SARS-CoV-2 Delta vaccine breakthrough transmissibility in Alachua County, Florida

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Summary

Genomic surveillance efforts have enabled thorough epidemiological and molecular analysis of COVID-19 vaccine breakthrough cases, revealing transmission involving vaccinated individuals that suggest limited levels of sterilizing immunity, despite effective mitigation of available COVID-19 vaccines against severe disease.

ABSTRACT

Background

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 4,439 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 three 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, three of which were confirmed by phylogenetic analysis. Delta breakthroughs exhibited broad viral RNA copy number values during acute infection (IQR 1.2 – 8.64 Log copies/ml), on average 38% lower than matched unvaccinated patients (3.29 – 10.81 Log copies/ml, $p<0.00001$). Nevertheless, 49-50% of all breakthroughs, and 56-60% of Delta-infected breakthroughs exhibited viral RNA levels above the transmissibility threshold (4 Log copies/ml) irrespective of time post 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.

INTRODUCTION

Over the course of the 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 (US); 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 03, 2021, 66.9% of Americans were fully vaccinated,[12] yet emergence of the Delta variant, which spread rapidly within the US 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.

According to clinical trial results, a two-dose regimen of Pfizer-BioNTech (BNT162b2) vaccine conferred 94.6% protection against severe disease,[6] while the Moderna (mRNA-1273) vaccine conferred 94.1% at the time of this study.[19]. While 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 US, these vaccine breakthrough cases linked to VOCs have been reported since January 2021.[21] In July 2021, following multiple large public events in a Barnsy, Massachusetts, town, an outbreak (90% Delta) of infection (albeit mild disease) was identified among Massachusetts residents traveling recently to the town, primarily comprised of 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 – 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, e.g., 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 three 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, 4,439 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 05, 2020, and August 06, 2021).

Following a trend similar to the rest of the US, 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 sub-variants in the following months (Figure 1A), while 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 seven months of 2021 (Figure 1B), including five Alpha variant cases, two Epsilon, five B.1, three B.1.2, one B.1.596, and one 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 due to low coverage of the Spike protein. The breakthrough cases exhibited an average time between full vaccination (defined as 14 days following final dose) and COVID-19 diagnosis of about 3 months (mean = 101.6 ± 57.7 days) (Figure 1C). Compared to 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, while 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%), ages 36.7 years on average (SD=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 (6.3%) related 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 infected with the Delta variant (N=56) averaged 4.66 Log copies/ml, with an interquartile range (IQR) of 1.2 – 10.62, overlapping to the one observed among breakthroughs infected by other variants (N=13) with average copy number of 5.39 Log copies/ml, and IQR 1.41 – 8.36 ($p = 0.35$ from a two tailed Mann–Whitney U test). For comparison with the non-vaccinated population, viral RNA burden was also evaluated in age- and gender- matched data sets, retrospectively assembled for the months of January – April and July, that included randomly selected independent samples (i.e., samples from patients not directly linked through known transmission events) from non-vaccinated 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 to vaccinated Delta or non-Delta breakthroughs (Figure 2), as well as to unvaccinated patients infected by other variants (mean 6.15 Log copies/ml, IQR 3.56 – 10.92), although effect size was modest (6% increase, $p = 0.17$ from a two tailed Mann–Whitney U test), probably due to 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 to unvaccinated Delta cases ($p<0.00001$, two tailed U-test, after Bonferroni correction), and 34% ($p<0.00001$) compared to 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 odd ratio (OR) and confidence interval (CI) 1.05 – 5.97 in the unadjusted analysis, and OR 3.04 and CI 1.16 – 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). Though 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 six putative transmission pairs, each one 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, while Donors D2 – D6 with BNT162b2 for 143 – 176 days, with all of them exhibiting > Log 4 RNA viral RNA copies/ml. Four out of six Recipients had also been vaccinated with BNT162b2 for 67 – 164 days at the time of symptom onset, thus classifying them as additional vaccine breakthrough cases. For three of these D-R pairs, we were able to obtain saliva samples collected approximately four 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 GISAID database (see Methods). Four non-monophyletic 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 interval of up to six days (see Methods). Though geographical sampling locations were intermixed throughout the phylogeny, clusters were primarily comprised of 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 receiving (R) individual within a transmission pair was observed directly adjacent to the corresponding putative donor (D) individual, lending validation to direct transmission between pairs. All six individuals belonging to the three 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. As 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 three D-R transmission pairs (Figure 3). Therefore, the three donor individuals were removed prior to regression analysis, leaving the three recipient individuals which 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 background population and remaining two in clusters c2 and c6) and could thus be included. Only one 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 two weeks after 2nd vaccination dose (in 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 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 nine-month genomic epidemiology surveillance program, represented approximately 2% of the sequenced cases, a 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 to unvaccinated patients. While other studies have reported similar patterns among vaccinated and unvaccinated individuals[13,22], such studies utilized 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. While the Center for Disease Control (CDC)-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 Barnsy[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 since only clinically affected subjects are usually tested for the virus. Nevertheless, assessing SARS-CoV-2 transmissibility in vaccine breakthrough cases is crucial, especially in light of 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, while additional observations based on larger numbers of patients are necessary, our work indicate 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 UF personnel, has been responsible for contact tracing efforts for University of Florida (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 UF Health Shands Hospital Gainesville during this time period, as well as from testing sites of UF Pathology Labs in Gainesville serving Alachua County residents (Supplementary Table S1). Samples from positive patients in the Tampa Bay are 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 Alachua County positive 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 PCR-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 de-identified before sample processing. The study was reviewed and approved under the category of Public Health practice by the University of Florida Institutional Review Board (IRB) and the Florida Department of Health IRB.*

