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Research article

A retrospective study suggests 55 days of persistence of SARS-CoV-2 during the first wave of the pandemic in Santiago de Chile☆

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ABSTRACT

Background: As the COVID-19 pandemic persists, infections continue to surge globally. Presently, the most effective strategies to curb the disease and prevent outbreaks involve fostering immunity, promptly identifying positive cases, and ensuring their timely isolation. Notably, there are instances where the SARS-CoV-2 virus remains infectious even after patients have completed their quarantine.

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Objective: Understanding viral persistence post-quarantine is crucial as it could account for localized infection outbreaks. Therefore, studying and documenting such instances is vital for shaping future public health policies.
Design: This study delves into a unique case of SARS-CoV-2 persistence in a 60-year-old female healthcare worker with a medical history of hypertension and hypothyroidism. The research spans 55 days, marking the duration between her initial and subsequent diagnosis during Chile's first COVID-19 wave, with the analysis conducted using RT-qPCR.
Results: Genomic sequencing-based phylogenetic analysis revealed that the SARS-CoV-2 detected in both Nasopharyngeal swab samples (NPSs) was consistent with the 20B clade of the Nextstrain classification, even after a 55-day interval.
Conclusion: This research underscores the need for heightened vigilance concerning cases of viral persistence. Such instances, albeit rare, might be pivotal in understanding sporadic infection outbreaks that occur post-quarantine.

1. Introduction

The COVID-19 pandemic has resulted in approximately 6 million death and has infected 445 million individuals globally [1]. This crisis has profoundly impacted the economic, social, and infrastructural systems of nations [2–4]. Notably, some individuals who recovered from the virus have reported enduring health complications post-SARS-CoV-2 infection [5]. Amidst this backdrop, the most potent defense against the pandemic is the immunity conferred by vaccines [6]. Additionally, large-scale proactive testing for COVID-19 and meticulous tracking of positive cases emerge as indispensable strategies. These methods facilitate the prompt identification of infections and monitor the persistence of the SARS-CoV-2 virus [7]. The persistence of SARS-CoV-2 after quarantine is currently related to the presence of viral RNA with no infection capacity [8]. However, there is evidence that viral persistence is even associated with an infective virus after more than 30 days [9], especially in patients with immune disorders [10]. Therefore, it is critical to design prevention policies to avoid viral outbreaks in the community, particularly concerning situations of persistence after completing the quarantine period. In this line, in a Qatari study, constant testing allowed the detection of six cases of COVID-19 that had persistently tested positive SARS-CoV-2 up to 9 weeks [11] or a continuous infection for up to 70 days after initial diagnosis [12]. These effects often are related to immunosuppressed patient's reduced antiviral response, evidenced by a decrease in antibody titers near 60 days of infection by SARS-CoV-2 [13,14]. The comorbidities are reducing the normal function of the immune system [15]. Furthemore, the evidence suggests that the persistence of infective SARS-CoV-2 could increase the probability of spread and contagion outbreaks. Thus, its study and evidence are of significant public interest. This retrospective study is the first report of a supposed case of SARS-CoV-2 persistence, identified in a health worker for 55 days undergoing mild symptomatology during Chile's first wave of COVID-19. This report seeks to increase the evidence of persistent cases of SARS-CoV-2 for further consideration in public health policies by the health authorities. These cases are often underestimated and, on occasion, could explain local outbreaks of infection, even after the end of the quarantine.

2. Methods

Study description. A 60-year-old female healthcare worker with a history of hypertension and hypothyroidism was admitted to Centro de Salud Familiar San José de Chuchunco, Santiago, Chile, on May 13th, 2020. The patient registered a persistent headache lasting for two days. The patient tested positive for SARS-CoV-2 by RT-qPCR in a nasopharyngeal swab sample (NPSs). The patient declared no close contact with COVID-19-positive individuals. The patient was quarantined for 14 days and remotely checked for disease progression. The patient announced no symptomatology during the quarantine and was discharged on June 18th without RT-qPCR on exit. Then, she returned to her usual activities. On July 7th, she tested positive for COVID-19 again, according to a routine RT-qPCR diagnosis. She was quarantined again and remotely checked, and she declared no symptoms during the second quarantine period. It is unknown if she caused any contagion through close contact during this period. She was discharged on July 21st.

Sample and RT-qPCR assay. NPSs were obtained from the patient according to the guide for handling and taking samples for COVID-19 described by the Center for Disease Control and Prevention [16]. Samples were transported to a diagnostic laboratory in a viral transport medium (VTM); Genosur (catalog number: DM0001VR; Genosur LLC, NW). Total RNA was extracted as previously described [17]. The viral detection of SARS-CoV-2 was carried out using the ORF1ab gene probe (TaqMan™ 2019 nCoV Assay Kit v1 (Thermo Fisher Scientific, Reference code. A47532) as previously reported [18].

Viral load determination. Using the positive control TaqMan 2019-nCoV Control Kit v1 (10^4 copies/uL, Thermo Fisher Scientific, Cat. No. A47533), we created a standard curve to determine the viral load of the samples. The viral load was determined using the antilogarithm of the following equation of the line ($y = -3.07*X+40.2)/2$. "X" substituted the Cq that was obtained from the NPSs. 2 μl of total RNA was used in the PCR reaction in the same way as previously reported [19].

Sample sequencing and analysis. The original virus sequence used to compare both samples was as follows: severe acute respiratory syndrome coronavirus 2 isolated Wuhan-Hu-1, ACCESSION MN908947, March 2020, from GeneBank database. cDNA synthesis was performed from the two-positive NPSs using the High-Capacity RNA-to-cDNA kit (Applied Biosystems) and amplified by ARTIC v3 protocol [20] using the 98-primer set with the GoTaq Green Master Mix enzyme (Promega). The genomic library was

constructed using the Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore) and processed and sequenced independently (without barcodes) to increase and determine the exact number of readings per sample. Sequencing reached 1 Gigabase (Gb) (approximately 3 h of sequencing) without base-calling process. The Nanopore sequencing was transformed to fastq format using the ONT Guppy-base caller [Guppy] https://nanoporetech.com (CPU mode). All reads were quality checked and assembled using [ARTICMINION] https://artic.network/ncov-2019. The Trimmomatic [21] tool was used to filter poor quality reads and trim poor-quality bases from these samples. The quality analyses were created with the MultiQC software. The consensus assembly genomes were aligned to the reference SARS-CoV-2 genome using Nucmer [MUMmer], and SNPs were predicted using Nucmer tool show-snps [22]. Then, SNPs were annotated using SnpEff [SNPEFF] within the SARS-CoV-2 database [23]. All computations were run at the National Laboratory for High-Performance Computing (www.nlhpc.cl) [24]. Sequence pairs were aligned to the SARS-CoV-2 reference genome using Bowtie 2 version 2.3. In addition, a clade assignment was performed using NextClade version 1.9.0 to compare and visualize the NPSs Clade [25]. Data are available in the NCBI BioSample database under accession numbers SAMN22047844 (for sample 1405–124) and SAMN22047845 (for sample 0807–052).

Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model [26]. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed [27]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test were shown next to the branches [27]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. This analysis involved 8 amino acid sequences. There was a total of 29,998 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [28,29].

Ethics statement. This study was authorized by the Ethical Committee of the University of Santiago of Chile (No. 226/2021) and the Scientific Ethical Committee of the Central Metropolitan Health Service, Ministry of Health, Government of Chile (No. 370/2021), and following the Chilean law in force.

3. Results

The patient tested positive for COVID-19 in the first instance on May 14th, 2020 (1405–124) (Fig. 1A). The RT-qPCR analyses yielded a Cq for ORF1ab = 17.45, indicating a viral load of 9×10^6 copies/μl. The internal control RNase P was Cq = 16.44 (Table 1). Then, 55 days later (on July 8th, 2020, sample ID 0807–052), the patient was diagnosed for a second time with COVID-19 (Fig. 1A), with a Cq for ORF1ab = 33.79 (viral load 6×10^1) and RNase P was Cq = 16.58 (Table 1). These Cq for ORF1ab indicate both positive

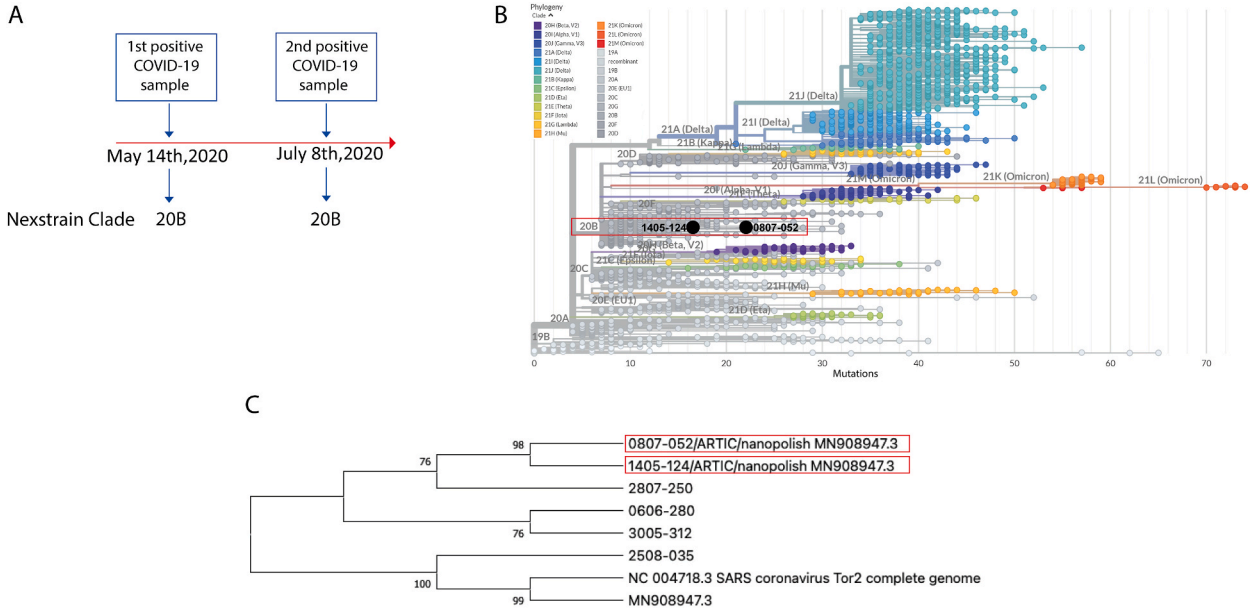

Fig. 1. Genomic classification of the first and second samples from a 60-year-old health worker. A. Chronology of both positive diagnoses with their respective Nextstrain classification. B. Phylogenetic location of samples 1405–124 and 0807–052 in the Nextstrain clade classification (of the Nextclade v1.14.0 software) concerning the rest of the SARS-CoV-2 clades and variants. C) Phylogenetic analysis for samples 0807–052 and 1405–124. Four NPSs were added, all belonging to the same Clade 20B (2807–250; 0606–280; 3005–312; 2508–035). This set of sequenced samples belong to the Biobank of samples at the Universidad de Santiago de Chile. The NPSs analyzed in this study are highlighted with a red box. Fig. 1B was built with NextClade version 1.9.0 enabled by GISAID data, filtering/country/Chile. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
RT-qPCR parameters from patient samples with viral persistence.

Sample	Rnase P (Cq)	ORF1ab (Cq)	Viral load (copies/μl)
1405–124	16.44	17.45	9×10^6
0807–052	16.58	33.79	6×10^1

results for SARS-CoV-2 according to the previously reported limit of detection [18]. The full sequence of 1405–124 and 0807–052 aligned against the SARS-CoV-2 reference sequence. They showed 99.55% and 87.31% identity, respectively, using the multiple sequence alignment tool Clustal Omega for genomic sequence alignment [30]. Sequence analysis by NextClade software indicated that 1405–124 and 0807-052 samples were members of the same 20B Nextstrain classification (Fig. 1B). However, some mutations were identified in the second sample (0807–052) regarding the first infection (1405–124). The sequencing quality of both NPSs is shown in Supplementary Fig. 1. Fig. 1C shows a phylogenetic analysis of the samples analyzed in this study and four other NPSs belonging to the same Clade 20B (2807–250, 0606–280, 3005–312, and 2508–035), diagnosed as COVID-19 positive in different dates. NPSs 1405–125 and 0807–052 (highlighted in the red box) are shown to be part of the same group and show slight divergence between them. The closest sample to the samples analyzed (0807–052 and 1405-124) is 2807–250, a sample from July 2020; however, sample 2508–035, despite belonging to the same Clade 20B, is the most distant phylogenetically. The Bootstrap Consensus was performed in a similar way to what was previously reported [31,32].

