

Notes from the Field

Whole-Genome Sequences Analysis Displays Relationship of SARS-CoV-2 Delta Variant Between Four Local Cases and Passengers of a Flight from South Africa — Shenzhen City, Guangdong Province, China, June 2021

Yaqing He^{1,2}; Renli Zhang^{1,2}; Qiuying Lyu³; Bo Peng^{1,2}; Shujiang Mei³; Ying Sun^{1,2}; Dongfeng Kong³; Chaoqiong Peng⁴; Ziquan Lyu⁵; Xinyi Wei^{1,2}; Can Zhu^{1,2}; Xiaoliang Xiao^{1,2}; Shimin Li^{1,2}; Qingju Lu^{1,2}; Jiancheng Chen^{1,2}; Hang Zhang⁴; Xuan Zou^{4,#}; Tiejian Feng^{2,4,#}; Long Chen^{1,2,#}

On June 14, 2021, a customs officer (Case A) went to the infirmary at Baoan International Airport in Shenzhen due to a runny nose and fever. He was admitted to the Central Hospital of Baoan immediately. This patient preliminarily tested positive for coronavirus disease 2019 (COVID-19) infection, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), using a quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) method in this hospital. Then, a mixed specimen of nasopharyngeal swab, oropharyngeal swab, and anal swab was sent to the virology laboratory of Shenzhen Center for Disease Control and Prevention (Shenzhen CDC) and was confirmed positive for SARS-CoV-2 by a qRT-PCR method simultaneously implemented in two commercial kits (Daan, Guangzhou, China and Bojie, Shanghai, China) (Supplementary Table S1, available in <http://weekly.chinacdc.cn/>). This patient tested negative for SARS-CoV-2 on June 8, 2021 and participated in an epidemiological investigation and sampling in a flight from South Africa that arrived at Baoan International Airport on June 10, 2021. Between June 10, 2021 and June 25, 2021, a total of 39 passengers (Case 1 to 39) from this flight were confirmed to be infected with SARS-CoV-2 in the virology laboratory of Shenzhen CDC.

On June 17, 2021, a third-party laboratory detected SARS-CoV-2 in a mixed specimen of 10 swabs from ten individuals by qRT-PCR method, and the preliminary result was positive. This mixed specimen and one (Case B) of ten nasopharyngeal swabs from ten individuals were confirmed positive for SARS-CoV-2 in the virology laboratory of Shenzhen CDC. Case B was a 22-year-old female who worked in a restaurant at Baoan International Airport. The third case (Case C) lived in Dongguan City and worked in

Nanshan District in Shenzhen City. He presented symptoms of chills, dry cough, diarrhea, and fever on June 12, 2021 and was confirmed to be infected with SARS-CoV-2 on June 18, 2021. The fourth case (Case D) lived and worked in Baoan District, Shenzhen. She was confirmed to be infected with SARS-CoV-2 during screening of key populations on June 20, 2021.

High-throughput sequencing was performed for 4 local SARS-CoV-2 strains and 39 imported SARS-CoV-2 strains by Illumina Sequencing Technology. First, viral RNA was extracted directly from 200- μ L swab samples using a High Pure Viral RNA Kit (Roche, Germany). Second, viral RNA was reverse-transcribed and amplified using ULSEN[®] 2019-nCoV Whole Genome Capture Kit V-090418 (Beijing MicroFuture Technology Co., Ltd, Beijing, China). Third, the sequencing libraries were prepared using the Nextera[®] XT Library Prep Kit FC-131-1001 (Illumina, Inc., San Diego, USA). The final viral-enriched libraries were sequenced using the MiSeq platform (Illumina, USA). The viral genome was assembled by MicronCoV[®] Analyzer M-881027 (Beijing MicroFuture Technology Co., Ltd, Beijing, China). Genome sequences of the 4 local SARS-CoV-2 strains and 28 of 39 imported SARS-CoV-2 strains were successfully determined. Virus strains from this study were genotyped using the online Pangolin COVID-19 Lineage Assigner (www.pangolin.cog-uk.io/). The evolutionary relationship of local SARS-CoV-2 strains and imported SARS-CoV-2 strains was inferred with the program MEGA version X (www.megasoftware.net) (1). Nucleotide difference between viral genome sequences from this study and the reference sequence Wuhan-Hu-1 (GenBank no. NC_045512.2) was analyzed using the programs BioEdit 7.1.9 (www.bioedit.software.informer.com) and MEGA version X.

