Outbreak Reports

Exploring the Bridge Cases' Role in the Transmission of the SARS-CoV-2 Delta Variant — Ruili City, Yunnan Province, China, July–September 2021

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Summary

What is already known about this topic?

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta variant has proved to have increased transmissibility, and mutations that can cause partial immune escape, which makes its transmission more insidious.

What is added by this report?

This study showed that probable cases who had negative results in nucleic acid testing but had positive IgM test result and/or IgG test value of over 20 S/CO in antibodies testing, might serve as bridges in the Delta variant's transmission chain.

What are the implications for public health practice?

In border inspection and quarantine, tests for SARS-CoV-2 IgM and IgG antibodies should be strengthened alongside nucleic acid tests to prevent probable cases with transmission potential from crossing the land border into China. In contact tracing investigations, the bridging role of probable cases should be considered to reconstruct the transmission chain.

On July 4, 2021, 3 confirmed coronavirus disease 2019 (COVID-19) patients infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) B.1.617.2 (Delta) variant were found through regular nucleic acid screening in Jiegao Community, Ruili City, Yunnan Province (*1*). During the epidemiological investigation conducted by local CDCs and the Yunnan Provincial CDC from July to September, 2021, many of the confirmed cases were found to have no clear history of contact to other cases, which made difficult the tracing of the source of cases and effective control of the recent outbreak. In a retrospective analysis of the epidemiological investigation and laboratory test data, we reported a phenomenon for the first time that the probable index

case, who had a negative nucleic acid test but had a positive IgM test result and/or IgG test value of over 20 S/CO in antibodies testing, might have acted as a bridging case for SARS-CoV-2 transmission. Based on this finding, probable cases were considered as clues of case tracing in the following epidemiological investigation, and serological antibody monitoring was strengthened to include the probable cases. Our evidence indicated that strengthening the management of probable cases is essential to effectively control transmission, especially in border areas that may have increased contact with COVID-19 prevalent regions.

INVESTIGATION AND RESULTS

The confirmed COVID-19 cases were diagnosed and classified according to severity (mild, moderate, severe, and critical) based on guidelines issued by the National Health Commission (*2*), which the local CDC confirmed. The biological samples of these cases were also sent to the China CDC for further virus genotyping. A detailed epidemiological investigation was conducted for these patients, including collecting their sociodemographic information, residential address, and history of travel, work, contacts, and activities. These patients were immediately transported to a designated quarantine site. Close contacts of these COVID-19 patients were found through epidemiological investigation and travel history big data and were quarantined for at least 14 days. Regular SARS-CoV-2 nucleic acid testing and antibody testing (IgM and IgG) was conducted on the COVID-19 patients and their close contacts. Their COVID-19 vaccination records were obtained from the vaccination database using ID numbers and names as unique identifiers. Compared with the confirmed COVID-19 cases, whose nucleic acid testing results were positive, we defined the probable cases as individuals who had negative results in nucleic acid testing but had positive

IgM test results and/or IgG test values of over 20 S/CO in antibody testing. We set a value limit of 20 S/CO for the IgG test because the positive IgG value was common among people who have been vaccinated against COVID-19, and the value of 20 S/CO was a conservative limit that was about two times higher than the 98% quantile value (10.810 S/CO) of the 117 confirmed COVID-19 cases' IgG test results in the one-month follow-up in Ruili City. Therefore, the probable cases had a high probability of infection but had not been detected by nucleic acid testing.

For laboratory testing, Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) targeting of ORF1ab and N genes was conducted on the viral RNA extracted from the nasopharyngeal swabs using the Novel Coronavirus 2019 Nucleic Acid Test Kit (manufactured by Bojie Medical Technology, Shanghai Municipality and Daan Gene Company, Guangzhou City, China). The detection limit of cycle threshold (Ct) of the above tests was 40, a Ct value of less than 40 was considered as a positive nucleic acid test result. The nucleic acid test reading positive or not was the key to distinguishing between the confirmed cases and the probable cases. Serum samples collected from confirmed COVID-19 patients and their close contacts were tested for antibodies IgM and IgG using Anti-SARS-CoV-2 Rapid Test Kit (manufactured by Antubio Diagnostics Company, Zhengzhou, China). A value of over 1 S/CO was considered a positive result for IgM and IgG tests.

