Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Clinical Virology Plus 1 (2021) 100015

Contents lists available at ScienceDirect

Journal of Clinical Virology Plus

journal homepage: www.elsevier.com/locate/jcvp

Short communication

Comparative genomic analysis demonstrates that true reinfection following SARS-CoV-2 infection is possible

Eamon O Murchu[a,b,*], Sinead O'Neill[a], Paula Byrne[a], Cillian De Gascun[c], Michelle O'Neill[a], Máirín Ryan[a,d,1], Patricia Harrington[a,1]

[a] Health Information and Quality Authority, George's Court, George's Lane, Dublin 7, Ireland
[b] Trinity College Dublin, Institute of Population Health, Tallaght, Dublin 24, Ireland
[c] UCD National Virus Reference Laboratory, Dublin, Ireland
[d] Department of Pharmacology & Therapeutics, Trinity College Dublin, Trinity Health Sciences, Dublin 8, Ireland

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Coronaviruses

ABSTRACT

Background: In recent months, multiple cases of confirmed SARS-CoV-2 reinfection have been reported. However, accurate epidemiological and virological data, including genomic analysis where possible, are required to differentiate cases of prolonged viral RNA shedding (i.e. intermittent detection) from true reinfection. The objective of this review was to systematically identify and summarise all cases of SARS-CoV-2 reinfection confirmed by comparative genomic analysis.

Methods: A protocol based on Cochrane rapid review methodology was employed. Databases and pre-print servers were searched until 9/11/2020.

Results: Ten studies, representing 17 patients, were identified (mean age=40; 71% male). The time interval between primary infection and reinfection ranged from 13 to 142 days (median: 60).

Comparative whole genome sequencing confirmed reinfection in 14 patients (the primary and secondary infections were caused by different viruses). A further three cases had strong, but not confirmed evidence of reinfection, as only partial genomes were retrieved on primary infection.

Across 12 studies that reported the number of single nucleotide polymorphisms (SNPs) comparing the first and second genomes, between 8 and 24 SNPs were discovered. With an average SARS-CoV-2 mutation acquisition rate of 1–2 per month, in all cases it is likely that the secondary infection was caused by a different SARS-CoV-2 virus, rather than prolonged shedding of viral RNA from the primary infection.

In five reinfection cases, the primary and secondary infections were caused by different SARS-CoV-2 lineages/clades, strongly indicating that infections were caused by different viruses.

Conclusion: Comparative genomic analyses from 14 patients confirm that SARS-CoV-2 reinfection can occur.

1. Background

Accurate epidemiological and virological data, including genomic analysis where possible, are required to differentiate cases of prolonged viral RNA shedding from true SARS-CoV-2 reinfection. In previous reviews conducted by our team at the Health Information and Quality Authority (HIQA), no true cases of reinfection were identified [1]. In recent months, however, multiple cases of confirmed reinfection have been reported. We therefore conducted a review of the literature to characterise all true cases of reinfection based on comparative genomic analysis.

2. Study design

A standardised protocol was employed [2], based on Cochrane rapid review methodology guidance. Electronic databases (PubMed, EMBASE and EuropePMC) and pre-print servers (medRxiv, bioRxiv) were searched until 9/11/2020.

* Corresponding author at: Health Information and Quality Authority, George's Court, George's Lane, Dublin 7, Ireland.
E-mail addresses: eomurchu@hiqa.ie (E. O Murchu), soneill@hiqa.ie (S. O'Neill), pbyrne@hiqa.ie (P. Byrne), cillian.degascun@ucd.ie (C. De Gascun), moneill@hiqa.ie (M. O'Neill), mryan@hiqa.ie (M. Ryan), pharrington@hiqa.ie (P. Harrington).
[1] Both authors are co-senior authors.

https://doi.org/10.1016/j.jcvp.2021.100015
Received 6 February 2021; Received in revised form 21 April 2021; Accepted 23 April 2021

3. Results

Database searches retrieved 3272 unique citations. Following screening and full text review (in duplicate), 10 studies, representing 17 patients, met our inclusion criteria (Table 1) [3–12]. These included two case series reporting on six patients in India [3,11], one case series reporting on four patients in Qatar [9], three case studies reporting on patients in the US [6,10,12] and four case studies reporting on patients in Belgium [8], Ecuador [5], Hong Kong [7] and the Netherlands [4].

