1 **Transmission event of SARS-CoV-2 Delta variant reveals multiple vaccine breakthrough**

2 **infections**

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27 **Key Points**

28 **Question:** Which SARS-CoV-2 variant is responsible for 6 cases of vaccine breakthrough, one

29 interventional monoclonal antibody treatment, and one death?

30 **Findings:** Viral sequencing revealed 6 vaccinated patients were infected with the Delta SARS-

31 CoV-2 variant. With no histories of vaccine breakthrough, this suggests Delta variant may

32 possess immune evasion in patients that received the Pfizer BNT162b2, Moderna mRNA-1273,

33 and Covaxin BBV152.

34 **Meaning:** Delta variant may pose the highest risk out of any currently circulating SARS-CoV-2

35 variants, with increased transmissibility over Alpha variant and possible vaccine breakthrough.

36

37 **Abstract**

38 **Importance:** Vaccine breakthrough by an emergent SARS-CoV-2 variant poses a great risk to

39 global public health.

40 **Objective:** To determine the SARS-CoV-2 variant responsible for 6 cases of vaccine

41 breakthrough.

42 **Design:** Nasopharyngeal swabs from suspected vaccine breakthrough cases were tested for

43 SARS-CoV-2 by qPCR for Wuhan-Hu1 and Alpha variant. Positive samples were then

44 sequenced by Swift Normalase Amplicon Panels to determine the causal variant.

45 **Setting:** Transmission event occurred at events surrounding a wedding outside of Houston, TX.

46 Two patients from India, likely transmitted the Delta variant to other guests.

47 **Participants:** Following a positive SARS-CoV-2 qPCR test at a third-party site, six fully

48 vaccinated patients were investigated. Three males and three females ranged from 53 to 69

49 years old. One patient suffered from diabetes while three others were classified as overweight.

50 No significant other comorbidities were identified. None of the patients had a history of failed

51 vaccination.

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53 **Introduction**

54 High numbers of global SARS-CoV-2 infections have led to the emergence of variants, notably

55 Alpha variant (B.1.1.7 UK), Beta (B.1.351 S. Africa), Gamma (P.1 Brazil), Epsilon (B.1.429

56 California), Iota (B.1.526 New York) and now, Delta and Kappa (B.1.617.2 and B.1.617.1 India).

57 Each of these strains gained advantageous mutations to become a dominant strain, e.g., Iota

58 first discovered November 23, 2020, represented 45% of new cases as of February 7, 2021[1].

59 Increased transmissibility results from genomic changes such as nonsynonymous mutations in

60 the receptor-binding domain (RBD) of the S-gene (encodes the spike protein) conferring higher

61 binding affinity to host angiotensin-converting enzyme 2 (ACE2) receptors or more efficient

62 cleavage by Transmembrane Serine Protease 2 (TMPRSS2) and subsequently, viral entry[2,3].

63 Mutations could also lead to vaccine breakthrough[4]. The spike protein's RBD is

64 immunodominant[5], targeted by convalescent sera and vaccine-elicited antibodies (Pfizer

65 BNT162b2[6]), though evidence suggest substantial role of the amino-terminal domain (NTD).

66 Mutations in the RBD therefore pose a risk of allowing immune evasion to one or more of the

67 current vaccines[4]. The Kappa and Delta variants emerged from the Indian state of Maharashtra

68 in December 2020, contributing to a resurgence of cases in the country, representing 70% of

69 daily new cases on May 2, 2021[7]. The B.1.617 lineage is now widely circulating in over 50

70 countries based on viral sequence data and is classified as a variant of concern by the CDC.

71 The Kappa and Delta variant lineages are defined by 7 and 8 nonsynonymous mutations in the

72 S protein respectively (Figure 1).

73 Emergent data suggests partial immunity to the Kappa variant, as convalescent sera and

74 vaccine-elicited (Pfizer BNT162b2 and Moderna mRNA-1273) antibodies show a 2.3- and 4-fold

75 reduction in neutralization in vitro respectively (noting that this study used protein-pseudotyped

76 lentiviruses lacking the T478K mutation found in Delta variant)[8]. A test negative case control

77 study estimated the effectiveness of vaccination (two weeks post second vaccination) against

78   symptomatic disease by Delta variant to be as high as 88% for Pfizer BNT162b2 in the UK

79   (compared to 93% for Alpha variant)[9].