Retrospective analysis was conducted based on the epidemiological investigation data from July to September 2021. Contact networks of confirmed COVID-19 patients and their close contacts were formed. The nodes in the contact networks represent the confirmed COVID-19 patients and their close contacts. Edges of the networks represent the contacts among these persons, including work and household contacts, environmental contacts, and so on. Statistical analysis was performed with IBM SPSS (version 21.0, IBM Corp., Armonk, NY, US). Network visualization was done with Cytoscape (version 3.5.1, NIH Biomedical Technology Research Center, Bethesda, MD, US).

In this study, we showed a representative example that 2 confirmed COVID-19 cases (C1 and C2) and their contact networks were linked by two probable cases (P1 and P2) that acted as bridges, which constituted a potential chain of Delta variant transmission (Figure 1). C1 was the first index case of the "July 4" COVID-19 outbreak in Ruili City, Yunnan Province and occurred in a 51-year-old Chinese man who lived in Jiegao Community. C2 occurred in a 25-year-old Chinese male diagnosed on August 2, 2021. The two confirmed cases were both infected with SARS-CoV-2 Delta variant, and the amino acid mutation sites in the S protein of the two patients' viruses were the same, including T19R, G142D, R158G, L452R, T478K, D614G, P681R, D950N, E156del, and F157del. The two patients all declared that they had no contact with other COVID-19 patients or anyone with suspected symptoms before they were diagnosed (Table 1). A total of 238 close contacts were found through epidemiological investigation, of whom 85 were close contacts of C1, and 153 were close contacts of C2. The mean age of the contacts was 31.2±13.5 years, and most of them were aged 18–45 years (73.9%). C1 had more contacts with people in Myanmar than C2 (50.6% vs. 1.3%); 75.2% of the close contacts were vaccinated against COVID-19 (Table 2).

P1 (a 34-year-old man from Myanmar) and P2 (a 25-year-old Chinese man) were two close contacts of the two patients. P1 was a close contact of C1, and P2 was a close contact of P1 and C2. All four patients had been vaccinated against COVID-19 before (Table 1, Figure 1). The detailed transmission chain was described in the following three stages (Figure 1).

Stage 1: P1's contact with C1

P1 had sustained environmental exposure to C1 due to sharing stairs until July 4, 2021. He had a high-level IgG value of 21.139 S/CO tested on July 5, 2021. The quarantine began on July 4, 2020, and he was repatriated to Myanmar on August 2, 2021. C1 also had extremely high antibody values tested on the same day (July 5, 2021), of which IgM value was 11.773 S/CO, and IgG value was 64.768 S/CO (Table 1). The potential transmission path might exist between the two cases (Figure 1).

Stage 2: P2's contact with P1

P2 had contact with P1 through shopping on June 27, 2021, and the quarantine period lasted from July 6 to 20, 2021. He had a positive IgM test result, and a relatively high IgG value of 11.552 S/CO tested on July 7, 2021. Considering that the infection of P1 occurred earlier (IgM negative and IgG positive) at the same period, there was a high probability that the virus transmitted from P1 to P2. (Table 1, Figure 1)
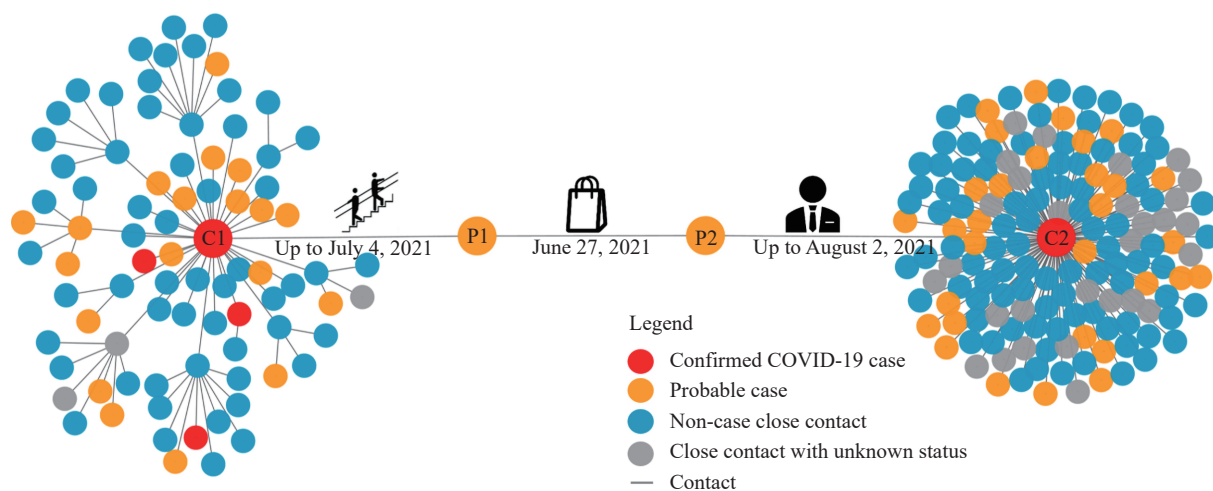
FIGURE 1. Contact network of two confirmed COVID-19 cases (C1 and C2) and two corresponding probable cases (P1 and P2) in Ruili City, Yunnan Province, China, July–September 2021.

