

# Impact of COVID-19 RT-PCR Testing of Asymptomatic Health Care Workers on Absenteeism and Hospital Transmission During the Pandemic

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# Abstract

Reducing the transmission of SARS-CoV-2 from asymptomatic and pre-symptomatic patients is critical in controlling the circulation of the virus. This study evaluated the prevalence of RT-PCR positivity in serial tests (every 20 days) in 429 asymptomatic health care workers (HCW) and its impact on absenteeism from May to August 2020. Asymptomatic HCW from a COVID-19 reference hospital in Campinas (1.2 million inhabitants), Brazil, were tested, screened, and placed on leave. A time-series segmented regression of weekly absenteeism rates was used, and cases of infection among hospitalized patients were analyzed. Viral gene sequencing and phylogenetic analysis were performed on samples gathered from professionals who had a positive result.

A significant decrease in absenteeism was detected 3–4 weeks after the intervention at a time of increased transmission within the city. The prevalence of RT-PCR positivity among asymptomatic professionals was 17.3%. Phylogenetic analyses of 59 samples detected nine clusters, two of them strongly suggestive of intra-hospital transmission with strains (75% B.1.1.28) circulating in the region during this period.

Testing and placing asymptomatic professionals on leave contributed to control strategy for COVID-19 transmission in the hospital environment, and in reducing positivity and absenteeism, which directly influences the quality of care and exposes professionals to an extra load of stress.

## Introduction

Health care workers (HCW) comprise a group that is particularly affected by SARS-CoV-2 and exposed to high viral loads, higher than the general population [1–3]. Transmissibility between asymptomatic or pre-symptomatic individuals is a challenge for the control of the pandemic, especially among HCW [4].

Absenteeism among HCWs is a major challenge for hospital management because it burdens the health services and promotes a decrease in the quality of care, exposing professionals to the extra load of stress, often associated with mental suffering.

A review identified different percentages (17–54%) and an estimated average of 40% of asymptomatic individuals with positive molecular tests for SARS-CoV-2 in different population groups, with evidence of silent viral circulation [5, 6].

Universal testing of asymptomatic patients has been adopted in several centers as a strategy to control intra-hospital infection of patients and HCWs with molecular tests [7, 8].

The objective of this study was to evaluate the impact of a universal RT-PCR testing strategy for asymptomatic HCWs in a university hospital to reduce the circulation of SARS-CoV-2 among health care workers, absenteeism, and consequently, nosocomial transmission between professionals and patients.

## Methods

This is a study of the prevalence of SARS-CoV-2 positivity and a time series of weekly rates of absenteeism due to COVID-19 among HCWs in a hospital during the first months of the epidemic, March to August 2020. The impact of implementing a universal serial RT-PCR testing program for asymptomatic HCW, tracking of contacts, and absenteeism were evaluated. Through the phylogenetic reconstruction of some of the positive samples, potential transmission clusters were investigated and correlated with information on function and place of work.

### Study location and period

The hospital has 325 beds, of which 196 are dedicated to public health, placing it as a regional clinical reference center (3 million inhabitants), and in the city of Campinas (1.2 million inhabitants), 100 km from the capital São Paulo. The hospital sectors enrolled in the research were the intensive care units (ICUs), COVID-19 wards, clinical and surgical wards, the adult emergency room, administrative, and cleaning and support sectors, with a total of 170 beds and 473 professionals.

Protocols for testing/tracking/absence from work on leave for symptomatic patients and personal protective equipment (PPE) were already implemented in the hospital according to the recommendations of the National Health Surveillance Agency (ANVISA) [09] and Centers for Disease Control and Prevention (CDC) [10].

### The hospital and the COVID-19 pandemic

The first imported case in Campinas was registered on March 12, 2020; the first patient with COVID-19 admitted to the PUC-Campinas Hospital was on March 15, and the first positive case in an HCW was on March 18. The increase in the incidence curves for COVID-19 in the city followed the transmission growth noted within the state of São Paulo, with a peak in epidemiological weeks 23–27 (June 2020), with a total of 8523 cases accumulated up to week 27 [11].

