



Occurrence of SARS-CoV-2 in Indoor Environments With Increased Circulation and Gathering of People

Radka Dziedzinska^{1,2}, Petr Kralik^{1,2} and Omar Šerý^{1,2,3*}

¹ Laboratory of Neurobiology and Pathological Physiology, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Liběchov, Czechia, ² Elisabeth Research Institute, Brno, Czechia, ³ Laboratory of DNA Diagnostics, Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czechia

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*Correspondence:

Omar Šerý
omarsery@sci.muni.cz

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At the time of sampling (2020/2021), the number of new cases of SARS-CoV-2-positive individuals in the Czech Republic significantly exceeded the numbers in neighboring countries and in the EU. In terms of the number of deaths, the country ranked near the top of the list. Legislative orders required wearing masks indoors, disinfecting surfaces in public places, and limiting the number of people per sales area in commercial spaces. Due to an situation, most schools and shops were closed. The entire country anticipated a total lockdown. To assess the risk to public health regarding SARS-CoV-2 transmission, air and surfaces were sampled in two public places: a post office and a shopping center. Samples were also collected at the COVID-19 unit at the local hospital. Neither air nor surface samples were positive for SARS-CoV-2 virus particles in the post office or shopping center. Positive results were found in the hospital ward, with floors being the most and highest contaminated surface. Based on our results, we believe that public places do not pose a risk in relation to SARS-CoV-2 transmission, especially when epidemiological measures to reduce transmission are followed, such as wearing masks, using disinfectant or limiting the number of customers per retail establishment.

Keywords: SARS-CoV-2, indoor, RT-qPCR, surface contamination, fomites, air

INTRODUCTION

The SARS-CoV-2 pandemic expanded at the turn of the years 2019 and 2020, erupting from a local outbreak in Wuhan, China to spread across the entire world. The speed with which the virus traveled, combined with the ease of transmission amongst patients with mild symptoms or no symptoms at all, meant that SARS-CoV-2 infected vast numbers of people in a relatively short time. Government and hygienic services in affected countries were nearly helpless to face the virus's rapid spread within the population. A series of implemented protective measures were blindly put in place, primarily focused on restricting the population's movement. This led to the partial or complete lockdown of the commercial sector in most of the affected countries, leaving only the essential stores open. All other segments of the commercial sector remained closed without any prospect of re-opening.

However, different countries' lockdowns in the commercial sector always occurred together with other measures such as school closures, restrictions on travel between regions, cancellations of group sports, mandates for wearing masks, etc. Therefore, it is difficult to evaluate the efficacy of each individual measure. Furthermore, different countries implemented these measures to varying

degrees. The main variables in the protective measures' efficacy were the motivation of citizens to adhere to the measures and adapt their behavior accordingly, as well as the impact of the virus variant itself, compounded by the overall epidemiological situation in a particular country.

The retail lockdowns were criticized as ineffective because the number of infections rose despite the retail closures. Various countries reported the same scenario. Retail representatives claimed that stores can easily restrict access and control the compliance with the hygienic measures like face mask wearing, social distancing or surface sanitization. Despite these assurances, stores remained closed, causing massive economic damage to the service and commercial sectors. Never in the past there has been such a massive restriction on the entire running of the country, involving both a restriction on the personal freedom of each individual and a restriction on the country's economy.

To help the decision-making bodies focus on effective preventive and protective measures in suppressing SARS-CoV-2 prevalence in the population, this study examined the screening of internal premises with a high movement of people to determine the risk of infection to patrons and visitors. For the purposes of the study, the central post office in Brno, Czech Republic (population 382,000) and a large shopping center in Olomouc, Czech Republic (population 100,000) were selected. The sampling was performed in the period of the SARS-CoV-2 epidemic when the percentage of positive tests exceeded 30% in the Czech Republic. The samples were taken by surface swabs from the internal environment as well as from the air. To compare the data from the publicly accessible internal premises, similar samples were collected from a local hospital's COVID-19 unit.

MATERIALS AND METHODS

Premises Selection

The study focused on the spread of the SARS-CoV-2 virus in enclosed premises with increased crowds and unavoidable social contact. The first sampling was performed on 17 December 2020 in the evening after 6 p.m. at the central post office in Brno city with a daily turnout of 2,600 people. Sampling was performed during the holiday season when the daily percentage of newly identified cases exceeded 30% (Ministry of Health of Czech Republic; <https://onemocneni-aktualne.mzcr.cz/api/v2/covid-19>) ending in early January 2021. The second sampling was performed on 17 February 2021 after 6 p.m. in the Šantovka shopping center in Olomouc, in which the supermarket and food court remained open. The samples were taken predominantly from the supermarket, however other places with a higher fluctuation of people were also screened due to movements of visitors through them (Table 1). The sampling was performed when the percentage of newly identified cases remained above 30% (Ministry of Health of the Czech Republic; <https://onemocneni-aktualne.mzcr.cz/api/v2/covid-19>). A total of over 10,000 people visited the Šantovka shopping center on the day of sampling. Of these 59% were pedestrians and 41% arrived by car. The last sampling was performed on 6 May, 2021 in a hospital unit set up for

TABLE 1 | Description of the samples collected at the post office and in the shopping center.

