ISSN 2096-7071
CN 10-1629/R1

CHINA CDC WEEKLY

Vol. 3 No. 21 May 21, 2021

中国疾病预防控制中心周报

COVID-19 ISSUE (13)

ISSN 2096-7071

China CDC Weekly

Cover Photo: Shanghai Customs entry checkpoint inspecting for COVID-19.

Outbreak Reports

COVID-19 Outbreak Caused by Contaminated Packaging of Imported Cold-Chain Products — Liaoning Province, China, July 2020

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Summary

What is known about this topic?

Few major outbreaks of coronavirus disease 2019 (COVID-19) have occurred in China after major non-pharmaceutical interventions and vaccines have been deployed and implemented. However, sporadic outbreaks that had high possibility to be linked to cold chain products were reported in several cities of China..

What is added by this report?

In July 2020, a COVID-19 outbreak occurred in Dalian, China. The investigations of this outbreak strongly suggested that the infection source was from COVID-19 virus-contaminated packaging of frozen seafood during inbound unloading personnel contact.

What are the implications for public health practice?

Virus contaminated paper surfaces could maintain infectivity for at least 17–24 days at -25 ℃. Exposure to COVID-19 virus-contaminated surfaces is a potential route for introducing the virus to a susceptible population. Countries with no domestic transmission of COVID-19 should consider introducing prevention strategies for both inbound travellers and imported goods. Several measures to prevent the introduction of the virus via cold-chain goods can be implemented.

On July 22, 2020, Dalian City, a major city in Liaoning Province, reported one confirmed coronavirus disease 2019 (COVID-19) case in a cold-chain products processing and storage company (Company K) who seek healthcare in hospital because of having a fever. Active case finding and management was conducted according to prevention and control protocols. On July 23, another 12 cases or asymptomatic infections with nucleic acid test positive results from Company K and 2 community cases who had no apparent relation to Company K were reported. On July 25, the affected streets in Ganjingzi District (approximately 1.83 million residents) were elevated to the "high-risk level" and locked down. Population quantitative real-time reverse transcription PCR (RT-PCR) screening was initiated for the high-risk region and was then conducted for other regions of Dalian.

This was the first local outbreak reported after having no new local infections in Dalian for 111 consecutive days. The Dalian CDC, Liaoning CDC, and China CDC together conducted a detailed epidemiological investigation to sort out the sources of the outbreak.

INVESTIGATION AND RESULTS

COVID-19 cases were diagnosed by a local hospital in Dalian according to the Protocol for Prevention and Control of COVID-19 (Edition 6) issued by China CDC (*1*). In this study, a confirmed case was defined as having a throat swab that tested positive for COVID-19 virus RNA by RT-qPCR; an undiscovered infected case was defined as having a sera sample that tested positive for COVID-19 antibodies but negative for COVID-19 virus RNA. Detailed epidemiological investigation for early cases were conducted through in-person interviews for their travel history, activity, work history, and contact history starting 14 days (incubation period) before the onset of illness. Environmental samples and cold-chain product samples collected from Company K were further tested with RT-qPCR. Individuals who had contact with the

positive environment samples and cold-chain products samples (mostly the dockworkers who had handled the cold-chain products) were quarantined and tested for COVID-19 virus nucleic acid and anti-COVID-19 virus antibodies.

Environmental smear samples were collected from the cold-chain seafood processing work area of Company K, which was the workplace of the first confirmed case of the Dalian outbreak, including samples from the operating table, floor, tools, sinks, sewers, and other environmental locations. According to records from the electronic cold storage management system of Company K, cold-chain products imported before the COVID-19 outbreak from the importing country were excluded. Surface swab samples of the inner and outer packaging of the cold-chain seafood imported by Company K from June 22 to July 8 (incubation period before the onset date of the first case) that were suspected to be contaminated by COVID-19 virus were carefully smeared and collected. In addition to the imported cold-chain seafood by Company K, all other imported cold-chain seafood products from the two cargo ships that were temporarily stored at Company K starting on June 1 were sampled and tested.

Viral RNA was extracted directly from 200-μL swab samples with a QIAamp Viral RNA Mini Kit (QIAGEN, Germany). RT-qPCR was conducted using the commercially available Novel Coronavirus 2019 Nucleic Acid Test Kit (Bojie, Shanghai, China) with an Applied Biosystems QuantStudio 5 instrument (Applied Biosystems, Hong Kong, China) following the manufacturers' instructions. A TaqMan probe-based kit was designed to detect the ORF1ab and N genes of COVID-19 virus in 1 reaction. The viral copy number was determined according to the Certified Reference Material of the COVID-19 virus Ribonucleic Acid Genome (No. GBW(E)091099) obtained from the National Institute of Metrology using RT-qPCR.

Corresponding serum samples were tested for anti-COVID-19 virus antibodies using a chemiluminescence immunoassay (CLIA, Bioscience, Chongqing, China). All these tests were conducted by the National Institute for Viral Disease Control and Prevention, China CDC.

Libraries were prepared using a Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA), and the resulting DNA libraries were sequenced on either a MiSeq or an iSeq platform (Illumina) using a 300-cycle reagent kit. Mapped assemblies were generated using the COVID-19 virus genome (accession number NC_045512) as a reference. Variant calling, genome alignment, and sequence illustrations were generated with CLCBio software (Qiagen, Hilden, Germany).

Each of the selected RT-qPCR-positive samples was seeded separately in Vero cells. The cells were monitored daily with light microscopy for cytopathic effects. COVID-19 virus genome sequences were submitted to GISAID database, accession numbers: EPI_ISL_2170885-2170894.

RESULTS

A total of 135 COVID-19 cases were identified in the present outbreak, 64 cases (47.4%) were employees of Company K and 67 (49.6%) were their contacts or residents living around Company K. The date of onset of the primary case (Case A) was July 9, and the infection was confirmed on July 25 by RT-qPCR testing. The index case was confirmed on July 23. A total of 4 early cases had onset by July 14, and the peak occurred from July 22 to 23 (Figure 1). Serum samples collected from Case A on August 5 tested positive for higher IgM level and lower IgG level (geometric mean titer=1:53.427 and 1:15.139, respectively).

Among 64 Company K employees, 63 were exposed to the environment of the cold-chain seafood processing workspace of Company K. On July 23, we collected 39 environmental smear samples from this location, including samples from the operating table, floor, tools, sinks, sewers, and other environmental areas. COVID-19 virus nucleic acid was tested in these samples, with a positive rate of 35.9% (14/39). In addition, the workers in the same and adjacent working areas to case A were identified as the first infected cases through work contact.

A total of 24 throat swab samples from confirmed cases and 2 environmentally positive samples from Company K were further sequenced, and full-length genomic sequences were acquired. Whole-genome analysis revealed that the sequences of 24 cases and two environmental samples were highly homologous. Compared with the reference sequence of COVID-19 virus, strain Wuhan-1 (GenBank No. NC_045512), all the sequences shared a total of 14 nucleotide mutations (C241T, C2091T, C3037T, A5128G, A8360G, C13860T, C14408T, T19839C, G19999T, A23403G, G28881A, G28882A, G28883C, and C28905T), belonging to PANGO lineage B.1.1.317 (*2*) (Figure 2).

From June 24 to July 8, which was the 14 days of

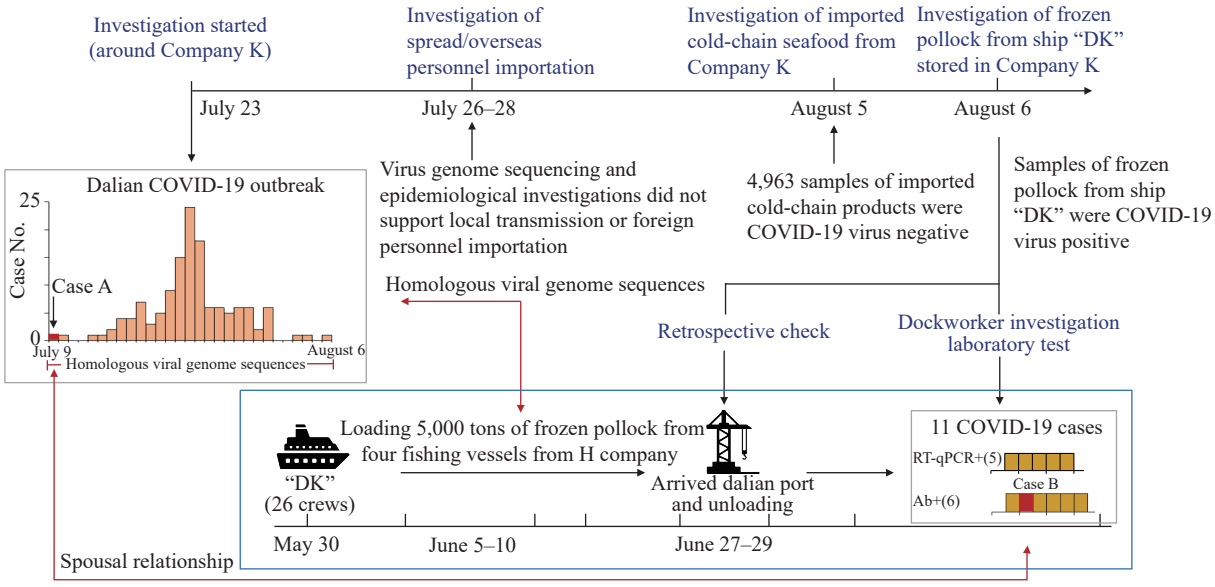

FIGURE 1. Investigation workflow and the Dalian COVID-19 outbreak. Imported cold-chain seafood products with COVID-19 virus infected wharf workers and then were introduced into the seafood product workshop of Company K before spreading further. The epidemiological investigation strategy was shown bolded in blue. COVID-19 case data were represented by a grey dotted box, and virus introduction from the DK ship was represented by a blue dotted box.