Genomic sequence analysis for both samples identified divergences located in the genes ORF1a, ORF1b, S, ORF3a, ORF6, ORF8, and N regarding the reference gene of SARS-CoV-2 (Table 2). The mutations detected and not detected in the first (1405–124) and second (0807–052) samples are shown in Table 2. The mutations N:I292T, ORF1b:R2308C, and ORF8:I121L, which were found in 1405–124 sample but not in 0807–052 sample, were indicated as undetermined, because these mutations coincided with a low-quality sequencing zone in unknown amino acid in sample 0807–052 (Supplementary Fig. 2). The second sample (0807–052) shared five Single-Nucleotide Variants (SNVs) (N: R203K, N: G204R, ORF1a: K737R, ORF6: I33T and S: D614G) with 1405–124 sample. By contrast, the second sample (0807–052) had seven SNVs (ORF1a: C341R, ORF1a: I3587V, ORF1a: I3663T, ORF1b:P314L, ORF1b: R2676S, ORF3a:S40L and S: F562L) that were absent in the first positive sample (1405–124). The SNVs were identified according to reference SARS-CoV-2 genome.

4. Discussion

This retrospective case study provides genomic evidence highlighting the persistence of SARS-CoV-2 for 55 days in a 60-year-old female health worker from Santiago, Chile. The findings lean towards a scenario of viral persistence rather than a reinfection event with the same variant, specifically the 20B Clade. For a case to be considered a reinfection, the second positive test must occur at least 90 days after the first positive diagnosis, criteria set by both the Pan American Health Organization (PAHO) and the Centers for Disease Control and Prevention (CDC) in 2023 [33,34]. Numerous studies have assessed the immunity a natural SARS-CoV-2 infection granted in the months following the illness. For instance, De Giorgi et al., 2021 found that 91.4% of 116 donors retained detectable IgG levels up to 11 months post-symptom recovery [35]. Other studies reported antibody levels for at least 90 days [36] and up to 8 months [37]. However, these levels can fluctuate based on factors like age, underlying health conditions, and individual genotypes. Natural viral infection typically offers temporary protection against reinfection. A study by Spicer et al., 2021, conducted in Kentucky, USA, revealed that innate immunity to SARS-CoV-2 reduced reinfection by 80% within 30–270 days for individuals aged 20 to 59. The protection rate for those aged 60 and above stood at 67% during the same timeframe [38]. Conversely, a Danish report showed 80% protection for patients under 65 years of age within 3–6 months [39]. In England, a comprehensive study involving over 25,000 health

Table 2
Detected, undetected, and undetermined mutations in the first (1405–124) and second (0807–052) samples relative to the SARS-CoV-2 reference genome.

MUTATIONS	1405–124	0807–052
N:R203K	Detected	Detected
N:G204R	Detected	Detected
N:I292T	Detected	Undetermined
ORF1a:C341R	Undetected	Detected
ORF1a:K737R	Detected	Detected
ORF1a:I3587V	Undetected	Detected
ORF1a:I3663T	Undetected	Detected
ORF1b:P314L	Undetected	Detected
ORF1b:R2308C	Detected	Undetermined
ORF1b:R2676S	Undetected	Detected
ORF3a:S40L	Detected	Undetected
ORF6:I33T	Detected	Detected
ORF8:I121L	Detected	Undetermined
S:D614G	Detected	Detected
S:F562L	Undetected	Detected

workers observed an 83% decrease in SARS-CoV-2 reinfections for at least five months post the initial infection [40]. Another study in Qatar, with a cohort of 43,000 patients, indicated a minimum of 7 months of protection against reinfection for 95% of those infected with SARS-CoV-2 [41]. While the protective capacity of natural immunity against reinfection remains a focal point of ongoing research, the existing studies on immunity duration against SARS-CoV-2 bolster the argument for viral persistence over reinfection in this study, especially since the second positive diagnosis transpired in under two months. In this context, a third positive RT-PCR test between the two samples would have been instrumental in confirming a persistence scenario.

Furthermore, several studies have documented the lingering presence of viral RNA post-SARS-CoV-2 infection. Li et al., 2020, for example, noted a 30-day persistence of viral RNA in the respiratory systems of certain Chinese patients [42]. Another study highlighted the continuous shedding of viral RNA in nasopharyngeal swabs for up to 46 days [43]. In the USA, it is posited that patients who remain positive ten days post-symptom onset due to SARS-CoV-2 infection are no longer contagious [44]. A striking report even cited a 386-day persistence in a health worker, although the patient's infectivity was not assessed [45]. However, evidence of SARS-CoV-2 infectivity after 30 days [9], 37 days [46], and 144 days [47] from the first infection has been reported. Regrettably, this study did not undertake such infectivity analyses.

Her existing health conditions might influence the patient's viral persistence. Thyroid hormones play a pivotal role in the proliferation and migration of immune cells, cytokine release, and antibody production [48,49]. Hypothyroidism could potentially disrupt this immune response. Additionally, hypertension has been linked to immune system deregulation and chronic inflammation, which might impact the initial defense against SARS-CoV-2 [50]. A study from Peru by Chambergo-Michilot et al., 2021 drew a connection between hypertension and immunosuppression [51].

The hypothesis of viral persistence is supported by the genomic identification of both samples in the same Nextstrain clade 20B classification. Although the sequencing quality of both samples is reasonable but not excellent, five identical mutations were identified in both NPSs, and seven new mutations in the sample 0807–052. It should be noted that the mutations present in NPSs 1405–124 that do not appear in sample 0807–052 may be because the low-sequencing quality of NPSs 0807–052 (Figure supplementary 2). However, at least five new mutations can be observed in 0807–052, which are not in the first positive sample (1405–124). The mutation rate of the SARS-CoV-2 genome reported previously was two SNPs per month in the population [52,53]. This aligns with the viral-genetic alterations observed in this patient, which averaged around three per month. Even though the analyses indicated the same clade, the samples were phylogenetically more similar than others from clade 20B. The 20B clade variant of SARS-CoV-2 was first identified in Chile in May 2020 and was reported until April 2021. Different variants like Eta, Mu, Alpha, and Gamma emerged during this period. As of 2023, the Omicron variant is predominant in Chile and globally. Notably, viral persistence of up to 14 days post Omicron infection has been observed in US children, with positive viral cultures [54].

