Clinical Infectious Diseases

MAJOR ARTICLE

Severe Acute Respiratory Syndrome Coronavirus 2 Delta Vaccine Breakthrough Transmissibility in Alachua County, Florida

Brittany Rife Magalis,[1,2] Shannan Rich,[3,4] Massimiliano S. Tagliamonte,[1,2] Carla Mavian,[1,2] Melanie N. Cash,[1,2] Alberto Riva,[5] Simone Marini,[2,3] David Moraga Amador,[5] Yanping Zhang,[5] Jerne Shapiro,[3,4] Amelia Horine,[4] Petr Starostik,[2] Maura Pieretti,[6] Samantha Vega,[6] Ana Paula Lacombe,[7] Jessica Salinas,[7] Mario Stevenson,[7] Paul Myers,[4] J. Glenn Morris Jr.,[1,8] Michael Lauzardo,[1,4,8] Mattia Prosperi,[3] and Marco Salemi[1,2]

[1]Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA; [2]Department of Pathology, University of Florida, Gainesville, Florida, USA; [3]Department of Epidemiology, University of Florida, Gainesville, Florida, USA; [4]Florida Department of Health, Alachua County, Gainesville, Florida, USA; [5]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida, USA; [6]BayCare Laboratories LLC, Tampa, Florida, USA; [7]University of Miami Miller School of Medicine, Miami, Florida, USA; and [8]Department of Medicine, College of Medicine, University of Florida, Gainesville, Florida

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta variant has caused a dramatic resurgence in infections in the United Sates, raising questions regarding potential transmissibility among vaccinated individuals.

Methods. Between October 2020 and July 2021, we sequenced 4439 SARS-CoV-2 full genomes, 23% of all known infections in Alachua County, Florida, including 109 vaccine breakthrough cases. Univariate and multivariate regression analyses were conducted to evaluate associations between viral RNA burden and patient characteristics. Contact tracing and phylogenetic analysis were used to investigate direct transmissions involving vaccinated individuals.

Results. The majority of breakthrough sequences with lineage assignment were classified as Delta variants (74.6%) and occurred, on average, about 3 months (104 ± 57.5 days) after full vaccination, at the same time (June-July 2021) of Delta variant exponential spread within the county. Six Delta variant transmission pairs between fully vaccinated individuals were identified through contact tracing, 3 of which were confirmed by phylogenetic analysis. Delta breakthroughs exhibited broad viral RNA copy number values during acute infection (interquartile range, 1.2-8.64 Log copies/mL), on average 38% lower than matched unvaccinated patients (3.29-10.81 Log copies/mL, $P < .00001$). Nevertheless, 49% to 50% of all breakthroughs, and 56% to 60% of Delta-infected breakthroughs exhibited viral RNA levels above the transmissibility threshold (4 Log copies/mL) irrespective of time after vaccination.

Conclusions. Delta infection transmissibility and general viral RNA quantification patterns in vaccinated individuals suggest limited levels of sterilizing immunity that need to be considered by public health policies. In particular, ongoing evaluation of vaccine boosters should specifically address whether extra vaccine doses curb breakthrough contribution to epidemic spread.

Keywords. SARS-CoV2, delta, transmission, Florida, vaccination, phylogenetic, contact tracing.

Over the course of the coronavirus disease 2019 (COVID-19) pandemic [1], several rapidly spreading variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have risen to the status of "variants of concern" (VOC), accumulating genomic mutations with respect to the original viral strain originating from the Wuhan province of China [2]. The spread of these and other VOCs across the globe, including the United States; evidence of increased transmissibility; and level of evolutionary divergence from the original strain have raised questions regarding the extent of protection of currently implemented vaccines against infection [3–7]. In 2020, lineage B.1.617.2 (renamed Delta) emerged from India [8]. Delta currently carries more than a dozen mutations, including L452R, also found in the "California variant" Epsilon [9], associated with increased Spike stability and viral fusogenicity, which results in enhanced viral replication and infectivity [10], as well as impaired immune response through antibody neutralization [11].

As of November 3, 2021, 66.9% of Americans were fully vaccinated [12], yet emergence of the Delta variant, which spread rapidly within the United States during 2021, caused a dramatic resurgence of infections and hospitalizations among unvaccinated individuals [13–16]. Approximately 74% of infections with the Delta variant are followed by symptomatic onset [17] and are characterized by relatively high viral RNA copy number linked to higher transmission rates than other lineages [18], explaining how this variant has outpaced other VOCs and become the predominant variant in several countries.

Received 30 November 2021; editorial decision 2 March 2022; published online 10 March 2022.
Correspondence: B. Rife Magalis, Emerging Pathogens Institute, University of Florida College of Medicine, 2055 Mowry Rd, Gainesville, FL 32610 (Brittany.rife@ufl.edu).

Clinical Infectious Diseases® 2022;75(9):1618–27

https://doi.org/10.1093/cid/ciac197

According to clinical trial results, a 2-dose regimen of Pfizer-BioNTech (BNT162b2) vaccine conferred 94.6% protection against severe disease [6], whereas the Moderna (mRNA-1273) vaccine conferred 94.1% at the time of this study [19]. Although vaccination continues to provide excellent protection against hospitalization and death, a gradual decline in vaccine efficacy against infection was observed in 2021 [20]. In the United States, these vaccine breakthrough cases linked to VOCs have been reported since January 2021 [21]. In July 2021, following multiple large public events in a Barnstable County, Massachusetts, town, an outbreak (90% Delta) of infection (albeit mild disease) was identified among Massachusetts residents traveling recently to the town, primarily comprising fully vaccinated persons [13]. Within the same month, a similar report of widespread Delta circulation (95% of sequenced samples by July 24) among individuals in Wisconsin (USA) was published in medRxiv [22]. Both studies reported similar viral RNA burden among vaccinated and unvaccinated individuals, which was estimated by using the number of polymerase chain reaction cycles required to quantify viral genetic sequence fragments (CT value) in a specimen. Previous work has shown that infectious SARS-CoV-2 can usually be recovered from specimens with CT < 25 to 30 [23]. Yet, CT values are only a proxy for the level of virus shed in the nasal passages and, while these data suggest vaccinated individuals are susceptible to infection by the Delta variant and harbor the level of infectious virus required for further transmission, definitive evidence of this transmission has not been presented. Moreover, CT values do not allow for a quantitative evaluation of the viral RNA copy number threshold required for potential transmission within a specific population (eg, the fraction of vaccine breakthrough cases harboring a viral RNA burden compatible with secondary transmissions, which both empirical data and theoretical studies have shown to be 4 Log copies/mL) [24, 25]. Finally, a recent comparison of Moderna (mRNA-1273) and Pfizer/BioNTech (BNT162b2) vaccines in cohorts from states with high prevalence of Delta infections (Minnesota, Wisconsin, Arizona, Florida, and Iowa) showed strong protection against disease but also a lower risk of infection after full vaccination with mRNA-1273 than after full vaccination with BNT162b2 [26], although the study did not provide direct virus genomic data of the breakthrough cases.