the COVID-19 incubation period before the onset date of Case A, a total of 8 imported cases were reported in Dalian. Based on the measures to prevent the risk of importation from overseas implemented in China, all the overseas arrivals were swabbed and tested for COVID-19 upon their entry at customs, were then transferred to the centralized quarantine location (usually a designated hotel) for 14 days for medical observation, and were tested again before their release. These 8 cases had no historical contact with early cases of the Dalian outbreak, and the whole-genome sequences from the samples of these cases were distinct from those of the Dalian outbreak (Figure 2). In addition, during this period, smeared swab samples were taken from the inner and outer packaging of a total of 2,119 suspicious packages of cold-chain seafood imported by Company K, and a total of 4,963 samples were collected for COVID-19 virus nucleic acid testing. The results revealed that all samples were negative.

In addition to importing and processing imported cold-chain seafood, Company K temporarily stored bonded cold-chain products for other companies. Imported bonded cold-chain products loaded from two overseas cargo ships were temporarily stored by Company K during that time. We traced the location of these cold-chain seafood products and conducted nucleic acid testing of their inner and outer packaging for COVID-19 virus and detected positive samples from the outer packaging of some cold-chain pollock loaded on one of the cargo ships (cargo ship DK). No positive samples were found from the other cargo ship. We further conducted RT-qPCR testing of the cold-chain pollock packaging that had been stored in four different freezers of DK, and the positive rates were 19.0% (19/100), 36.3% (37/102), 43.9% (29/66), and 49.0% (49/100).

DK was a large ocean-going refrigerated transport ship. From June 5 to 10, the ship DK carried 5,000 tons of cold-chain pollock from four fishing vessels that belonged to the same overseas company H. During the marine operation, the crews of the four overseas fishing vessels participated in processing and loading the cold chain pollock into the freezers of ship DK (temperature minus 25 degrees Celsius), while the crews of ship DK neither participated in cargo handling nor contacted the overseas crew. When the cargo ship arrived at the Dalian terminal on June 27–29, a total of 216 dockworkers from Company K were divided into three shifts; each shift spent 10 hours handling the cold-chain products, while the crews from ship DK did not participate in handling. According to custom inspection records, the ship DK crew's temperatures were normal, and they presented no symptoms of infection, but they were not tested with RT-qPCR while in China. All of them were quarantined on the ship until the ship left the port, which was confirmed by video from the dock around

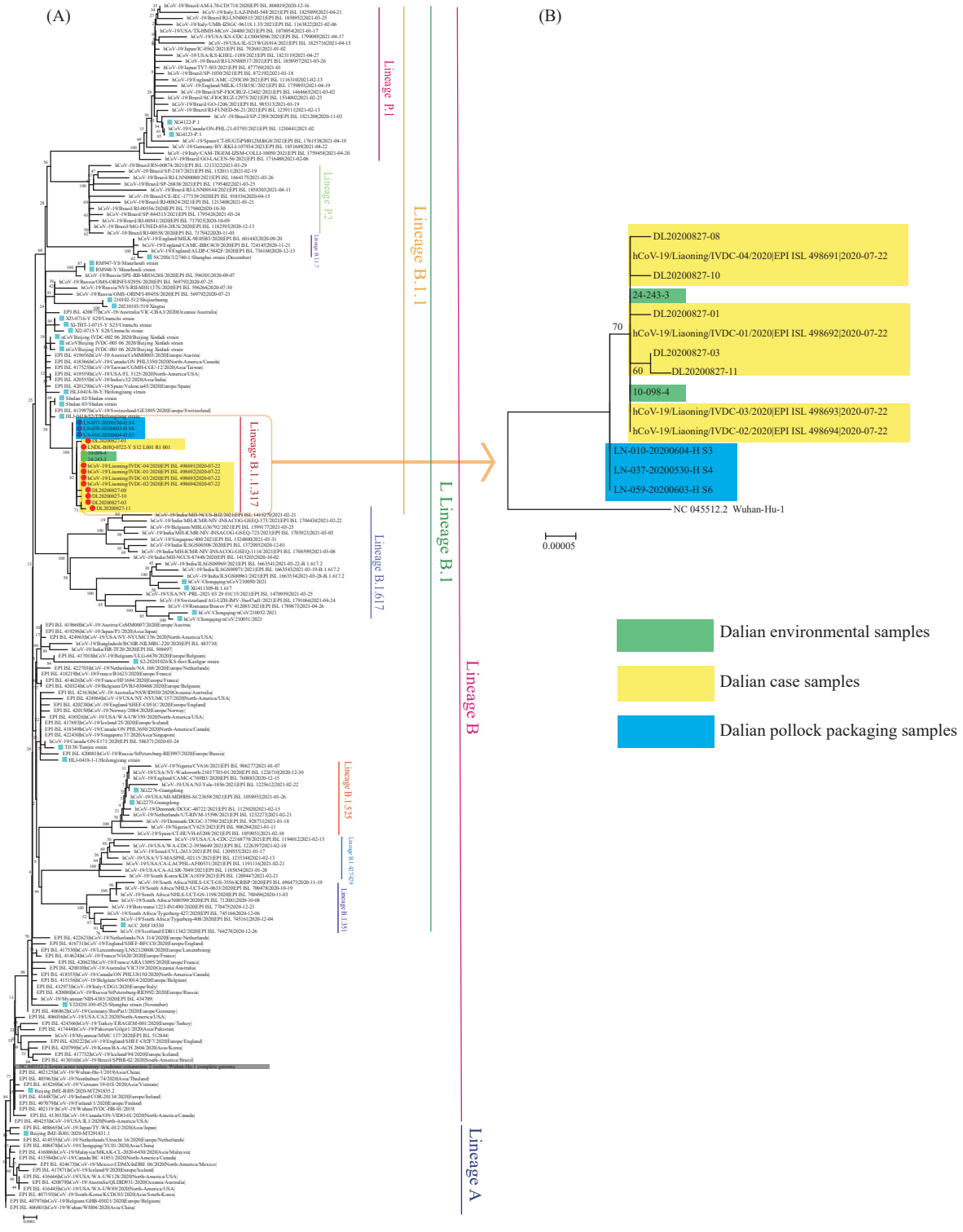

FIGURE 2. Phylogenetic tree based on Dalian outbreak-related samples. (A) Phylogenetic tree based on the representative full-length genome sequences of the COVID-19 virus. The genomes of the COVID-19 virus from Dalian case samples were highlighted in yellow, environmental samples from cold-chain seafood processing work area of Company K were highlighted in green and pollock packaging samples were highlighted in blue. The genome of the reference COVID-19 virus from Wuhan in December 2019 was shaded in grey. Strains associated with specific outbreaks in China were indicated by blue squares. The PANGO lineages were marked and colored on the right. The tree was rooted using strain WH04 (EPI_ISL_406801) in accord with the root of PANGO tree. (B) Phylogenetic tree based on the full-length genome sequences of Dalian COVID-19 virus samples, rooted using the Wuhan reference strain.

the ship. We also interviewed the crew of the DK ship, who maintained a distance of at least 10 metres from overseas crews; they had neither talked with the overseas crews nor used the toilet on their vessels. All the dockworkers of Company K wore labour gloves and disposable masks but carried the seafood products close to their faces and drank water and wiped sweat with their face mask off (Figure 1).

We further investigated the COVID-19 virus infection status of the workers who handled the cold-chain pollock. From July 23 to August 6 (14 days since the index case was confirmed), a total of 245 dockworkers and 13 cold storage stevedores of Company K who were exposed to DK cargo were quarantined and screened with RT-qPCR several times until August 5. Among these 258 potential contacts, five dock workers tested positive for COVID-19 virus nucleic acid, and all the others were negative. In addition, on August 11, sera samples of 195 dock workers and 13 cold storage stevedores of Company K were collected for antibody detection, and 50 dock workers were not sampled because they had been released from quarantine and were lost to follow up. Among the 208 potential contacts, 6 dockworkers who tested negative for nucleic acid were positive for COVID-19 IgG and IgM antibodies. Among the 5 confirmed cases and 6 undiscovered cases who had been infected with COVID-19, 2 of them reported physical soreness or cold symptoms at the end of June and early July, and the other nine were asymptomatic. These 11 people were all among the 216 dockworkers who participated in the actual handling of cold-chain pollock carried by the overseas DK cargo ship, and the infection rate was 5.1% (11/216). It is worth noting that 1 of the 6 dockworkers who were positive for COVID-19 IgG and IgM antibodies was the husband of Case A (designated Case B). The geometric mean titer of anti COVID-19 virus IgM and IgG of Case B were 1:3.899 and 1:13.677, respectively (Figure 1).