Stage 3: C2's contact with P2

After P2 left the first quarantine on July 20, 2021, he was quarantined a second time on August 3, 2021 because he had work and daily life contact with C2. The last contact between P2 and C2 was on August 2, 2021. C2 was diagnosed as a confirmed COVID-19 case on August 2, 2021, and the first antibody test results were negative for both IgM and IgG tested on August 3, 2021, but then both were revealed to be positive in subsequent tests. The three antibodies test results of P2 during this quarantine were all double-positive for IgM and IgG until August 17, 2021. It was possible that P2's latent infection status continued from July to August after the contact with P1, in which the virus was transmitted to C2. (Table 1, Figure 1)

In the ongoing outbreak of COVID-19 in Ruili, antibody tests were carried out among almost all of the close contacts to identify potential probable cases. The epidemiological investigation and case tracing was carried out using the clue of probable cases. In addition, serological antibody monitoring and follow-up were strengthened for the probable cases.

DISCUSSION

In this study, we constructed a hidden chain of transmission between two confirmed COVID-19 patients connected by two probable cases acting as bridges. This finding could help solve the difficulties faced in the front-line epidemiological investigation in tracing the source of cases. Both confirmed patients had no contact history with other COVID-19 patients and anyone with suspected symptoms before, but the virus they were infected with had the same amino acid mutation sites. Without considering the probable cases' effect, it was possible that the two cases were separate, and the transmission links were hard to be established.

Of the two probable cases, P2 was the most critical bridge case in the transmission chain. His double-positive antibodies status lasted for over one month and covered his first 14-day quarantine after he had contact with P1. A previous study had indicated that the SARS-CoV-2 Delta variant contained mutations that could cause partial immune escape (3). A special confirmed COVID-19 case with virus variation has been reported to have continuous negative results for routine PT-PCR detection in Guangzhou and was finally diagnosed using Nanopore Sequencing combined with antibody testing (4). P2 might have a similar situation with the special case. A study from Todsen et al. also indicated that the oropharyngeal swabs showed relatively lower sensitivity for lower respiratory tract infections of SARS-CoV-2 (5). It was possible that the virus existed in the lower respiratory tract infections of P2. In addition, the intermittent virus shedding between two regular nucleic acid tests, which were conducted every three days, made it possible for P2 to transmit the virus to other persons but could not be detected by regular nucleic acid tests. Another potential reason is that the SARS-CoV-2 variant might have a longer latent period than previously thought. Recent evidence provided by Guangzhou's outbreak showed a latent period of 4.0 days and an incubation period of 5.8 days (6). However, the report of the Hebei's outbreak showed an incubation period of more than 22.0 days (7).

TABLE 1. Characteristics of the two confirmed COVID-19 cases and two probable cases in Ruili City, Yunnan Province, China, July–September 2021.

Case*	Sex	Age (years)	Community	Nationality	Date of symptom onset	Date of positive nucleic acid test	Severity	Date of COVID-19 vaccination		Antibody test results (S/CO)			
								First Dose	Second Dose	First time		Last time	
										Date	Results	Date	Results
C1	Male	51	Jiegao	China	Jul 2, 2021	Jul 3, 2021	Moderate	May 5, 2021†	-	Jul 5, 2021	IgM: 11.773(+); IgG: 64.768(+)	Aug 5, 2021	IgM: 7.684(+); IgG: 7.154(+)
C2	Male	25	Jiegao	China	Aug 2, 2021	Aug 2, 2021	Mild	Mar 18, 2021	Apr 1, 2021	Aug 3, 2021	IgM: 0.032(−); IgG: 0.343(−)	Aug 23, 2021	IgM: 24.428(+); IgG: 8.227(+)
P1	Male	34	Jiegao	Myanmar	-	-	-	Apr 25, 2021	May 26, 2021	Jul 5, 2021	IgM: 0.373(−); IgG: 21.139(+)	-	-
P2	Male	25	Jiegao	China	-	-	-	May 6, 2021†	-	Jul 7, 2021	IgM: 1.695(+); IgG: 11.139(+)	Aug 17, 2021	IgM: 2.426(+); IgG: 5.001(+)

* C1=confirmed COVID-19 case 1; C2=confirmed COVID-19 case 2; P1=probable case 1, P2=probable case 2.
† The COVID-19 vaccine they received has only one dose.