The present study analyzes data generated as part of the SARS-CoV-2 genomic epidemiology surveillance program in Alachua County, Florida, from October 2020 to August 2021, to answer 3 main questions: (1) Did the emergence of the Delta variant result in increases in vaccine breakthroughs? (2) Do fully vaccinated people, infected by the Delta variant (or other variants), transmit the infection? (3) What fraction of vaccine breakthrough patients exhibit a viral RNA copy number estimate above the transmissibility threshold during acute infection?

RESULTS

Between October 2020, and first week of August 2021, 4439 SARS-CoV-2 samples were sequenced from patients in Alachua County (Table S1), Florida, representing approximately 22% of reported positive cases during the same period (20 612 cases between October 5, 2020, and August 6, 2021). Following a trend similar to the rest of the United States, the first VOC circulating in the county, Alpha, was detected in December 2020. Alpha became the dominant variant in March 2021 but decreased in favor of VOC Gamma and other B.1 subvariants in the following months (Figure 1A), whereas the number of infections significantly dwindled due in part to a major vaccination effort [12], which began in Alachua County in December 2020. Sporadic vaccine breakthroughs were identified in the first 7 months of 2021 (Figure 1B), including 5 Alpha variant cases, 2 Epsilon, 5 B.1, 3 B.1.2, 1 B.1.596, and 1 B.1.377. During the third week of June, we detected the first Delta infection in a fully vaccinated patient, followed by a rapid increase in Delta vaccine breakthroughs through the end of July, coinciding with Delta becoming the most prevalent variant (Figure 1A and 1B). Overall, 109 vaccine breakthrough cases were identified, including 58 Delta infections and 34 unknowns because of low coverage of the Spike protein. The breakthrough cases exhibited an average time between full vaccination (defined as 14 days following the final dose) and COVID-19 diagnosis of about 3 months (mean = 101.6 ± 57.7 days) (Figure 1C). Compared with the number of vaccinations, the proportion of diagnosed breakthrough infections remained extremely low throughout our surveillance (Supplementary Figure S1), in line with several other studies that have shown the effectiveness of currently available vaccines. However, although the spike in breakthrough cases during the month of July did coincide with a significant vaccination scale up in Alachua County [18], the majority (71%) of these patients had already been fully vaccinated for more than 90 days (Figure 1C) and became infected by the Delta variant in July, while its frequency was exponentially increasing among the unvaccinated population (Figure 1B), indicating that the increase in breakthroughs was indeed linked with the emergence of the Delta variant.

The breakthrough population consisted largely of White individuals (73.4%), aged 36.7 years on average (standard deviation = 14.2), all of whom had mild symptoms with low prevalence of associated comorbidities (Table 1). None of the individuals within this population required hospitalization. Approximately 44% of breakthrough cases could be traced to known exposures, identifying household transmission (54.2%) as the primary source of putative infection, followed by community- (37.5%) and healthcare-related (6.3%) exposures. A supplemental saliva sample was collected from a subset of the breakthrough cases (N = 83), on average 4.2 days after disease onset (Table 1), to measure the viral RNA burden during acute infection. Viral RNA copy number among breakthrough cases