The mean age of patients was 40 (range: 25 to 89) and 71% (*N*=12) were men. The time interval between infection events ranged from 13 to 142 days (median: 60). This interval represented the time from recovery from primary infection (i.e. first negative RT-PCR test) to the onset of the secondary infection (i.e. first documented symptoms or first RT-PCR positive test in asymptomatic cases); when detailed information was not provided, the time interval reported by study authors was used. Across all cases, severity ranged from asymptomatic to severe on both primary infection and on reinfection. There was one fatality [4] which occurred in a patient who was severely immunocompromised. All other patients appeared to be immunocompetent. Of the four studies that reported cycle threshold (Ct) values from both episodes of infection, three patients had lower Ct values on reinfection [3,4] and two had higher Ct values [6,10].

Whole genome sequencing (WGS) confirmed that primary and secondary infections were caused by different viruses in 14 cases, although the degree of separation between infections varied (Table 1). Three apparent reinfections could not be confirmed by sequencing due to insufficient genetic material extracted from the primary infection (partial genomes). However, genetic evidence consistent with reinfection was still present.

With the exception of one study [9], all confirmed cases included a quantification of the number of single nucleotide polymorphisms (SNPs) comparing primary and secondary infections (range: eight [11] to 24 [7] differences, Table 1). In addition, five studies presented stronger evidence of reinfection through phylogenetic analysis; in each case the primary and secondary infections belonged to different SARS-CoV-2 lineages or clades [5,7,8,10,11].

Mutations that result in the D614G amino acid change in the spike protein were present on reinfection in four studies [7,9,10,12]. Additionally, rare mutations leading to an amino acid (AA) change in a single viral lineage were identified in some studies, such as NSP6 and L142F [7].

In terms of antibody testing, only two studies performed IgG testing at both infection events. In the first case, anti-SARS-CoV-2 IgG was not detected at primary infection, or at four days post-symptom onset; however, IgG was detected at reinfection and 30 days post-symptom onset [5]. The timing of testing may have impacted the findings, however, as the first sample may have been taken prior to seroconversion taking place. In the second case, anti-SARS-CoV-2 IgG was not detected at the initial infection, or at 10 days post-symptom onset [7]. However, seroconversion occurred following the reinfection event, with IgG not detected at serial testing on days 1–3 post-hospitalisation, but detected on day five post-hospitalisation. No study investigated neutralising antibody profiles or cell-mediated immunity.

Only one study assessed the rate of reinfection [9]. In this study, potential reinfection cases were identified among a larger cohort of 133,266 laboratory-confirmed SARS-CoV-2 infections. The authors estimated the risk of reinfection to be 0.01% (95% CI: 0.01–0.02%).

4. Discussion

While clinical and epidemiological factors are important in the assessment of possible SARS-CoV-2 reinfections, comparative genomic analysis provides the best evidence. This review identified 14 individual patients with confirmed SARS-CoV-2 reinfection.

Strong evidence of reinfection exists if sequences recovered from the two infectious events belong to different genetic clades or lineages [13]. However, even if viral strains are from the same clade or lineage, differences in the number of SNPs may indicate different viruses. The virus is known to mutate by 1–2 nucleotides per month [14]. Therefore, if infecting viruses differ by more mutations than this over a given timeframe, it increases the likelihood that the infections are from genuinely different origins, rather than a virus that has evolved in the setting of a persistent infection. All reinfections in this review recorded a greater number of mutations than would have been expected to occur through natural viral evolution.