### The intervention: universal testing and placing on leave of asymptomatic HCW

On May 11 (epidemiological week 20), the intervention began by testing all asymptomatic health professionals who agreed to participate in the research, from all the hospital sectors enrolled in the study.

After signing the informed consent form, professionals were invited to answer a questionnaire and then a biological sample was collected using a nasopharynx swab to perform the RT-PCR Fleury test (Charité [12] and CDC [10] protocols) and/or Gene-X-pert (Cepheid-USA) for faster results. If positive, HCW were placed on leave. Three serial collections were performed on each professional who had been negative in previous tests, with an interval of 20 days between them. Symptomatic workers were not included in the study; that is, those who presented at least one of the following symptoms: headache, fever, cough, sore throat, anosmia, ageusia, myalgia, chills, cough, or runny nose.

## Study variables and data source

Using a structured questionnaire, data on demographics (gender and age), workplace, and role performed in the hospital, as well as symptoms (in the last 14 days) were obtained.

RT-PCR test positivity rates were calculated according to the variables of interest (gender, age, professional category, work place in the hospital). The number of daily leaves and the total days of leave for any reason and for COVID-19 were obtained from the Department of Human Resources and Occupational Medicine of the hospital from January 1 to September 12, 2019, and 2020 (epidemiological weeks 1–37).

The impact of the testing intervention in asymptomatic individuals was evaluated based on the temporal trend of weekly leave rates by COVID-19 (number of leaves per week/total number of employees scheduled for the week) from March 8 to September 12, 2020 (epidemiological weeks 11–37).

Data on the COVID-19 epidemic (confirmed cases of COVID-19 severe acute respiratory syndrome) of the population of Campinas and HCW were obtained from publications of the Health Surveillance Department of the Municipal Health Department of Campinas, allowing for comparison of the incidence curves in the study institution and in the community (<https://covid-19.campinas.sp.gov.br/>).

Health care-associated COVID-19 was considered when the onset of symptoms occurred at least 7 days after admission [13].

## Viral genetic sequencing and phylogenetic analysis

Biological samples from professionals with positive RT-PCR results collected in different sectors of the hospital were sequenced to assess clusters and the lineage of SARS-CoV-2.

After extraction of viral RNA, cDNA synthesis was performed using the Protoscript II First Strand transcription kit (New England Biolabs, UK) and random hexamers (Thermo Fisher Scientific, Waltham, MA, USA). Amplification of the total genome was carried out using the multiplex PCR reaction with specific primers for SARS-CoV-2 (<https://artic.network/ncov-2019>), together with the Q5 High-Fidelity DNA Polymerase Kit (New England Biolabs, UK). The PCR conditions have been described previously (<https://artic.network/ncov-2019>). Amplicons were purified using magnetic beads (1× AMPure XP, Beckman Coulter, UK). After purification, the product was quantified using fluorometry techniques with Qubit dsDNA high sensitivity reagents, and reading was performed using the Qubit 3.0 instrument (Life Technologies, Carlsbad, CA, USA).

## Complete genome sequencing of SARS-CoV-2

To achieve good coverage of the genome, only samples with more than 4 ng/μL of DNA were used to prepare the library for sequencing. The amplicons of each sample were normalized to be equimolar. After

this process, the normalized amplicons were processed to continue preparing the library for sequencing according to the previously published protocol [14] using the EXP-NBD104 (1–12) and EXPNBD114 (13–24) kits (Oxford Nanopore Technologies, Oxford, UK). The libraries were loaded into a flow cell and sequenced by MinION for 8–24 h using the SQK-LSK109 kit (Oxford Nanopore Technologies). To monitor the sequencing in real time and estimate the depth of coverage (200 times target), ARTIC Network RAMPART software (<https://artic.network/ncov-2019>) was used. Minimap2 v2.28.0 software was used to obtain the consensus sequence and structure the fast five files against the reference genome of the SARS-CoV-2 Wuhan-Hu 1 isolate (GenBank accession number MN908947).