The majority of mutations detected in the second sample (0807–052) that were absent in the first (1405–124) were located in the Open Reading Frame 1 (ORF1) gene. Research has identified mutations in the SARS-CoV-2 ORF1 gene, with some linked to enhanced transmissibility. For instance, the ORF1a: P314L mutation, exclusive to the second sample, is believed to augment the virus's infectivity and replication capabilities. This mutation, in conjunction with the S: D614G mutation, appears in over 70% of more evasive SARS-CoV-2 variants in European patients [55,56]. Other mutations in the second sample, like ORF8: I121L, are associated with reduced MHC-I expression in cells, complicating their recognition by T lymphocytes [57]. However, the precise consequences of ORF mutations in SARS-CoV-2 are complicated and depend on the Single-Nucleotide Variants (SNVs) and the environment in which they arise (reviewed in ([58]). While some ORF1 amino acid substitutions might have a negligible effect on the virus, others could alter its replication efficiency, pathogenicity, or immune evasion capabilities. Comprehensive studies are essential to grasp the impact of ORF1 mutations on SARS-CoV-2 and its potential to propagate the disease.

The patient's low viral load during the second infection is noteworthy, especially given her asymptomatic presentation. This diminished viral load might also correlate with the sequencing quality of the analyzed NPSs. In this study, unlike others, the patient's viral load or infective potential post-quarantine was not assessed. Hence, it remains uncertain if the patient could have been a viral carrier upon resuming work, posing a transmission risk. This underscores the importance of RT-qPCR tests post-quarantine to confirm the cessation of viral infection, especially in regions with a significant unvaccinated populace. While SARS-CoV-2 persistence is atypical [59], it should be widely considered because the R0 factor (number of average infections per person in a COVID-19 positive patient) of SARS-CoV-2 reaches 5.8 at least in EE. UU and 6.1 in other countries [60]. An asymptomatic individual with viral persistence could inadvertently cause infections and local SARS-CoV-2 outbreaks, potentially undermining current public health strategies. Given the current pandemic landscape dominated by highly evasive variants like Omicron, increasing evidence of viral persistence should prompt health authorities to reconsider and adapt public health policies.

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Ethics approval: The experimental procedures included in this study were authorized by the Ethical Committee of the University of Santiago of Chile (No. 226/2021) and the Scientific Ethical Committee of the Central Metropolitan Health Service, Ministry of Health, Government of Chile (No. 370/2021) and following the Chilean law in force.

Consent for publication: Informed consent was obtained from the patient involved in this study.

Availability of data and materials: The data that support the findings of this study are available in the NCBI database BioSample

(https://www.ncbi.nlm.nih.gov/biosample/); SAMN22047844 (for sample 1405–124) and SAMN22047845 (for sample 0807–052).

CRediT authorship contribution statement

Claudio Acuña-Castillo: Writing – original draft, Funding acquisition, Conceptualization. **Mabel Vidal:** Investigation, Formal analysis, Data curation. **Eva Vallejos-Vidal:** Investigation. **Roberto Luraschi:** Investigation, Formal analysis. **Carlos Barrera-Avalos:** Writing – review & editing, Writing – original draft, Investigation. **Ailen Inostroza-Molina:** Investigation. **Sonia Molina-Cabrera:** Investigation. **Daniel Valdes:** Validation, Supervision. **Carolina Schafer:** Investigation. **Kevin Maisey:** Investigation. **Mónica Imarai:** Visualization, Validation, Project administration. **Rodrigo Vera:** Investigation. **Sergio Vargas:** Investigation. **Leonel E. Rojo:** Investigation. **Elías Leiva-Salcedo:** Investigation. **Alejandro Escobar:** Investigation. **Sebastián Reyes-Cerpa:** Investigation. **Alexis Gaete:** Investigation. **Ricardo Palma-Vejares:** Investigation. **Dante Travisany:** Investigation. **Claudio Torres:** Investigation. **Felipe E. Reyes-López:** Writing – review & editing, Writing – original draft, Validation, Project administration, Data curation, Conceptualization. **Ana María Sandino:** Visualization, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24419.

