Received: 29 June 2021 | Revised: 15 July 2021 | Accepted: 24 July 2021

DOI: 10.1002/jmv.27239

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

Sequencing on an imported case in China of COVID-19 Delta variant emerging from India in a cargo ship in Zhoushan, China

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Funding information
Zhoushan Science and Technology Project, Grant/Award Numbers: 2020C31006, 2020C31004, 2020C31005; Young Talents Project of Zhoushan Medical and Health Science and Technology Plan, Grant/Award Number: 2020RC02

Abstract

A cluster of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections was found in a cargo ship under repair in Zhoushan, China. Twelve of 20 crew members were identified as SARS-CoV-2 positive. We analyzed four sequences and identified them all in the Delta branch emerging from India with 7–8 amino acid mutation sites in the spike protein.

KEYWORDS
coronavirus, disease control, genetics, mutation, virus classification

The current coronavirus disease 2019 (COVID-19) pandemic is still threatening global health. New variants with higher transmission rates have been spreading around the world, such as the variants of concerns (VOCs) Alpha from the UK, Beta from South Africa, and Gamma from Brazil.[1,2] The "double mutated" variant Delta in linage B.1.617 with the spike mutations of L452R and E484Q, is becoming the most prevalent virus among all COVID-19 variants in India, and has been found in more than 21 countries throughout the world.[3]

On April 23, 2021, three crew members on an under repair cargo ship at a shipyard in Zhoushan, Zhejiang Province, China, developed fever symptoms and were reported to the Customs. All the 20 crew members' throat swabs were collected for COVID-19 detection. On April 25, the Zhoushan Center for Disease Prevention and Control (Zhoushan CDC) received the samples and confirmed 11 of them were COVID-19 nucleic acid positive. The 11 confirmed patients were transferred to the designated hospital for further treatment. After consultation by the expert groups, 10 patients were diagnosed as confirmed cases of COVID-19, including nine cases of normal type and one case of mild type. In addition, one patient without symptoms was determined as asymptomatic infection. According to the expert consultant's opinion, relevant treatment plans were formulated for isolation treatment. On April 28, through clinical symptoms and expert consultation, two patients were judged to be "severe," and another two had a tendency to be severe. All close contacts were isolated and nucleic acid will be tested regularly, while prevention and control measures were determined based on the test results. After hospitalization, one turned to be "severe" (three in total), one remained asymptomatic, and the rest were of the normal type. On April 29, on the second round of detection, another case was detected positive without symptoms.

The epidemiological investigation indicated that all the 20 crew members boarded the ship at Nansha Port in Guangzhou, China, on November 6, 2020. All of them were tested negative for COVID-19 nucleic acid and had not been vaccinated for COVID-19. The ship is mainly engaged in the marble shipping business from India to Fujian, China. It has made 16 port calls in the last 6 months, of which the last one was from April 19 to April 21 at Xiamen, Fujian, to unload. From December 24, 2020, to March 22, 2021, there were two Bangladeshi crew members working on the ship, who provided the negative certificates of nucleic acid test for COVID-19 when they boarded the ship, and no suspected symptoms such as fever, cough, or diarrhea were found during the working period on the ship. During the

Bing Wu and Hui Zhang contributed equally to this study.

TABLE 1 Nucleotide substitutions of 4 SARS-CoV-2 genome sequences compared with the reference strain of SARS-CoV-2 (GenBank No. NC_045512)

Nucleotide position	Reference	Zhoushan01	Zhoushan02	Zhoushan03	Zhoushan04	Amino acid	Gene region
210	G	T	T	T	T	Noncoding	UTR
241	C	T	T	T	T	Noncoding	UTR
1191	C	T	T	T	T	P309L	ORF1ab
1267	C	T	T	T	T	syn	ORF1ab
2144	G	T	T	T	T	V627F	ORF1ab
3037	C	T	T	T	T	syn	ORF1ab
5184	C	T	T	T	T	P1640L	ORF1ab
6539	C	C	T	C	T	H2092Y	ORF1ab
9891	C	T	T	T	T	A3209V	ORF1ab
11418	T	C	C	C	C	V3718A	ORF1ab
12946	T	C	C	C	C	syn	ORF1ab
14408	C	T	T	T	T	P4715L	ORF1ab
15451	G	A	A	A	A	G5063S	ORF1ab
16466	C	T	T	T	T	P5401L	ORF1ab
20262	A	G	G	G	G	syn	ORF1ab
20320	C	T	T	T	T	H6686Y	ORF1ab
21618	C	G	G	G	G	T19R	S
21987	G	A	A	A	A	G142N	S
22028	GAGTTCA	G	G	G	G	F157del, R158del	S
22917	T	G	G	G	G	L452R	S
22995	C	A	A	A	A	T478K	S
23403	A	G	G	G	G	D614G	S
23604	C	G	G	G	G	P681R	S
24410	G	G	G	G	A	D950N	S
24745	C	T	T	T	T	syn	S
25469	C	T	T	T	T	S26L	ORF3a
26767	T	C	C	C	C	I82T	M
27638	T	C	C	C	C	V82A	ORF7a
27739	C	T	T	T	T	L116F	ORF7a
27752	C	T	T	T	T	T120I	ORF7a
28247	AGATTTC	A	A	A	A	D119del, F120del	ORF8
28249	A	T	A	A	T	D119V	ORF8
28253	C	A	A	A	A	F120L	ORF8
28461	A	A	A	A	G	D63G	N
28881	G	T	T	T	T	R203M	N
29402	G	T	T	T	T	D377Y	N
29427	G	A	A	A	A	R385K	N
29742	G	T	T	T	T	Noncoding	UTR