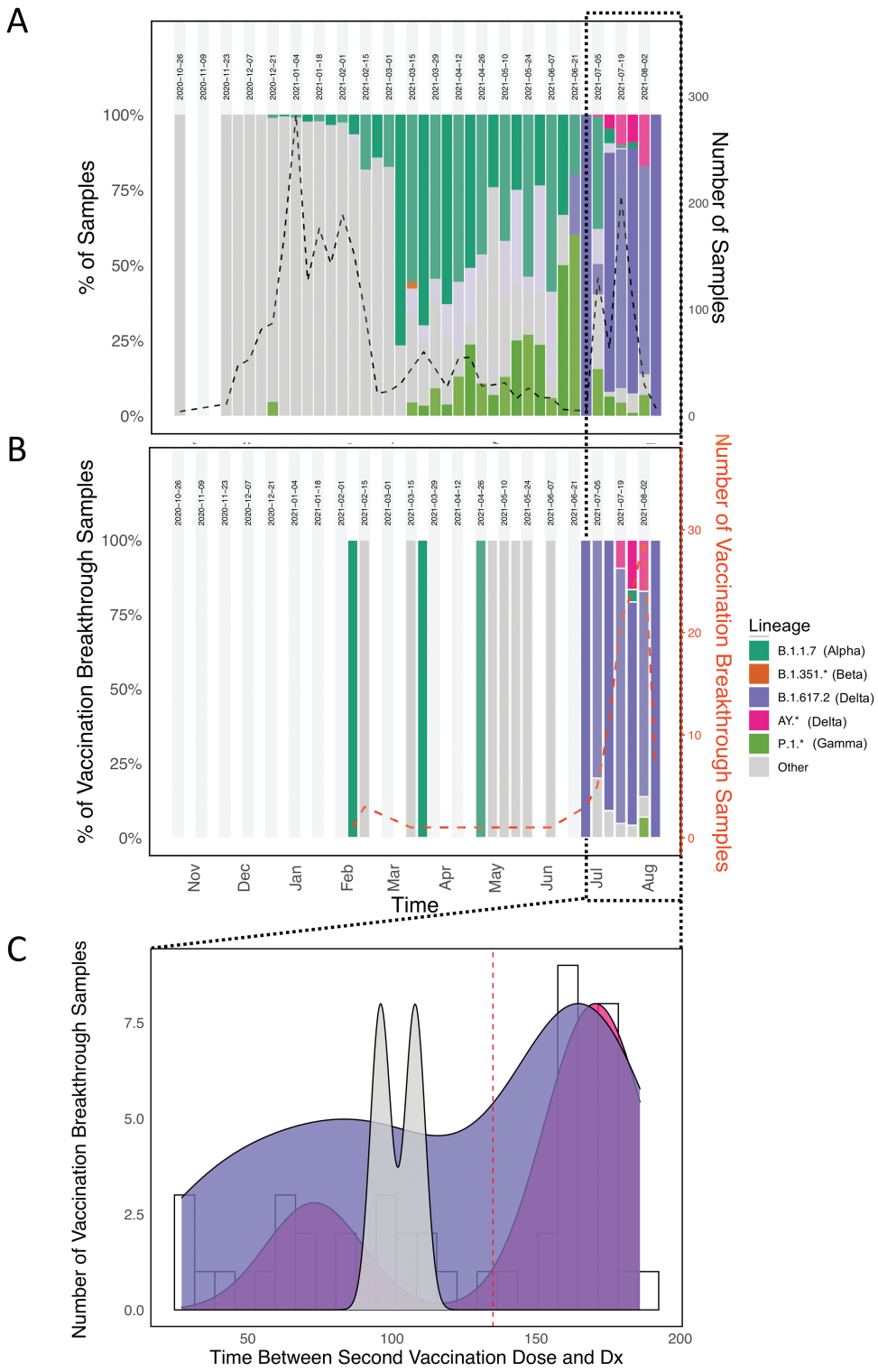

Figure 1. SARS-CoV-2 variants distribution in Alachua County, Florida, over time. *A*, Lineage distribution (y-axis) versus time (x-axis) among sequenced samples. Total number of samples successfully sequenced is represented by the black, dotted line (right y-axis). *B*, Lineage distribution (y-axis) versus time in vaccine breakthrough cases. Total number of samples successfully sequenced is represented by the red, dotted line (right y-axis). *C*, number of vaccine breakthrough cases (x-axis) versus time between second vaccination dose and diagnosis (y-axis). Abbreviations: Dx, diagnosis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

infected with the Delta variant (N = 56) averaged 4.66 Log copies/mL, with an interquartile range (IQR) of 1.2 to 10.62, overlapping the one observed among breakthroughs infected by other variants (N = 13) with an average copy number of 5.39 Log copies/mL, and IQR 1.41 to 8.36 ($P = .35$ from a 2-tailed Mann-Whitney *U* test). For comparison with the nonvaccinated

Table 1. Summary of Vaccine Breakthrough Population (N = 109) in Alachua County, Florida, from January to August 2021

Age, y	36.7 (14.2)
Sex, n (%)	
Female	68 (62.4%)
Male	41 (37.6%)
Race, n (%)	
White	80 (73.4%)
African American/Black	10 (9.2%)
Asian/Pacific Islander	11 (10.1%)
Other/Unknown	8 (7.3%)
Ethnicity, n (%)	
Non-Hispanic	91 (83.5%)
Hispanic	18 (16.5%)
Symptoms, n (%)	96 (88.1%)
Dry cough	47 (43.1%)
Productive cough	18 (16.5%)
Dyspnea	14 (12.8%)
Anosmia	35 (32.1%)
Ageusia	34 (31.2%)
Sore throat	40 (36.7%)
Headache	53 (48.6%)
Runny nose	79 (72.5%)
Fatigue	54 (49.5%)
Comorbidities, n (%)	
Asthma	11 (10.1%)
Diabetes	3 (2.8%)
Hypertension	15 (13.8%)
BMI	Mean = 27.0 (SD = 6.3)
Known exposure, y, n (%)	48 (44.0%)
Household	26 (54.2%)
Community	18 (37.5%)
Healthcare	3 (6.3%)
Vaccine, n (%)	
BNT162b2 (Pfizer/BioNTech)	83 (76.1%)
mRNA-1273 (Moderna)	11 (10.1%)
Ad.26.COV2.S (Johnson & Johnson/Janssen)	14 (12.8%)
NVX-CoV2373 (Novavax)	1 (0.92%)
Time between vaccination and disease onset,[a] d	Mean = 104.0 (SD = 57.5)
Time between disease onset[a] and sample collection date (d) for viral RNA copy number measurement (N = 83)	Mean = 4.2 (SD = 2.4)

Numbers report frequencies and (percentages) unless otherwise stated.

Abbreviations: BMI, body mass index; SD, standard deviation.

[a]Disease onset is defined as the date of symptom onset for symptomatic individuals or the original date of laboratory collection for asymptomatic individuals.

population, viral RNA burden was also evaluated in age- and gender- matched data sets, retrospectively assembled for the months of January through April and July, which included randomly selected independent samples (ie, samples from patients not directly linked through known transmission events) from nonvaccinated individuals infected with Delta (N = 36) or other variants (N = 75). In agreement with previous reports [27], unvaccinated patients infected with the Delta variant exhibited, on average, the highest viral RNA copy number (mean 7.36 Log copies/mL, IQR 3.29-10.81) compared with vaccinated Delta or non-Delta breakthroughs (Figure 2), as well as with unvaccinated patients infected by other variants (mean 6.15 Log copies/mL, IQR 3.56-10.92), although effect size was modest (6% increase, $P = .17$ from a 2-tailed Mann-Whitney U test), probably because of small sampling size. Contrary to other reports [13, 22], however, Delta-infected breakthrough cases in Alachua County exhibited an average 38% viral RNA copy number reduction compared with unvaccinated Delta cases ($P < .00001$, 2-tailed U test, after Bonferroni correction), and 34% ($P < .00001$) compared with unvaccinated non-Delta cases (Figure 2). The multivariable analysis did not find any association between viral RNA burden and age, gender, race, ethnicity, or vaccine type in the sample (Table S2). Yet, Delta infections had a strong association with viral RNA burden above the transmissibility threshold of Log 4 RNA copies/mL [24, 25], with a 2.46 odds ratio and confidence interval of 1.05 to 5.97 in the unadjusted analysis, and odds ratio 3.04 and confidence interval of 1.16 to 8.54 in the adjusted analysis (Table S3). In particular, the majority of vaccine breakthrough cases infected with the Delta variant (58.5%) exhibited a viral RNA copy number estimate above the required threshold for potential transmission (Figure 2). Although this threshold was based on viral burden measured from nasopharyngeal swabs, saliva samples have shown to be more sensitive to the detection of SARS-CoV-2 [28, 29], indicating the potential for underestimating the viral burden in these individuals.