## Phylogenetic analysis

Consensus sequences were initially submitted to a quality control check using Nextclade. To retain the maximum amount of information possible, sequences with coverage above 80% were used for further analysis. Our final dataset consisted of 49 original SARS-CoV-2 complete genomes and nine sequences collected in Campinas downloaded from GISAID as of July 31, 2020.

Fast multiple sequence alignment was applied in our dataset with MAFFT v. 7.450. A maximum likelihood tree was reconstructed using IQ-TREE version 1.6.12 [15] under the TIM + F + I nucleotide substitution model (ModelFinder). Statistical support was evaluated using 1000 bootstrap replicates. Internal nodes with > 90% statistical support were assigned on the tree. To infer the existence of potential transmission clusters, we used Phydentity v. 2.1. SARS-CoV-2 lineages were identified using Pangolin software.

## Statistical analysis

The prevalence of positivity and the respective 95% confidence intervals were calculated by the ratio of positive tests to the population tested at the three testing moments, according to the variables of interest. Proportions were compared using the chi-squared test with Yates' correction, with a significance level of 5% ( $P < 0.05$ ).

After verifying the assumption of homoscedasticity, absence of autocorrelation, and normality of residuals, a segmented regression of weekly absenteeism rates was adjusted. The percentage and mean variations in the rates and their statistical significance were obtained using the Joint Point Regression Program version 4.5.0.1.

The median and quartiles (1st and 3rd) of daily leaves for all causes of health care workers from January 1 to September 12, 2019, and 2020 were also evaluated. The daily averages of HCW sick leave from 2019 and 2020 were compared using analysis of variance analysis (ANOVA) and the F test, considering a significance level of 5% ( $P < 0.05$ ).

All methods were carried out in accordance with relevant guidelines and regulations and this study was approved by Pontifical Catholic University of Campinas Medical Ethics Committee of Pontifical Catholic University of Campinas, under protocol no. 31042120.4.0000.5481.

## Results

From May to August 2020, 429 asymptomatic HCWs were tested with RT-PCR three times at a mean interval of 20 days. The total prevalence of positive RT-PCR tests was 74 of 429 (17.3%; 95% CI, 13.7–20.3), with 11.9% (95% CI, 8.8–14.9) in the first collection, 6.9% (95% CI, 3.8–9.9) in the second, and 2.9% (95% CI, 0.4–5.4) in the third. Fifty-two RT-PCR positive nasopharyngeal secretion samples were sequenced. The analysis of the strains revealed that approximately 75% of the sequences belonged to the B.1.1.28 strain, 21.15% to the B.1.1.143 strain, and 3.84% to the B.1 strain. The most frequent amino acid substitutions were L3930F from the ORF 1ab region (open reading frame), D614G and V1176F from the Spike protein region, and R203K and G204R from the Nucleocapsid protein. According to PROVEAN analysis, all these substitutions can be classified as neutral.

The phylogenetic tree shows potential putative transmission clusters among the health professionals (Fig. 1). A total of nine clusters were inferred through the patristic distance distribution on the tips of the tree. Among them, five clusters shared samples belonging to professionals who worked in the same occupation. Two clusters also shared the same workplace inside the hospital, strongly suggesting intra-hospital transmission; cluster C was found in two nursing technicians in the COVID-19 ward and collected on the same day (June 19, 2020) and cluster H collected from two cleaning staff of the same unit, on different dates (June 22 and 29, 2020).

Figure 2 shows the temporal trend of weekly rates of work leave due to COVID-19 and their respective variations by time segment. From the intervention (week 20), at first there was an increase in HCW leave, still in segment 1; and after 3–4 weeks, there was a significant change in the trend and a decrease in absenteeism from the epidemiological week onward. (Weekly Percent Variation:  $p < 0.05$ )

The epidemic curve of COVID-19 in Campinas (in the general population and among health professionals) does not present a decrease concomitant with the reduction of absenteeism in the hospital; on the contrary, this reduction occurs in the weeks of peak incidence of infection in the city (Fig S1).