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 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 Global Initiative for Sharing All Influenza Data (GISAID) database associated with the state of Florida were extracted up to August 03, 2021, and added to the collection of high-coverage in-house-produced genome sequences (N=3,110 from Alachua County, N=126 from Fort Myers, N=791, from Miami Dade), totaling 20,117 sequences, ranging from February 28, 2020, to August 03, 2021. Each Floridian sequence within this concatenated dataset was then used in a local alignment (BLAST)[35] search for putative epidemiologically linked non-Floridian sequences, as described in the Supplementary Methods, totaling 5,074 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 CDC 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), gender (male, female, unknown), and days from January 2021, as described in the Supplementary Methods. Patients were considered above the transmissibility threshold (i.e., potentially infectious) if they had a viral RNA copy number greater than or equivalent to Log 4 copies per ml of sample. [31]

NOTES

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FIGURE LEGENDS

Figure 1. SARS-CoV-2 variants distribution in Alachua County, Florida, over time. (A) Lineage distribution (y-axis) *vs.* 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) *vs.* 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) vs. time between 2nd vaccination dose and diagnosis (y-axis).

Figure 2. Viral RNA copy number distribution in vaccinated and unvaccinated SARS-CoV-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 two-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.

Figure 3. Phylogenetic reconstruction and transmission characterization of SARS-CoV-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 prior to diagnosis are represented with asterisks. Branches are scaled in substitutions/site.

Figure 4. Viral RNA copy number vs. 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 two weeks after 2nd 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 SARS-CoV-2 variants (N=89). The bottom panel includes only patients infected with the Delta variant (N=53).

Table 1. Summary of vaccine breakthrough population (N=109) in Alachua County, Florida from January to August 2021.

Numbers are present as frequencies and (percentages) unless otherwise stated

Age (years)	36.7 (14.2)
Sex	
Female	68 (62.4%)
Male	41 (37.6%)
Race	
White	80 (73.4%)
African American/Black	10 (9.2%)
Asian/Pacific Islander	11 (10.1%)
Other/Unknown	8 (7.3%)
Ethnicity	
Non-Hispanic	91 (83.5%)
Hispanic	18 (16.5%)
Symptoms	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	
Asthma	11 (10.1%)
Diabetes	3 (2.8%)

Hypertension	15 (13.8%)
BMI [b]	Mean = 27.0 (SD = 6.3)
Known exposure (Y)	48 (44.0%)
Household	26 (54.2%)
Community	18 (37.5%)
Healthcare	3 (6.3%)
Vaccine	
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] (days)	Mean = 104.0 (SD = 57.5)
Time between disease onset [a], and sample collection date (days) for viral RNA copy number measurement (N=83)	Mean = 4.2 (SD = 2.4)

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

[b] BMI: body mass index.

Table 2. Vaccine and Viral load information for identified transmission pairs

Pair	Individual	Vaccine	Days since vaccination*	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

* Time since vaccination was defined as two weeks after administration of 2nd dose of either Moderna or Pfizer vaccine.

