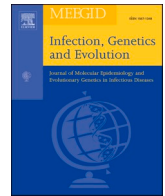




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Short communication

Two prolonged viremic SARS-CoV-2 infections with conserved viral genome for two months



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ABSTRACT

We document two cases of viremic and prolonged active infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) where the viral genome was conserved for two months, but infection was with little or no symptoms. The first infection persisted for 80 days and the second for 62 days. Clearance of infection occurred 40 and 41 days, respectively, after development of detectable antibodies. Both cases were identified incidentally in an investigation of reinfection in a cohort of 133,266 laboratory-confirmed infected persons.

Characterized by high infectiousness (He et al., 2020a; MIDAS Online COVID-19 Portal, 2020), the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to disruptive epidemics globally (De Walque et al., 2020; COVID-19 Outbreak Live Update, 2020). The epidemic in Qatar, a peninsula in the Arabian Gulf region, was pervasive with broad exposure to the infection and >60,000 laboratory-confirmed infections per million population (Abu-Raddad et al., 2020a; Al Kuwari et al., 2020; Jeremijenko et al., 2020).

Marked by a centralized and standardized data-capture system of all SARS-CoV-2 data and a well-resourced Coronavirus Disease 2019 (COVID-19) healthcare, Qatar provides an opportunity for conducting epidemiological investigations at a national level that are difficult to do

elsewhere. Recently, we completed an assessment of the risk of reinfection and confirmation of reinfection with viral genome sequencing in a cohort of 133,266 SARS-CoV-2 laboratory-confirmed infected persons (Abu-Raddad et al., 2020b). The study incidentally identified two cases of prolonged active infection with conserved virus for about two months (Abu-Raddad et al., 2020b).

In-depth retrospective case investigation was completed for these two cases. We retrieved their SARS-CoV-2 testing and hospitalization records from the centralized and standardized national database maintained at Hamad Medical Corporation (HMC), the nationally-designated provider for all COVID-19-related healthcare needs. The study was approved by HMC and Weill Cornell Medicine-Qatar Institutional

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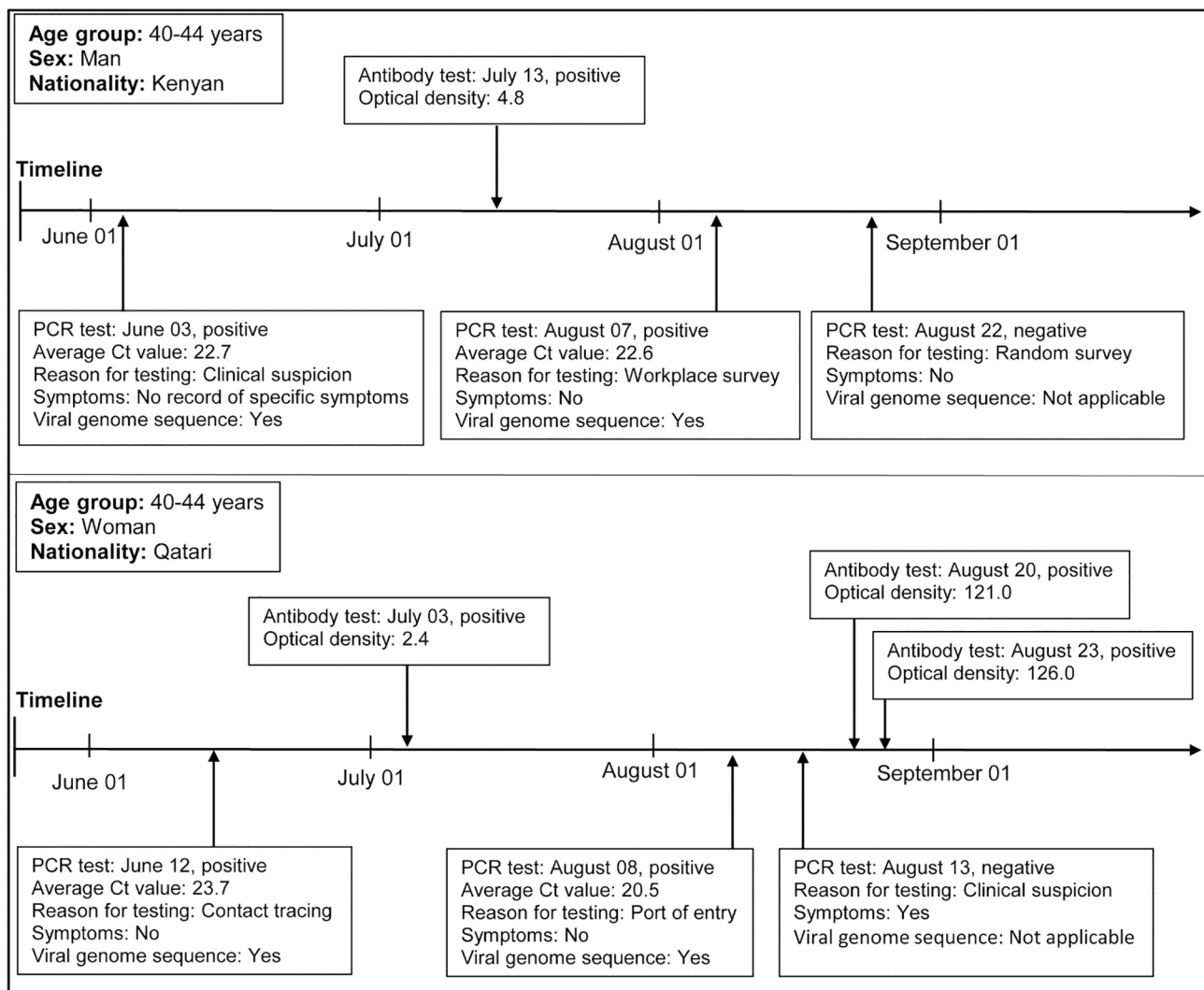