References

[1] World Health Organization, WHO Coronavirus (COVID-19) Dashboard, 2022.
[2] T.-T. Rao, S.-L. Yang, X. Zhu, How does social class affect need for structure during the COVID-19 pandemic? A moderated mediating model analysis, Int. J. Environ. Res. Publ. Health 19 (2022) 932, https://doi.org/10.3390/ijerph19020932.
[3] Y. Shang, H. Li, R. Zhang, Effects of pandemic outbreak on economies: evidence from business history context, Front. Public Health 9 (2021), https://doi.org/10.3389/fpubh.2021.632043.
[4] S. Khetrapal, R. Bhatia, Impact of COVID-19 pandemic on health system & sustainable development goal 3, Indian J. Med. Res. 151 (2020) 395, https://doi.org/10.4103/ijmr.IJMR_1920_20.
[5] Centers for Disease Control and Prevention, Long COVID or Post-COVID Conditions, 2022. Https://Www.Cdc.Gov/Coronavirus/2019-Ncov/Long-Term-Effects/Index.Html.
[6] L. Grigoryan, B. Pulendran, The immunology of SARS-CoV-2 infections and vaccines, Semin. Immunol. 50 (2020), https://doi.org/10.1016/j.smim.2020.101422.
[7] T.R. Mercer, M. Salit, Testing at scale during the COVID-19 pandemic, Nat. Rev. Genet. 22 (2021) 415–426, https://doi.org/10.1038/s41576-021-00360-w.
[8] R. Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. Müller, D. Niemeyer, T.C. Jones, P. Vollmar, C. Rothe, M. Hoelscher, T. Bleicker, S. Brünink, J. Schneider, R. Ehmann, K. Zwirglmaier, C. Drosten, C. Wendtner, Virological assessment of hospitalized patients with COVID-2019, Nature 581 (2020) 465–469, https://doi.org/10.1038/s41586-020-2196-x.
[9] M. Davies, L.R. Bramwell, N. Jeffery, B. Bunce, B.P. Lee, B. Knight, C. Auckland, J.A. Masoli, L.W. Harries, Persistence of clinically relevant levels of SARS-CoV2 envelope gene subgenomic RNAs in non-immunocompromised individuals, Int. J. Infect. Dis. 116 (2022) 418–425, https://doi.org/10.1016/j.ijid.2021.12.312.
[10] S.A. Dolan, J. Mulcahy Levy, A. Moss, K. Pearce, M. Butler, S. Jung, S.R. Dominguez, E. Mwangi, K. Maloney, S. Rao, SARS-CoV-2 persistence in immunocompromised children, Pediatr. Blood Cancer 68 (2021), https://doi.org/10.1002/pbc.29277.
[11] A.E. Alsaud, A.P. Nair, A.S. Matarneh, S. Sasi, R. el Hassan, F. Khan, P. Coyle, M.A. Khattab, M.F.H. Mohamed, Case report: prolonged viral shedding in six COVID-19 patients, Am. J. Trop. Med. Hyg. 104 (2021) 1472–1475, https://doi.org/10.4269/ajtmh.20-0933.
[12] V.A. Avanzato, M.J. Matson, S.N. Seifert, R. Pryce, B.N. Williamson, S.L. Anzick, K. Barbian, S.D. Judson, E.R. Fischer, C. Martens, T.A. Bowden, E. de Wit, F. X. Riedo, V.J. Munster, Case study: prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer, Cell 183 (2020) 1901–1912.e9, https://doi.org/10.1016/j.cell.2020.10.049.
[13] D. Blanco-Melo, B.E. Nilsson-Payant, W.C. Liu, S. Uhl, D. Hoagland, R. Møller, T.X. Jordan, K. Oishi, M. Panis, D. Sachs, T.T. Wang, R.E. Schwartz, J.K. Lim, R. A. Albrecht, B.R. tenOever, Imbalanced host response to SARS-CoV-2 drives development of COVID-19, Cell 181 (2020) 1036–1045.e9, https://doi.org/10.1016/j.cell.2020.04.026.
[14] W.H. Self, M.W. Tenforde, W.B. Stubblefield, L.R. Feldstein, J.S. Steingrub, I. Shapiro Nathan, A.A. Ginde, E. Prekker Matthew, M. Brown Samuel, I.D. Peltan, M. N. Gong, M.S. Aboodi, A. Khan, C. Exline, D. Clark, K.W. Gibbs, J. Christopher, Lindsell, W. Todd, I. Rice, D. Jones, N. Halasa, H Keipp Talbot, C.G. Grijalva, J.D, D. Casey, N. Hager, N. Qadir, J. Daniel, M.M. Henning, J. Coughlin, V. Schiffer, H. Semenova, Li N.J. Thornburg, M.M. Patel, Morbidity and mortality weekly report decline in SARS-CoV-2 antibodies after mild infection among frontline health care personnel in a multistate hospital network-12 states, april-august 2020, morbidity and mortality weekly report, MMWR (Morb. Mortal. Wkly. Rep.) 69 (2020) 1762–1766, https://doi.org/10.15585/mmwr.mm6947a2.
[15] L.A. Callender, M. Curran, S.M. Bates, M. Mairesse, J. Weigandt, C.J. Betts, The impact of pre-existing comorbidities and therapeutic interventions on COVID-19, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.01991.
[16] Centers for Disease Control and Prevention, Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing, 2021.
[17] C. Barrera-Avalos, R. Luraschi, E. Vallejos-Vidal, M. Figueroa, E. Arenillas, D. Barría, F. Hernández, C. Mateluna, J. Mena, C. Rioseco, C. Torrent, C. Vergara, G. Gutiérrez, J. Quiroz, J. Alarcón, J. Cartagena, J. Cayunao, A. Mella, Á. Santibañez, S. Tapia, A. Undurraga, D. Vargas, V. Wong, A. Inostroza, D. Valdés, M. Imarai, C. Acuña-Castillo, F.E. Reyes-López, A.M. Sandino, Analysis by real-time pcr of five transport and conservation medium of nasopharyngeal swab samples to covid-19 diagnosis in Santiago of Chile, J. Med. Virol. (2021) 1–8, https://doi.org/10.1002/jmv.27446.
[18] R. Luraschi, Á. Santibáñez, C. Barrera-Avalos, E. Vallejos-Vidal, C. Mateluna-Flores, J. Alarcón, J. Cayunao, A. Mella-Torres, F. Hernández, A. Inostroza-Molina, D. Valdés, M. Imarai, C. Acuña-Castillo, F.E. Reyes-López, A.M. Sandino, Evaluation and comparison of the sensitivity of three commercial RT-qPCR kits used for the detection of SARS-CoV-2 in Santiago, Chile, Front. Public Health 10 (2022), https://doi.org/10.3389/fpubh.2022.1010336.

[19] C. Barrera-Avalos, R. Luraschi, E. Vallejos-Vidal, A. Mella-Torres, F. Hernández, M. Figueroa, C. Rioseco, D. Valdés, M. Imarai, C. Acuña-Castillo, F.E. Reyes-López, A.M. Sandino, The rapid antigen detection test for SARS-CoV-2 underestimates the identification of COVID-19 positive cases and compromises the diagnosis of the SARS-CoV-2 (K417N/T, E484K, and N501Y) variants, Front. Public Health 9 (2022), https://doi.org/10.3389/fpubh.2021.780801.

[20] J. Quick, nCoV-2019 Sequencing Protocol V3 (LoCost) V. 3 2020, 2021. https://www.protocols.io/view/ncov-2019-sequencingprotocol-v3-locost-bh42j8ye.

[21] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.

[22] G. Marçais, A.L. Delcher, A.M. Phillippy, R. Coston, S.L. Salzberg, A. Zimin, MUMmer 4: a fast and versatile genome alignment system, PLoS Comput. Biol. 14 (2018) 1–14, https://doi.org/10.1371/journal.pcbi.1005944.

[23] D.M. Cingolani, P, A. Platts, l. Wang, M. Coon, T. Nguyen, L. Wang, S.J. Land, X. Lu, Ruden, A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118, Fly 6 (2012) 80–92, https://doi.org/10.4161/fly.19695, iso-2; iso-3.

[24] I. Letunic, P. Bork, Interactive Tree of Life (iTOL) v4: recent updates and new developments, Nucleic Acids Res. 47 (2019) 256–259, https://doi.org/10.1093/nar/gkz239.

[25] I. Aksamentov, C. Roemer, E. Hodcroft, R. Neher, Nextclade: clade assignment, mutation calling and quality control for viral genomes, J. Open Source Softw. 6 (2021) 3773, https://doi.org/10.21105/joss.03773.

[26] D.T. Jones, W.R. Taylor, J.M. Thornton, The rapid generation of mutation data matrices from protein sequences, Bioinformatics 8 (1992) 275–282, https://doi.org/10.1093/bioinformatics/8.3.275.

[27] J. Felsenstein, Confidence limits on phylogenies: an approach using the bootstrap, Evolution 39 (1985) 783–791.