80   Here we describe a transmission of a Delta variant containing SARS-CoV-2 strain, between

81   family members associated with events surrounding a wedding with 92 attendees, near

82   Houston, Texas. Attendance required guests be fully vaccinated and took place outdoors in a

83   large, open-air tent. To date, 6 individuals have tested positive for SARS-CoV-2, all patients

84   were symptomatic, one patient severely enough to receive monoclonal antibody infusion

85   treatment (Regeneron Pharmaceuticals Inc.) and one patient has died. Encounter timings and

86   viral sequence similarities suggest the strain containing the Delta variant was transmitted to

87   wedding guests from two patients travelling from India. With no history of vaccine failure in

88   these patients, our observations suggest these are true cases of vaccine breakthrough,

89   mediated by the Delta variant.

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91   **Results**

92   In early April, 2021, Patient 0a, a man with no comorbidities, and Patient 0b, a woman with

93   diabetes, travelled from India to attend a wedding in Texas (designated 0a and 0b due to

94   difficulty establishing true patient 0). Both tested negative for SARS-CoV-2 by qPCR as part of

95   the pre-flight criteria. Formal wedding events were held outdoors and in a large open-air tent

96   and attendance required full vaccination (Patient 0a and 0b travelled to Houston 10 days after

97   their second doses of Covaxin BBV152, Table 1). Patients 1-5 confirmed having close

98   encounters with Patient 0a and 0b at the wedding. Events were attended by fewer than 100

99   guests.

100   On the evening of the first night of the wedding, Patient 0b complained of fatigue but associated

101   it with diabetes and jet lag. Patient 0a developed a cough two days after the wedding and both

102   him and Patient 0b developed a fever three days after. Patient 0a and 0b tested positive for

103   SARS-CoV-2 by nasal swab qPCR 4 days after the wedding at a third-party site. Patient 0a's

104   symptoms progressed over the following days and was admitted to the hospital on day 6 post

105   wedding. He was transferred to Baylor St. Luke's hospital in the Texas Medical Center with

106   worsening symptoms. A month after the wedding, Patient 0a died from complications of COVID-

107   19.

108   Five other guests have tested positive for SARS-CoV-2 following confirmed interactions with

109   Patient 0a and 0b. All positive patients received Pfizer BNT162b2, Moderna mRNA-1273, or

110   Covaxin BBV152 (Table 1). Six of these have experienced symptoms of COVID-19 (Table 1).

111   Patient 1, who received the Pfizer BNT162b2 vaccine developed severe symptoms and was

112   admitted to Baylor St. Luke's hospital for monoclonal antibody infusion treatment (Regeneron

113   Pharmaceuticals Inc.) on ten days after the wedding. The density of vaccine breakthrough

114   resulting in COVID-19 symptoms suggested the patients were carrying a SARS-CoV-2 variant.

115   To characterize the variant, total RNA was extracted from nasopharyngeal swabs of each of the

116   6 patients. All positive for SARS-CoV-2 Wuhan-Hu-1 and negative for Alpha variant by qPCR

117   (Table 1). Human RNase P (RP) gene control values suggested sampling of patients and RNA

118   isolation were performed optimally. Amplicon libraries were successfully prepared from all 6

119   qPCR positive samples (N1 Ct value 17 – 29), with 900,754 – 2,381,756 pass-filter reads

120   generated using Swift Biosciences Sarscov2 analysis pipeline (sTable 1). Median sequence

121   coverage ranged from 2085x to 12,932x with >99.7% of the genome covered at 40x or greater.

122   All 6 samples were identified as the SARS-CoV-2 Delta variant based on the presence of the 10

123   mutations listed by the CDC's 'Selected Characteristics of SARS-CoV-2 Variants of Interest'.

124   These mutations, located on the S protein were T19R, (G142D), 156del, 157del, R158G,

125   L452R, T478K, D614G, P681R, D950N. 156del, 157del, R158G are annotated as a single

126   mutation (S:GAGTTCA22028G:Glu156_Arg158delinsGly) due to their proximity (Figure 1).

127   Phylogenetic analysis places each patient sample in a subclade of the Delta variant (white box,

128   Figure 2).

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130 **Discussion**

131 Ending the current SARS-CoV-2 pandemic requires limiting the spread through continued

132 vigilance of masking, social distancing, and vaccination. Variants emerge from areas

133 experiencing uncontrolled viral spread and display increased transmissibility due to mutations in

134 the spike protein. These mutations may occur in the antigenic region of the RBD, altering

135 binding sites for vaccine-elicited antibodies. Mutations such as the ones found in the Kappa

136 variant, provide partial resistance to antibody neutralization (Pfizer BNT162b2, Moderna mRNA-

137 1273, Regeneron[8], and Covaxin BBV152[10]), likely due to changes in epitope sequence.