Most importantly, the COVID-19 whole-genome sequences from 7 nucleic acid-positive samples of the packaging of cold-chain pollock from the ship DK were obtained, all of which had very high homology with the sequences of the Dalian cases, also belonging to PANGO lineage B.1.1.317, only lacking a mutation site (A5128G), which was strongly suggestive of the parental virus of the Dalian cases (Figure 2).

We tried to isolate the virus from COVID-19 virus nucleic acid-positive samples detected in the cold-chain pollock from the DK cargo ship, but the virus was not successfully isolated. However, the result of quantitative fluorescence RT-qPCR detection was 1,090 copies, which was equivalent to 13,080 copies of the gene in the 200-μL environmental swab. Even though the virus was not isolated, such high viral copy numbers indicated that the viral load was large.

DISCUSSION

COVID-19 virus is transmitted from person to person through respiratory droplets and close contact during coughing, sneezing and speaking. In China, there have been some reports of COVID-19 virus nucleic acid detection on imported cold-chain chicken and cold-chain shrimp packaging material (*3*). Based on epidemiological investigations, laboratory testing (nucleic acid and antibody detection) and full-length genome sequencing, our study strongly suggested that the Dalian COVID-19 outbreak was caused by COVID-19 virus-contaminated imported cold-chain seafood products, sugggesting the possibility that COVID-19 virus can be transmitted through cold-chain products.

We investigated the 14-day incubation period of COVID-19 before the onset date of the first case (June 24 to July 8), first ruling out the possibility that the COVID-19 virus that caused the Dalian COVID-19 outbreak was from overseas personnel on the ground based on epidemiological investigations and whole-genome sequence differences. Second, 4,963 suspicious imported and processed frozen white shrimp samples from Company K tested negative for COVID-19 virus nucleic acid, which excluded the possibility that COVID-19 virus came from cold-chain goods imported and processed by Company K.

After detecting COVID-19 virus nucleic acid-positive cold-chain pollock samples from ship DK, an overseas cargo ship that had temporarily stored sealed imported cold-chain seafood for Company K, the investigation was clearer. The relatively high COVID-19 virus nucleic acid positivity rate in the four different freezers of the ship DK indicated a high proportion of virus contamination, and the 216 dockworkers who participated in the actual handling of cold-chain pollock carried by the overseas ship DK had a COVID-19 virus infection rate of 5.1% (11/216). This finding suggested that the source of COVID-19 virus infection in the Dalian outbreak was very likely the outer packaging of cold-chain pollock loaded on the DK cargo ship. Furthermore, the serological results of Cases B (high IgG level and low IgM level) and A (high IgM level) strongly suggested that Case A was

infected after Case B, which further indicated that Case A was infected through family transmission from contact with Case B.

The timeline of the Dalian outbreak could be well summarized based on the detailed retrospective investigation. Case B was asymptomatically infected with COVID-19 virus while handling the COVID-19 virus contaminated packaging of imported cold-chain seafood from the ship DK from June 27 to 29 and then transmitted COVID-19 virus to his wife, Case A. Case A presented symptoms of fatigue on July 9, which was 10 to 12 days since her husband was infected. Case B tested negative for nucleic acid from July 23 to August 5, indicating a transient infection. The serological evidence further demonstrated that Case B had been infected for some time (higher IgG level and low IgM level) but that Case A was newly infected (positive for nucleic acid and very high IgM level). After becoming infectious, Case A transmitted COVID-19 to her co-workers who had been working with Case A from July 6 to 23 in the cold-chain seafood processing workspace of Company K and contaminated the work environment. The specialized environment, with poor ventilation, humidity and low temperature in the work space, made it easier to contaminate with the virus (*4*–*6*). Therefore, the rapid spread through human-to-human transmission and environmental exposure triggered the outward spread of COVID-19 virus in the Company K, which led to the Dalian outbreak. The whole-genome sequences from positive pollock packaging samples lacked a unique mutation site compared with the sequences from the Dalian case samples, indicating that COVID-19 virus from pollock packaging was the originator of COVID-19 virs for the Dalian cases.

The results of this study have important public health significance for international organizations and countries for taking measures to prevent and control the importation of COVID-19 virus. The seafood product industry and trading market could act as a "virus amplifier" that causes COVID-19 outbreaks. COVID-19 virus was more stable on plastic and stainless steel than on copper and cardboard, and live virus was detected within 72 hours after application to these surfaces (*7*). Therefore, frequent exposure to contaminated surfaces in public places is a potential route for the spread of COVID-19 virus. One study found that when COVID-19 virus was added to chicken, salmon, and pork slices, the virus could survive (*8*). After 21 days of storage at 4 °C (standard refrigeration) and –20 °C (standard freezing), the titre of infectious virus did not decrease (*9*). According to a study published by the British Food Standards Agency on April 29, 2020, although consumers are less likely to be infected with the virus by eating food or handling packaging, COVID-19 virus in cold-chain or refrigerated transport and storage products may still be infectious.

Virus isolation is usually difficult, and it is not easy to isolate live virus even from respiratory tract samples of COVID-19 patients (*10*). Although the virus was not successfully isolated in this study, when the viral load of the contaminated goods was high and the virus was stored and transported in the cold chain, the survival rate of the virus was very high.

The key to strengthening the prevention and control of cold-chain seafood product import of the virus is to regularly carry out nucleic acid screening for COVID-19 virus in dockworkers engaged in the import of cold-chain goods and special personnel engaged in seafood product production and sales; carry out health education for dockworkers and seafood product production and sales personnel; and take protective and hygiene measures to reduce the potential risk of the recurrence of the epidemic.

Acknowledgements: We thank Dalian, Liaoning and China CDC for the epidemiological investigation.

Conflicts of interest: No conflicts of interest were reported.

Funding: The National Key Research and Development Project, Ministry of Science and Technology of the People's Republic of China (2021YFC0863000, 2018ZX10713002, and 2020YFC0846900), National Natural Science Foundation of China (No.72042012, and No. 82041032) and National Key Technology R&D Program of China (2017ZX10104001).

doi: 10.46234/ccdcw2021.114

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Submitted: May 09, 2021; Accepted: May 20, 2021

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Methods and Applications

Development of a PDRA Method for Detection of the D614G Mutation in COVID-19 Virus — Worldwide, 2021

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ABSTRACT

Background: COVID-19 infection is a major public health problem worldwide, and the D614G mutation enhances the infectivity of COVID-19.

Methods: A probe-directed recombinase amplification (PDRA) assay was discussed to detect the D614G mutation at 39 ℃ for 30 min. The sensitivity, specificity, and reproducibility of the PDRA were evaluated by D614 and G614 recombinant plasmids. The clinical performance of PDRA assay was validated by testing of 53 previously confirmed COVID-19 positive RNAs and 10 negative samples. Direct sequencing was carried out in parallel for comparison.

Result: With good reproducibility and specificity, the PDRA assay worked well with the concentration in the range of 10^3–10^7 copies/reaction. Compared with direct sequencing as a reference, the recombinase-aided amplification (RAA) assay obtained 100% sensitivity and 100% specificity using clinical samples.

Conclusions: A rapid, convenient, sensitive, and specific method to detect D614G mutation was developed, which offers a useful tool to monitor mutations in COVID-19 virus RNA.

INTRODUCTION

At the end of 2019, coronavirus disease 2019 (COVID-19) caused by COVID-19 virus, also known as SARS-CoV-2, was first discovered and quickly began to spread around the world (*1*). Some researchers evaluated and compared the whole genome sequences of circulating COVID-19 and found mutations associated with the infectivity of the virus. High-frequency mutations in the COVID-19 genome were found in nsp6, RNA polymerase, helicase, membrane glycoprotein, RNA primer, nucleocapsid phosphoprotein, and spike protein genes (*2*). One of the most critical mutations was the D614G of the spike protein gene (S), which is a replacement of aspartic acid (D) with glycine (G). The transmission of S-G614 mutants was stronger than that of S-D614 mutants because a newly-formed serine protease called elastase-2 in the S-G614 mutant could lead to an increase in enzyme cleavage efficiency and infectivity (*3*–*4*).

The D614G mutation brings new challenges to the prevention and control of the epidemic, and there are few reported molecular detection methods for D614G mutation (*5*). Therefore, developing a detection method to distinguish S-G614 mutants from S-D614 mutants is highly important. Many conventional methods are available for detecting single nucleotide polymorphism (SNP), such as real-time PCR (*6*), DNA sequencing (*7*), restriction fragment length polymorphism (RFLP) (*8*). However, these methods are either time-consuming and laborious or require more sophisticated instruments and skillful personnel.