Figure 1

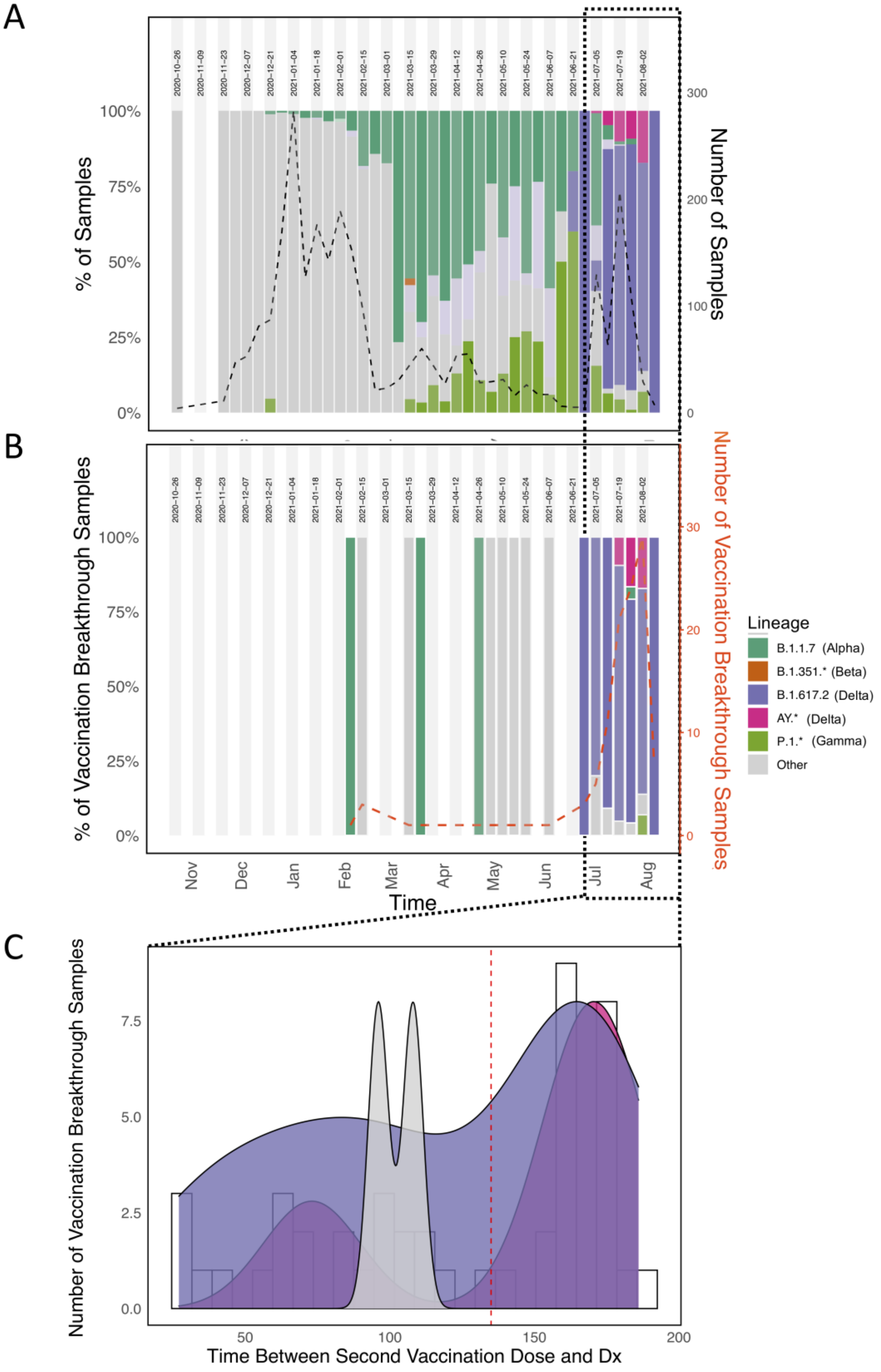

A
% of Samples
Number of Samples
100%
75%
50%
25%
0%
300
200
100
0
2020-10-26
2020-11-09
2020-11-23
2020-12-07
2020-12-21
2021-01-04
2021-01-18
2021-02-01
2021-02-15
2021-03-01
2021-03-15
2021-03-29
2021-04-12
2021-04-26
2021-05-10
2021-05-24
2021-06-07
2021-06-21
2021-07-05
2021-07-19
2021-08-02
B
% of Vaccination Breakthrough Samples
Number of Vaccination Breakthrough Samples
100%
75%
50%
25%
0%
30
20