138 Vaccine breakthrough by highly transmissible variants (Delta variant up to 50% more

139 transmissible than Alpha variant, SAGE) could lead to significant setbacks in pandemic control

140 efforts, requiring renewed social distancing and masking efforts. Significant vaccine

141 breakthrough could necessitate vaccine boosters or targeted lockdowns to reduce spread of

142 infection. An analysis of spike protein epitopes found several antigenic regions (IDa-IDi, Zhange

143 et al.). Delta variant spike protein contains mutations in three of these regions (450–469 IDf,

144 480–499 IDg, and 522–646 IDh, Figure 1) possibly resulting in decreased neutralization by

145 vaccine-elicited antibodies.

146 According to the cases presented in this study, antibodies elicited in patients receiving Pfizer

147 BNT162b2, Moderna mRNA-1273, and Covaxin BBV152 may provide decreased immunity to

148 the Delta variant. It is possible that some individuals in this study failed to produce an effective

149 immune response to their immunization, however, none of the patients had a history of vaccine

150 failure.

151 Our observations support continued efforts to generate SARS-CoV-2 genomic sequences from

152 positive patient samples, in order to identify possible vaccine breakthrough mutations. The

153 continued effectiveness of vaccine-elicited antibodies towards SARS-CoV-2 variants highlights

154 the importance of vaccination efforts. Partial efficacy of current vaccines (70% at 75% coverage)

155 is theoretically sufficient to stop a pandemic[11]. Slowing the spread could prevent emergence of

156 future variants, hastening the end of this pandemic.

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158 **Materials and Methods**

159 **Specimen collection and ethical considerations**

160 All individuals were initially tested by third party SARS-CoV-2 testing sites. To confirm,

161 nasopharyngeal swabs were collected by a physician or nurse as close to the first positive test

162 as possible. Samples were submitted to the Alkek Center for Metagenomics and Microbiome

163 Research for RNA extraction and the Human Genome Sequencing Center for qPCR

164 confirmation. Protocols for collection, qPCR testing, and whole genome sequencing were

165 approved by the Baylor College of Medicine Institutional Review Board (H-47423).

166

167 **cDNA synthesis and amplicon libraries.**

168 RNA extracted from nasopharyngeal swabs of six individuals that tested positive for SARS-

169 COV-2 using qPCR was converted to 1st strand cDNA using SuperScript™ IV First-Strand

170 Synthesis System (Thermo Fisher, Cat. No. 18091050). The 1st strand cDNA reaction was

171 performed starting with 10 µl of total RNA in 25 µl reaction mix, which was incubated at 23°C for

172 10 minutes followed by 50°C for 50 minutes. The resulting 1st strand cDNA was then diluted with

173 DEPC treated water, where for two samples with Ct<24 cDNA was diluted 20 times and for the

174 remaining four samples, with Ct>24, cDNA was diluted 2 times, respectively. This diluted 1st

175 strand cDNA (10 ul) was used as input for amplification of the SARS-CoV-2 viral genome, using

176 the SARS-CoV-2 Additional Genome Coverage Panel (Cat#COVG1V2-96). This panel was

177 designed against SARS-CoV-2 Wuhan-Hu-1 strain (NC_045512.2) and has 345 amplicons of

178 116-255 bp (average 150 bp) that cover 99.7% (29,828 of 29,903 total bases) of the genome.

179 These amplicons come in a single tube, and the workflow involves two rounds of PCR, a

180 multiplex PCR (4 + 18 cycles) and the indexing PCR (9 cycles) to generate sequence ready

181   libraries. The reaction mixes and the thermocycler conditions were performed according to the

182   Swift Normalase® Amplicon Panels (SNAP) Workflow. Libraries were barcoded with 8bp unique

183   dual indices at the Indexing PCR. For library normalization, the 2 nM Normalase I protocol was

184   performed on libraries individually, followed by pooling 5 µl of each post-Normalase I library to

185   perform Normalase II reaction, which results in sequence ready library pool. Before sequencing,

186   the normalized library pool concentration was measured using qPCR with KAPA Library

187   Quantification Kits (Roche, KK4835, 07960204001).

188

189   **Illumina Sequencing.**

190   The pooled SARS-CoV-2 amplicon libraries were sequenced on Illumina NovaSeq 6000 S4

191   flowcell to generate 2x150bp reads.