Isothermal DNA amplification technology offers a good alternative to mutation detection due to its simplicity and rapidity. Among them, loop-mediated isothermal amplification (LAMP) is the most common method for SNP detection, but the design of LAMP primers is complex, and its application is limited by the requirement of a high temperature (65 °C) (*9*). There are also some mutation detection studies on recombinase polymerase amplification (RPA) (*10*), its sensitivity and specificity are similar with PCR, but expensive (7.7 USD/test). Recombinase-aided amplification (RAA) is a new isothermal amplification assay for various pathogens (*11*) and uses specific enzymes and proteins to quickly detect nucleic acid in less than 30 minutes at 39 °C, and it is cheaper (4.6 USD/test). Recently, a modified RAA assay, probe-directed recombinase amplification (PDRA), was developed in our laboratory for detection of SNPs (*12*–*13*). In this study, a PDRA method for simple and quick differentiation of D614G mutation in COVID-19 was reported.

MATERIALS AND METHODS

Clinical Sample Collection

A total of 53 previously confirmed COVID-19 virus positive RNAs and 10 negative RNAs (from other respiratory viral pathogen-positive swab samples) were all stored in the National Institute for Viral Disease Control and Prevention of China CDC in Beijing, China.

Direct Sequencing of Clinical Samples

The QIAGEN OneStep RT-PCR Kit for PCR and sequencing were used for comparison. The PCR forward primer sequence was 5-AATCTATCAGGCCGGTAGCAC-3. The reverse sequence was 5-CACCAATGGGTATGTCACACT-3. (*14*) The PCR assay was performed in a 25 μL reaction system containing 5 μL of 5×PCR buffer, 2 μL of One Step RT-PCR Enzyme Mix (QIAGEN), 1 μL dNTP mix, 0.1 μL of RNase inhibitor (RRI), 2 μL of primer mix (10 μmol/L), and 2 μL of template nucleic acid using a CFX96 Real-Time PCR System (Bio-Rad, USA) under the following conditions: a 30 min reverse transcription step at 50 °C, a 15 min denaturation step at 95 °C, and followed by 35 cycles at 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min, a 10 min final extension step at 72 °C, and finally kept at 4 °C. The PCR products were then sent to Sangon Biotech (Shanghai, China) for sequencing.

Design of Primer & Probes for the PDRA Assay

The PDRA assay included two real-time RAA reactions (A and G) to detect the A and G nucleotides of the D614G mutation (A23403G polymorphism), respectively. Each individual PDRA reaction contained a primer and a probe. The forward primers of the two reactions were the same, and there was only one nucleotide difference in the polymorphic site between the two probes. the primers and probe design were based on the S protein sequence of SARS-CoV-2 with accession number MT252819.1. A forward primer and two specific reverse probes for A and G nucleotides of A23403G polymorphism were designed using Oligo7 software. The primer is usually 30–35 bp in length and the probe is usually 46–52 nucleotides in length, of which at least 30 nt is located at the 5' end, adjacent to the internal base-free site mimic (tetrahydrofuran [THF]) site, flanked by dT-fluorophore and the corresponding dT-quencher group, and at least 3 nucleotides at the 3' end. At the same time, the mutation site was designed to be just before the THF site of each probe (Figure 1). The forward primer sequence was 5-TTGAGATTCTTGACATTACACC ATGTTCTTT-3. The reverse probe D sequence was 5-TAGCAACAGGGACTTCTGTGCAGTT AACATCCTGATAAAGAACAGC-3. The reverse probe G sequence was 5-TAGCAACAGGGACTTCT GTGCAGTTAACACCCTGATAAAGAACAGC-3. All the primers and probes were synthesized and purified by Sangon Biotech (Shanghai, China) using high-performance liquid chromatography (HPLC).

PDRA Assay

PDRA assay was performed using RAA kits (Jiangsu Province Qitian Biology Co., Ltd., China), and the reaction system was partially modified. The A and G reactions were divided into two tubes (A and G). A reaction was carried out in a 50 μL lyophilized reaction

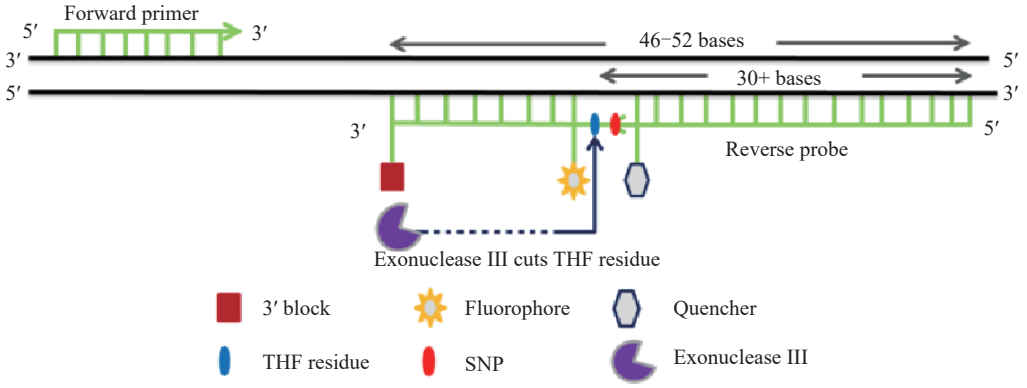

FIGURE 1. Design of the PDRA assay probe.
Abbreviations: SNP=single nucleotide polymorphism; THF=tetrahydrofuran.

tube containing a dried enzyme precipitation 0.2 mL, 25 μL rehydration buffer, 18.2 μL ddH_2O, 1.3 μL forward primers (10 μmol/L), 1 μL A nucleotide specific PDRA probe (10 μmol/L), 2 μL DNA template, and 2.5 μL magnesium acetate (280 mmol/L). G reaction was carried out in a 50 μL lyophilized reaction tube containing a dried enzyme precipitation 0.2 mL, 25 μL rehydration buffer, 17.9 μL ddH2O, 1.6 μL forward primers (10 μmol/L), 1 μL G nucleotide specific PDRA probe (10 μmol/L), 2 μL DNA template, and 2.5 μL magnesium acetate (280 mmol/L). The test tube was then transferred to the test tube rack of RAA fluorescence detection equipment QT-RAA-F7200 (Jiangsu Province Qitian Biology Co., Ltd., China) and incubated at 39 °C for 30 min. Negative controls (water without nuclease) were included in each run.

Interpretation of the PDRA Results

When the slope of the fluorescence curve is >20, it is judged to be a positive result by the detector, and when the positive result of probe D appears earlier than that of probe G and the time difference ≥3 min, it is interpreted as S-D614 strain. When the positive result of probe G appeared earlier than that of probe D and the time difference ≥3 min, it was interpreted as S-G614 mutant.

Analytical Sensitivity, Specificity, and Reproducibility of PDRA Assay

The recombinant plasmids of S-G614 mutant and S-D614 strain with a concentration in the 10-fold range of 10^2 copies/μL to 10^7 copies/μL were used to validate the sensitivity and specificity of PDRA test. In different 5 days, 20 repeated experiments were carried out to verify its repeatability.

Detection of Clinical Samples by PDRA Assay

PDRA was used to detect 53 COVID-19 positive nucleic acid samples and 10 negative samples the results were compared with those of the sequencing results.

RESULTS

The sensitivity of PDRA for the detection of two recombinant plasmids was 10^3 copies (Figure 2) and the reproducibility was shown in Table 1. No cross reaction between two recombinant plasmids (G and D) was observed within at least 3-minute interval by corresponding probes in the detection range (Figure 3). A total of 63 clinical samples were typed by PDRA and direct sequencing. The sequencing results of 63 clinical samples were compared with the results of PDRA (Figure 4), and the coincidence rate was 100% (Table 2). Among the 63 clinical samples, there were 22 S-D614 mutants, 31 S-G614 mutants, and 10 negative samples.

DISCUSSION

At present, COVID-19 is still spreading rapidly all over the world. The virus spike protein is one of the best-targeted molecules for the development of vaccines or monoclonal antibodies against this virus (*15–16*). Therefore, the mutation of S protein has special clinical significance. As one of the key mutations of S protein, the D614G mutation has been concerned by many researchers. D614G mutation was first found in Germany (*17*). After that, Becerra-Flores et al. suggested that S-G614 mutants are more pathogenic and have higher mortality (*18*). These characteristics make S-G614 mutants rapidly become the dominant species and expand around the world (*3*).

Few studies reported D614G detection. The D614G detection method designed by Hashemi et al. (*14*), actually detected the mutation to V615V (*19*). Whole-genome sequencing is a powerful but costly tool to identify D614G mutants, which cannot be applied in primary laboratories for sequencing a large number of samples. Considering the importance of D614G compared with other mutations, a simple and rapid method to detect this mutation is favorable. Prior to this, PDRA has been successfully used to detect heart disease-related SNP and prostate cancer-related SNP (*12–13*).