The analysis of specific genetic mutations provides additional insight into reinfection events, such as mutations that result in the D614G AA change in spike protein. The D614G AA change was found on reinfection in four studies [7,9,10,12]. This AA change defines the SARS-CoV-2 variant with greater replicative fitness [15] and is now present in most circulating SARS-CoV-2 lineages [7]. Another genetic variation, 22882T>G (S:N440K) within the receptor-binding domain of the spike protein which possibly confers resistance to neutralising antibodies, was detected in one study [3]. The presence of rare mutations, based on published sequence data, strengthens the case for reinfection. In the study by To et al. [7], the secondary genome also contained the mutation NSP6 L142F, which was only rarely reported (only 0.009% of genomes deposited into GISAID contained this mutation on 20/8/2020 [16]).

One of the primary limitations of included studies was their inability to definitively exclude false-positive RT-PCR results, either at primary or secondary infection events. While RT-PCR testing is the operational gold standard for SARS-CoV-2 diagnosis [17], technical problems at some point during the testing process may result in false-positives. Indeed, the problem of contamination can be a real concern; for example, the CDC in the US had to withdraw SARS-CoV-2 RT-PCR testing kits in March 2020 after a high rate of false-positives due to reagent contamination [18]. Nonetheless, the fact that SARS-CoV-2 WGS requires a given sample to be processed – typically in separate pathways – from the primary sample at least twice, the risk of false positive results, if not contamination, is significantly reduced if not eliminated.

Methods to exclude false-positive RT-PCR results across studies included excluding RT-PCR results with high Ct values and/or using cut-offs for the interval between infection events. All patients had a minimum interval of 45 days, except one patient who developed symptoms just 13 days after initial recovery [11]. Study authors suggested that this patient's primary infection may have occurred days to weeks before his first positive RT-PCR test, as he was asymptomatic. However, the risk of contamination must be considered, especially when clinical or epidemiological data suggest otherwise.

Another limitation of our findings is the inability to calculate a population-level reinfection rate due to the extremely low number of confirmed reinfections identified. As confirmation of reinfection necessitates WGS of both events, our findings may represent a significant underestimation of all reinfections. Nonetheless, these data suggest that reinfections can occur, but are a rare phenomenon, suggesting strong protective immunity following primary infection.

The phenomenon of SARS-CoV-2 reinfection has significant policy implications and suggests that immunity following primary SARS-CoV-2 infection is not universal. Although there were no documented cases of onward transmission from these reinfected cases, knowledge is evolving. Infection prevention and control, isolation and contact tracing considerations are not likely to differ for the reinfections compared with the primary infection.

Table 1
Summary of included studies.