Direct virus transmission among vaccinated individuals infected by the Delta variant was, indeed, confirmed by contact tracing and phylogenetic analysis of SARS-CoV-2 sequence data. Contact tracing allowed us to identify 6 putative transmission pairs, each involving a fully vaccinated donor (D), part of our initially detected breakthrough cases, and a recipient (R) with no other known infection exposure history (Table 2). At the time of symptom onset, donor D1 had been fully vaccinated with mRNA-1273, for 120 days, whereas donors D2 through D6 had been fully vaccinated with BNT162b2 for 143 to 176 days, with all of them exhibiting >Log 4 RNA viral RNA copies/mL. Four of 6 recipients had also been vaccinated with BNT162b2 for 67 to 164 days at the time of symptom onset, thus classifying them as additional vaccine breakthrough cases. For 3 of these D-R pairs, we were able to obtain saliva samples collected approximately 4 days following initial diagnosis and obtain the full genome sequence of the virus. Sequences for individuals involved in transmission pairs (all Delta variants) were evaluated in the context of Delta sequences derived from other parts of Florida and of closely related Delta sequences retrieved from the Global Initiative for Sharing All Influenza Data (GISAID) database (see Methods). Four nonmonophyletic transmission clusters were identified using a depth-first search algorithm applied to the phylogeny, identified as groups of individuals sharing minimal genetic sequence differences and pairwise sampling time differences within the reported typical serial time

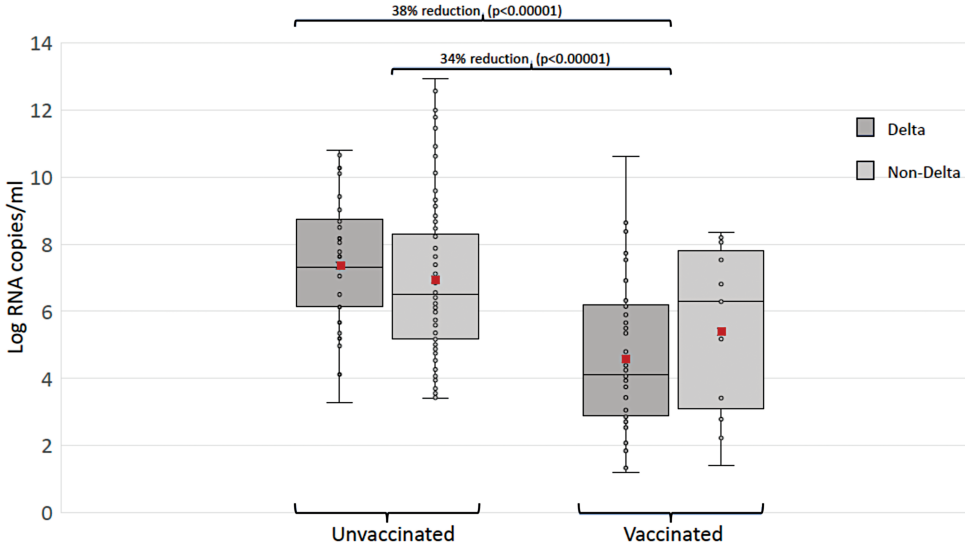

Figure 2. Viral RNA copy number distribution in vaccinated and unvaccinated severe acute respiratory syndrome coronavirus 2–infected patients. Each box plot (with line at median and red dot indicating the mean) represents a group of patients infected with either the Delta variant or other variants, vaccinated or unvaccinated according to the legend in the figure. For each group pair, a 2-tailed Mann-Whitney *U* test was executed. *P* value and effect size are shown on top for those comparisons between groups that were significant at the 5% level after Bonferroni correction for multiple tests.

interval of up to 6 days (see Methods). Though geographical sampling locations were intermixed throughout the phylogeny, clusters primarily comprised local transmissions (Figure 3A). Vaccination breakthroughs were also intermixed throughout the phylogeny; however, all contact tracing-identified transmission pairs belonged to cluster c3 (Figure 3B). Each R individual within a transmission pair was observed directly adjacent to the corresponding putative D individual, lending validation to direct transmission between pairs. All 6 individuals belonging to the 3 successfully sequenced transmission pairs were fully vaccinated, supporting the transmission capability of fully vaccinated individuals.

To examine further SARS-CoV-2 Delta variant potential transmissibility in breakthrough infections in the context of the specific vaccination received by the patients, we investigated the correlation between viral RNA copy number and time since full vaccination in individuals who received the Pfizer-BioNTech (N = 72 assigned variants, N = 53), the Johnson & Johnson/Janssen (N = 9, N = 6 Delta), or the Moderna (N = 8, N = 6 Delta) vaccine. The only patient in our population sample vaccinated with Novavax was excluded. Because regression analyses assume random sampling of the population, individuals included in transmission clusters are considered to be potentially linked epidemiologically and should not be considered independent. Minimal branch support within cluster c3 could not rule out epidemiological linkage among the 3 D-R transmission pairs (Figure 3). Therefore, the 3 donor individuals were removed before regression analysis, leaving the 3

Table 2. Vaccine and Viral Load Information for Identified Transmission Pairs

Pair	Individual	Vaccine	Days Since Vaccination[a]	Viral RNA Copy Number (Log copies/mL)
D1-R1	D1	Moderna	120	4.53
	R1	Pfizer	139	2.08
D2-R2	D2	Pfizer	162	6.81
	R2	Pfizer	164	NA
D3-R3	D3	Pfizer	143	8.64
	R3	Pfizer	67	3.94
D4-R4	D4	Pfizer	157	4.12
	R4 (uncollected)	NA	NA	NA
D5-R5	D5	Pfizer	173	4.07
	R5 (No sequence)	Pfizer	86	NA
D6-R6	D6	Pfizer	176	NA
	R6 (uncollected)	NA	NA	NA

Abbreviations: D, donor; NA, not available; R, recipient.

[a]Time since vaccination was defined as 2 weeks after administration of second dose of either Moderna or Pfizer vaccine.