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193   **Swift amplicon data analysis**.

194   Swift amplicon data were analyzed using Swift Biosciences Sarscov2 analysis pipeline

195   (https://github.com/swiftbiosciences/sarscov2analysis_docker) with minimum read coverage

196   depth of 3. The GATK variants were next filtered with allele fraction >=80, and min read depth

197   30x[12,13]. Swift analysis pipeline produced variant vcf file, consensus genome, pangolin lineage

198   and Nextclade assignment (https://clades.nextstrain.org/). Variant vcf from Swift amplicon data

199   was also annotated using SnpEff[13].

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201   **Phylogenetic Analysis**.

202   Sequences for the designated variants of concern and variants of interest by centers for disease

203   control (CDC), was downloaded from GISAID on 2nd June 2021[15]. All samples downloaded from

204   GISAID, were analyzed using Pangolin V3.0.3 with pangoLEARN 2021-05-27[16] to ensure that

205   the variant designation assigned by GISAID is accurate. Global alignment of 334 sequences

206   including the sequences from the current study was done using MAFFT v7.480[17]. Maximum-

207 likelihood phylogenetic tree with boot strap (5,000) was generated using IQ-Tree V2.1.2[18,19].

208 Annotation and visualization of the tree was carried out by using FigTree v1.4.4

209 (http://tree.bio.ed.ac.uk/software/figtree/). Clades were labeled with the WHO nomenclature.

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233   **Figure 1.** Spike protein mutation prevalence in SARS-CoV-2 variant lineages. Only mutations

234   found in greater than 50% in at least one lineage are displayed. Green text denotes a mutation

235   found in all patients in this report. Figure modified from Outbreak.info[5]

236

237   **Figure 2.** Phylogenetic analysis of SARS-CoV-2 variants. All patients (white box) cluster in a

238   sub clade of the Delta variant (red). Sequences obtained from GISAID.

239

240   **Supplementary Figure 1.** Mutation metrics from sequencing results for all 6 samples. Figure

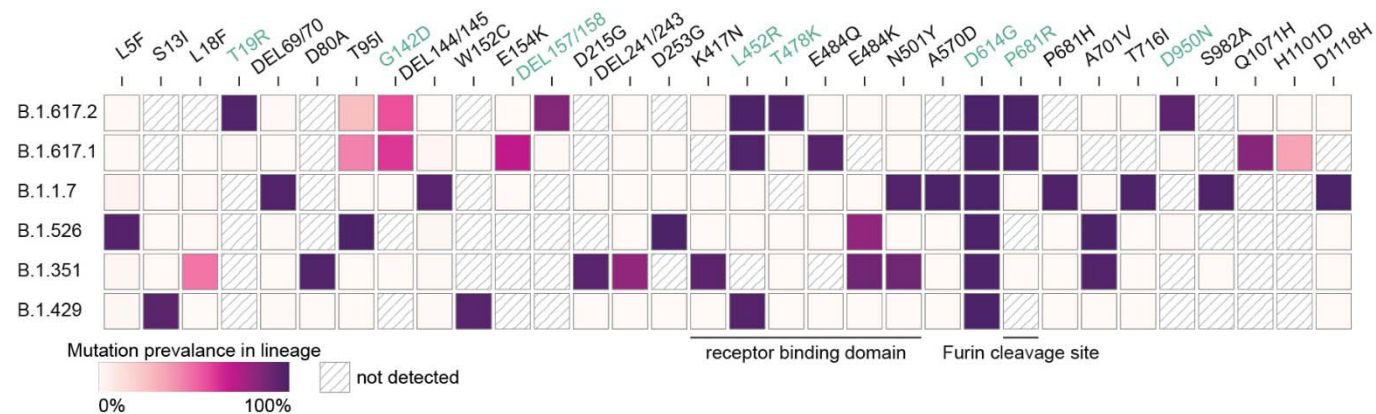241   generated using Coronapp[9]
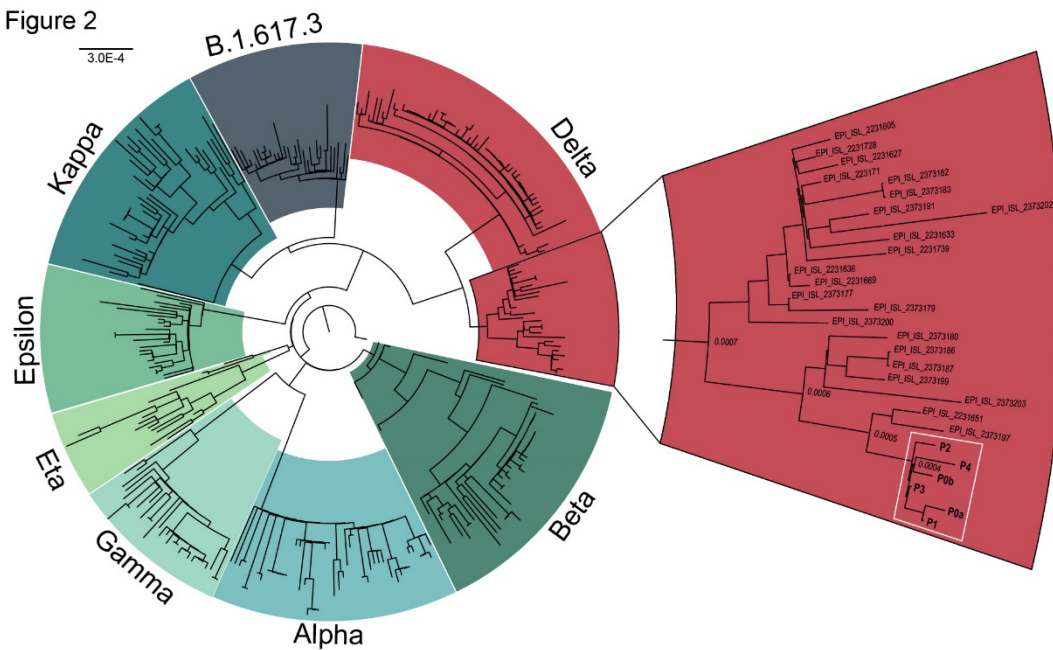
Figure 1

