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Case Report

# First Case of Genetically Confirmed Travel Related COVID- 19 Re Infection in Oman

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## Abstract

A 45 years old Omani female, living in Muscat Governorate presented to Al Khuwair Health Center on February 23, 2020 with symptoms of dry cough and sore throat which started soon after she returned back from Iran (Qom). She has no history of fever, runny nose or loss of smell. Swabs were taken on 23 February 2020 and sent to Central Public Health Laboratory in Darsait, Muscat. She was tested positive for SARS-CoV-2 and announced to be the 1st case of COVID 19 in Oman. In October 2020, she travelled to Iraq and tested positive for COVID 19 on arrival to Oman which was confirmed to be a different variant of SARS-CoV-2 using whole genome sequencing method (WGS). Reporting and studying the potential of re-infection is important to facilitate our understanding on the degree of protective immunity.

## Background

The Covid-19 pandemic has severely disrupted the healthcare system and socioeconomic activities. COVID-19 continues to spread worldwide despite strict control measures since it started in Wuhan, China in December 2019 [1]. Moreover, resurgence of COVID-19 cases is seen in many areas after relaxation of social distancing policies [1] and emerging of new SARS-CoV-2 variants all of which raised a high level of concern all over the world.

It was expected that individuals develop protective immunity after recovering from COVID-19 infection, however, the first case of documented reinfection of COVID-19 in August 2020 showed the opposite [2]. Up to date, the evidence regarding duration and level of protection after SARS-CoV-2 infection is still under studying. Worldwide there are many cases reported with re-infection. However, many cases are lacking genomic confirmation of re-infection with different strains. To our knowledge, we are reporting the first genetically confirmed case of COVID 19 re-infection in Oman.

## Case Presentation

A 45 yr old Omani female, living in Muscat Governorate presented to Al Khuwair Health Center on February 23, 2020 with symptoms of dry cough and sore throat which started soon after she returned back from Iran (Qom). She has no history of fever, runny nose or loss of smell. On examination, a febrile, pulse 74 beat/minutes, respiratory rate 16, saturation 99% in room air, chest examination revealed no abnormal finding All other systemic examinations were normal. Swabs were taken on 23 February 2020 and sent to Central Public Health Laboratories, the national reference laboratory for

COVID-19 testing in Darsait, Muscat. She was tested positive for SARS-CoV-2 and announced to be the 1st case of COVID 19 in Oman. She was kept on home quarantine/ isolation for 14 days, ran a mild course of the disease, managed with symptomatic treatment and close monitoring. As part of de-isolation policy at that time, PCR test was repeated on 14th March 2020 and reported negative.

Later, she travelled to Iraq and pre-travel SARS-CoV-2 PCR test in Oman, on 5th October, was negative and she was tested again before leaving Iraq on 10th October and again was negative. On arrival to Oman, the test was done in Muscat International Airport as a requirement for country entry and reported positive on 12th October, despite being asymptomatic. This was seven months and 18 days after the initial positive test. Patient presented to the health center on 18th October 2020 with runny nose but with no history of fever, cough or shortness of breath. On examination, temperature 37 C, BP 150/90, respiratory rate 20, saturation 99 % in room air and chest examination was normal. Swabs taken and reported positive Timeline of the case presentation and laboratory results are shown in **figure 1**.

