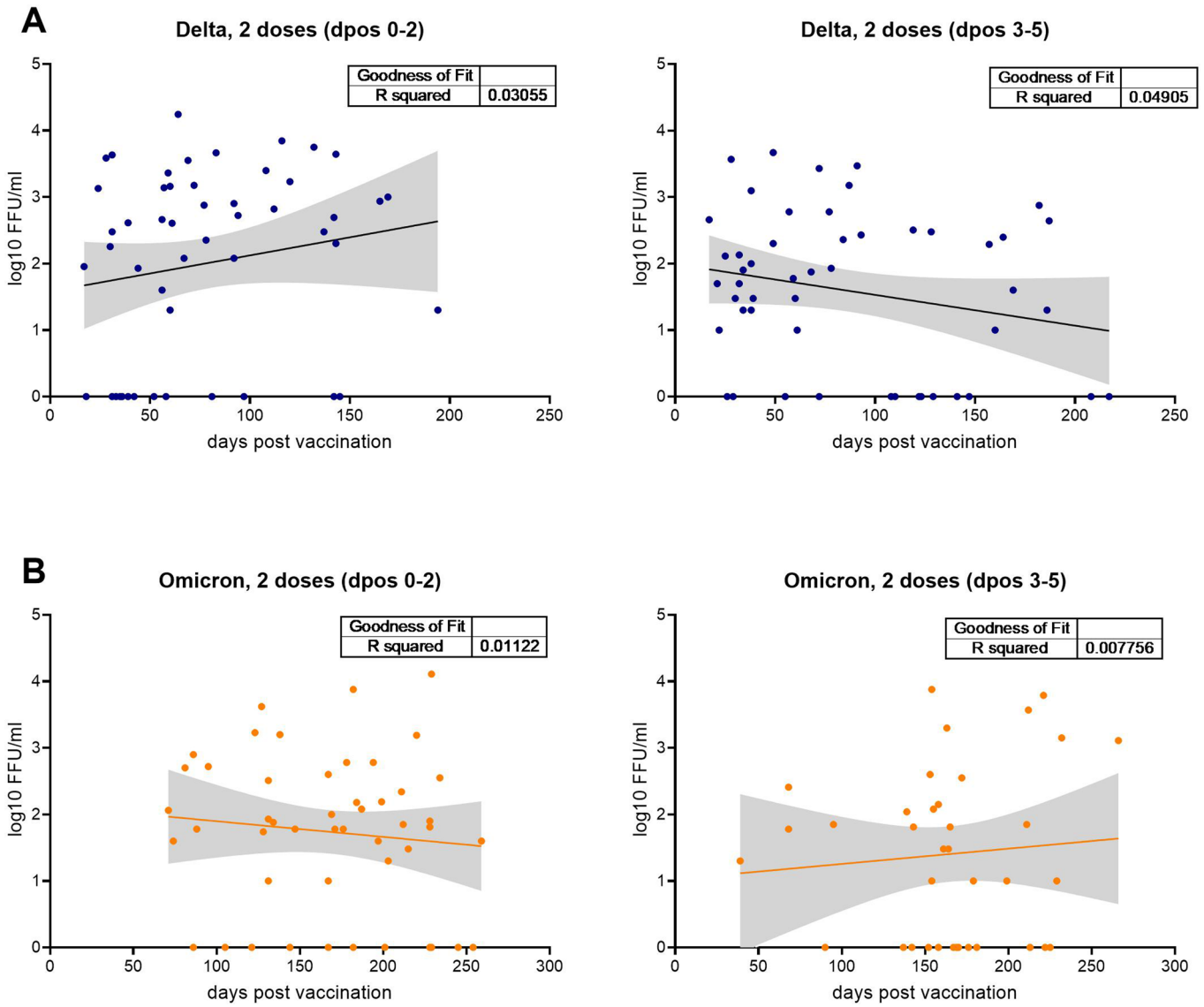
Extended Data Fig. 2 | Correlation between age and infectious viral loads. Linear regression analysis of SARS-CoV-2 titers in FFU/ml and the corresponding age of the patient. Error bars represent 95% confidence bands of the best-fit line.



Extended Data Fig. 3 | Infectious viral loads by sex. Comparison of infectious viral shedding measured in female and male patients. Error bars indicate mean ± SD. Two-tailed t-test was used to determine differences of means. ns= nonsignificant. Each graph contains following number of patients: 24 female and 38 male (A), 26 female and 30 male (B), 35 female and 28 male (C), 30 female and 34 male (D), 23 female and 30 male (E), 30 female and 21 male (F), 26 female and 27 male (G), 22 female and 16 male (H).



Extended Data Fig. 4 | Infectious viral load in matched samples. (a) Flow chart demonstrating the algorithm used for matching of the samples. The samples were matched first by DPOS, then by sex and finally by age group. SARS-CoV-2 infectious viral loads detected in unvaccinated patients infected with pre-VOC or Delta **(b)**, unvaccinated and vaccinated patients infected with Delta **(c)**, vaccinated patients infected with Delta or Omicron BA.1 **(d)** matched by age, sex, and dpos. Flow charts on the left side of each graph represent the numbers of samples in each category that were matched. Error bars indicate mean ± SD. Two-tailed paired t-test was used to determine differences of means for each group. **p = 0.001; ***p = 0.00028.



Extended Data Fig. 5 | Correlation between days post vaccination with infectious viral load. Linear regression analysis of infectious viral shedding and time since the completion of two vaccine doses in Delta **(a)** and Omicron BA.1 **(b)** infected patients. Error bars represent 95% confidence bands of the best-fit line.

nature portfolio

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Last updated by author(s): Mar 31, 2022

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data collection was done using Excel 2019
Data analysis	Data analysis was performed using GraphPad Prism Version 9.3.1 and R Statistical Software version 4.1.1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data is included in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	565 patients. Sample size was restricted by the availability of breakthrough infections for Delta and Omicron that have a CT value below 27.
Data exclusions	Patients with a CT >27 were excluded from the study as they typically do not shed infectious virus.
Replication	Observational study, no randomization. All clinical samples were titrated in duplicates, all attempts at replication were successful.
Randomization	Observational study, no randomization was done
Blinding	Observational study, no blinding was done

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	SARS-CoV-2 anti-nucleocapsid monoclonal antibody (Geneva Antibody facility, JS02; ABCD_AV865 in the ABCD Database, URL: https://web.expasy.org/abcd/ABCD_AV865). Secondary anti-human IgG antibodies (Jackson ImmunoResearch, 109-036-098).
Validation	The antibody was validated in the following publication: https://www.frontiersin.org/articles/10.3389/fimmu.2020.595970/full

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero-E6 cells received from Volker Thiel Lab, Vero-TMPRSS from National Institute for Biological Standards and Controls.
Authentication	Cell lines were not authenticated recently.
Mycoplasma contamination	Cells were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Commonly misidentified lines have not been used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	We inlcuded a total of 565 SARS-CoV-2 patients, among them 280 females and 285 males, median age was 38 years. 118

Population characteristics	unvaccinated patients were infected with pre-VOC strains, 127 unvaccinated and 166 vaccinated subjects were infected with Delta VOC and 33 unvaccinated and 121 vaccinated patients were infected with Omicron VOC. Median age and female/male ration were similar between all groups.
Recruitment	This was an observational study in which leftover specimens were used
Ethics oversight	Cantonal Ethics Committee (CCER) at the University Hospitals of Geneva approved the protocol and the research plan for this study

Note that full information on the approval of the study protocol must also be provided in the manuscript.