Fig. 1. History of SARS-CoV-2 infection in the two prolonged active-infection cases with high viral RNA concentration and conserved viral genome.

Review Boards.

Viral genome sequencing was conducted using an amplicon-based strategy. Viral genome differences were detected using standard single nucleotide polymorphism (SNP) calling in order to collect information on allele balances and ensure that quasi-species sites were well documented. Nucleotide differences between strains were determined using a collection of samples from the cohort of SARS-CoV-2 laboratory-confirmed infected persons as a reference (Abu-Raddad et al., 2020b). Differences between the viral strains collected from the same patient at different time points (deviations over the course of infection) was the main goal in this study rather than to investigate evolutionary changes across the population of patients. Each of the two cases reported here had only two polymerase chain reaction (PCR) positive samples; no sequential samples were available for further analysis. To ensure that time-course viral samples indeed derived from the same patient, we sequenced residual DNA in the samples from both time-points (prior to DNase treatment) using Accel-Amplicon® Panel for 15 genes of the human genome (Swift Biosciences, Ann Arbor, MI, USA; Cat. No. CP-CQ6110-1008) as per the manufacturers' recommended protocol. The residual human DNA in each swab serves as an internal control system to identify the patient from whom the swab was collected. Sequencing of the 15 genes and documentation of variable sites (SNPs) in the amplicons showed that there was high correlation between the human DNA sequences of the first and second swabs for the patients tested. Given that only 17 SNPs were compared, this cannot completely exclude swabs

originating from other patients, however, the chances given these internal control results are not likely.

Fig. 1 summarizes the investigation's key results. Case 1 was a Kenyan man in the 40–44 years age group, with a history of controlled hypertension, who first underwent PCR testing for SARS-CoV-2 on June 3, 2020 based on clinical suspicion of infection, but no record was found of specific symptoms. His test result was positive with an average cycle threshold (Ct) value of 22.7 across the different target proteins using the Roche cobas® SARS-CoV-2 Test (Roche, Switzerland). Per guidelines, the person was in isolation for two weeks, but was not retested during this period. Serological testing performed 40 days later (on July 13, 2020) using the Roche Elecsys® Anti-SARS-CoV-2 (Roche, Switzerland) yielded an anti-SARS-CoV-2 positive result, but with a low optical density of 4.8 (result interpretation per manufacturer's instructions: reactive at optical density cut-off index ≥ 1.0 and non-reactive at cutoff index < 1.0) (The Roche Group, 2020).