The total number of days of leave for all causes from January to August 2019 and 2020 were 733 days (daily average, 4; SD, 2.6) and 3469 days (daily average, 18.9; SD, 9.7) ( $P < 0.001$ ), respectively, which represented an increase of 473%. An excess of leave for all causes was observed when comparing the 2019 and 2020 (Fig. 3).

## Discussion

Several studies report wide circulation of SARS-CoV-2 among health workers, identified as one of the most affected segments in the COVID-19 epidemic, both in developed countries and in poorer regions [2, 3, 16]. We found a high prevalence of positive RT-PCR tests of 17.3% (95% CI, 13.7–20.3), decreasing in the successive tests of asymptomatic professionals. Surprisingly, the high prevalence in the non-COVID-19 clinical/surgical and ICU cardiology sectors of 20.3% (95% CI, 10.8–29.8) and 28.0% (95% CI, 17.8–

38.2), respectively, highlight the importance of universal testing of professionals, regardless of their locations with supposedly less exposure to the virus. The phylogenetic identification of hospital clusters of cases with the same occupation and in the same work environment requires specific prevention and training measures, both of which occurred in COVID-19 units.

The B.1.1.28 strain identified in the hospital, which came from Europe (clade 1) in the first months of the pandemic [16], was the most predominant strain in the state of São Paulo. In April 2020, SARS-CoV-2 with the D614G mutation in the Spike protein became dominant in the pandemic in Brazil and in various parts of the world, associated with higher transmissibility and a higher viral load, however, without a change in the pathogenicity [18].

Some reports of tracking symptomatic [19, 20] and asymptomatic HCW [21] have identified areas of risk for infection in specific hospital care sectors for COVID-19 and in others. Particularly at the beginning of the epidemic, with less access to protective equipment and testing resources and with no available vaccines, HCW were especially exposed by SARS-CoV-2 [1]. Outbreaks, both among patients and among HCW, puts individuals with comorbidities who are hospitalized for other causes at risk and depletes the work teams in outpatient and inpatient services [22]. In addition to investigating the molecular test positivity, genomic surveillance could qualify the epidemiological investigation allowing the identification of clusters, transmission sites, and timely actions for the control and prevention of hospital infection [23]. In the UK, a study carried out on the general population [25], showed a variation in percentages of asymptomatic individuals of 15–66% (on average, one third of those detected by RT-PCR) depending on the timing in the pandemic.

Up to the start of the screening of asymptomatic HCW (week 20), the increase in leaves due to COVID-19 followed the growth of COVID-19 diagnoses among professionals and the general population in the municipality (outpatient clinics and admissions for severe acute respiratory syndrome). However, the temporal distribution of HCW leaves due to COVID-19 showed a significant reversal of the trend 3–4 weeks after the beginning of the testing of asymptomatic professionals, suggesting a reduction in hospital exposure after the intervention.

Absenteeism due to COVID-19, in addition to posing a risk to HCWs, worsens inpatient care, exposes the rest of the team to an exhaustive and, consequently, less safe workday [8, 25]. Work overload in small teams causes insecurity and stress in health professionals and is related to a wide spectrum of mental health problems [24].

Among the limitations of this study, the short period of post-intervention analysis makes it difficult to obtain more robust estimates of the impact, although there is already a significant reduction in intra-hospital transmission between professionals and patients. We used different databases to compare the temporal evolution of indicators in the city and in the hospital, however these were used as proxies for the local epidemiological situation in order to contextualize the downward trend in absenteeism in the institution. Besides, external influences due to community-related intervention measures, such as social isolation, may have interfered with viral circulation, reducing the community risk of transmission.

However, the study was carried out in the weeks with the highest transmission peak during the city's first wave of the epidemic and when hospital occupancy rates were more than 80% [11].