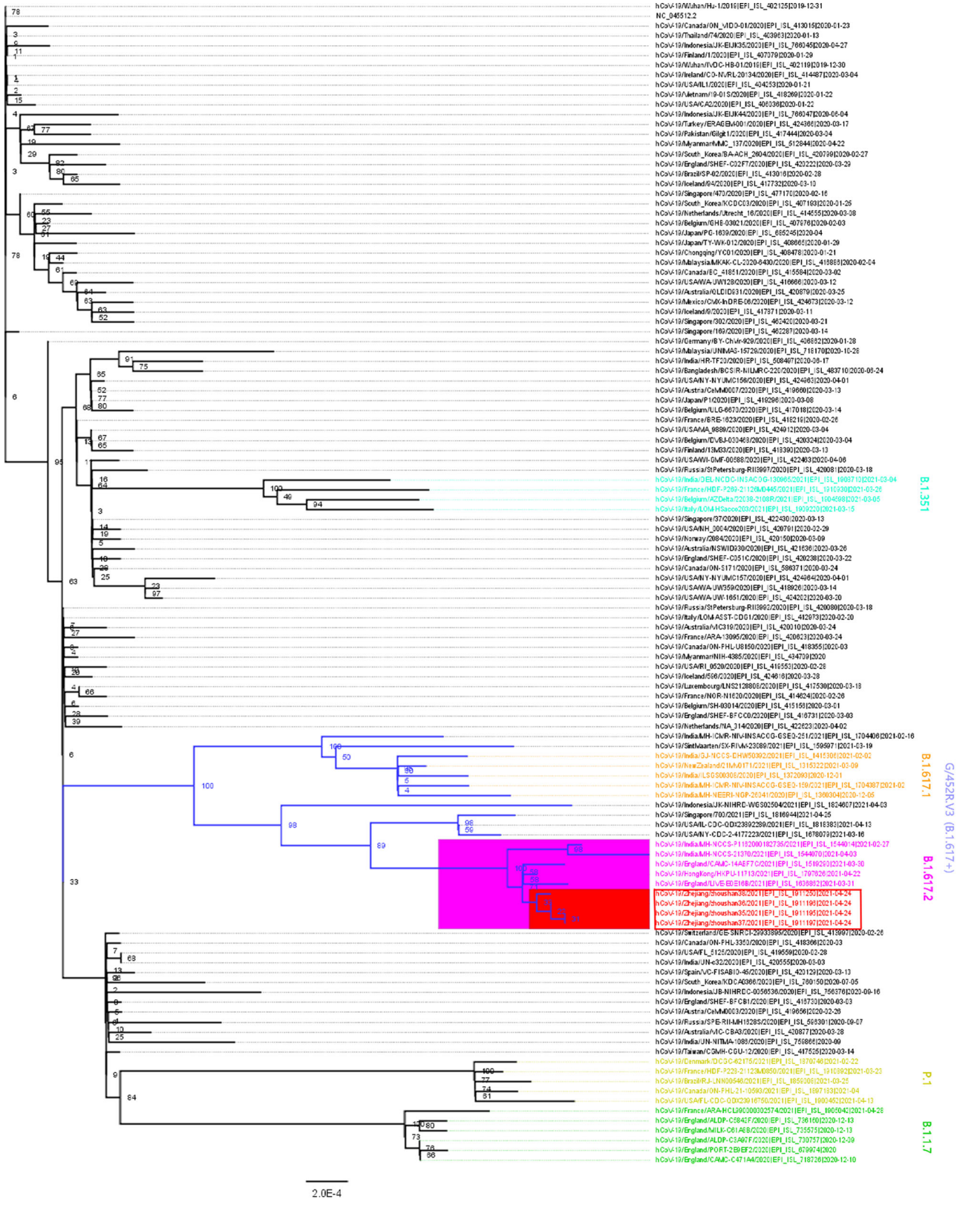

FIGURE 1 Phylogenetic tree of 114 published full-length genomes from GISAID and 4 Zhoushan genomes (Red box). Main severe clades are colored. GISAID, Global Initiative on Sharing All Influenza Data