Therefore, more comprehensive epidemiological and laboratory data collection is needed to verify this phenomenon. In addition, it has been proved that the Delta variant had increased transmissibility (8). Though the virus was not detected in P2's respiratory tract samples, it did not rule out that he transmitted the virus to other people or the environment in some other routes. Considering that he was vaccinated against COVID-19, the total shedding period of the virus might be longer than the evidence provided by Chen et al. (9).

Although we only showed a representative example, the phenomenon that probable cases acted as bridges in the transmission chain is not rare. This study also finds other potential transmission between two probable cases regardless of P1 and P2's connection in C1's contact network (Figure 1). A previous study in Liaoning Province also showed a phenomenon in an outbreak that made it difficult to determine the first index case, persons who were double-positive for IgM and IgG but negative for nucleic acid testing were also found, which supported our finding (10).

Our finding has strong practical implications. Great importance needs to be attached to the role of latent infections and probable cases in the transmission chain. First, considering that Ruili City is an important border port city bordering Myanmar in western Yunnan Province, refugees caused by the war in north Myanmar and border trade increased the difficulty of COVID-19 control and management in China's border. In this study, P1, who was a man from Myanmar, became an important link in the chain of transmission. Therefore, in border inspection and quarantine, tests for SARS-CoV-2 antibodies (IgM and IgG) should be carried out alongside nucleic acid tests to prevent probable cases with transmission potential from crossing the land border into China. Second, in case tracing investigations, not only the connections among confirmed COVID-19 patients and asymptomatic cases, but also the bridging role of probable cases should be considered to reconstruct the transmission chain. In addition, in the process of close contacts management, the probable cases need to be treated the same as confirmed cases to reduce the risk of transmission.

In conclusion, this is a pioneering study to reveal the bridging role of latent infection in the spread of the SARS-CoV-2 Delta variant. However, there were some limitations in the process of building the transmission chain. First, because the situation of the border port in Ruili City was so complex, there might be some

TABLE 2. Characteristics of close contacts of the two confirmed COVID-19 cases in Ruili City, Yunnan Province, China, July–September 2021.

Characteristics	Total (n=238), n (%)	C1* (n=85), n (%)	C2† (n=153), n (%)
Age (years)			
Mean±SD	31.2±13.5	30.7±17.7	31.4±10.4
<18	22/234 (9.4)	19/84 (22.6)	3/150 (2.0)
≥18 to <45	173/234 (73.9)	49/84 (58.3)	124/150 (82.7)
≥45 to <65	37/234 (15.8)	14/84 (16.7)	23/150 (15.3)
≥65	2/234 (0.9)	2/84 (2.4)	0/150 (0.0)
Sex			
Male	164/236 (69.5)	35/84 (41.7)	129/152 (84.9)
Female	72/236 (30.5)	49/84 (58.3)	23/152 (15.1)
Nationality			
China	193 (81.1)	42 (49.4)	151 (98.7)
Myanmar	45 (18.9)	43 (50.6)	2 (1.3)
COVID-19 vaccination			
Yes	179 (75.2)	60 (70.6)	119 (77.8)
No	59 (24.8)	25 (29.4)	34 (22.2)
Infection status			
Confirmed COVID-19 case	3 (1.3)	3 (3.5)	0 (0)
Probable case	53 (22.3)	21 (24.7)	32 (20.9)
Non-case close contact	150 (63.0)	58 (68.2)	92 (60.1)
Unknown status	32 (13.4)	3 (3.5)	29 (19.0)

* C1=confirmed COVID-19 case 1.
† C2=confirmed COVID-19 case 2.

incompleteness in the process of close contact tracing and epidemiological investigations. The bridging role of the probable cases needed to be verified by strengthening investigations of the detailed co-exposure history of close contacts in further case tracing because the possible co-exposure with confirmed cases during one's infectious period might not imply a definite transmission chain linkage. Second, though we set a very conservative limit for the antibody test results to prevent the inclusion of unreasonable probable cases, the standard of selecting probable cases from confirmed cases' close contacts needed to be further explored in deliberately designed representative research. In future studies, more abundant and detailed epidemiological investigations should be carried out to give a more precise definition of probable case with latent infection and verify their possible bridging role. It is also needed to strengthen basic virology research to explain the biological mechanism of latent infections' transmission capacity.

Conflicts of interest: No conflicts of interest.

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