The two PDRA reactions were carried out separately for two reasons: 1) in order to achieve good sensitivity for two different mutants; 2) in order to make the results of PDRA easier to interpret. Through the experiment, the detection limit of the PDRA was found to be in the range of 10^3–10^7 copies/reaction. PDRA assay was not able to specifically distinguish the genotypes of the sample in the higher sample concentration (>10^7 copies) or led to false-negative results in the lower sample concentration (<10^3

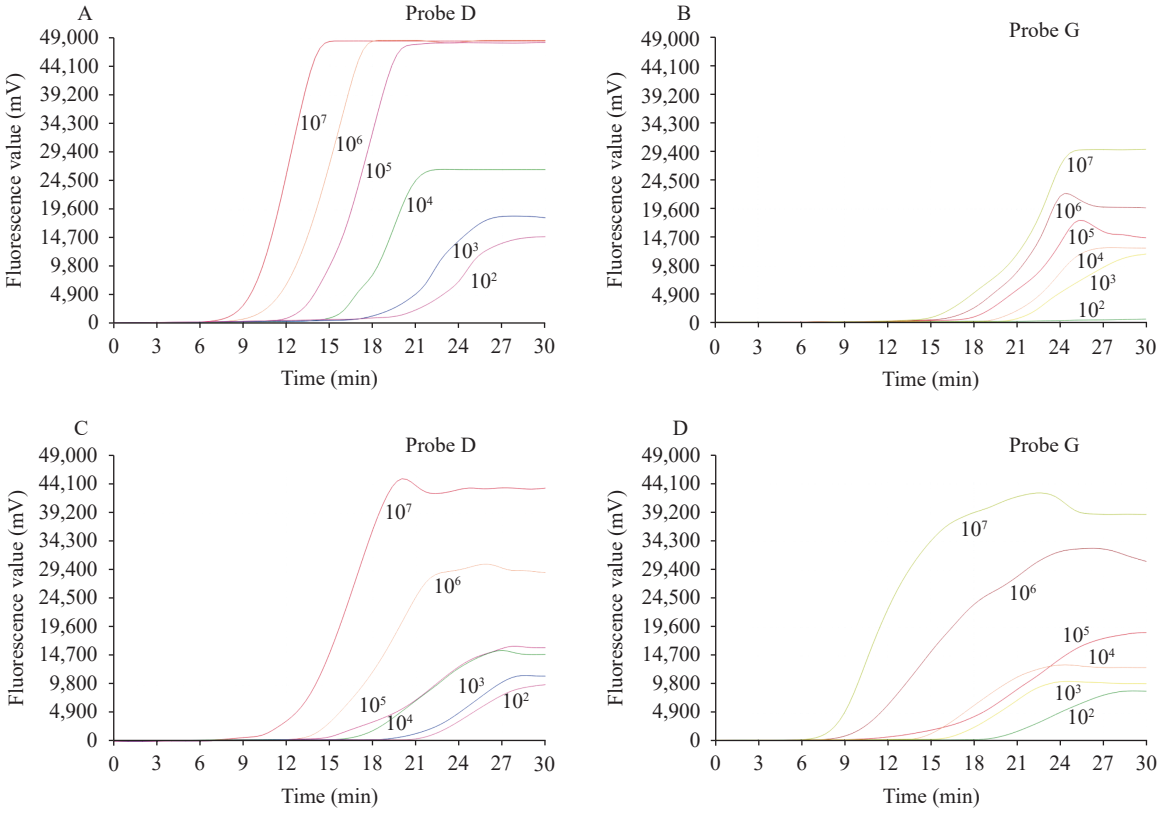

FIGURE 2. Sensitivity result of PDRA with recombinant plasmids. (A) Probe D tested S-D614 mutant recombinant plasmids; (B) Probe G tested S-D614 mutant recombinant plasmid; (C) Probe D tested S-G614 mutant recombinant plasmids; (D) Probe G tested S-G614 mutant recombinant plasmids.
Note: When the S-D614 strain recombinant plasmid was detected, the sensitivity of Probe D and Probe G was 10^2 copies/reaction and 10^3 copies/reaction, respectively, and the time to positivity by probe D at each concentration was less (at least 3 min interval) than that by probe G. When the S-G614 mutant recombinant plasmid was detected, the sensitivity of both Probe D and Probe G was 10^2 copies/reaction. The time to positivity by probe G at each concentration was less (at least 3 min interval) than that by probe D.

TABLE 1. The reproducibility of probe-directed recombinase amplification (PDRA) assay.

Serial diluted DNA	No. of replicates tested	No. detected	Detection rate (%)
10^7	20	20	100
10^6	20	20	100
10^5	20	20	100
10^4	20	20	100
10^3	20	20	100
10^2	20	2	10

copies). Adding two reaction systems in one tube was attempted for saving time and cost but failed in the end. A possible reason is that there might be a competition mechanism between the two probes that makes them unable to react.

Although the PDRA method has some limitations, compared with direct sequencing, PCR and LAMP, PDRA possess the advantages of simple experimental conditions, short turnaround time and appropriate sensitivity and specificity. PDRA assay needs only 30 minutes to complete the detection and requires no sophisticated equipment. The detection process of PDRA is also simple and easy to learn. In view of the characteristics of PDRA and further improvement, PDRA will facilitate the detection of D614G mutations in resource-limited settings, particularly in locations where the contamination of the vaccine strain (D614) needs to be monitored and differentiated with

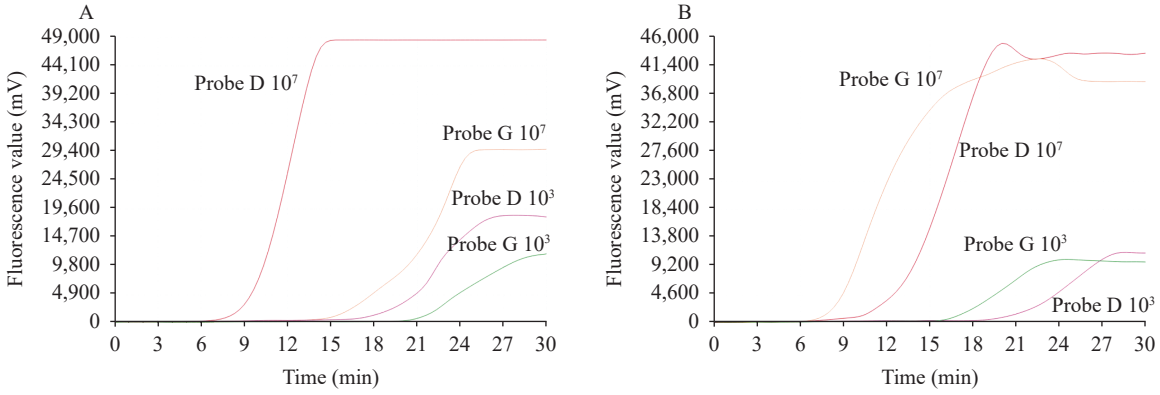

FIGURE 3. Specificity result of PDRA with recombinant plasmids. (A) S-D614 strain recombinant plasmid; (B) S-G614 mutant recombinant plasmid.
Note: When the 10^7 copies of S-D614 strain recombinant plasmid were detected, the time to positivity by Probe D was less than that of Probe G by 6 min. When the 10^3 copies of recombinant plasmid were detected, the time to positivity by Probe D was less than that of Probe G by 4 min. When the 10^7 copies and 10^3 copies of S-G614 mutant recombinant plasmids were detected, the time to positivity by Probe G was less than that of Probe D by 3 min.

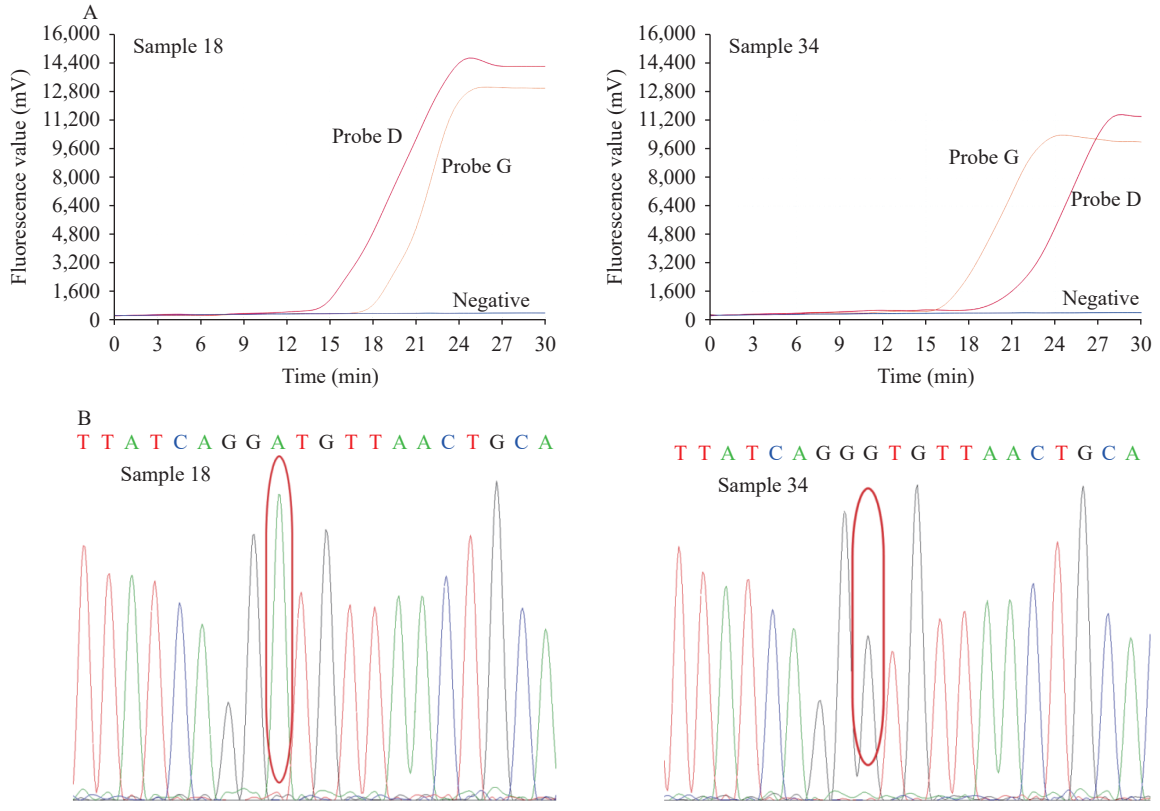

FIGURE 4. Comparison of the PDRA assay and direct sequencing results. (A) The PDRA results of sample 18 and sample 34; (B) The direct sequencing results of sample 18 and sample 34.
Note: Sample 18 was S-D614 mutant, and sample 34 was S-G614 mutant. Sample 18 was S-D614 mutant, and sample 34 was S-G614 mutant.

non-local circulating strains (G614) in China.