The 4 local SARS-CoV-2 strains and 26 of 28 imported SARS-CoV-2 strains from this study were assigned to lineage B.1.617.2 (Supplementary Table S2, available in <http://weekly.chinacdc.cn/>), which was the fourth variant of concern (VOC) (Delta variant). The other two imported SARS-CoV-2 strains were assigned to lineage B.1.351 and lineage C.1.2. Molecular phylogeny indicated that 30 SARS-CoV-2 Delta variants (lineage B.1.617.2) from this study formed 2 distinct clades (Figure 1). The imported

strain hCoV-19/Shenzhen/IVDC-0610-01/2021 (Case1) clustered in clade A and showed the closest relationship to the Indian strain hCoV-19/India/GJ-NCDC-NIV-INSACOG-24095/2021 by an Audacity Instant search in Global initiative on sharing all influenza data (GISAI). The 4 local SARS-CoV-2 strains of lineage B.1.617.2 and 25 imported SARS-CoV-2 strains of lineage B.1.617.2 clustered in clade B and showed the closest relationship or showed only one nucleotide difference to the British strain hCoV-

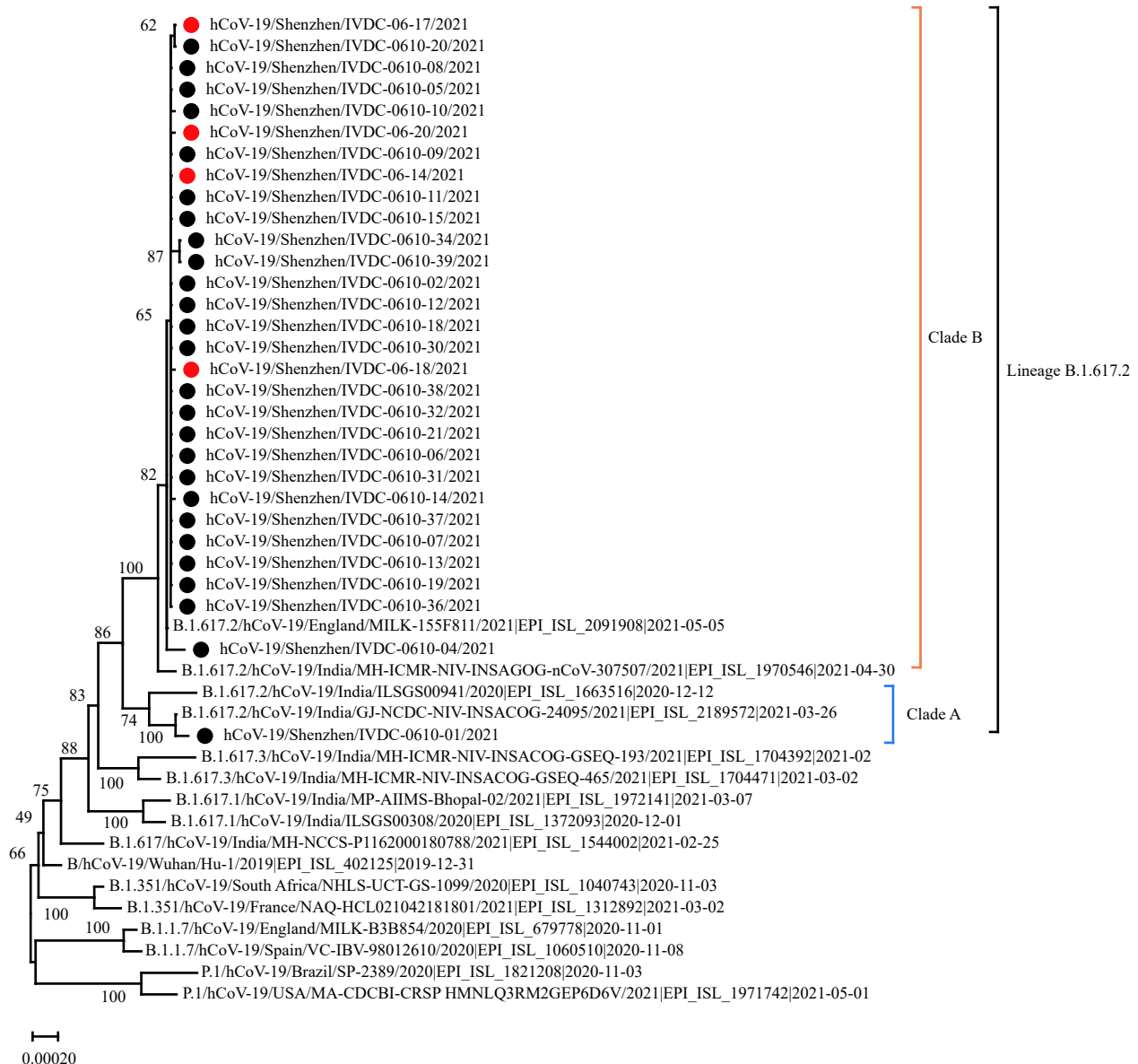


FIGURE 1. Evolutionary relationships of 30 SARS-CoV-2 strains of lineage B.1.617.2 from this study.

Note: Maximum likelihood phylogenetic tree was reconstructed for 30 SARS-CoV-2 strains from this study and 16 reference strains based on the whole-genome sequences. The best fit nucleotide substitution model used for phylogenetic reconstruction was TN93+G5+I. The scale bar represents a genetic distance of 0.0002 nucleotide substitutions per site. Bootstrap analysis (1,000 replicates) was used for statistical support of the tree. The 4 local strains were indicated by red dots, and 26 imported strains were indicated by black dots.