Post office		Shopping center	
Type of sample	Number	Type of sample	Number
Customer retrieval terminal	3	Self-service checkout display	6
Post counter	9	Shopping cart and basket handles	20 [†]
Door handles	2	Escalator handrail	4
Writing desk for customers	2	Toilet door handle	5
Hands of operators	4	Hands of cashiers	6
		Elevators buttons	8
		Buttons of the parking terminal	2
Sum	20		51

[†] One sample consist of swab from 3 shopping cart or basket handles.

treatment of patients who tested positive for SARS-CoV-2 (Table 2). The hospital was situated in Blansko district, ~30 km from Brno.

Sample Collection

Two types of samples - swabs and air - were gathered in all three locations. Swab samples were taken from surfaces which come into direct contact with people's hands as well as directly from the hands themselves (Table 1). The flocked swabs (FLOQSwabs Genetics, Copan, Italy) were moistened in a 0.9% saline solution and area of ~100 cm² was wiped. The swab sample was immediately placed into 600 µl of Lysis buffer (EliGene[®] Viral DNA/RNA FAST Isolation Kit, Elisabeth Pharmacon) diluted to a 1:1 aqueous solution.

The air was sampled by the commercially available air washer LW220 (Beurer, Ulm, Germany). The principle of the air washer is based on the slow turning of a segmented wheel in water. The tank intended for the water was filled with ~4l of the Lysis buffer (EliGene[®] Viral DNA/RNA FAST Isolation Kit, Elisabeth Pharmacon) diluted in a 1:1 aqueous solution and the speed of the air flow was set to Maximum. The air washer was able to exchange 60 m³ of air per hour. This volume was determined experimentally by measuring the air flow when the machine was running at the Maximum setting.

The air washer was placed ~1 m above the floor in the location where people gathered. The main entrance door of the post office and the counter area of the supermarket were selected. On the COVID-19 unit, the air washer was placed on the window sill of a room with a covid patient. The operation time of the air washer in all three premises was 12 h, which means that 720 m³ flowed through the washer during each sampling. Afterwards, the remaining liquid for the air washer was collected and transferred into high-volume flasks.

TABLE 2 | Description of the samples collected at the COVID-19 unit.

Location	Type of sample*	Average quantity of virus [†]
Room 1	Patient—hands	–
	Patient—mouth	–
	Tabletop by the window	–
	Floor next to the bed	6
	Cup + mobile phone	–
	Door handles	–
	Washbasin	–
	Sink drain	–
	Bedside table	–
	Balcony handles + window handles	56
	Floor in front of Room 1	16
Room 2	Patient 1—mouth	–
	Patient 1—hands	–
	Patient 2—mouth	–
	Patient 2—hands	–
	Patient 3—mouth	4
	Patient 3—hands	–
	Tabletop	1
	Floor between the beds	–
	Edge of the bed	5
	Door handle	–
	Washbasin	3
Examination room	Sink drain	–
	Balcony handles	3
	Floor in front of Room 2	–
	PC keyboard	–
	Shared mobile phone	–
Staff equipment	Floor	33
	Shoe sole	42
	Face shield	–
	Overall	–
Changing room	Phonendoscope—ear attachments	–
	Floor	1

*One sample was collected from each location ($n = 33$).

[†]The quantity of virus (viral genomes) is related to the 5 μ l of qPCR reaction.
–, negative.

RNA Isolation

The RNA from swab samples was isolated according to the original protocol from EliGene[®] Viral DNA/RNA FAST Isolation Kit (Elisabeth Pharmacon, Brno, Czech Republic). Two hundred microliters of the suspension with the swab was mixed with the Lysis Buffer with the Solution M added. In this step, 5 μ l of internal amplification control (IAC) from the EliGene[®] COVID-19 CONFIRM RT (Elisabeth Pharmacon) was pipetted into the suspension. The whole volume was loaded onto the column, washed, and eluted to 50 μ l of Elution buffer.