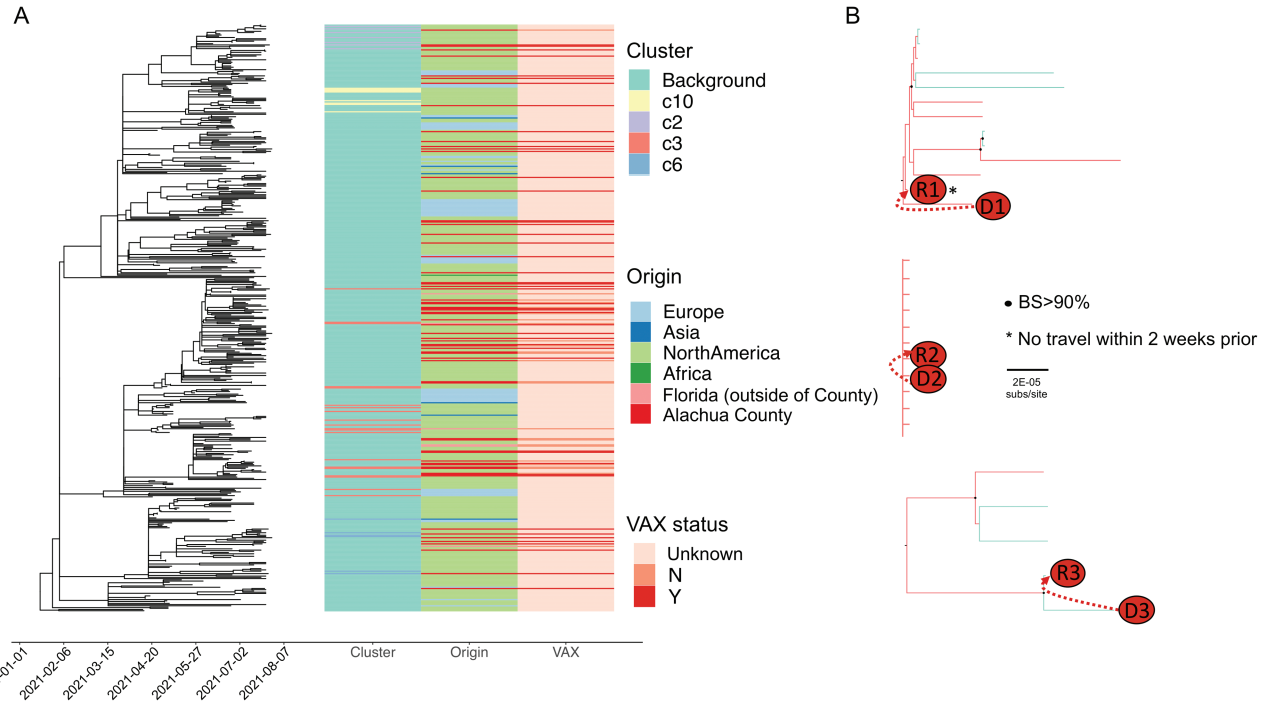

Figure 3. Phylogenetic reconstruction and transmission characterization of severe acute respiratory syndrome coronavirus 2 Delta sequences obtained from Alachua County, Florida, and epidemiologically relevant outside locations. *A*, Phylogeny of sequences with heatmap depicting cluster origin, geographical origin, and vaccination status of each sampled sequence. *B*, Phylogenetic relationships of sequences from donor (D)-recipient (R) pairs linked via exposure using contact tracing (branches are colored according to cluster origin). Bootstrap support (BS) > 90% within clades containing transmission pairs is represented by a black dot. Individuals with confirmed no known travel within 2 weeks before diagnosis are represented with asterisks. Branches are scaled in substitutions/site.

recipient individuals that we know to be unrelated via direct transmission. Remaining Delta vaccination breakthroughs with viral RNA copy number data were sparsely placed throughout the tree (45 in the background population and the remaining 2 in clusters c2 and c6) and could thus be included. Only 1 unvaccinated Delta individual with viral RNA copy number data was observed within a transmission cluster (c2) and was considered to be distinct from the vaccinated individual in this cluster by significant branch support and so was included in regression analysis. Non-Delta sequences were not included in the phylogeny; however, saliva samples from this population were chosen for viral RNA burden analysis so that a wide range of collection times and variants were included, minimizing the probability of epidemiological linkage and the need for phylogenetic assessment of independence. Multiple regression analysis had already found no evidence of association between viral RNA burden and time since full vaccination (8-186 days), time interval between symptom onset and sampling, or vaccine type using either linear or logistic methods (Supplementary Table S2). There was also no correlation between distribution of viral RNA copy number over time elapsed since full vaccination, defined as the time interval between 2 weeks after second vaccination dose (in the case of Pfizer-BioNTech or Moderna) or after single dose (Johnson & Johnson/Janssen). Importantly, the proportion of patients with viral RNA copy numbers above the theoretical transmission threshold (>4 Log copies/mL) was essentially the same when looking at patients infected <100 days (48.8% all, 55.6% Delta) or >100 days (50% all, 60% Delta) after full vaccination (Figure 4). Given the small number of Delta vaccine breakthrough patients who received Johnson & Johnson/Janssen (N = 6) or Moderna (N = 6), no conclusions could be drawn, although the few Moderna vaccinated patients were the only ones who showed a strong linear correlation ($R^2 = 0.86$) between viral RNA burden, and time elapsed since full vaccination (Supplementary Figure S2).

DISCUSSION

The rapid emergence Delta infections in the summer of 2021 correlated with a major spike in reported cases, hospitalizations, and deaths in Florida and in Alachua County. Though primarily among unvaccinated persons [30], Delta's emergence also coincided with a sudden spike in vaccine breakthrough cases. Our results demonstrate that direct transmission from vaccine breakthroughs can occur, with more than one-half of the Delta breakthroughs in our sample harboring sufficient viral RNA copies to transmit the virus during acute infection.

Overall, the number of breakthrough cases detected during our nearly 9-month genomic epidemiology surveillance program, represented approximately 2% of the sequenced cases, a