TABLE 1. Nucleotide differences between 30 SARS-CoV-2 strains of lineage B.1.617.2 from this study and the reference strain Wuhan-Hu-1 (NC_045512.2).

Clade	Case	Nucleotide variation	Nucleotide deletion mutation
Clade A	Case 1	G210T, C241T, C799T, C3037T, G4181T, C6402T, C7124T, C8986T, G9053T, C10029T, A11201G, A11332G, G13812T, C14408T, G15451A, C16466T, C19220T, C21618G, G21987A, T22917G, C22995A, A23403G, C23604G, G24410A, C25413T, C25469T, T26767C, T27638C, C27752T, C27874T, A28461G, G28881T, G28916T, G29402T, and G29742T	del22029-22034, del28248-28253, del28271
	Case A, Case 2, Case 5–9, Case 11–15, Case 18, Case 19, Case 21, Case 30–32, Case 36, Case 38	31 variation sites: G210T, C241T, G410T, C3037T, C5184T, A5584G, T9429C, C9891T, T11418C, C11514T, C13019T, C14408T, G15451A, C16466T, C21618G, G21987A, C22227T, T22917G, C22995A, A23403G, C23604G, G24410A, C25469T, T26767C, T27638C, A27677C, C27752T, A28461G, G28881T, G29402T, and G29742T	del510-518, del22029-22034, del28248-28253, del28271
Clade B	Case B, Case 20	31 variation sites + C21575T	del510-518, del22029-22034, del28248-28253, del28271
	Case C	31 variation sites + C18431T	del510-518, del22029-22034, del28249, del28253, del28271
	Case D	31 variation sites + C10605A	del510-518, del22029-22034, del28248-28253, del28271
	Case 4	31 mutations – G210T – G410T – G21987A0 – C22227T + G174T + C1059T + A5839G	del28249, del28253, del28271
	Case 10	31 mutations + C11665T	del510-518, del22029-22034, del28248-28253, del28271
	Case 34, Case 39	31 mutations + G3875A + G6476T	del510-518, del22029-22034, del28248-28253, del28271
	Case 37	31 mutations + A28249T + C28253A	del510-518, del22029-22034, del28271