This person was PCR re-tested for SARS-CoV-2 infection on August 7, 2020 (65 days after the first positive swab) as part of a workplace testing. Although asymptomatic at this time, he was found PCR positive with an average Ct value of 22.6 using the TaqPath™ COVID-19 Combo Kit (Thermo Fisher Scientific, USA) on a ABI 7500 FAST (Thermo Fisher, USA). Of note that the choice of platform for PCR testing (Roche cobas® SARS-CoV-2 Test versus TaqPath™ COVID-19 Combo Kit) depended on instrument availability within the laboratory—test results for this analysis were retrieved retrospectively. Viral genome sequencing of the

Case	Positive swab	Swab date	241	373	1302	2844	2788	2878	3037	3373	3695	6181	6817	7733	9841	10995	12695	14408	14805	15315	15486	15672	16537	16989	17193	17550	17907	20870	21712	21737	21846	22015	23003	24675	24926	25207	25490	25552	25563	25704	26144	26801	26963	27341	27476	27769	28054	28087	29144	29370	29554	29642
1	First	3-Jun	C	T	C	C	C	T	C	C	T	A	A	G	T	C	T	C	C	A	G	R	C	G	N	G	A	T	C	T	B	A	G	C	C	G	T	T	G	N	A	A	C	C	C	C	C	T	C			
	Last	7-Aug	C	T	C	C	C	T	C	C	T	A	A	G	T	C	T	C	C	A	G	R	C	G	N	G	A	T	C	T	B	A	G	C	C	G	T	T	G	N	A	A	C	C	C	C	C	T	C			
2	First	13-Jun	C	T	C	C	C	T	C	C	T	A	A	G	T	C	T	C	C	A	G	R	C	G	N	G	A	T	C	T	B	A	G	C	C	G	T	T	G	N	A	A	C	C	C	C	C	T	C			
	Last	8-Aug	C	T	C	C	C	T	C	C	T	A	A	G	T	C	T	C	C	A	G	R	C	G	N	G	A	T	C	T	B	A	G	C	C	G	T	T	G	N	A	A	C	C	C	C	T	C				

Fig. 2. Viral genome sequencing analysis of the paired viral specimens of the first and last positive swabs for the two active-infection cases with high viral RNA concentration for two months.

first and subsequent positive swabs indicated infection with the same virus with no evidence for reinfection (Fig. 2). A third PCR test performed two weeks later (on August 22, 2020) was conducted as part of a random testing survey, but he was PCR negative. No specific COVID-19 treatment was administered and no hospitalization occurred at any time indicating the infection was of mild severity. No other clinical or contact-tracing data relevant to explain the prolonged infection or its infection transmission implications were available.

Case 2 was a Qatari woman in the 40–44 years age group who was on treatment for hypertension using bisoprolol and more recently amlodipine/perindopril (post-infection), hypothyroidism using levothyroxine, and pre-diabetes using metformin. She was first identified as PCR positive on June 12, 2020 during contact tracing, with an average Ct value of 23.7 across the different target proteins, assessed using the TaqPath™ COVID-19 Combo Kit (Thermo Fisher Scientific, USA) on a ABI 7500 FAST (Thermo Fisher, USA). She was asymptomatic. Per guidelines, the person was in isolation for two weeks, but was not retested during this period. Serological testing performed 21 days later (on July 03, 2020) using the Roche Elecsys® Anti-SARS-CoV-2 (Roche, Switzerland) identified detectable antibodies with a low optical density of 2.4.

A subsequent PCR test performed on August 8, 2020 (57 days after the first positive swab) at port of entry (upon returning from travel abroad) yielded a positive result, with absence of symptoms, but with an average Ct value of 20.5 using the Thermo Fisher platform. Viral genome sequencing of the first and subsequent positive swabs indicated infection with the same virus with no evidence for reinfection (Fig. 2). The woman was re-tested few days later (on August 13, 2020) for clinical suspicion with symptoms suggestive of an upper respiratory tract infection, but was found PCR negative. No further case investigation (PCR testing or clinical follow-up) was performed. Two subsequent serological tests performed on August 20 and August 23 yielded anti-SARS-CoV-2 positive results with high optical densities of 121.0 and 126.0, respectively. No specific COVID-19 treatment was administered and no hospitalization occurred at any time indicating that infection was of mild severity. No other clinical or contact-tracing data relevant to explain the prolonged infection or its infection transmission implications were available.