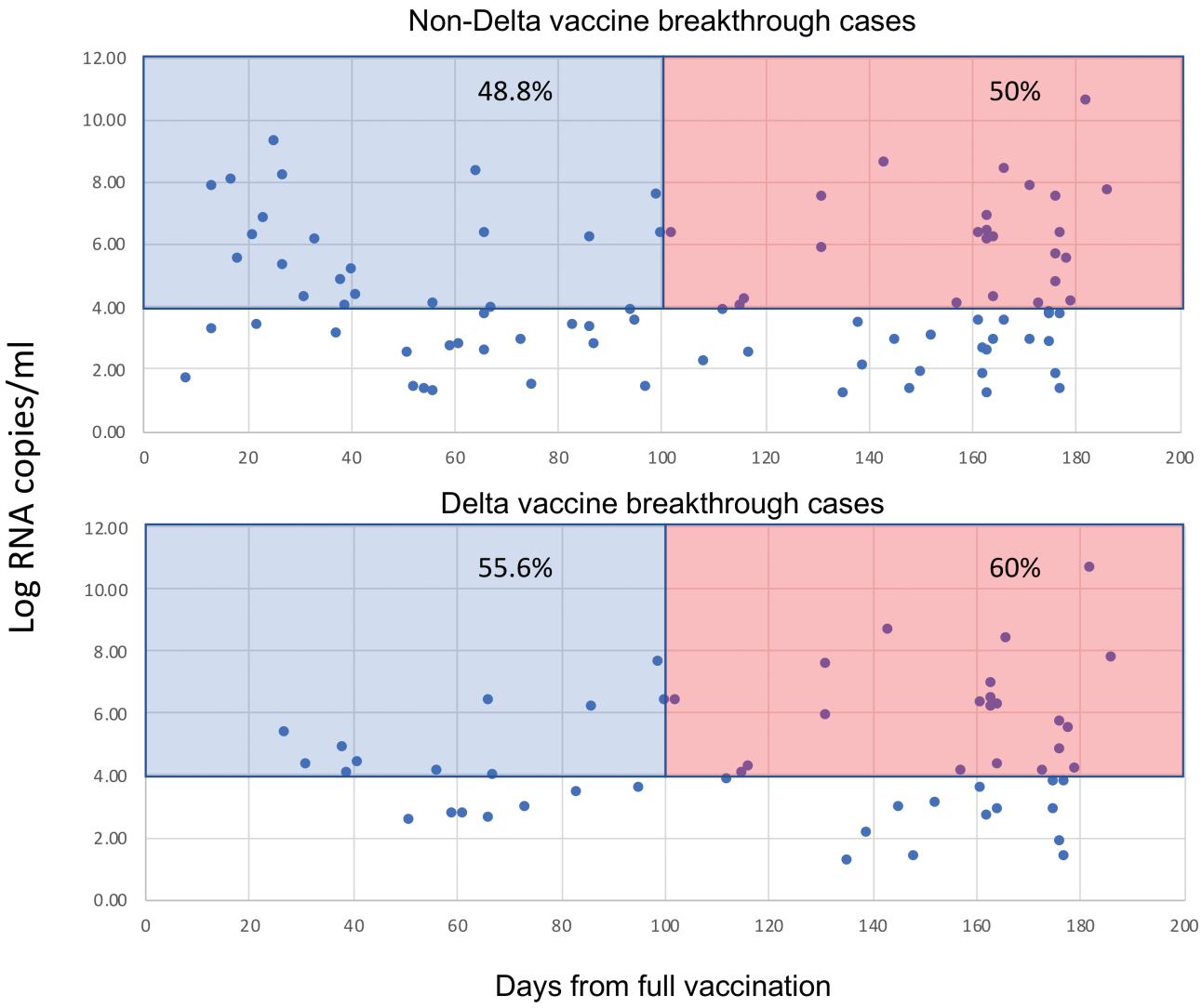

Figure 4. Viral RNA copy number versus time from full vaccination in vaccine breakthrough cases at the time of symptoms onset. Each dot in the scatterplots represents a single patient fully vaccinated with either Pfizer/BNT162b2, Moderna/mRNA-1273 or Johnson & Johnson/Janssen vaccine. The y-axis reports Log viral RNA copies/mL. The x-axis represents the time (in days) between full vaccination, defined as 2 weeks after second vaccination dose (in case of Pfizer-BioNTech or Moderna) or after single dose (Johnson & Johnson/Janssen), and time of sampling, which occurred for each patient on average 4.2 days after symptoms onset (see Table 1). Shaded areas highlight proportions patients (reported inside each area) with viral RNA copy number above the transmissibility threshold (Log 4 copies/mL) who were full vaccinated for <101 days (cyan) or >100 (pink). The top panel includes vaccine breakthrough cases infected with different severe acute respiratory syndrome coronavirus 2 variants (N = 89). The bottom panel includes only those patients infected with the Delta variant (N = 53).

low number of vaccine failures in agreement with the known effectiveness of the vaccines according to randomized clinical trials. Even assuming that we may have missed a substantial number of vaccine breakthrough infections in asymptomatic individuals, we have no records of severe/fatal COVID-19 cases that involved vaccinated patients between December 2020 (when vaccination began in Alachua County) and end of July 2021. Our viral RNA copy number data also show that, despite Delta variant high transmissibility—as evidenced by its ability to become the majority variant within a month since its emergence in the county—vaccination is associated with lower viral replication in breakthrough cases compared with unvaccinated patients. Although other studies have reported similar patterns among vaccinated and unvaccinated individuals [13, 22], such studies used CT values as a proxy for viral burden among groups of patients rather than the actual number of RNA copies, which may in part explain the discrepancy. Although the Centers for Disease Control and Prevention–recommended method of viral RNA quantification used in this study does not differentiate individual viral RNA genomes from RNA transcripts (may vary from patient to patient despite similar viral load), it does provide a more precise estimate of the viral burden than CT alone. Moreover, studies such as the one in Barnstable County [13], Massachusetts, focused on outbreaks within local communities likely characterized by related transmission clusters that may confound statistical comparisons under the assumption of

independent sampling (because samples from different patients can be related through a transmission chain), unless adjusted by using phylogenetic regression methods [31, 32]. Unfortunately, none of those studies investigated the phylogenetic relationships among the samples to exclude potentially linked individuals. Our quantitative viral RNA analysis, on the other hand, was filtered on individuals that were considered independent transmission events through careful sampling and transmission cluster identification within the phylogeny.

Development of sterilizing immunity does not commonly occur for most human and animal vaccines, and current vaccine trials are not designed to address it because only clinically affected subjects are usually tested for the virus. Nevertheless, assessing SARS-CoV-2 transmissibility in vaccine breakthrough cases is crucial, especially considering the current spread of the Delta variant, not only to break the cycle of viral transmission, thus resulting in fewer cases of severe COVID-19 and death, but also to reduce the likelihood of emergence of more pathogenic or potentially vaccine-resistant viral variants. The continuous circulation of SARS-CoV-2 among both unvaccinated and vaccinated individuals provides the virus the chance to continue exploring the wide fitness landscape available to fast-evolving viruses and to accumulate mutations that may eventually result in the emergence of even more transmissible/pathogenic or vaccine-resistant strains. Therefore, although additional observations based on larger numbers of patients are necessary, our work indicates that the differential level of sterilizing immunity, or lack of thereof, that may be present in the vaccinated population is an important factor to be considered for the implementation of the next phase of vaccination and intervention policies. In particular, the ongoing evaluation of vaccine boosters [33, 34] should address, besides effectiveness against disease, whether the administration of extra vaccine doses may reduce the number of breakthrough cases, or at least decrease the proportion of breakthroughs exhibiting viral RNA copy number above the transmissibility threshold during the acute phase of infection, thus improving sterilizing immunity.

