

False Coronavirus Disease 2019 Cases due to Contamination by Inactivated Virus Vaccine

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A false-positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse-transcription polymerase chain reaction result can lead to unnecessary public health measures. We report 2 individuals whose respiratory specimens were contaminated by an inactivated SARS-CoV-2 vaccine strain (CoronaVac), likely at vaccination premises. Incidentally, whole genome sequencing of CoronaVac showed adaptive deletions on the spike protein, which do not result in observable changes of antigenicity.

Keywords. inactivated COVID-19 vaccine; contamination; false positive.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can spread rapidly even in low-incidence areas, especially with the novel variants of concern [1,2]. Hence, aggressive public health measures, such as prompt isolation of patients,

quarantine of close contacts, or even city lockdown, are implemented even when a few cases are found.

With stringent public health measures for both incoming travelers and in the local community [3], the fourth wave of coronavirus disease 2019 (COVID-19) in Hong Kong ended in April 2021. However, in late May, 2 individuals without recent travel tested positive for SARS-CoV-2 by reverse-transcription polymerase chain reaction (RT-PCR). Investigation showed that the specimens from these 2 patients were likely contaminated by inactivated SARS-CoV-2 vaccine (CoronaVac). Here, we report the details of these 2 cases.

METHODS

Archived deep throat saliva (DTS) specimens that tested positive for SARS-CoV-2 by RT-PCR were retrieved. Environmental swabs from a general practitioner's (GP) clinic and a community vaccination center were collected as previously described [4]. CoronaVac (lot number A2021010034) and other vaccine batches were retrieved for whole genome sequencing (WGS) or viral load testing. As part of an emergency public health investigation, institutional review board approval was exempted for this study. Real-time RT-PCR, sequencing, bioinformatics analysis, phylogenetic analysis, and enzyme immunoassay were performed as we described previously [1, 2, 5, 6] (see [Supplementary Methods](#) for details).

RESULTS

Case 1

A 35-year-old man presented to a GP with 1-day history of cough, sore throat, and rhinorrhea in May 2021 ([Supplementary Table 1](#)). On the day of symptom onset, he visited a community vaccination center but was denied vaccination due to active respiratory symptoms. He had no epidemiologic link to any COVID-19 patients.

He was given a DTS collection pack by his GP ([Supplementary Figure 1](#)). The DTS specimen tested positive by N gene RT-PCR in a commercial laboratory (cycle threshold [Ct] = 16.5 [N1] and 17.2 [N2]). This specimen also tested positive by the Public Health Laboratory Services Branch (PHLSB) with RT-PCR against the RNA-dependent RNA polymerase (RdRp) gene (Ct = 18). The patient was hospitalized on day 3 post-symptom onset. All respiratory specimens collected after hospitalization tested negative for SARS-CoV-2 ([Supplementary Table 1](#)). His specimen also tested negative for other respiratory viruses by multiplex PCR panel; his daughter's specimen tested positive for parainfluenza virus 3 and rhinovirus/enterovirus, and his son's specimen tested positive for rhinovirus/enterovirus.

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Whole viral genome nanopore sequencing was performed for the positive DTS specimen. Four single-nucleotide variants (SNVs) were found in the patient's specimen, including C8782T (synonymous), C13170T (nsp10-T49I), C15480T (synonymous), and T28144C (ORF8-L84S) (Figure 1A). In addition, the patient's sequence contained 2 deletions in the spike protein, including the spike amino acid (aa) residues 68–76 (Δ 68–76) and 679–688 (Δ 679–688). Phylogenetic analysis showed that the patient's sequence clustered with the sequences of CoronaVac (Figure 1B). The patient's sequence was identical to the CoronaVac genome of SARS-CoV-2/human/CHN/CN2/2020 (MT407650.1) with 4 signature mutations [7], and 1 nucleotide different from SARS-CoV-2/human/CHN/CN1/2020 (MT407649.1) [8]. However, SARS-CoV-2/human/CHN/CN2/2020 and SARS-CoV-2/human/CHN/CN1/2020 do not have any spike gene deletions.

Because spike gene deletions are frequently found in SARS-CoV-2 isolates passaged in VeroE6 cells [9], we suspected that the deletion may also be found in the CoronaVac vaccine. WGS was performed on 1 vial of CoronaVac (lot number A2021010034). The sequence from this CoronaVac vaccine vial was 100% identical to the patient's sequence, containing all 4 SNVs and also the 2 spike deletions. Using RdRp/Hel quantitative RT-PCR, the viral load in the CoronaVac vaccine vial was found to be 1.02×10^{11} copies/mL.

Results from whole genome analysis suggested the possibility of contamination of the patient's DTS specimen with the CoronaVac vaccine strain. Environmental sampling was performed at the GP's clinic on day 4 post-symptom onset. Of the 18 environmental samples (Supplementary Table 2), only the swab from a sharps box tested positive by RdRp gene RT-PCR with a Ct value of 36, which is too low for WGS.