19/England/MILK-155F811/2021. These 29 SARS-CoV-2 strains of lineage B.1.617.2 showed at least 23 nucleotide differences to the strain hCoV-19/Shenzhen/IVDC-0610-01/2021 (Case 1). In clade B, the imported strain hCoV-19/Shenzhen/IVDC-0610-04/2021 (Case 4) showed a certain degree of genetic distance to the other 28 virus strains from this study, which was also indicated by comparative analysis of genome sequences in Table 1. The local B.1.617.2 strain hCoV-19/Shenzhen/IVDC-06-14/2021 (Case A) showed 100% sequence identity to 19 imported B.1.617.2 strains (Table 1). These 20 SARS-CoV-2 strains of lineage B.1.617.2 shared 31 nucleotide variation sites and 22 deletion mutations compared with the reference strain Wuhan-Hu-1. Except for the variation sites above, an additional variation site was observed in genomes of the 3 local strains hCoV-19/Shenzhen/IVDC-06-17/2021 (Case B), hCoV-19/Shenzhen/IVDC-06-18/2021 (Case C), and hCoV-19/Shenzhen/IVDC-06-20/2021 (Case D). The local strain hCoV-19/Shenzhen/IVDC-06-17/2021 (Case B) showed 100% sequence identity to the strain hCoV-19/Shenzhen/IVDC-0610-20/2021 from imported Case 20.

Among imported SARS-CoV-2 strains, genome sequences of 19 SARS-CoV-2 strains from imported passengers showed 100% sequence identity to each

other, and these 19 passengers had no common exposure history before boarding. It suggested that at least one virus transmission occurred among the 19 passengers after boarding. The only strain from Case 1 belonged to clade A within lineage B.1.617.2, which indicated that Case 1 was not related to the infection in the cabin. Based on phylogenetic relationship and comparative analysis of genome sequences, we deduced that 3 virus strains from local cases (Cases B to D) and 5 virus strains from imported passengers (Cases 10, 20, 34, 37, and 39) were progeny viruses.

According to the report by Li et al (2), SARS-CoV-2 Delta variant strains from this study showed 18 to 28 nucleotide differences to those from the first outbreak of COVID-19 in Guangzhou, May 2021, which suggested that the SARS-CoV-2 Delta variant that caused local outbreaks of COVID-19 in Guangzhou and Shenzhen came from different sources. At present, the SARS-CoV-2 Delta variant is becoming the dominant variant worldwide and has been detected in at least 142 countries as of August 10, 2021. The SARS-CoV-2 Delta variant is posing new challenges on the control and prevention of COVID-19 due to its increased transmissibility compared with Alpha variant (lineage B.1.1.7) and capacity of immune escape (3–7).

In conclusion, whole-genome sequencing (WGS) confirmed that 4 local SARS-CoV-2 strains and 26 of

28 imported SARS-CoV-2 strains from this study were Delta variants, and phylogenetic and comparative genome analyses showed close relationship between the four local SARS-CoV-2 strains of Delta variant and imported SARS-CoV-2 strains of Delta variant introduced from South Africa.

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Corresponding authors: Long Chen, chen_l_2011@163.com; Tiejian Feng, fengtiej@126.com; Xuan Zou, 914494557@qq.com.

¹ Institute of Pathogen Biology, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, China; ² Shenzhen Center for Infectious Disease Control and Prevention, Chinese Academy of Medical Sciences, Shenzhen, Guangdong, China; ³ Department of Communicable Diseases Control and Prevention, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, China; ⁴ Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, China; ⁵ Laboratory of Molecular Epidemiology, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, China.