berthing of the ship at several foreign docks, there were close contacts between the crew and local foreign workers, and the local workers did not regulate personal protection when they boarded the ship. From April 2 to April 7, when berthing the Indian port of Kakinada, close interaction between local workers and crew members also happened.

We chose four qualified samples (Zhoushan01–04) to be sequenced for whole-genome using the Illumina iSeq. 100™ system in the laboratory of Zhoushan CDC. These sequencing data have been deposited to the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) with accessions EPI_ISL_1911195, EPI_ISL_191196, EPI_ISL_1911197, and EPI_ISL_1911250. Compared with the Wuhan reference sequence (NC_045512.2), the homology of the four novel coronavirus genome sequences was 99.3%, and the average depth was 233X for all four severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes with 35 same nucleotide variation sites (Table 1). The common nucleotide mutation site (C6539T) was found in samples Zhoushan02 and Zhoushan04, while one additional nucleotide mutation site (G24410A) was in the sample Zhoushan04. The four samples' virus genomes have a strong genetic association and high homology with EPI_ISL_1544014, which belonged to Pangolin lineage B.1.617.2 (Delta), a new COVID-19 variant first detected in India. Furthermore, seven amino acid mutation sites (spike T19R, spike G142D, spike del 157/158, spike L452R, spike T478K, spike D614G, and spike P681R) were detected in the spike protein. There was an additional D950N amino acid mutation in sequence Zhoushan04.

For the phylogenetic analysis, 114 full-genome SARS-CoV-2 sequences were retrieved from GISAID, along with the Wuhan reference sequence NC_045512.2 from GenBank. The sequences were aligned in MAFFT v.7.271[4] with default parameters. The maximum likelihood phylogenetic tree was implemented in IQTREE v.2.1.2,[5] and the result was visualized in Figtree v1.4.4 (Figure 1).

The result indicated that Zhoushan sequences were in the same transmission chain belonging to the B.1.617.2 branch. Notably, in all four Zhoushan sequences, we found no virus with the E484Q Spike gene mutation, which was excluded from the "double mutant" Delta branch. According to the reported research results, the amino acid mutation at position 484 of S protein is one of the major mutations affecting the world. Gamma, Beta, and Eta variants in Brazil,[6] South Africa,[7] and the United States,[8] respectively, all share E484K mutation at this site, which can reduce the effectiveness of antibodies in the human body.[9,10] Mutations in E484Q may enhance the immune escape ability of the virus.[11]

In conclusion, all the four Zhoushan sequences were genetically related to the sequence that originated from India, belonging to branch Delta, and were in the same transmission chain. The cargo ship in Zhoushan had the first imported B.1.617.2 variant in China and this poses a high potential threat to the prevention and control of COVID-19 in China.

ACKNOWLEDGMENTS

We thank all the persons involved in the response to this outbreak. This study was supported by the Zhoushan Science and Technology Project (Grant nos. 2020C31006 and 2020C31004, and 2020C31005) and Young Talents Project of Zhoushan Medical and Health Science and Technology Plan (2020RC02).

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Bing Wu and Hui Zhang contributed equally to this study. Bing Wu, Hui Zhang, and Jia-bei Chen carried out experiments. Bing Wu and Hui Zhang contributed to data collection. Bing Wu analyzed sequencing data. Yu-chao Wang, An Tang, Ke-feng Li, and Peng Li carried out epidemiological investigations. Bing Wu and Hui Zhang wrote the manuscript. Hong-ling Wang and Jian-bo Yan reviewed and edited the manuscript. All authors reviewed the manuscript and edited it for intellectual content and gave final approval for this version to be published.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Global Initiative on Sharing All Influenza Data (GISAID) at https://www.gisaid.org/, reference number EPI_ISL_1911195, EPI_ISL_191196, EPI_ISL_1911197, EPI_ISL_1911250.

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How to cite this article: Wu B, Zhang H, Wang Y-c, et al. Sequencing on an imported case in China of COVID-19 Delta variant emerging from India in a cargo ship in Zhoushan, China. *J Med Virol*. 2021;93:6828-6832. https://doi.org/10.1002/jmv.27239