METHODS

SARS-COV-2 genomic epidemiology surveillance in Alachua County, Florida

The Alachua County Department of Health, with assistance from designated University of Florida (UF) personnel, has been responsible for contact tracing efforts for UF students, faculty, staff, and other UF-affiliated people, including the UF Health Academic Medical Center (~123 000 total UF affiliates) since the beginning of the epidemic. SARS-CoV-2 positive samples were collected for virus full genome sequencing, as part of this program, between October 2020 and August 2021 from patients hospitalized at the UF Health Shands Hospital Gainesville during this period, as well as from testing sites of UF Pathology Laboratories in Gainesville serving Alachua County residents (Supplementary Table S1). Samples from positive patients in the Tampa Bay and Miami Dade (provided by BayCare and University of Miami, respectively), which had been collected for other studies, were included in the sequence analysis for comparison purposes (Table S1). Full epidemiological investigations were conducted on positive Alachua County cases to collect exposure information, trace contacts, and provide disease transmission education.

Vaccine Breakthrough Cases Involvement

Vaccine breakthrough cases were defined as individuals who were polymerase chain reaction–positive for SARS-CoV-2 and ≥ 14 days after the second dose of Pfizer or Moderna or first dose of Janssen/J&J. Repeat saliva samples were collected from possible breakthrough cases for sequencing. Patient samples and linked data were fully deidentified before sample processing. The study was reviewed and approved under the category of Public Health practice by the University of Florida institutional review board and the Florida Department of Health institutional review board.

Sample Processing and Next-generation Sequencing

Viral RNA from viral transport medium, nasopharyngeal swabs, or saliva was extracted for each sample and used in complementary DNA synthesis as described in the Supplementary Methods. Viral DNA library preparation for next-generation sequencing was performed using the COVIDSeq Test kit (Illumina, San Diego, CA) and Mosquito HV Genomics Liquid Handler (SPT Labtech Inc., Boston MA). Constructed libraries were pooled and sequenced using the Illumina NovaSeq 6000 Sequencing platform. Illumina's DRAGEN pipeline was used to derive sample consensus sequences, which were filtered based on a minimum of 70% coverage of the genome and 20X sequencing depth.

Database Sequence Retrieval, Sequence Alignment, and Masking

For each in-house sequencing run, sequences within the GISAID database associated with the state of Florida were extracted up to August 3, 2021, and added to the collection of high-coverage in-house-produced genome sequences (N = 3110 from Alachua County, N = 126 from Fort Myers, N = 791 from Miami Dade), totaling 20 117 sequences, ranging from February 28, 2020, to August 3, 2021. Each Floridian sequence within this concatenated dataset was then used in a local alignment (Basic Local Alignment Search Tool) [35] search for putative epidemiologically linked non-Floridian sequences, as described in the Supplementary Methods, totaling 5074 sequences, ranging from February 21, 2020, to July 27, 2021. Sequences were aligned and filtered based on quality as described in the Supplementary Methods.

Phylogenetic Tree Reconstruction and Optimization

Subsequent sequencing resulting in sufficient-quality genome sequences were used in updating the maximum likelihood

phylogeny as described in the Supplementary Methods. Lineages for all sequences were determined using the PangoLEARN model (updated daily), which is trained using GISAID SARS-CoV-2 sequences to classify incoming sequences based on molecular and epidemiological criteria [36, 37].

SARS-CoV-2 Delta Transmission Cluster Identification

The subtree containing sequences classified as Delta was pruned from the full tree for transmission cluster analysis as described in the Supplementary Methods. Briefly, transmission cluster identification within the resulting pruned tree was performed using a depth-first search among nodes restricted to minimal evolution (branch lengths) and sampling time difference of 6 days, based on meta-analysis performed by Rai et al for SARS-CoV-2 [38].

Viral RNA Quantification

Levels of SARS-CoV-2 RNA were determined using the 2019-nCoV_N1 assay (primer and probe set) with 2019-nCoV_N_ positive control (IDT, Coralville, Iowa) per Centers for Disease Control and Prevention guidelines [39]. Briefly, viral RNA was extracted using the Qiagen QIAamp Viral RNA Mini Kit then subjected to first strand synthesis using ProtoScript II Reverse Transcriptase according to the manufacturer's instructions (NEB, Ipswich, MA). Additional details on viral RNA quantitation can be found in the Supplementary Methods.

Relationship Between Viral RNA Burden, Vaccination Status, and SARS-CoV-2 Lineage

We performed a multiple regression analysis of viral RNA copy number, CT, or binary transmissibility with patient and/or viral characteristics, including but not limited to vaccination status (yes/no), Delta lineage (yes/no, age [<21, 21-28, 28-44, >44 years old or unknown], sex [male, female, unknown], and days from January 2021, as described in the Supplementary Methods). Patients were considered above the transmissibility threshold (ie, potentially infectious) if they had a viral RNA copy number greater than or equivalent to Log 4 copies per milliliter of sample [31].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank Maureen Long for her critical reading of the manuscript.

Financial support. This work was supported by the Stephany W. Holloway University of Florida Chair and by funds of the University of Florida Office of Research and Health Science Center with resources from the Interdisciplinary Center for Biotechnology Research Gene Expression Core https://scicrunch.org/resources/Any/source/nlx_144509-1/search?q=SCR_019145&l=SCR_019145 (RRID:SCR_019145), NextGen Sequencing Core (RRID:SCR_019152) https://scicrunch.org/resources/Any/source/nlx_144509-1/search?q=SCR_019152&l=SCR_019152 and Bioinformatics Core (RRID:SCR_019120). Funding for this work was also provided by National Science Foundation, Division of Environmental Biology, award no. 2028221; The AIDS HealthCare Foundation Award no. OS00000633; and The Rockefeller Foundation Award 2021 HTH 012.