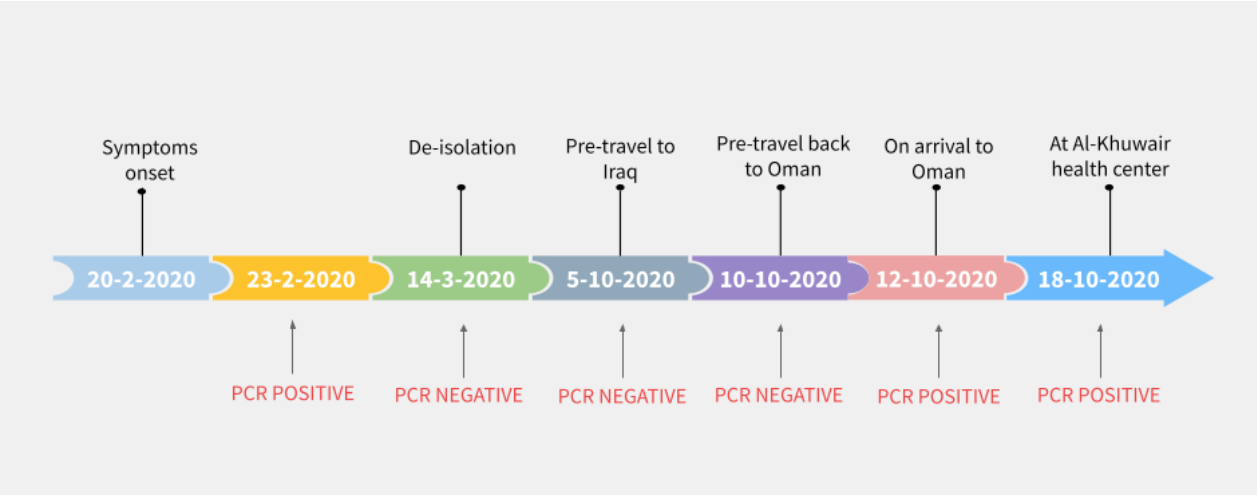


**Figure 1:** Timeline of SARS COV2 PCR Results

The patient made a good recovery after 14 days of home quarantine with close follow up by AL-Khuwair health center team.

### Past medical problem

Known case of Asthma on prophylaxis, Hypertension, Hyperlipidemia, CKD stage 2 on treatment.

## Investigations

Nasopharyngeal swabs were collected and transported in viral transport medium to Central public Health laboratories. There, the viral RNA was extracted using Viral RNA Isolation Kit with Liferiver EX3600 (Liferiver Biotech, Hangzhou Bay, China), following manufacturer protocol. A SARS-CoV-2 real time RT-PCR test was performed on the RNA extract and found to be RT-PCR positive. For the first infection, which was performed on a sample collected on 23rd Feb 2020, the specimen tested positive with cycle threshold values for N-gene, E-gene and RdRp-gene, 22.3, 19.1 and 22.6, respectively. The LightMix Modular SARS-CoV-2 test (TIB Molbiol) was used on the QuantStudio5 instrument following manufacturer instructions, adopted from Drosten et al. [3]. For the second infection, specimen tested positive for N2 gene and ORF1 ab genes with cycle threshold values; 24.7 and 25.6, respectively using SANSURE BIOTECH Novel Coronavirus (2019-nCoV) kit from Sansure Biotech Inc (Hunan, China).

Whole genome sequencing (WGS) of the sample obtained from the first infection was carried out using the Illumina Nextseq 500 platforms (Macrogen, Korea). Next generation sequencing for the second sample was performed using the Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific, Waltham, MA, USA).

SARS-CoV-2 genome sequences of both specimens were aligned to the reference SARS-CoV-2 genome (GenBank NC_045512.2) and other SARS-CoV-2 genomes available in the GlSAID using Clustal-W multiple alignments (BioEdit software). The accession IDs are included in Supplementary Table 1.

For phylogenetic analysis, phylogenetic tree was inferred with the maximum likelihood method using the Tamura-Nei substitution model and 1000 bootstrap replicates using MEGA X [4].

symptomatic individuals during the acute phase. In addition, forty percent of asymptomatic individuals became seronegative during the early convalescent phase. In addition, it was reported that patients with severe COVID-19 both seroconvert earlier and develop higher concentrations of SARS-CoV-2-specific IgG than patients with mild symptoms [5]. This could be a possible reason for asymptomatic/mild recovered individuals to become susceptible to reinfection.

Moreover, the characteristics of the reported cases of COVID 19 reinfection vary in disease severity between the first and the second episode. Among these, 68.8% (11/16) had similar severity, 18.8% (3/16) had worse symptoms and 12.5% (2/16) had milder symptoms during the second episode compared with the first episode. In this report, the patient was apparently immune-competent which is similar to the 17 reported cases of covid19 reinfection, published in BMJ March 2021with only one of the cases was immunocompromised [2].

Cases of reinfection were reported in Qatar [6], Ecuador [7] and USA [8,9] with increased severity of symptoms. However, Cases from Hongkong [2], Belgium [10] and Netherlands did not show difference in severity of symptoms.

We report the first reinfection case that was confirmed by RT-PCR as well as the first reported COVID-19 case in the country. This patient was back in a trip from Iran where two family members were also infected. Strict public health measures were undertaken and patient was home quarantined for 14 days, which did not lead to further transmission of infection (Intisar to comment). Our patient had mild course of the disease during both episodes. She showed complete recovery after both infections with three negative PCR tests between the two episodes. Both episodes were travel related.

Whole genomic sequencing was performed which confirmed infection with two different viral strains. Seven months and 18 days was the time frame between the first and second episode.

Further investigation on the immune response posts the first infection is encouraged to explain Why do some reinfections result in a milder disease and others in more severe disease. In addition, host and viral genetic interaction may play a role contributing to the disease course post infection with SASRS-CoV-2.