## **Conclusion**

Screening and isolation of asymptomatic health care workers using RT-PCR possibly had an impact on intra-hospital viral circulation, significantly reducing tests positivity and leave among HCWs due to COVID-19 at a time of increased community transmission in the city and in hospitalized patients. The reduction in absenteeism indirectly reflects a decrease in the circulation of SARS-CoV-2 in the hospital environment, detected in nine clusters within the institution. There was also a trend toward a reduction in the transmission of the infection to hospitalized patients.

Therefore, this is an important strategy for the prevention and control of outbreaks of COVID-19 in the hospital environment, given the insufficiency of screening based on symptoms. Antigen tests could also be used for this purpose, with lower cost and faster results, although with less sensitivity.

## **Declarations**

### **Author contributions**

Each author has substantially contributed to the study conducting the underlying research and drafting this manuscript. Authors have no conflict of interest, financial, or otherwise.

### **Availability of data and material**

All the data analyzed in this study will be made available upon reasonable request to the corresponding author.

### **Ethical approval**

This study was approved by the Medical Ethics Committee under CAE protocol no. 31042120.4.0000.5481.

### **Competing interests**

All authors declare they have no conflicts of interests, financial or otherwise, to declare.

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## Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

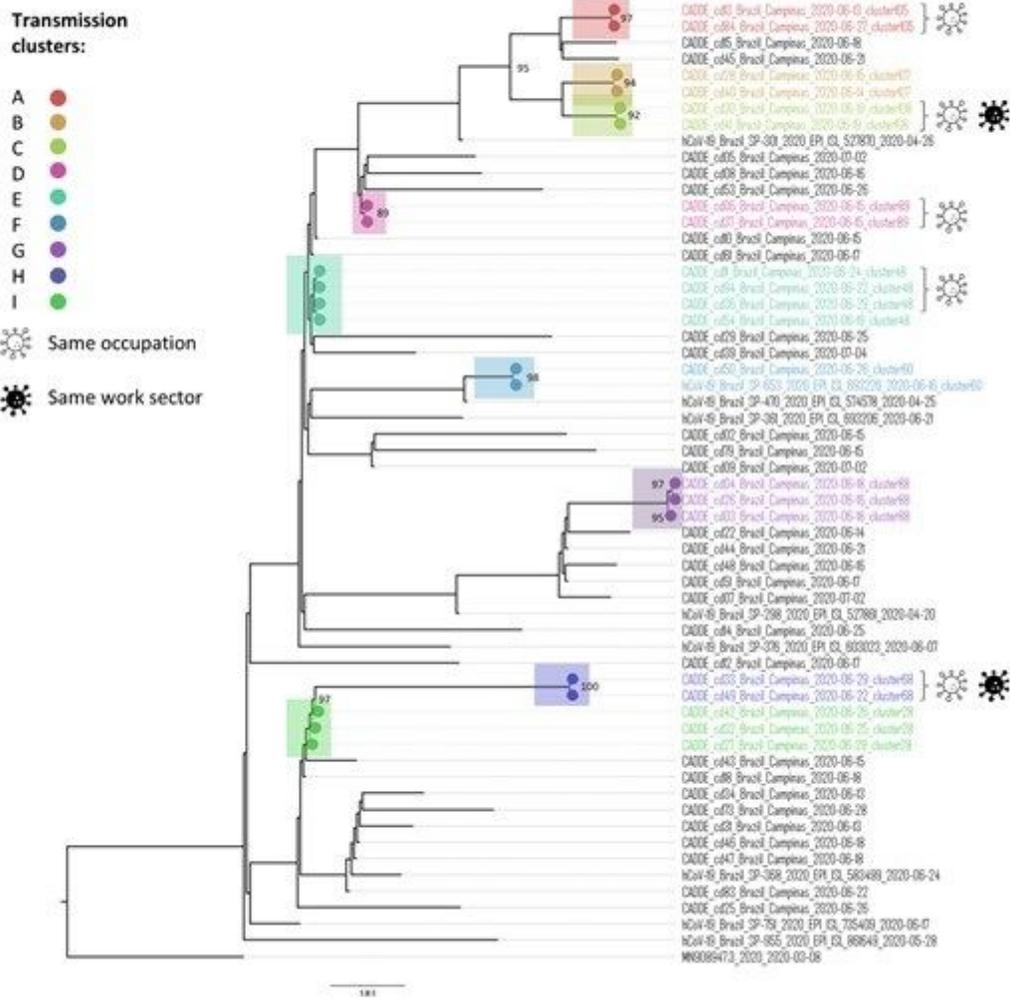
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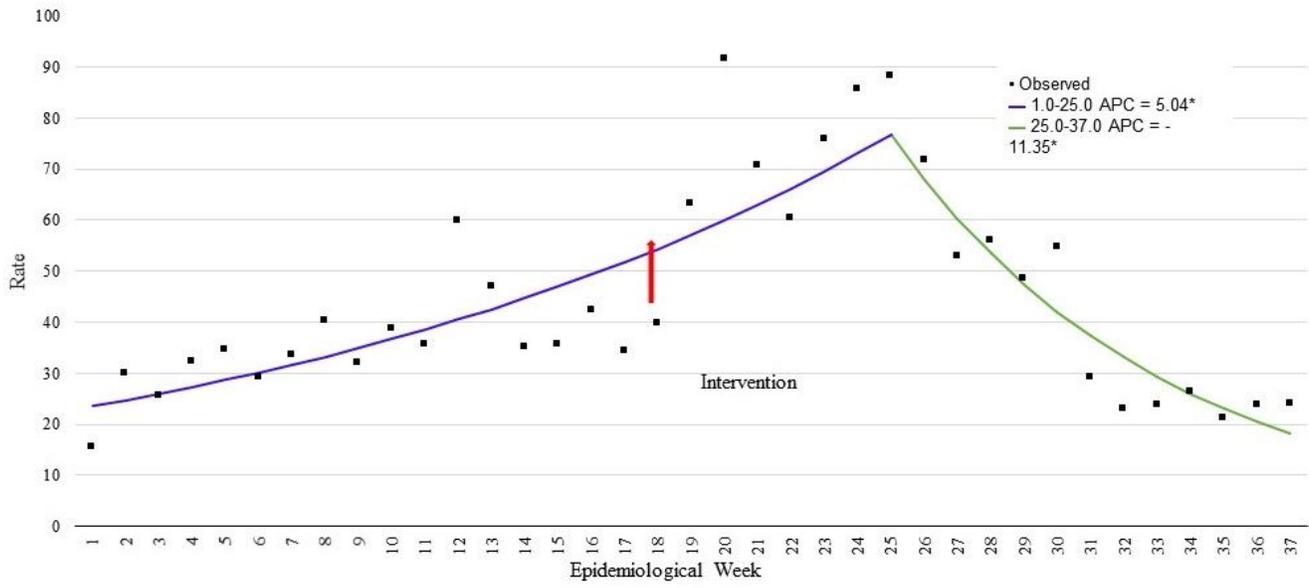
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## Figures