Author, Study type	Patient demographics	Location	Severity of illness	Interval between infection events[a]	Whole genome sequencing differentiation	
					Single nucleotide polymorphisms	Lineage/ clade comparison
Abu-Raddad 2020 [9] Case series (*N*=4 reinfection cases out of a cohort of *N*=133,266 infections)	Patient 1: 25–29 year old male Patient 2: 40-44 year old male Patient 3: 45-49 year old female Patient 4: 25-29 year old male	Qatar	Most patients asymptomatic[b], however clinical course of the reinfection cases not reported	Patient 1: 45 days Patient 2: 70 days Patient 3: 87 days Patient 4: 54 days	*N*=2 patients: due to "multiple changes in allele frequency" and presence of the D614G mutation (23403bp A>G) considered confirmed reinfection cases by study authors) *N*=2 patients: presence of D614G mutation considered 'supportive' evidence by study authors	N/R
Goldman 2020 [10] Case study	60-69 year old male	United States	Severe initial infection with hospitalisation, mild on reinfection	118 days	10 nucleotide differences (confirmed reinfection)	1st infection: clade 19B, 2nd infection: clade 20A
Gupta 2020 [3] Case series (*N*=2)	Patient 1: 25 year old male	India	Patient 1: Asymptomatic on initial infection and reinfection	Patient 1: 108 days	Patient 1: 9 nucleotide differences (confirmed reinfection)	N/R
	Patient 2: 28 year old female	India	Patient 2: Asymptomatic on initial infection and reinfection	Patient 2: 111 days	Patient 2: 10 nucleotide differences (confirmed reinfection)	
Larson 2020 [12] Case study	42 year old male	United States	Mild initial infection, severe reinfection	51 days	'Several variations' noted, however only partial genome recovered from 1st infection (supportive evidence)	2nd infection: Lineage B.1.26 (not available for 1st infection)
Mulder 2020[4] Case study	89 year old female	The Netherlands	Severe initial infection, more severe reinfection and subsequent death	59 days	10 nucleotide differences (confirmed reinfection)	Sequences did not cluster in phylogenetic tree
Prado-Vivar 2020 [5] Case study	46 year old male	Ecuador	Mild initial infection, more severe reinfection	63 days	18 nucleotide differences (confirmed reinfection)	1st infection: lineage B.1.p9 lineage, clade 20A 2nd infection: lineage A.1.1, clade 19B
Shastri 2020 [11] Case series (*n*=4)	Patient 1: 27 year old male	India	Patient 1: Mild initial infection, mild/moderate reinfection	Patient 1: 60 days	8 nucleotide differences (confirmed reinfection)	3 patients infected on both occasions with lineage B.1.1, clade A2a One patient had a shift in lineage from B.1 to B In terms of subclades, one patient clustered in different subclades on reinfection
	Patient 2: 31 year old male		Patient 2: Asymptomatic initial infection, mild reinfection	Patient 2: 59 days	9 nucleotide differences (confirmed reinfection)	
	Patient 3: 27 year old male		Patient 3: Asymptomatic initial infection, mild reinfection	Patient 3: 13 days	9 nucleotide differences (confirmed reinfection)	
	Patient 4: 24 year old female		Patient 4: Mild initial infection, mild/moderate reinfection	Patient 4: 48 days	12 nucleotide differences (confirmed reinfection)	
Tillett 2020 [6] Case study	25 year old male	US	Mild initial infection, severe reinfection with hospitalisation	48 days	11 nucleotide differences (confirmed reinfection)	1st and 2nd infections from same clade (20C)
To 2020 [7] Case study	33 year old male	China	Mild initial infection, asymptomatic reinfection	142 days	24 nucleotide differences (confirmed reinfection)	1st infection: GISAID clade V, Nextstrain clade 19A, Pangolin lineage B.2 2nd infection: GISAID clade G, Nextstrain clade 20A, Pangolin lineage B.1.79
Van Elslande 2020 [8] Case study	51 year old female	Belgium	Moderate initial infection, mild reinfection	93 days	11 nucleotide differences (confirmed reinfection)	1st infection: lineage B.1.1 2nd infection: lineage A

[a] This interval represents the time from recovery from primary infection (first negative RT-PCR test) until the onset of the secondary infection (first documented symptoms or first RT-PCR positive test in asymptomatic cases); when detailed information was not provided, the time interval reported by study authors was used.
[b] Most of those infected were identified through random testing campaigns, surveys or contact tracing.N/R – not reported.

Funding statement

This research was funded in part by the Health Research Board under grant no. HRB-CICER-2016-1871.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Executive Assistant Debra Spillane (HIQA).

References

[1] HIQA. Immune Response Following SARS-CoV-2 Infection. 06 August 2020. Available at: https://www.hiqa.ie/sites/default/files/2020-08/Evidence-summary_SARS-CoV-2-immune-response.pdf

[2] HIQA. Protocol: Evidence Summary of the Duration of Immunity and Reinfection Following SARS-CoV-2 Infection; 11 November 2020. Available at: https://www.hiqa.ie/sites/default/files/2020-11/Protocol-for-evidence-summary-of-immunity-and-reinfection.pdf. 2020.

[3] V Gupta, RC Bhoyar, A Jain, et al., Asymptomatic reinfection in two healthcare workers from India with genetically distinct SARS-CoV-2, Clin. Infect. Dis. (2020) ciaa1451, doi:10.1093/cid/ciaa1451.

[4] M Mulder, DSJM van der Vegt, BB Oude Munnink, et al., Reinfection of SARS-CoV-2 in an immunocompromised patient: a case report, Clin. Infect. Dis. (2020) ciaa1538, doi:10.1093/cid/ciaa1538.