Based on current understanding of the natural history and infectiousness profile of SARS-CoV-2 infection, individuals typically develop latent infection for about 4 days (Lauer et al., 2020; Li et al., 2020b; Rothe et al., 2020; Zou et al., 2020), and then transition into an infectious period of about four days (He et al., 2020b; Li et al., 2020b; Rothe et al., 2020; Zou et al., 2020). A fraction of individuals develop symptoms typically about two days after transitioning into the infectious period, the time around which SARS-CoV-2 viral RNA concentration starts declining (Centers for Disease Control and Prevention, 2020; He et al., 2020b). The infectious period is sometimes followed by a prolonged PCR positivity period at high Ct value (>30) that may extend for several weeks, but such PCR positivity reflects very low level of viral replication, or more often non-viable remnants of the virus (Sethuraman et al., 2020; Wajnberg et al., 2020).

However, our cases had evidence for viremic prolonged PCR positivity with a conserved virus at high enough viral concentration that was conducive for quality genome sequencing (Fig. 2). Clearance of infection occurred 40 and 41 days, respectively, after first evidence of detectable antibodies (Fig. 1). With the low optical density in the first serological test, but very high optical density weeks later (Fig. 1), development of detectable antibodies appears to have been a slow process, particularly

for case 2 for whom there were records for three serological tests. Our findings concur with observations from other studies documenting viremic and prolonged PCR positivity in similarly immunocompetent patients (Hong et al., 2020; Li et al., 2020a).

In conclusion, we documented two cases of viremic and prolonged active SARS-CoV-2 infection with a conserved viral genome for over two months, but with little or no symptoms. Although this is of concern given implications on current guidelines for isolation and discharge from isolation (World Health Organization, 2020), these occurrences are likely rare, as they were identified incidentally in an investigation of reinfection in a cohort of 133,266 SARS-CoV-2 laboratory-confirmed infected persons (Abu-Raddad et al., 2020b). Onward infection transmission and other public health consequences of such cases remain unknown.

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Author contributions

LJA conceived and designed the study and led the analyses. HC performed the data analyses and wrote the first draft of the article. JAM led the viral genome sequencing analyses and AAA, YAM, and SY conducted these analyses. All authors contributed to data collection and acquisition, database development, discussion and interpretation of the results, and to the writing of the manuscript. All authors have read and approved the final manuscript.

Credit authorship contribution statement

Laith J. Abu-Raddad: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Project administration. Hiam Chemaitelly: Formal analysis, Investigation, Data curation, Writing - original draft. Joel A. Malek: Formal analysis, Investigation, Writing - review & editing. Ayeda A. Ahmed: Formal analysis, Investigation, Writing - review & editing. Yasmin A. Mohamoud: Formal analysis, Investigation, Writing - review & editing. Shameem Younuskuju: Formal analysis, Investigation, Writing - review & editing. Zaina Al Kanaani: Data curation, Writing - review & editing. Abdullatif Al Khal: Data curation, Writing - review & editing. Einas Al Kuwari: Data curation, Writing - review & editing. Adeel A. Butt: Data curation, Writing - review & editing. Peter Coyle: Data curation, Writing - review & editing. Andrew Jeremijenko: Data curation, Writing - review & editing. Anvar Hassan Kaleeckal: Data curation, Writing - review & editing. Ali Nizar Latif: Data curation, Writing - review & editing. Riyazuddin Mohammad Shaik: Data curation, Writing - review & editing. Hanan F. Abdul Rahim: Data curation, Writing - review & editing. Hadi M. Yassine: Data curation, Writing - review & editing. Mohamed G. Al Kuwari: Data curation, Writing - review & editing. Hamad Eid Al Romaihi: Data curation, Writing - review & editing. Mohamed H. Al-Thani: Data curation, Writing - review & editing. Roberto Bertollini: Data curation, Writing - review & editing.

Declaration of Competing Interest

We declare no competing interests.

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