[28] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, X. Mega, Molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547–1549, https://doi.org/10.1093/molbev/msy096.

[29] G. Stecher, K. Tamura, S. Kumar, Molecular evolutionary genetics analysis (MEGA) for macOS, Mol. Biol. Evol. 37 (2020) 1237–1239, https://doi.org/10.1093/molbev/msz312.

[30] F. Sievers, A. Wilm, D. Dineen, T.J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Söding, J.D. Thompson, D.G. Higgins, Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega, Mol. Syst. Biol. 7 (2011) 1–6, https://doi.org/10.1038/msb.2011.75.

[31] N.-N. Bui, Y.-T. Lin, S.-H. Huang, C.-W. Lin, Haplotype distribution of SARS-CoV-2 variants in low and high vaccination rate countries during ongoing global COVID-19 pandemic in early 2021, Infect. Genet. Evol. 97 (2022) 105164, https://doi.org/10.1016/j.meegid.2021.105164.

[32] H.K. Kim, S.-W. Yoon, D.-J. Kim, B.-S. Koo, J.Y. Noh, J.H. Kim, Y.G. Choi, W. Na, K.-T. Chang, D. Song, D.G. Jeong, Detection of severe acute respiratory syndrome-like, Middle East respiratory syndrome-like bat coronaviruses and group H rotavirus in faeces of Korean bats, Transbound Emerg Dis 63 (2016) 365–372, https://doi.org/10.1111/tbed.12515.

[33] The Pan American Health Organization/World Health Organization (Paho/Who), Interim Guidelines for Detecting Cases of Reinfection by SARS-CoV-2, 2020, p. 2020. Https://Www.Paho.Org/En/Documents/Interim-Guidelines-Detecting-Cases-Reinfection-Sars-Cov-2.

[34] Centers for Disease Control and Prevention (Cdc), What is COVID-19 Reinfection? (2023) 2023. Https://Www.Cdc.Gov/Coronavirus/2019-Ncov/Your-Health/Reinfection.Html.

[35] V. De Giorgi, K.A. West, A.N. Henning, L.N. Chen, M.R. Holbrook, R. Gross, J. Liang, E. Postnikova, J. Trenbeath, S. Pogue, T. Scinto, H.J. Alter, C.C. Cantilena, Naturally acquired SARS-CoV-2 immunity persists for up to 11 Months following infection, J. Infect. Dis. 224 (2021) 1294–1304, https://doi.org/10.1093/infdis/jiab295.

[36] J.P. Townsend, H.B. Hassler, Z. Wang, S. Miura, J. Singh, S. Kumar, N.H. Ruddle, A.P. Galvani, A. Dornburg, The durability of immunity against reinfection by SARS-CoV-2: a comparative evolutionary study, Lancet Microbe 2 (2021), https://doi.org/10.1016/S2666-5247(21)00219-6 e666–e675.

[37] J.M. Dan, J. Mateus, Y. Kato, K.M. Hastie, E.D. Yu, C.E. Faliti, A. Grifoni, S.I. Ramirez, S. Haupt, A. Frazier, C. Nakao, V. Rayaprolu, S.A. Rawlings, B. Peters, F. Krammer, V. Simon, E.O. Saphire, D.M. Smith, D. Weiskopf, A. Sette, S. Crotty, Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection, Science (2021) 371, https://doi.org/10.1126/science.abf4063.

[38] K.B. Spicer, C. Glick, A.M. Cavanaugh, D. Thoroughman, Protective immunity after natural infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) – Kentucky, USA, 2020, International Journal of Infectious Diseases 114 (2022) 21–28, https://doi.org/10.1016/j.ijid.2021.10.010.

[39] C.H. Hansen, D. Michlmayr, S.M. Gubbels, K. Mølbak, S. Ethelberg, Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study, Lancet 397 (2021) 1204–1212, https://doi.org/10.1016/S0140-6736(21)00575-4.