Conflicts of interest: No conflicts of interest were reported.

Availability of data and materials: The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Funding: Grants from the China Mega-Projects for Infectious Disease (2017ZX10302301-004-002), and Academician Hou Yunde Research Youth Fund Project (2019HYDQNJJ03).

TABLE 2. Clinical sample typing results.

Type	Sequencing	PDRA	Coincidence rate (%)
S-D614	22	22	100
S-G614	31	31	100
Negative	10	10	100

Abbreviation: PDRA=probe-directed recombinase amplification.

doi: 10.46234/ccdcw2021.115

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Submitted: April 01, 2021; Accepted: May 19, 2021

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Notes from the Field

The First Reported Case of COVID-19 and *Plasmodium ovale* Malaria Coinfection — Guangdong Province, China, January 2021

Muxin Chen[1,2,&]; Shitong Gao[3,&]; Lin Ai[2]; Jiaxu Chen[4]; Tiejian Feng[5]; Zhigao Chen[3]; Xiaomin Zhang[1]; Shaoyu Deng[1]; Zidan Lin[1]; Yijun Tang[1]; Qian Zhang[1]; Gengcheng He[1]; Huawei Xiong[3]; Xiao-nong Zhou[2,#]; Renli Zhang[5,#]; Dana Huang[1,#]

The current coronavirus disease 2019 (COVID-19) pandemic has posed an unprecedented challenge to global health (*1*–*3*). The clinical symptoms of COVID-19 are similar to those of malaria, such as fever, myalgia, fatigue, headache, and gastrointestinal symptoms. Due to the heavy burden of medical services and the possible shortage of resources caused by the long-term COVID-19 pandemic, the risk of coinfection of malaria and COVID-19 is a matter of particular concern. Here we reported the first case of COVID-19 coinfection with *Plasmodium ovale* (*P. ovale*) malaria in a 47-year-old man who had been previously diagnosed with *Plasmodium* infection and incompletely treated with antimalarial drugs.

The 47-year-old man worked from October 30, 2018 to December 16, 2020 in Masindi, Uganda, in an area dually affected by malaria and COVID-19. On December 19, 2020, the nasopharyngeal swab for COVID-19 virus testing using reverse transcriptase polymerase chain reaction (RT-PCR) and malaria rapid diagnostic testing (RDT) both had negative results when the patient returned from Uganda and entered Shenzhen City, Guangdong Province, China. However, on December 25, the nasopharyngeal swab for COVID-19 virus test result was positive, and the patient was admitted to a COVID-19 ward for further observation and symptom control on the next day. On December 29, the patient experienced chills, fever, muscle pain, and other clinical symptoms. On December 31, the results of blood smear test showed that the different blood stages of *P. ovale*, including trophozoites and schizonts, were consistent with *P. ovale* (Figure 1). In addition, the blood smear identified trophozoites of *P. ovale* with a parasitemia of 0.01%. The plasmodial ssrRNA (MW768131) and *P. ovale* sp. tryptophan-rich antigen (*potra*) (MW872056) gene were amplified by nested PCR, respectively (*4*–*6*). After sequencing, the protozoan was identified as *P. ovale wallikeri* (*P. o. wallikeri*).

The patient's symptoms were mild and resolved completely after treatment with primaquine for 24 hours. The result of RT-PCR detection of COVID-19 virus swab tested negative on January 1, 2021. During hospitalization, the patient did not have cough, shortness of breath, or other respiratory symptoms including decreased oxygen levels or COVID-19 infection, and did not require any oxygen supply. On January 6, 2021, the blood smear test results were negative for *P. ovale*. According to his epidemiological history, clinical manifestations, and laboratory test results, this patient was preliminarily identified as an imported case of COVID-19 and *P. o. wallikeri* malaria coinfection in Uganda.

The patient was recently diagnosed with malaria on December 10, 2020 in Uganda, after which he left this area. During treatment on December 16, there was a possibility of reinfection, which suggested that COVID-19 might induce the current relapse of *P. ovale*. Similarly, a case of COVID-19 and *P. vivax* coinfection was reported by Kishore et al., which also highlighted the possibility of COVID-19 infection in inducing current malarial relapse (*7*). Associated cytokine response with systematic illness has been postulated in *P. vivax* relapse (*8*). The COVID-19 infection, with its cytokine response, was assumed to induce the *P. ovale* relapse of patient. More trials are needed to illustrate the mechanism of COVID-19 in inducing malaria relapse.

During the COVID-19 pandemic, consistent diagnosis of malaria and other infectious diseases should still be prioritized (*7*,*9*–*11*), especially in the areas with endemic infectious diseases such as those in Africa. In addition, invested funds related to malaria elimination were reduced or interrupted and might have lead to the interruption of malaria-related diagnostic tools and drug supply, which might have delayed targets and implementation of the malaria control strategies.

In conclusion, it is necessary to further study the risk factors, clinical outcomes, treatment challenges, and prognosis of cases on COVID-19 coinfection with other pathogens. Given the risk of these diseases, the

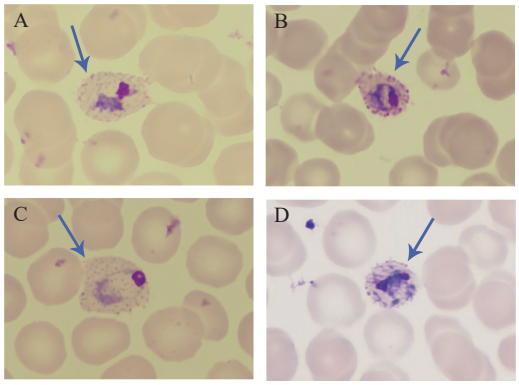

FIGURE 1. Giemsa-stained thin blood smears from the peripheral blood of the patient (×1,000). (A) *P. ovale* trophozoites parasitizing distorted erythrocytes; (B) *P. ovale* trophozoites parasitizing distorted erythrocytes; (C) *P. ovale* trophozoites parasitizing enlarged erythrocytes; (D) Early shizont of *P. ovale*.

following comprehensive prevention and control strategies were formulated: 1) 14 days of centralized isolation medical observation for entry personnel in medium and high-risk areas should be implemented, as well as screening for pathogens such as *Plasmodium*; 2) publicity and education on COVID-19 prevention and control of malaria and other diseases should be continued to reduce the burden of disease or death due to misdiagnosis and missed diagnoses; 3) it is necessary to build human health center communities that supplement existing prevention and control strategies for endemic infectious diseases including COVID-19, malaria, etc. Protecting the health and safety of the global community can only be accomplished with joint effort.

Funding: Key scientific and technological project of Shenzhen Science and Technology Innovation Committee (JSGG20200207161926465 and KCXFZ 202002011006190), Youth Science Foundation of Chinese Center for Disease Control and Prevention (2018A105), Shenzhen San-Ming Project for prevention and research on vector-borne diseases (SZSM201611064), Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2020-PT330-006), Shenzhen Key Medical Discipline Construction Fund (SZXK064), Natural Science Foundation of Shanghai (18ZR1443500), General Program Shanghai Municipal Commission of Health and Family Planning of China (201840286), Open project of Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education (2019kfkt02), and Key Laboratory of Echinococcosis Prevention and Control (2020WZK 2002).

doi: 10.46234/ccdcw2021.101

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Submitted: March 18, 2021; Accepted: April 25, 2021

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Notes from the Field

Two Imported Cases of New Variant COVID-19 First Emerging from Brazil — Guangdong Province, China, April 30, 2021

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On April 15, 2021, a 62-year-old male (Patient A, XG4122) and a 58-year-old female (Patient B, XG4123) travelled from Canada via airplane and were transferred to a central isolation hotel after being tested by the laboratory of Guangzhou Customs using nose swab tests for coronavirus disease 2019 (COVID-19). The results for both patients returned COVID-19 positive and the couple were transported to the Guangzhou Eighth People's Hospital for isolated treatment by ambulance on April 16, 2021. Patient A had inflamed lungs while Patient B had mild inflammation in her lungs according to the chest computed tomography (CT) examination. Both of their 28 close contacts underwent centralized isolation and medical observation.