Four other individuals received CoronaVac vaccine on the same day before our patient attended the clinic. The GP would prepare the vaccine at the treatment area next to the refrigerator, and administer the vaccine at the consultation area. After vaccination, the nurse would peel off the label from the vaccine vial and stick it onto the patient's record. The same nurse prepared a DTS pack for our patient. Both the DTS packs and patient records were stored in a trolley inside the consultation room. Single-use gloves were not worn by either the doctor or the nurses, but hand hygiene with alcohol-based hand scrub was performed. Alcohol was used for disinfection of the working surface afterward.

Case 2

A 64-year-old female nurse, who worked in a community vaccination center, was found to have indeterminate result on her regular SARS-CoV-2 testing in late May 2021. The combined nasal and throat swab tested positive by RT-PCR targeting the ORF1ab (Ct = 41.29) and N (Ct = 39.4) genes in a commercial

laboratory. The same specimen tested positive at PHLSB by RdRp gene RT-PCR with a Ct value of 33.7.

The nurse remained asymptomatic throughout. All specimens collected after hospitalization, including combined nasopharyngeal–throat swab and DTS, tested negative by RT-PCR (Supplementary Table 3).

Environmental sampling was conducted at the community vaccination center where the patient worked. Among 43 environmental swabs taken from the vaccine storage rooms and injection station, 8 were RdRp gene RT-PCR positive with Ct values of >30 , and 3 were tested indeterminate (Supplementary Table 4 and Supplementary Figure 2). Alcohol wipes were used to clean the equipment and working environment while bleach was used to disinfect the floor and seats after each shift.

Since the viral load of the patient's specimen was too low, WGS was not performed. An SNV real-time RT-PCR showed that the patient had aspartic acid (D) at spike aa residue 614, suggesting that this is more related to the ancestral strain from Wuhan and vaccine virus. Partial sequencing of the RdRp/Hel region (nucleotide position 16240–16329) showed that the sequence was 100% identical to the CoronaVac vaccine strain.

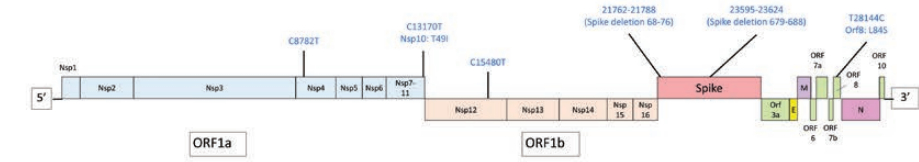
Vaccine Virus Mutation Investigations

Illumina sequencing of different batches of vaccine virus demonstrated that fractions of the 2 deletions (Δ 68–76 and Δ 679–688) were similar among different vaccine batches, implicating the relative stability of the vaccine variants (Supplementary Figure 3). The 2 deletions exhibit high fractions in the vaccines used for the phase 1–3 clinical trials, indicating that the safety and efficacy of the resulting vaccine should be consistent with the published report by SinoVac. Enzyme immunoassay showed that the Δ 68–76 mutation does not alter the binding the spike protein by most N-terminal domain-targeting antibodies or convalescent serum spike-targeting antibodies (Supplementary Figure 4).

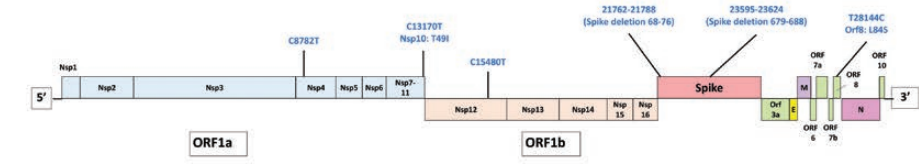
DISCUSSION

In this study, we presented 2 “false-positive” cases of COVID-19 due to contamination of the specimens by an inactivated virus vaccine strain. We postulated that the contamination of the patients' specimens occurred at the time of specimen collection. Air is often injected into the vaccine ampoule to create a positive pressure for easier withdrawal of vaccine into the syringe. When the syringe needle was withdrawn from the vaccine ampoule, a small jet of vaccine could be created, which contaminated the cap and outer surface of the vial. Since the viral load is very high in the vaccine vials, even a microliter amount would contain a large amount of viral genomic RNA. The vaccine virus may have been transferred to specimen collection bottles via the hands of nurses who handled the vaccine vial. In both situations, alcohol was used for disinfection of the operators' hands