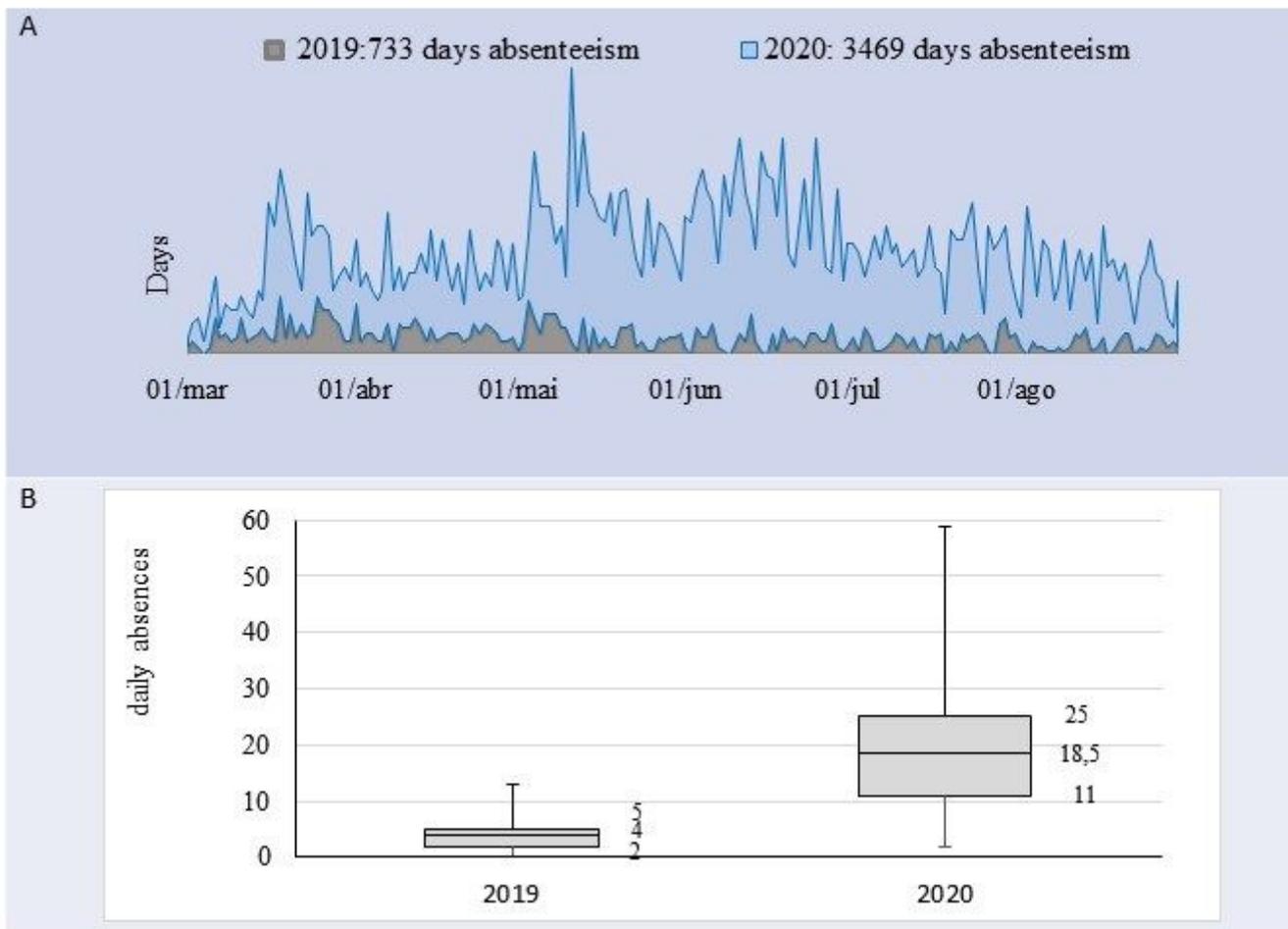
**Figure 1**

**Phylogenetic tree of SARS-CoV-2 detected in samples of nasopharyngeal secretion (n = 59) from health care professionals at the PUC-Campinas hospital from May to September 2020. Cluster C, both nursing technicians in the COVID ward collected on the same day (June 19); cluster H, cleaning staff in the COVID ward collected on June 22 and 29.**



**Figure 2**

**Temporal trend of weekly rates of absence from work by COVID-19.** In PUC-Campinas Hospital, epidemiological weeks 1–37, 2020. \*APC refers to weekly percent variation of rates, which are significantly different from zero at alpha = 0.05.



**Figure 3**

**Absence of health professionals for all causes at Hospital PUCG, Campinas.** (A) Temporal trend of absence from work from March to August 2019 and 2020. (B) Daily absenteeism (median and 1st and 3rd quartiles) for all causes from January 1 to August 30, 2019, and 2020.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [HCWtests.xlsx](#)
- [MicroreactTreeCampinas.png](#)
- [MolecularanalysisHCWd1.xlsx](#)
- [suplgrafic.tif](#)