10
0
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Time
Lineage
B.1.1.7 (Alpha)
B.1.351.* (Beta)
B.1.617.2 (Delta)
AY.* (Delta)
P.1.* (Gamma)
Other
C
Number of Vaccination Breakthrough Samples
7.5
5.0
2.5
0.0
50
100
150
200
Time Between Second Vaccination Dose and Dx

Figure 2

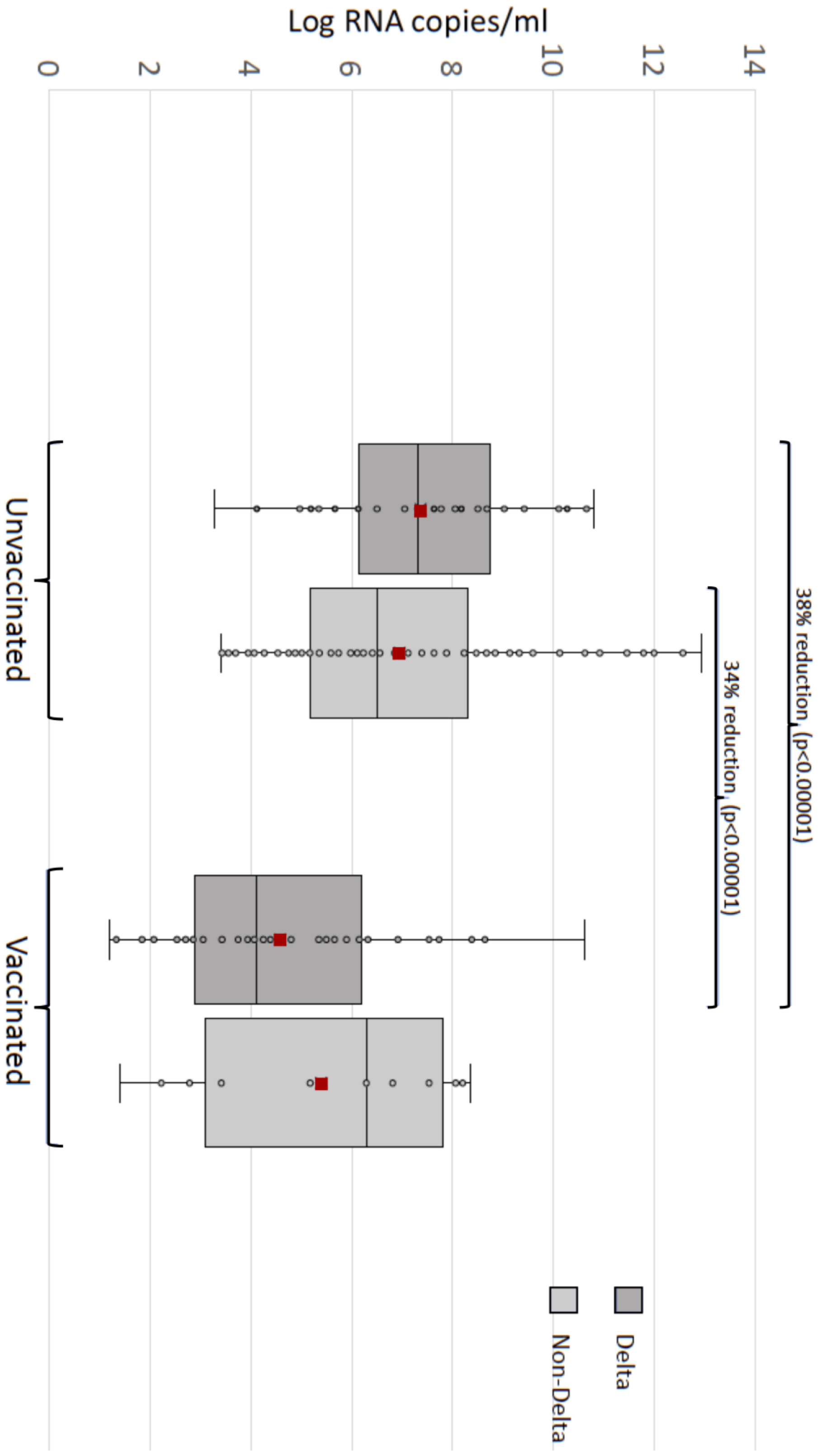

Log RNA copies/ml
0
2
4
6
8
10
12
14
38% reduction, (p<0.00001)
34% reduction, (p<0.00001)
Unvaccinated
Vaccinated
Delta
Non-Delta