On April 21, 2021, Guangdong Provincial CDC received the samples and began virus isolation and gene sequencing analysis. On April 27, 2021, the 2 samples were sequenced using Nanopore GridION. On April 30, 2021, the sequencing analysis concluded that the 2 virus genomes belonged to lineage P.1, 20J/501Y.V3, which was first found in four Brazilians after a routine check at Japan's Haneda Airport in late December 2020. The variant was then widely spread to Manaus in the northern State of Amazona in Brazil. The Brazil variant has been reported in 40 different countries as of April 30, 2021(*1*).

Compared with the Wuhan reference sequence (EPI_ISL_402119) (*2*–*3*), the strain from Patient A (XG4122) displayed 33 nucleotide variation sites (C241T, T733C, C2749T, C3037T, C3828T, A5648C, A6319G, A6613G, G9105A, C12778T, C14408T, G17259T, C21614T, C21621A, C21638T, G21974T, G22132T, A22812C, G23012A, A23063T, C23380T, A23403G, C23525T, C24642T, G25088T, T26149C, G28167A, C28512G, A28877T, G28878C, G28881A, G28882A, and G28883C) belonged to the Pangolin lineage P.1 (Figure 1). Patient B (XG4123) strain displayed 34 nucleotide variation sites (C241T, T733C, C2749T, C3037T, C3828T, A5648C, A6319G, A6613G, G9105A, C12778T, C13860T, C14408T, G17259T, C21614T, C21621A, C21638T, G21974T, G22132T, A22812C, G23012A, A23063T, C23380T, A23403G, C23525T, C24642T, G25088T, T26149C, G28167A, C28512G, A28877T, G28878C, G28881A, G28882A, and G28883C).

Furthermore, 23 amino acid mutation sites (N:P80R, N:R203K, N:G204R, ORF1a:S1188L, ORF1a:K1795Q, ORF1a:S2947N, ORF1b:P314L, ORF1b:E1264D, ORF3a:S253P, ORF8:E92K, ORF9b:Q77E, S:L18F, S:T20N, S:P26S, S:D138Y, S:R190S, S:K417T, S:E484K, S:N501Y, S:D614G, S:H655Y, S:T1027I, and S:V1176F) and 3 amino acid deletions (ORF1a:S3675-, ORF1a:G3676-, and ORF1a:F3677-) were detected in the protein that corresponded to the features of the Brazil variant (P.1) (*4*). Both variants have 12 mutations to the spike protein, including 3 mutations of concern in common with 20H/501Y.V2 (K417N/T, E484K, and N501Y) which may affect transmissibility and host immune response (*4*–*5*).

The P.1 lineage and the B.1.1.7 first described in the United Kingdom share the spike N501Y mutation and a deletion in ORF1b (del 3675-3677 SGF). The P.1 lineage and the B.1.351 (also known as 501Y.V2) lineage described in South Africa share 3 mutation positions in common in the spike protein (K417N/T, E484K, and N501Y). Both the P.1 and the B.1.351 lineage also have the ORF1b deletion (del 3675-3677 SGF) (*6*).

This is the fourth recent detection of a major international variant following the detection of the United Kingdom 501Y.V1 variant, the South African 501Y.V2 variant, and the Nigerian B.1.525 variant in Guangdong (*7*–*9*). The transmissibility and pathogenicity of these mutant variants urgently need further study.

doi: 10.46234/ccdcw2021.110

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Submitted: May 06, 2021; Accepted: May 07, 2021

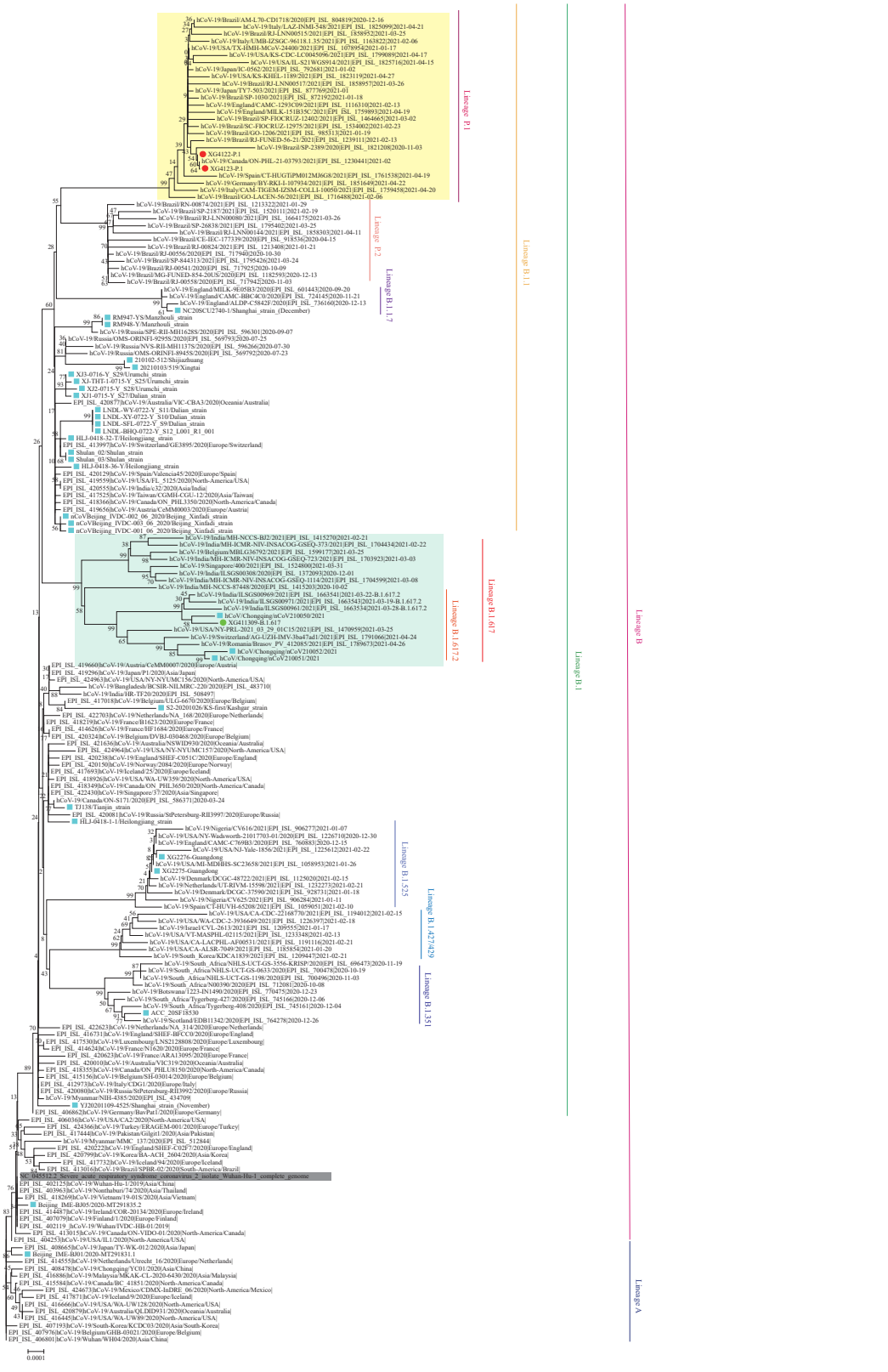

FIGURE 1. Phylogenetic tree based on the full-length genome sequences of the COVID-19 virus. The Brazil variants (P.1) are highlighted in yellow and the Guangdong imported P.1 variants are marked with red dots. The strains associated with specific outbreaks in China are marked with blue squares. The Wuhan reference strain is shaded in gray. The seven distinguished COVID-19 mutants are marked and colored on the right. The A or B lineage of the COVID-19 virus were marked and colored on the right.

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Recommendations

Technical Vaccination Recommendations for COVID-19 Vaccines in China (First Edition)

COVID-19 Vaccine Technical Working Group

To date, five coronavirus disease 2019 (COVID-19) vaccines have been conditionally approved for market authorization or granted emergency use authorization in China by the National Medical Products Administration (NMPA). Three inactivated COVID-19 vaccines and one adenovirus-vectored vaccine were granted conditional approval, as phase Ⅲ clinical trial data for these four conditionally-approved vaccines have demonstrated protective efficacy levels that meet requirements of NMPA's *Guidelines for Clinical Evaluation of COVID-19 vaccine (interim version)* and also meet requirements recommended by the World Health Organization (WHO) in their *COVID-19 Vaccines Target Product Profile*. Adverse event surveillance data from clinical trials, emergency use, and large-scale vaccination of key populations have demonstrated that the approved COVID-19 vaccines have excellent safety profiles. One recombinant subunit COVID-19 vaccine was approved for emergency use, as phase Ⅱ clinical trial results showed good immunogenicity and safety of the recombinant subunit COVID-19 vaccine (CHO cell).