Figure 2

Table 1. Patient demographics, vaccine history, and symptoms

Sample	Sex	Age	Height (cm)	Weight (kg)	Vaccine received	Symptoms	Comorbidities†	N1 qPCR Ct
Patient 0a	Male	67-69	177.8	81.6	Covaxin BBV152	Fever, cough, body aches, fatigue, loss of taste and/or smell, Shortness of breath at rest, Shortness of breath with activity	None	29
Patient 0b	Female	65-70	152.4	53	Covaxin BBV152	Fever, cough, body aches, fatigue, loss of taste and/or smell, Shortness of breath at rest, Shortness of breath with activity	Diabetes	27
Patient 1	Male	60-66	172	68	Pfizer BNT162b2	Cough, fatigue	None	17
Patient 2	Male	59-64	170.1	77	Pfizer BNT162b2	Fever, cough, fatigue	Hypertension	24
Patient 3	Female	51-56	155	63.5	Moderna mRNA-1273	Fever, cough, body aches, fatigue, loss of taste and/or smell	Overweight	25
Patient 4	Female	51-56	162.6	88.5	Moderna mRNA-1273	Fatigue, loss of taste and/or smell	Overweight	22

† - As defined by the Centers for Disease Control and Prevention

Supplementary table 1. Swift Amplicon Normalase Panel sequencing metrics

Sample	Reads passed filter	Yield (Mb)	Mean	Stdev	Median	Maximum	Bases without coverage	0x %	>=5x %	>=10x %	>=20x %	>=40x %
Patient 0a	900,754	272	2941.96	3405.34	2085	216863	46	0.15	99.85	99.84	99.83	99.8
Patient 0b	2,381,756	719	15470.4	11416.18	12932	108010	43	0.14	99.85	99.85	99.84	99.84
Patient 3	1,458,602	440	9711.2	8845.54	7534	81585	42	0.14	99.83	99.81	99.8	99.72
Patient 1	1,424,114	430	8651.92	14635.48	4457	159997	40	0.13	99.84	99.84	99.83	99.72
Patient 4	1,501,130	453	9067.53	8965.74	6690	119267	42	0.14	99.86	99.84	99.83	99.8
Patient 2	1,710,539	517	11420.22	10814.68	8203	91027	41	0.14	99.85	99.84	99.83	99.72

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