Figure 3

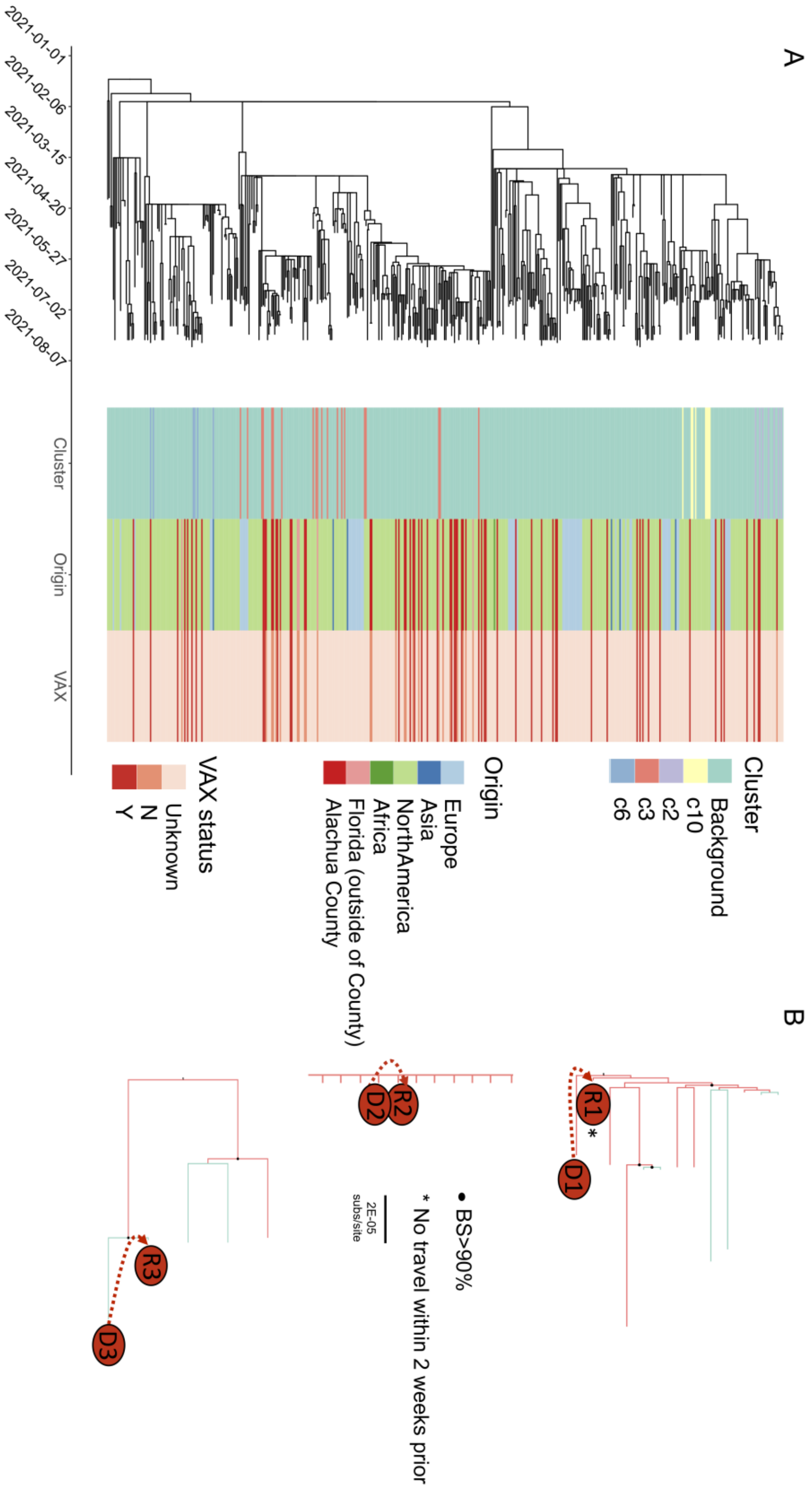
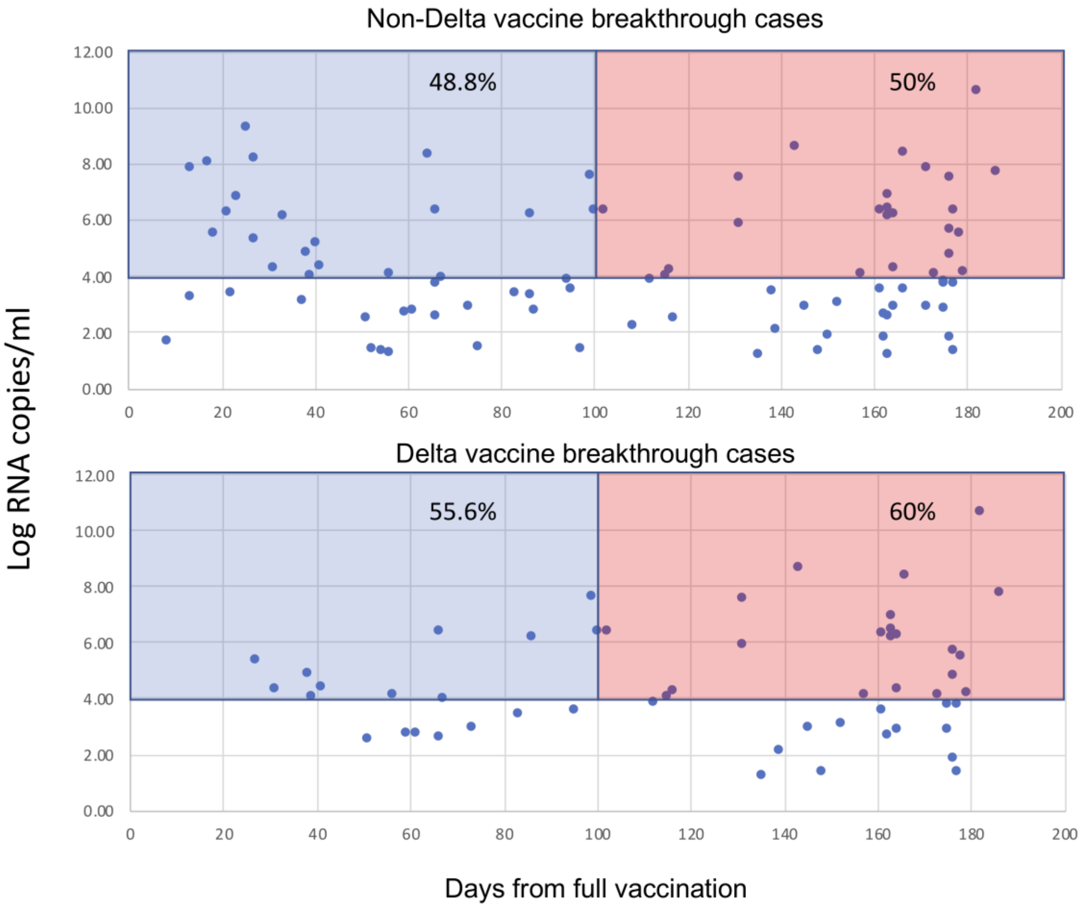

A
2021-01-01
2021-02-06
2021-03-15
2021-04-20
2021-05-27
2021-07-02
2021-08-07
Cluster
Origin
VAX
Cluster
Background
c10
c2
c3
c6
Origin
Europe
Asia
NorthAmerica
Africa
Florida (outside of County)
Alachua County
VAX status
Unknown
N
Y
B
R1
*
D1
R2
D2
R3
D3
● BS>90%
* No travel within 2 weeks prior
2E-05 subs/site

Figure 4


Non-Delta vaccine breakthrough cases
48.8%
50%
Delta vaccine breakthrough cases
55.6%
60%
Log RNA copies/ml
Days from full vaccination
0.00
2.00
4.00
6.00
8.00
10.00
12.00
0
20
40
60
80
100
120
140
160
180
200