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SUPPLEMENTARY TABLE S1. Demographic characteristics of 4 local cases and 39 imported cases, and specimen testing information.

Case	Gender	Age (years)	Date of first positive detection of COVID-19 virus	Ct value (ORF1ab/N) of specimen used for sequencing	
				Daan	Bojie
Case A	Male	30	June 14, 2021	14/18	16/18
Case B	Female	21	June 17, 2021	23/21	22/22
Case C	Male	35	June 18, 2021	24/22	22/24
Case D	Female	64	June 20, 2021	30/30	35/28
Case 1	Male	36	June 11, 2021	31/31	30/31
Case 2	Male	35	June 11, 2021	31/31	26/28
Case 3	Female	42	June 11, 2021	33/34	34/34
Case 4	Female	58	June 11, 2021	30/27	27/28
Case 5	Male	53	June 13, 2021	17/16	16/17
Case 6	Male	32	June 14, 2021	24/23	24/23
Case 7	Male	52	June 14, 2021	17/17	16/17
Case 8	Male	33	June 15, 2021	17/15	15/17
Case 9	Male	47	June 15, 2021	16/16	15/16
Case 10	Male	64	June 15, 2021	21/20	20/21
Case 11	Male	40	June 15, 2021	15/14	14/15
Case 12	Male	50	June 15, 2021	18/17	16/18
Case 13	Male	33	June 15, 2021	28/26	27/28
Case 14	Female	31	June 15, 2021	19/17	18/17
Case 15	Male	61	June 15, 2021	14/14	15/15
Case 16	Female	38	June 15, 2021	35/34	36/33
Case 17	Male	39	June 15, 2021	37/40	Undet/35
Case 18	Female	50	June 15,2021	14/15	14/14
Case 19	Male	35	June 15, 2021	17/16	16/17
Case 20	Male	58	June 15, 2021	16/15	15/16
Case 21	Female	47	June 15, 2021	29/25	26/26
Case 22	Male	29	June 15, 2021	35/35	38/Undet
Case 23	Male	32	June 15, 2021	38/36	Undet/35
Case 24	Male	63	June 15, 2021	35/34	35/32
Case 25	Female	63	June 15, 2021	35/33	33/33
Case 26	Male	45	June 15, 2021	38/35	Undet/Undet
Case 27	Male	44	June 15, 2021	Undet/38	Undet/35
Case 28	Male	61	June 15, 2021	Undet/37	37/Undet
Case 29	Male	58	June 15, 2021	37/Undet	Undet/36
Case 30	Male	9	June 15, 2021	21/21	22/22
Case 31	Female	45	June 15, 2021	28/28	27/28
Case 32	Male	42	June 15, 2021	15/15	14/15
Case 33	Male	35	June 16, 2021	26/24	23/26
Case 34	Male	1	June 16, 2021	30/27	27/29
Case 35	Female	24	June 17, 2021	34/30	32/30
Case 36	Male	25	June 17, 2021	19/18	19/18
Case 37	Female	32	June 19, 2021	22/19	19/20
Case 38	Male	38	June 19, 2021	31/29	28/30
Case 39	Female	28	June 25, 2021	18/16	17/18

Note: There were 43 subjects in total, and the male/female ratio and mean age of subjects was 2.58 : 1 and 40.9 years, respectively. Abbreviations: Undet=Undetected; Ct=Cycle threshold; COVID-19=Coronavirus disease 2019.

SUPPLEMENTARY TABLE S2. Genome sequence information for 30 SARS-CoV-2 strains of lineage B.1.617.2 from this study.