Financial Support. S. R. reports support for this work from the Florida Department of Health in Alachua County. J. S. reports support from the Florida Department of Health—Alachua, outside the scope of this work (contact tracing for COVID-19). M. L. reports support from the Centers for Disease Control and Prevention and the Florida Department of Health outside the scope of this work and participation on a Data Safety Monitoring Board or Advisory Board for a 2-month rifampin clinical trial for McGill University (Dick Menzies, principal investigator). S. M. reports contracts or grants outside the scope of this work from the Consortium for Medical Marijuana Clinical Outcomes related to cannabidiol-induced biomarkers of inflammation reduction in people living with human immunodeficiency virus at the single-cell level. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. CDC. SARS-CoV-2 Variant Classifications and Definitions. Available at: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html. Accessed 9 August 2021.
2. Rambaut A, Loman N, Pybus O, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. Virologicalorg 2020. Available at: https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563. Accessed 9 August 2021.
3. Madhi SA, Baillie V, Cutland CL, et al. Efficacy of the ChAdOx1 nCoV-19 Covid-19 vaccine against the B.1.351 variant. N Engl J Med **2021**. doi: 10.1056/NEJMoa2102214.
4. Buss LF, Prete CA, Abrahim CMM, et al. Three-quarters attack rate of SARS-CoV-2 in the Brazilian Amazon during a largely unmitigated epidemic. Science **2021**;371:288–92.
5. Sabino BD, Alonso FOM, Oliveira MSC, Venceslau MT, Guimarães MAAM, Varella RB. Long-term intermittent detection of SARS CoV 2 in the upper respiratory tract: what is the meaning of it? Infect Dis (Lond) **2021**;53:151–3.
6. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med **2020**;383:2603–15.
7. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 vaccines against the B.1.617.2 (Delta) variant. N Engl J Med **2021**;385:585–94. doi: 10.1056/NEJMoa2108891.
8. Adam D. What scientists know about new, fast-spreading coronavirus variants. Nature **2021**;594:19–20.
9. Zhang W, Davis BD, Chen SS, Sincuir Martinez JM, Plummer JT, Vail E. Emergence of a novel SARS-CoV-2 variant in Southern California. JAMA **2021**;325:1324–6.
10. Motozono C, Toyoda M, Zahradnik J, et al. SARS-CoV-2 spike L452R variant evades cellular immunity and increases infectivity. Cell Host Microbe **2021**;29:1124–36.e11.e11. doi: 10.1016/j.chom.2021.06.006.
11. Starr TN, Greaney AJ, Dingens AS, Bloom JD. Complete map of SARS-CoV-2 RBD mutations that escape the monoclonal antibody LY-CoV555 and its cocktail with LY-CoV016. Cell Rep Med **2021**; 2:, doi: 10.1016/j.xcrm.2021.100255
12. CDC. COVID-19 Vaccinations in the United States. Available at: https://covid.cdc.gov/covid-data-tracker/#vaccinations_vacc-total-admin-rate-total
13. Brown CM, Vostok J, Johnson H, et al. Outbreak of SARS-CoV-2 infections, including COVID-19 vaccine breakthrough infections, associated with large public gatherings - Barnstable County, Massachusetts, July 2021. MMWR Morb Mortal Wkly Rep **2021**;70:1059–62.
14. Dougherty K, Mannell M, Naqvi O, Matson D, Stone J. SARS-CoV-2 B.1.617.2 (Delta) variant COVID-19 outbreak associated with a gymnastics facility - Oklahoma, April-May 2021. MMWR Morb Mortal Wkly Rep **2021**;70:1004–7.
15. Farinholt T, Doddapaneni H, Qin X, et al. Transmission event of SARS-CoV-2 delta variant reveals multiple vaccine breakthrough infections. BMC Med **2021**;19:255.

16. Mallapaty S. Delta's rise is fuelled by rampant spread from people who feel fine. Nature **2021**. doi: 10.1038/d41586-021-02259-2.
17. Kang M, Xin H, Yuan J, et al. Transmission dynamics and epidemiological characteristics of Delta variant infections in China. [Preprint]. medRxiv **2021**.08.12.2.21261991. Posted August 13, 2021.
18. Zhang M, Xiao J, Deng A, et al. Transmission dynamics of an outbreak of the COVID-19 Delta variant B.1.617.2 - Guangdong Province, China, May-June 2021. China CDC Wkly **2021**;3:584–6.
19. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med **2021**;384:403–16.
20. Thomas SJ, Moreira ED, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine through 6 months. N Engl J Med **2021**. doi: 10.1056/NEJMoa2110345.
21. CC-VBCI T. COVID-19 vaccine breakthrough infections reported to CDC - United States, January 1-April 30, 2021. MMWR Morb Mortal Wkly Rep **2021; 70**:792–3.
22. Riemersma KK, Grogan BE, Kita-Yarbro A, et al. Shedding of infectious SARS-CoV-2 despite vaccination when the Delta variant is prevalent - Wisconsin, July 2021. medRxiv **2021**.
23. Singanayagam A, Patel M, Charlett A, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill **2020**;25. doi: 10.2807/1560-7917.ES.2020.25.32.2001483.
24. Goyal A, Reeves DB, Cardozo-Ojeda EF, Schiffer JT, Mayer BT. Viral load and contact heterogeneity predict SARS-CoV-2 transmission and super-spreading events. Elife **2021**;10. doi: 10.7554/eLife.63537.
25. Marks M, Millat-Martinez P, Ouchi D, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. Lancet Infect Dis **2021**;21:629–36. doi: 10.1016/S1473-3099(20)30985-3.
26. Puranik A, Lenehan PJ, Silvert E, et al. Comparison of two highly-effective mRNA vaccines for COVID-19 during periods of Alpha and Delta variant prevalence. [Preprint]. medRxiv **2021**. doi: 10.1101/2021.08.06.21261707.
27. Giovanetti M, Cella E, Benedetti F, et al. SARS-CoV-2 shifting transmission dynamics and hidden reservoirs potentially limit efficacy of public health interventions in Italy. Commun Biol **2021**;4:489.
28. Beyene GT, Alemu F, Kebede ES, et al. Saliva is superior over nasopharyngeal swab for detecting SARS-CoV2 in COVID-19 patients. Sci Rep **2021**;11:22640.
29. Teo AKJ, Choudhury Y, Tan IB, et al. Saliva is more sensitive than nasopharyngeal or nasal swabs for diagnosis of asymptomatic and mild COVID-19 infection. Sci Rep **2021**;11:3134.
30. CDC. COVID-19 Case Surveillance Public Use Data with Geography. Available at: https://data.cdc.gov/Case-Surveillance/COVID-19-Case-Surveillance-Public-Use-Data-with-Ge/n8mc-b4w4. Accessed 10 August 2021.
31. Grafen A. The phylogenetic regression. Philos Trans R Soc Lond B Biol Sci **1989**;326:119–57. :
32. Adams DC. A method for assessing phylogenetic least squares models for shape and other high-dimensional multivariate data. Evolution **2014**;68:2675–88.
33. Levine-Tiefenbrun M, Yelin I, Katz R, et al. Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine. Nat Med **2021**;27:790–2.
34. Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. N Engl J Med **2021**;385:1393–400.
35. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol **1990**;215:403–10.
36. Rambaut A, Holmes EC, O'Toole Á, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol **2020**;5:1403–7.
37. Rambaut A, Holmes EC, O'Toole Á, et al. Addendum: a dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol **2021**;6:415.
38. Rai B, Shukla A, Dwivedi LK. Estimates of serial interval for COVID-19: a systematic review and meta-analysis. Clin Epidemiol Glob Health **2021**;9:157–61.
39. CDC. CDC real-time reverse transcriptase PCR (rRT-PCR) diagnostic test for COVID-19. Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html/. Accessed 9 August 2021.