[5] Prado-Vivar B., Becerra-Wong M. and Guadalupe, J.J. and Marquez, S. and Gutierrez, B. and Rojas-Silva, P. and Grunauer, M. and Trueba, G. and Barragan, V. and Cardenas, P.. COVID-19 Re-Infection by a Phylogenetically Distinct SARS-CoV-2 Variant, First Confirmed Event in South America. (September 3, 2020). Available at SSRN: https://ssrn.com/abstract=3686174 or http://dx.doi.org/10.2139/ssrn.3686174. 2020.

[6] Tillett R., Sevinsky J. and Hartley, P. and Kerwin, H. and Crawford, N. and Gorzalski, A. and Laverdure, C. and Verma, S. and Rossetto, C. and Jackson, D. and Farrell, M. and Van Hooser, S. and Pandori, M.. Genomic Evidence for a Case of Reinfection With SARS-CoV-2 (August 25, 2020). Available at SSRN: https://ssrn.com/abstract=3680955 or http://dx.doi.org/10.2139/ssrn.3680955. 2020.

[7] KK To, IF Hung, JD Ip, et al., COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing, Clin. Infect. Dis. (2020) ciaa1275, doi:10.1093/cid/ciaa1275.

[8] J Van Elslande, P Vermeersch, K Vandervoort, et al., Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain, Clin. Infect. Dis. (2020) ciaa1330, doi:10.1093/cid/ciaa1330.

[9] LJ Abu-Raddad, H Chemaitelly, JA Malek, et al., Assessment of the risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfection in an intense reexposure setting, Clin. Infect. Dis. (2020) ciaa1846, doi:10.1093/cid/ciaa1846.

[10] Goldman JD, Wang K, Roltgen K, et al. Reinfection With SARS-CoV-2 and Failure of Humoral Immunity: A Case Report. *medRxiv*.2020:2020.2009.2022.20192443.

[11] Jayanthi Shastri SP, Sachee Agarwal, Nirjhar Chatterjee. Whole Genome Sequencing Confirmed SARS-CoV-2 Reinfections Among Healthcare Workers in India with Increased Severity in the Second Episode. Available at SSRN: https://ssrn.com/abstract=3688220 or http://dx.doi.org/10.2139/ssrn.3688220. 2020.

[12] D Larson, SL Brodniak, LJ Voegtly, et al., A case of early re-infection with SARS–CoV-2, Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. (2020) ciaa1436.

[13] A Rambaut, EC Holmes, O'Toole Á, et al., A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology, Nat. Microbiol. 5 (2020) 1403–1407, doi:10.1038/s41564-020-0770-5.

[14] New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG). Is There Evidence for Genetic Change in SARS-CoV-2 and if so, do Mutations Affect Virus Phenotype? 23 October 2020. Available at: https://www.gov.uk/government/publications/nervtag-is-there-evidence-for-genetic-change-in-sars-cov-2-and-if-so-do-mutations-affect-virus-phenotype-30-september-2020. 2020.

[15] Q Li, J Wu, J Nie, et al., The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity, Cell 182 (5) (2020) 1284–1294 e1289.

[16] WHO. Coronavirus Disease 2019 (COVID-19). External Situation Report #18. 2 September 2020. Available at: https://iris.wpro.who.int/bitstream/handle/10665.1/14519/covid19-20200831.pdf. Accessed 16.10.2020 2020.

[17] E Surkova, V Nikolayevskyy, F. Drobniewski, False-positive COVID-19 results: hidden problems and costs, Lancet Respir. Med. 8 (12) (2020) 1167–1168.

[18] The New York TimesC.D.C. Labs Were Contaminated, Delaying Coronavirus Testing, Officials Say, Sheila Kaplan, 2020 Published April 18Available at: https://www.nytimes.com/2020/04/18/health/cdc-coronavirus-lab-contamination-testing.html Accessed: 14.4.2021.