Case	Virus name	Length in nt (% GC content)	Closest strain by an audacity instant search in GISAID
Case A	hCoV-19/Shenzhen/IVDC-06-14/2021	29,813nt (37.97)	hCoV-19/England/MILK-155F811/2021
Case B	hCoV-19/Shenzhen/IVDC-06-17/2021	29,562nt (37.98)	hCoV-19/England/MILK-155F811/2021
Case C	hCoV-19/Shenzhen/IVDC-06-18/2021	29,428nt (37.98)	hCoV-19/England/MILK-155F811/2021
Case D	hCoV-19/Shenzhen/IVDC-06-20/2021	29,612nt (37.98)	hCoV-19/England/MILK-155F811/2021
Case 1	hCoV-19/Shenzhen/IVDC-0610-01/2021	29,702nt (37.96)	hCoV-19/India/GJ-NCDC-NIV-INSACOG-24095/2021
Case 2	hCoV-19/Shenzhen/IVDC-0610-02/2021	29,813nt (37.97)	hCoV-19/England/MILK-155F811/2021
Case 4	hCoV-19/Shenzhen/IVDC-0610-04/2021	29,836nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 5	hCoV-19/Shenzhen/IVDC-0610-05/2021	29,555nt (37.99)	hCoV-19/England/MILK-155F811/2021
Case 6	hCoV-19/Shenzhen/IVDC-0610-06/2021	29,657nt (38.00)	hCoV-19/England/MILK-155F811/2021
Case 7	hCoV-19/Shenzhen/IVDC-0610-07/2021	29,814nt (37.97)	hCoV-19/England/MILK-155F811/2021
Case 8	hCoV-19/Shenzhen/IVDC-0610-08/2021	29,824nt (37.97)	hCoV-19/England/MILK-155F811/2021
Case 9	hCoV-19/Shenzhen/IVDC-0610-09/2021	29,831nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 10	hCoV-19/Shenzhen/IVDC-0610-10/2021	29,833nt (37.95)	hCoV-19/England/MILK-155F811/2021
Case 11	hCoV-19/Shenzhen/IVDC-0610-11/2021	29,830nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 12	hCoV-19/Shenzhen/IVDC-0610-12/2021	29,830nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 13	hCoV-19/Shenzhen/IVDC-0610-13/2021	29,710nt (37.99)	hCoV-19/England/MILK-155F811/2021
Case 14	hCoV-19/Shenzhen/IVDC-0610-14/2021	29,551nt (37.98)	hCoV-19/England/MILK-155F811/2021
Case 15	hCoV-19/Shenzhen/IVDC-0610-15/2021	29,834nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 18	hCoV-19/Shenzhen/IVDC-0610-18/2021	29,820nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 19	hCoV-19/Shenzhen/IVDC-0610-19/2021	29,828nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 20	hCoV-19/Shenzhen/IVDC-0610-20/2021	29,820nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 21	hCoV-19/Shenzhen/IVDC-0610-21/2021	29,574nt (37.99)	hCoV-19/England/MILK-155F811/2021
Case 30	hCoV-19/Shenzhen/IVDC-0610-30/2021	29,827nt (37.97)	hCoV-19/England/MILK-155F811/2021
Case 31	hCoV-19/Shenzhen/IVDC-0610-31/2021	29,829nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 32	hCoV-19/Shenzhen/IVDC-0610-32/2021	29,849nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 34	hCoV-19/Shenzhen/IVDC-0610-34/2021	29,809nt (37.97)	hCoV-19/England/MILK-155F811/2021
Case 36	hCoV-19/Shenzhen/IVDC-0610-36/2021	29,819nt (37.96)	hCoV-19/England/MILK-155F811/2021
Case 37	hCoV-19/Shenzhen/IVDC-0610-37/2021	29,827nt (37.97)	hCoV-19/England/MILK-155F811/2021
Case 38	hCoV-19/Shenzhen/IVDC-0610-38/2021	29,553nt (37.99)	hCoV-19/England/MILK-155F811/2021
Case 39	hCoV-19/Shenzhen/IVDC-0610-39/2021	29,797nt (37.94)	hCoV-19/England/MILK-155F811/2021

Abbreviation: nt=Nucleotide.