Based on clinical trial data of the NMPA-approved domestic vaccines and the epidemiological characteristics of COVID-19, the COVID-19 Vaccine Technical Working Group of China CDC drafted technical vaccination recommendations. The National Immunization Advisory Committee reviewed, discussed, and refined the recommendations. The National Health Commission accepted and published these recommendations (*1*).

These recommendations can be used by health departments and disease control institutions at all levels in China to guide COVID-19 vaccination.

VACCINE TYPES

Inactivated COVID-19 Vaccines

The three conditionally-approved inactivated COVID-19 vaccines are manufactured by Beijing Institute of Biological Products Co., Ltd, Sinopharm (Beijing Institute), Wuhan Institute of Biological Products Co., Ltd, Sinopharm (Wuhan Institute), and Sinovac Research & Development Co., Ltd (Sinovac). All three use Vero cells to culture and grow COVID-19 virus and β -propionolactone to inactivate the virus while retaining antigenic components that induce immune responses. All three are adjuvanted with aluminum hydroxide to improve immunogenicity.

Adenovirus Vectored COVID-19 Vaccine

The conditionally-approved adenovirus-5 vectored COVID-19 vaccine is manufactured by CanSinoBIO (CanSino). This vaccine is made by recombining the spike-glycoprotein (S protein) gene of COVID-19 virus into the genetic material of a replication-deficient human adenovirus-5. After injection of the vaccine, the recombinant adenovirus brings the S-protein gene into the person's cells that then use the gene to make S-protein antigen, which induces an immune response in the person vaccinated.

Recombinant Subunit COVID-19 Vaccine

The recombinant subunit COVID-19 vaccine (CHO cell) that has been approved for emergency use is manufactured by Anhui Zhifei Longcom Biopharmceutical Co., Ltd. This vaccine is made by recombining the S-protein receptor binding domain (RBD) gene with a gene in Chinese hamster ovary (CHO) cells. The CHO cells make RBD protein dimer that are purified and concentrated for use in producing the vaccine. Aluminum hydroxide adjuvant is added to improve immunogenicity.

RECOMMENDED IMMUNIZATION SCHEDULE

Target Population

The five COVID-19 vaccines approved for

conditional marketing or emergency use are suitable for persons aged 18 years and above.

Schedules

Inactivated COVID-19 vaccines (Vero cell): Two doses are recommended with a minimum 3-week interval between doses. The second dose should be given at the earliest possible opportunity after 3 weeks, within 8 weeks.

Adenovirus vector COVID-19 vaccine (Ad5): One dose is recommended.

Recombinant subunit COVID-19 vaccine (CHO cell): Three doses are recommended with 4-week minimum intervals between doses. The second dose should be given at the earliest possible opportunity between 4 weeks and 8 weeks after the first dose. The third dose should be given within 6 months after the first dose, at least 4 weeks after the second dose.

Route and Site

All doses are recommended to be given by intramuscular injection in the deltoid muscle.

SPECIAL CONSIDERATIONS

Delayed and Catch-Up Doses and Minimum Interval Violations

Individuals who did not complete vaccination in the recommended time frame are recommended to receive the delayed dose(s) at the earliest possible opportunity. Delayed doses do not need to be repeated.

Individuals administered inactivated vaccine whose second dose was inadvertently administered less than 14 days after the first dose are recommended to be given one additional dose no earlier than 3 weeks after the second dose. Individuals administered inactivated vaccine who received a second dose between 14 and 21 days after the first dose do not need an additional dose.

Booster Doses

Currently, booster doses are not recommended.

Co-administration with Other Vaccines

At present, it is not recommended to administer COVID-19 vaccines with other vaccines. With the exception of rabies post-exposure vaccination and tetanus wound management, there should be a minimum interval of 14 days between administration of a COVID-19 vaccine and other vaccines. If rabies vaccine or tetanus toxoid needs to be administered due to animal bites, trauma, and any other emergency indication, there is no minimum interval following COVID-19 vaccination for administering rabies vaccine or tetanus toxoid.

Interchangeability of COVID-19 Vaccines

At present, it is suggested to use the same manufacturer's COVID-19 vaccine to complete a series once started. In special circumstances such as stockouts or changing vaccination sites, vaccines from other manufacturers that are made with the same technology platform can be used to complete the schedule.

Infection and Antibody Tests

Testing for COVID-19 virus infection with PCR or antibody tests prior to COVID-19 vaccination is not recommended. Routinely testing for antibodies after vaccination to determine protection is not recommended.

Contraindications

COVID-19 vaccination is contraindicated for (a) people who are allergic to any of the components or excipients in the vaccine or substances used in the production process, or who have had allergic reactions after vaccination with similar vaccines; (b) people with a history of severe allergic reaction to any vaccine (e.g., acute allergic shock, angioneurotic edema, dyspnea, or laryngeal edema, etc.); (c) people with uncontrolled epilepsy and other serious progressive neurological diseases (e.g., transverse myelitis, GBS, or demyelinating diseases, etc.); (d) people with fever, acute illness, acute exacerbation of a chronic disease, or uncontrolled severe chronic disease; and (e) pregnant women.

COVID-19 vaccine package inserts (prescribing instructions) specify precautions and contraindications for several specific groups of people and these specific population can be considered as follows.

VACCINATION OF SPECIFIC POPULATIONS

Persons Aged 60 Years and Over

The risk of severe disease and death due to COVID-19 is greatest in persons aged 60 years and

over. The Phase III efficacy trials of the four conditionally approved COVID-19 vaccines had insufficient elderly subjects to make robust estimates of efficacy. Efficacy or effectiveness estimates for these vaccines are anticipated to be available in the future. Phase Ⅰ/Ⅱ clinical trial data for the five vaccines that have conditional approval or emergency use authorization showed that these vaccines were safe for this age group. Neutralizing antibody levels were slightly lower among elderly subjects than among subjects aged 18–59 year. However, seroconversion rates were similar, suggesting that these vaccines will be efficacious and effective in individuals aged 60 years and over. Therefore, these COVID-19 vaccines are recommended for use among people 60 years of age and older.

Children and Adolescents Below the Age of 18 Years

There are currently no efficacy or safety data for children or adolescents below the age of 18 years. Until such data are available, vaccination for individuals below 18 years of age is not recommended.

Persons with Comorbidities

Certain comorbidities have been identified as increasing the risk of severe COVID-19 disease and death. Vaccination is recommended for persons with comorbidities who are healthy or have conditions that are controlled well with medicine.

Childbearing Age and Lactating Women

Although these vaccines are currently contraindicated in pregnancy due to lack of clinical data and experience, they are anticipated to be safe for use in pregnancy. Pregnant women, at any stage of pregnancy, who are inadvertently vaccinated should not take any special action, including termination, because of the vaccination. Women who become pregnant after vaccination also should not take any specific action. Routine physical examinations during pregnancy and follow-up are recommended. Women planning pregnancy after getting vaccinated do not need to delay pregnancy.

Although there are currently no data about the impact of COVID-19 vaccine on breastfeeding child, on the basis of the safety profile of the above vaccines, a lactating woman who is otherwise recommended for vaccination, such as healthcare workers, should be offered vaccination. In reference to international practices, breastfeeding after vaccination is recommended as routine.

Immunocompromised Persons

Immunocompromised persons are at higher risk of severe disease and death due to COVID-19. Available data are currently insufficient to assess vaccine efficacy or safety in immunocompromised persons (such as persons with malignancies, nephrotic syndrome, and AIDS) and persons with HIV infection. It is possible that the immune response to vaccination may be reduced, lowering clinical effectiveness. Given the safety profiles of similar types of vaccines, inactivated COVID-19 vaccines and recombinant subunit vaccines are recommended for use. Regarding the adenovirus vectored COVID-19 vaccine, although the adenovirus is replication defective, there are no safety data for similar vaccines in this population. Immunocompromised persons may receive adenovirus vectored COVID-19 vaccine after assessing benefits and risks of the vaccine with the help of a health care provider.

Persons Who Have Previously Had COVID-19

Currently available data indicate that symptomatic reinfection within the first six months after an initial infection is rare. Persons who have previously had COVID-19 (symptomatic or asymptomatic) may receive one dose of any of the COVID-19 vaccines at least six months after infection.

OTHER CONSIDERATIONS

These recommendations will be updated upon approval of additional COVID-19 vaccines as more information is available from clinical trials, post-marketing surveillance, and evaluations, and as changes in the COVID-19 epidemiological situation warrant.

doi: 10.46234/ccdcw2021.083

Submitted: March 29, 2021; Accepted: March 30, 2021

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The inauguration of *China CDC Weekly* is in part supported by Project for Enhancing International Impact of China STM Journals Category D (PIIJ2-D-04-(2018)) of China Association for Science and Technology (CAST).

CCDC weekly

Vol. 3 No. 21 May 21, 2021

Responsible Authority
National Health Commission of the People's Republic of China

Sponsor
Chinese Center for Disease Control and Prevention

Editing and Publishing
China CDC Weekly Editorial Office
No.155 Changbai Road, Changping District, Beijing, China
Tel: 86-10-63150501, 63150701
Email: weekly@chinacdc.cn

CSSN
ISSN 2096-7071
CN 10-1629/R1