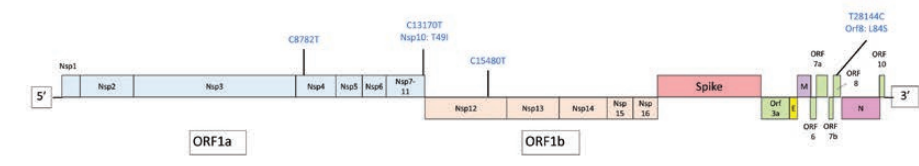
A
Case 1



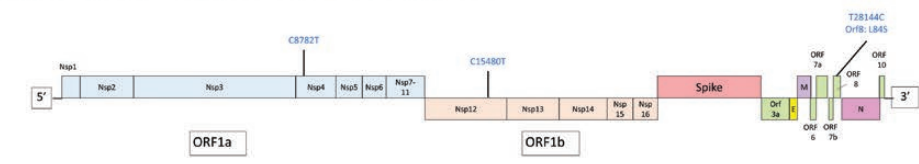
CoronaVac



SARS-CoV-2/human/CHN/CN2/2020



SARS-CoV-2/human/CHN/CN1/2020



B

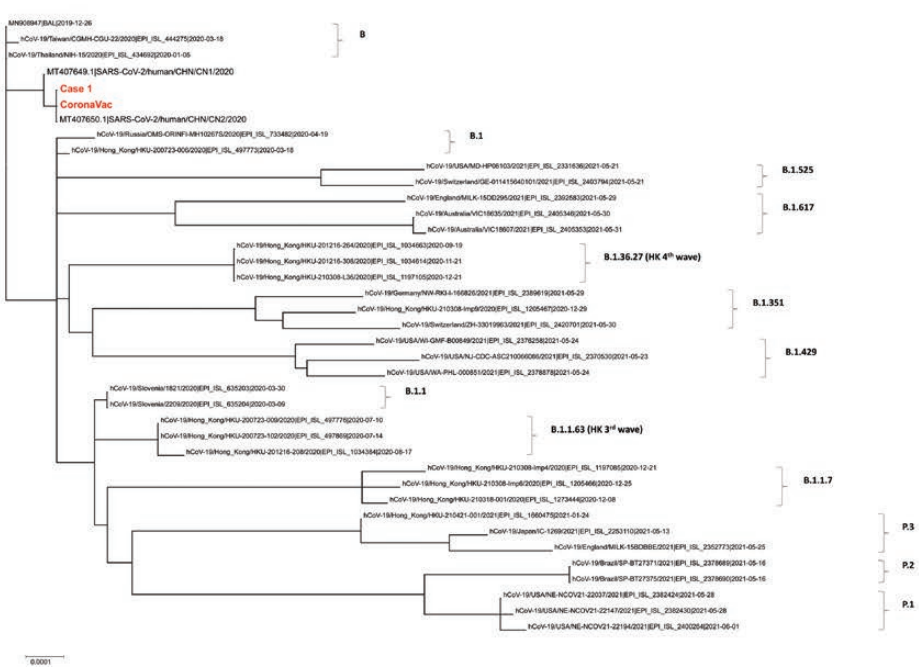


Figure 1. Whole viral genome analysis of case 1. *A*, Schematic diagram showing the mutations of case 1, CoronaVac vaccine strain (sequenced in this study), and published sequences of CoronaVac (SARS-CoV-2/human/CHN/CN2/2020 and SARS-CoV-2/human/CHN/CN1/2020). Wuhan-Hu-1 (GenBank accession number MN908947.3) was used as the reference genome. *B*, Whole genome phylogenetic analysis showing the relationship between case 1, CoronaVac vaccine sequences, and genomes from different Pango lineages. The trees were constructed by maximum likelihood method. The reference genome Wuhan-Hu-1 (GenBank accession number MN908947.3) is used as the root of the tree. Abbreviations: HK, Hong Kong; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

and working surfaces, which was unable to destroy the nucleic acid residues, causing biologically true-positive tests but clinically false-positive cases.

The absence of spike protein D614G mutation in the positive specimens from both cases is highly suggestive of their origin from the vaccine strain, since almost all currently circulating SARS-CoV-2 strains contain the D614G mutation [10]. Interestingly, the positive specimen from case 1 contained 2 deletions in the spike protein, which were not reported in the CoronaVac genome sequences but were present when we did WGS of a vial of CoronaVac vaccine. The spike Δ 679–688 is located at the S1/S2 junction. The N501Y lineage with Δ 69/70 has been circulating in the United Kingdom since September 2020. Previous studies showed that the S1/S2 deletion is readily seen during passage in VeroE6 cells and conferred enhanced growth and stability in vitro [9]. However, our investigations showed that the mutations in the vaccine virus should not affect the antigenicity of the virus.

False-positive diagnosis caused by COVID-19 vaccine has not been previously reported. However, there have been several reports of asymptomatic researchers who handled noninfectious SARS-CoV-2 nucleic acids and subsequently tested positive on surveillance screening [11]. The transfer of nucleic acids can occur via contaminated surface or objects, with recent research showing large quantities of highly stable DNA amplicons in the workspaces used for SARS-CoV-2 nucleic acid amplification, shared equipment, refrigerators, and freezers [12]. Such positive results will lead to unnecessary follow-up investigations, hospitalization, contact tracing, and patient and societal anxiety. Similar incidents might occur with messenger RNA or DNA vaccines. Adequate hygienic measures should be taken during vaccine preparation and administration to avoid contamination of hands if medical personnel need to handle specimens for SARS-CoV-2 testing. Chlorine-based disinfectant should be used for environmental disinfection after each shift to destroy the nucleic acid residues.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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