[40] V.J. Hall, S. Foulkes, A. Charlett, A. Atti, E.J.M. Monk, R. Simmons, E. Wellington, M.J. Cole, A. Saei, B. Oguti, K. Munro, S. Wallace, P.D. Kirwan, M. Shrotri, A. Vusirikala, S. Rokadiya, M. Kall, M. Zambon, M. Ramsay, T. Brooks, C.S. Brown, M.A. Chand, S. Hopkins, N. Andrews, A. Atti, H. Aziz, T. Brooks, C.S. Brown, D. Camero, C. Carr, M.A. Chand, A. Charlett, H. Crawford, M. Cole, J. Conneely, S. D'Arcangelo, J. Ellis, S. Evans, S. Foulkes, N. Gillson, R. Gopal, L. Hall, V. J. Hall, P. Harrington, S. Hopkins, J. Hewson, K. Hoschler, D. Ironmonger, J. Islam, M. Kall, I. Karagiannis, O. Kay, J. Khawam, E. King, P. Kirwan, R. Kyffin, A. Lackenby, M. Lattimore, E. Linley, J. Lopez-Bernal, L. Mabey, R. McGregor, S. Miah, E.M.J. Monk, K. Munro, Z. Naheed, A. Nissr, A.M. O'Connell, B. Oguti, H. Okafor, S. Organ, J. Osbourne, A. Otter, M. Patel, S. Platt, D. Pople, K. Potts, M. Ramsay, J. Robotham, S. Rokadiya, C. Rowe, A. Saei, G. Sebbage, A. Semper, M. Shrotri, R. Simmons, A. Soriano, P. Staves, S. Taylor, A. Taylor, A. Tengbe, S. Tonge, A. Vusirikala, S. Wallace, E. Wellington, M. Zambon, D. Corrigan, M. Sartaj, L. Cromey, S. Campbell, K. Braithwaite, L. Price, L. Haahr, S. Stewart, E.D. Lacey, L. Partridge, G. Stevens, Y. Ellis, H. Hodgson, C. Norman, B. Larru, S. Mcwilliam, S. Winchester, P. Cieciwa, A. Pai, C. Loughrey, A. Watt, F. Adair, A. Hawkins, A. Grant, R. Temple-Purcell, J. Howard, N. Slawson, C. Subudhi, S. Davies, A. Bexley, R. Penn, N. Wong, G. Boyd, A. Rajgopal, A. Arenas-Pinto, R. Matthews, A. Whileman, R. Laugharne, J. Ledger, T. Barnes, C. Jones, D. Botes, N. Chitalia, S. Akhtar, G. Harrison, S. Horne, N. Walker, K. Agwuh, V. Maxwell, J. Graves, S. Williams, A. O'Kelly, P. Ridley, A. Cowley, H. Johnstone, P. Swift, J. Democratis, M. Meda, C. Callens, S. Beazer, S. Hams, V. Irvine, B. Chandrasekaran, C. Forsyth, J. Radmore, C. Thomas, K. Brown, S. Roberts, P. Burns, K. Gajee, T.M. Byrne, F. Sanderson, S. Knight, E. Macnaughton, B.J.L. Burton, H. Smith, R. Chaudhuri, K. Hollinshead, R.J. Shorten, A. Swan, C. Favager, J. Murira, S. Baillon, S. Hamer, K. Gantert, J. Russell, D. Brennan, A. Dave, A. Chawla, F. Westell, D. Adeboyeku, P. Papineni, C. Pegg, M. Williams, S. Ahmad, S. Ingram, C. Gabriel, K. Pagget, G. Maloney, J. Ashcroft, I. del Rosario, R. Crosby-Nwaobi, C. Reeks, S. Fowler, L. Prentice, M. Spears, G. McKerron, K. McLelland-Brooks, J. Anderson, S. Donaldson, K. Templeton, L. Coke, N. Elumogo, J. Elliott, D. Padgett, A. Cross, J. Price, S. Joyce, I. Sinanovic, M. Howard, T. Lewis, P. Cowling, D. Potoczna, S. Brand, L. Sheridan, B. Wadams, A. Lloyd, J. Mouland, J. Giles, G. Pottinger, H. Coles, M. Joseph, M. Lee, S. Orr, H. Chenoweth, C. Auckland, R. Lear, T. Mahungu, A. Rodger, K. Penny-Thomas, S. Pai, J. Zamikula, E. Smith, S. Stone, E. Boldock, D. Howcroft, C. Thompson, M. Aga, P. Domingos, S. Gormley, C. Kerrison, L. Marsh, S. Tazzyman, L. Allsop, S. Ambalkar, M. Beekes, S. Jose, J. Tomlinson, A. Jones, C. Price, J. Pepperell, M. Schultz, J. Day, A. Boulos, E. Defever, D. McCracken, K. Gray, A. Houston, T. Planche, R. Pritchard Jones, D. Wycherley, S. Bennett, J. Marrs, K. Nimako, B. Stewart, N. Kalakonda, S. Khanduri, A. Ashby, M. Holden, N. Mahabir, J. Harwood, B. Payne, K. Court, N. Staines, R. Longfellow, M.E. Green, L.E. Hughes, M. Halkes, P. Mercer, A. Roebuck, E. Wilson-Davies, L. Gallego, R. Lazarus, N. Aldridge, L. Berry, F. Game, T. Reynolds, C. Holmes, M. Wiselka, A. Higham, M. Booth, C. Duff, J. Alderton, H. Jory, E. Virgilio, T. Chin, M.Z. Qazzafi, A.M. Moody, R. Tilley, T. Donaghy, K. Shipman, R. Sierra, N. Jones, G. Mills, D. Harvey, Y.W.J. Huang, J. Birch, L. Robinson, S. Board, A. Broadley, C. Laven, N. Todd, D.W. Eyre, K. Jeffery, S. Dunachie, C. Duncan, P. Klenerman, L. Turtle, H. Baxendale, J.L. Heeney, SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN), Lancet 397 (2021) 1459–1469, https://doi.org/10.1016/S0140-6736(21)00675-9.

[41] L.J. Abu-Raddad, H. Chemaitelly, P. Coyle, J.A. Malek, A.A. Ahmed, Y.A. Mohamoud, S. Younuskunju, H.H. Ayoub, Z. al Kanaani, E. al Kuwari, A.A. Butt, A. Jeremijenko, A.H. Kaleeckal, A.N. Latif, R.M. Shaik, H.F. Abdul Rahim, G.K. Nasrallah, H.M. Yassine, M.G. al Kuwari, H.E. al Romaihi, M.H. Al-Thani, A. al Khal, R. Bertollini, SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy, EClinicalMedicine 35 (2021), https://doi.org/10.1016/j.eclinm.2021.100861.

[42] N. Li, X. Wang, T. Lv, Prolonged SARS-CoV-2 RNA shedding: not a rare phenomenon, J. Med. Virol. 92 (2020) 2286–2287, https://doi.org/10.1002/jmv.25952.

[43] J. Sun, J. Xiao, R. Sun, X. Tang, C. Liang, H. Lin, L. Zeng, J. Hu, R. Yuan, P. Zhou, J. Peng, Q. Xiong, F. Cui, Z. Liu, J. Lu, J. Tian, W. Ma, C. Ke, Prolonged persistence of SARS-CoV-2 RNA in body fluids, Emerg. Infect. Dis. 26 (2020) 1834–1838, https://doi.org/10.3201/eid2608.201097.

[44] D. Owusu, M.A. Pomeroy, N.M. Lewis, A. Wadhwa, A.R. Yousaf, B. Whitaker, E. Dietrich, A.J. Hall, V. Chu, N. Thornburg, K. Christensen, T. Kiphibane, S. Willardson, R. Westergaard, T. Dasu, I.W. Pray, S. Bhattacharyya, A. Dunn, J.E. Tate, H.L. Kirking, A. Matanock, L.M. Duca, E. Rabold, R. Gharpure, H. Njuguna, P. Dawson, E.E. Conners, V. Fields, P. Salvatore, P. Marcenac, H.E. Reses, M. Fajans, R.L. Laws, S. Yin, D. Ye, E. Pevzner, K. Battey, C. Tran, M. O'Hegarty, J. Vuong, R.J. Chancey, C.J. Gregory, M. Banks, J. Rispens, S. Lester, L. Mills, A. Fry, S. Nabity, B. Freeman, S. Buono, Persistent SARS-CoV-2 RNA shedding without evidence of infectiousness: a cohort study of individuals with COVID-19, J. Infect. Dis. 224 (2021) 1362–1371, https://doi.org/10.1093/infdis/jiab107.

[45] C. Acuña-Castillo, K. Maisey, M. Vidal, C. Barrera-Avalos, A. Inostroza-Molina, R. Luraschi, E. Vallejos-Vidal, D. Valdés, M. Imarai, F.E. Reyes-López, A. M. Sandino, Genomic evidence suggests viral persistence of SARS-CoV-2 for 386 Days in health worker: a case report from Santiago of Chile, Infect. Dis. Rep. 14 (2022) 971–978, https://doi.org/10.3390/idr14060096.