Genome sequences from the two samples were deposited in the Global Initiative on Sharing All Influenza Data database (GISAID) with accession numbers EPI_ISL_457701 and EPI_ISL_1517192.

Phylogenetic analysis in figure 2 revealed the existence of two distinct lineages of the two specimens where the first specimen belonged to clade B4 (O) and the second sample clustered with sequences belonged to clade B1.36 (GH). Further analysis of the variant profile for both genomes; have shown that the two specimens had two distinct variant distributions with no common variants between the two samples. The first sample had only four amino acid changes when compared with the hCoV-19/Wuhan/WIV04/2019 reference strain; NSP6 L37H, NSP2 R27C & V198I and NSP4 M33I. Indicating a close similarity when compared with the viral strains identified early during the pandemic.

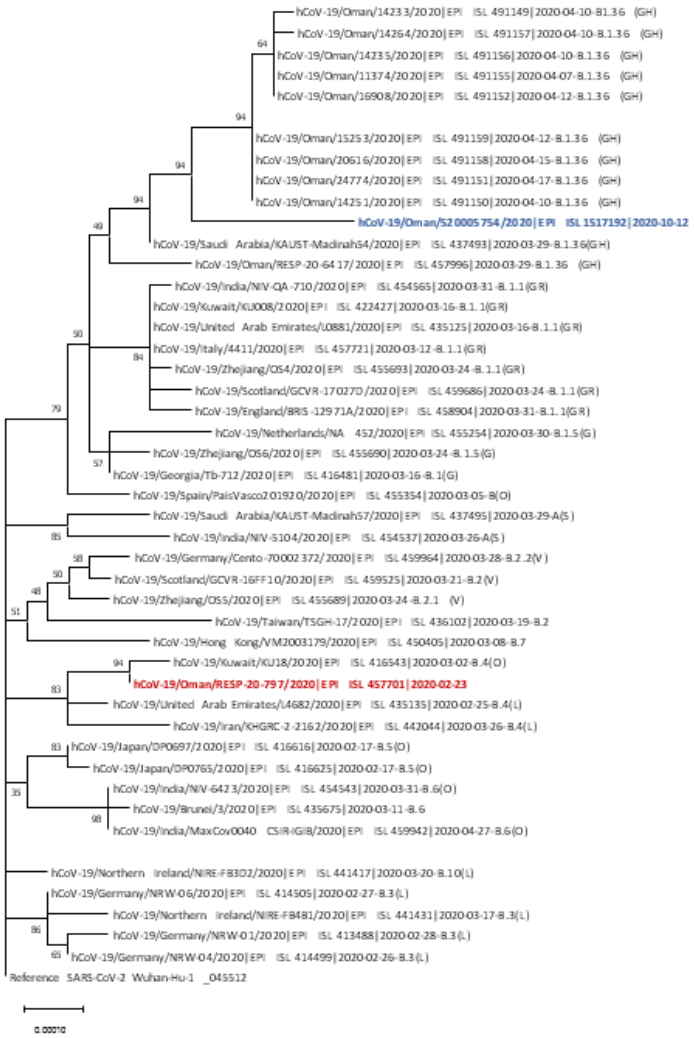


**Figure 2:** Phylogenetic analysis of full-length SARS-CoV-2 genomes including the first infection episode (in red) and the second episode (in blue), other representative of different viral clades and reference genome (SARS-CoV-2 _045512).

During the second episode, the isolated viral strain belonged to clade (GH), with 14 variants compared with the hCoV-19/Wuhan/WIV04/2019 reference strain. In Particular, the D614G substitution in the spike protein, the Q57H substitution in the ORF-

3a and the P323L in the NSP12 which corresponds to another clade (GH). The phylogenetic analysis included also sequences obtained from a cluster reported in April 2020 [14], it was observed from the analysis that the reinfection strain was not very closely related to the outbreak viral strains of the same clade (GH).

Unfortunately, serum sample was not obtained after the first episode of infection, therefore we could not assess the immune response as well as estimate the effectiveness of the immune response.

From this case, its evident that reinfection is possible in the presence of different strains that has the potential to evade immunity elicited by either natural infection or by vaccination. Furthermore, the level and duration of immunity after infection still uncertain. For better understanding of post infection immunity, further future surveillance is required.

**Take Home Messages**

1. Confirming re-infection requires genomic analysis.
2. This case approves that re-infection with COVID19 is possible as there are different strains of SARS-CoV-
3. It's needed to continue the precaution measures and social distancing even if a person had recovered from COVID19 infection.

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