[46] T. Zahn, I. Mhedhbi, S. Hein, J. Raupach, C. Miskey, Y. Husria, K. Bayanga, D. Bartel, S. Vieths, Z. Ivics, D. Oberle, B. Keller-Stanislawski, M. Herrlein, T.J. Maier, E. Hildt, Persistence of infectious SARS-CoV-2 particles for up to 37 days in patients with mild COVID-19, Allergy 77 (2022) 2053–2066, https://doi.org/10.1111/all.15138.

[47] T.T. Truong, A. Ryutov, U. Pandey, R. Yee, L. Goldberg, D. Bhojwani, P. Aguayo-Hiraldo, B.A. Pinsky, A. Pekosz, L. Shen, S.D. Boyd, O.F. Wirz, K. Röltgen, M. Bootwalla, D.T. Maglinte, D. Ostrow, D. Ruble, J.H. Han, J.A. Biegel, M. Li, C. Huang, M.K. Sahoo, P.S. Pannaraj, M. O'Gorman, A.R. Judkins, X. Gai, J. Dien Bard, Increased viral variants in children and young adults with impaired humoral immunity and persistent SARS-CoV-2 infection: a consecutive case series, EBioMedicine 67 (2021) 103355, https://doi.org/10.1016/j.ebiom.2021.103355.

[48] A.J. Klecha, A.M. Genaro, A.E. Lysionek, R.A. Caro, A.G. Coluccia, G.A. Cremaschi, Experimental evidence pointing to the bidirectional interaction between the immune system and the thyroid axis, Int. J. Immunopharm. 22 (2000) 491–500, https://doi.org/10.1016/S0192-0561(00)00012-6.

[49] R. de Luca, P.J. Davis, H.-Y. Lin, F. Gionfra, Z.A. Percario, E. Affabris, J.Z. Pedersen, C. Marchese, P. Trivedi, E. Anastasiadou, R. Negro, S. Incerpi, Thyroid hormones interaction with immune response, inflammation and non-thyroidal illness syndrome, Front. Cell Dev. Biol. 8 (2021), https://doi.org/10.3389/fcell.2020.614030.

[50] M. Peng, J. He, Y. Xue, X. Yang, S. Liu, Z. Gong, Role of hypertension on the severity of COVID-19: a review, J. Cardiovasc. Pharmacol. 78 (2021), https://doi.org/10.1097/FJC.0000000000001116 e648–e655.

[51] D. Chambergo-Michilot, I.P. Chambergo Campos, Association between hypertension and immunosuppression in SARS-CoV-2 infection, Electronic Journal of General Medicine 18 (2021) em297, https://doi.org/10.29333/ejgm/10867.

[52] M. Worobey, J. Pekar, B.B. Larsen, M.I. Nelson, V. Hill, J.B. Joy, A. Rambaut, M.A. Suchard, J.O. Wertheim, P. Lemey, The emergence of SARS-CoV-2 in europe and north America, Science 370 (2020) 564–570, https://doi.org/10.1126/science.abc8169.

[53] S. Duchene, L. Featherstone, M. Haritopoulou-Sinanidou, A. Rambaut, P. Lemey, G. Baele, Temporal signal and the phylodynamic threshold of SARS-CoV-2, Virus Evol 6 (2020), https://doi.org/10.1093/ve/veaa061.

[54] Z.M. Lohse, J.J. Shapiro, J.A. Lednicky, M.N. Cash, I. Jun, C.N. Mavian, M.S. Tagliamonte, C. Saleem, Y. Yang, E.J. Nelson, M. Salemi, K.A. Ryan, J.G. Morris, Persistence of severe acute respiratory syndrome coronavirus 2 Omicron variant in children and utility of rapid antigen testing as an indicator of culturable virus, Clin. Infect. Dis. 76 (2023), https://doi.org/10.1093/cid/ciac693 e491–e494.

[55] D. Haddad, S.E. John, A. Mohammad, M.M. Hammad, P. Hebbar, A. Channanath, R. Nizam, S. Al-Qabandi, A. Al Madhoun, A. Alshukry, H. Ali, T.A. Thanaraj, F. Al-Mulla, SARS-CoV-2: possible recombination and emergence of potentially more virulent strains, PLoS One 16 (2021) e0251368, https://doi.org/10.1371/journal.pone.0251368.

[56] J.A. Plante, Y. Liu, J. Liu, H. Xia, B.A. Johnson, K.G. Lokugamage, X. Zhang, A.E. Muruato, J. Zou, C.R. Fontes-Garfias, D. Mirchandani, D. Scharton, J.P. Bilello, Z. Ku, Z. An, B. Kalveram, A.N. Freiberg, V.D. Menachery, X. Xie, K.S. Plante, S.C. Weaver, P.-Y. Shi, Spike mutation D614G alters SARS-CoV-2 fitness, Nature 592 (2021) 116–121, https://doi.org/10.1038/s41586-020-2895-3.

[57] L.C. S.M.V, I.A. Moriyama M, SARS-CoV-2 Omicron Subvariants Evolved to Promote Further Escape from MHC-I Recognition, BioRxiv, 2022.

[58] A.I. Abulsoud, H.M. El-Husseiny, A.A. El-Husseiny, H.A. El-Mahdy, A. Ismail, S.Y. Elkhawaga, E.G. Khidr, D. Fathi, E.A. Mady, A. Najda, M. Algahtani, A. Theyab, K.F. Alsharif, A. Albrakati, R. Bayram, M.M. Abdel-Daim, A.S. Doghish, Mutations in SARS-CoV-2: insights on structure, variants, vaccines, and biomedical interventions, Biomed. Pharmacother. 157 (2023) 113977, https://doi.org/10.1016/j.biopha.2022.113977.

[59] J. Ringlander, J. Olausson, K. Nyström, T. Härnqvist, H.E. Jakobsson, M. Lindh, Recurrent and persistent infection with SARS-CoV-2–epidemiological data and case reports from Western Sweden, 2020, Inf. Disp. 53 (2021) 900–907, https://doi.org/10.1080/23744235.2021.1957143.

[60] R. Ke, E. Romero-Severson, S. Sanche, N. Hengartner, Estimating the reproductive number R0 of SARS-CoV-2 in the United States and eight European countries and implications for vaccination, J. Theor. Biol. 517 (2021), https://doi.org/10.1016/j.jtbi.2021.110621.