The liquid from the air washer was diluted with water to the original volume (4l). Using a vacuum manifold, four separate spin columns, and special extensions for higher volume

processing, a total of 500 ml of the liquid was gradually loaded onto the columns, processing an average of 125 ml per column. IAC was applied to each column in a similar way as the RNA isolation from swabs. The washing and elution to 50 μ l was performed according to the manufacturer's recommendation from the EliGene[®] Viral DNA/RNA FAST Isolation Kit (Elisabeth Pharmacon).

RT-qPCR Analysis

The RT-qPCR analysis was performed by the EliGene[®] COVID-19 CONFIRM RT (Elisabeth Pharmacon) in total volume of 20 μ l with 5 μ l of the extracted RNA added. The qPCR protocol comprised an RT step at 55°C for 15 min, followed by the initial denaturation at 95°C for 3 min and 45 cycles of amplification at 95°C for 5 s, 55°C for 15 s, and 67°C for 15 s. The experiments were performed at CFX Touch qPCR (Bio-Rad, Hercules, CA, USA) cyclers and the analysis of the results was performed by the CFX Maestro 2.0 software (version 5.0.021.0616).

To estimate an approximate number of viral particles in the samples, a simplified calibration curve was included in each run. According to the declaration of the kit's manufacturer, the positive control from the detection kit contains 10³ synthetic DNA molecules of the SARS-CoV-2 targets per microliter. The undiluted and 10 \times - and 100 \times -diluted positive controls were used for the construction of the calibration curve in a range 10³–10¹ DNA molecules/ μ l, i.e., 5 \times 10³–5 \times 10¹/qPCR reaction. The LOD of the qPCR kit was determined by the manufacturer to be 15 genomic copies per qPCR reaction. Each isolated RNA molecule was processed as a technical triplicate. Samples were considered positive if they presented as positive in at least one repeat.

RESULTS

Analysis of the Central Post-office and Shopping Center Premises

A total of 71 swab samples of various surfaces, including hands of the operators/employees, were collected at the post office and in the shopping center (Table 1). All of them were found to be negative for the presence of SARS-CoV-2 virus. After 12 h of operating, the SARS-CoV-2 was detected neither in liquid from the air washer located at the post-office ($n = 1$) nor at shopping center ($n = 1$).

Presence of SARS-CoV-2 in the Environment of the Hospital COVID-19 Unit

Altogether 33 swab samples were collected from different premises within the hospital COVID-19 unit. Eleven samples (33%) were found to be positive for the presence of SARS-CoV-2 (Table 2). The quantity of virus in the positive samples was rather low, in units or tens of viral genomes in qPCR reaction. The quantity was not absolute and served only for the rough estimation of virus particle counts. The floor and various places that come into physical contact with patients' hands were the most contaminated with the virus. Samples taken from the floor or sole were positive for the presence of virus in five cases.

The air in the room with a single patient was found to be negative for the presence of the virus.

DISCUSSION

Today, the potential risk of SARS-CoV-2 transmission through touching fomites is known to be negligible. In the past, a number of studies demonstrated the presence of SARS-CoV-2 virus on surfaces with a higher or lower percentage of positive samples (1–3). As simple RT-qPCR was used in most of them, they could not provide any information about the viability of viral particles or their ability to cause infection. Based on prediction models, the estimated risk of probability of SARS-CoV-2 infection from contaminated surfaces through hands was determined to be low and ranged from $<10^{-5}$ (2) to 10^{-6} (4). In fact, the real prevalence would be probably even lower due to voluntary/ordered quarantine of an unknown fraction of positive people and due to people not coughing directly to their hands, factors which the predictive models rely upon to work (4).

In our case, positive samples were not found in the central post office nor in the public area of the mall. Negative results were obtained despite an increase of over 30% of newly identified cases per day in both sampling periods (mid-December 2020 and mid-February 2021) in the Czech Republic. At the time of sampling, the Czech Republic had implemented a requirement for wearing masks in indoor places and the mandatory disinfecting of public places. Both of these factors could have contributed to an inability to detect the virus. Only a few studies focused on monitoring the virus in different facilities others than hospitals. SARS-CoV-2 RNA appeared in 6% of samples in Italian tourist and recreational facilities (3), 8.3% of indoor and outdoor high-touch surfaces in the city of Somerville, Massachusetts (2) and in 12.3% of samples collected from public sites and households with inhabitants who had active, recovered, and negative disease statuses as confirmed by PCR tests (5). Ardura et al. (6) found low positivity only at two out of 20 urban fomites—wood playground slide and sanitizer dispenser, while any positive sample was found out of 368 samples collected from high-touch surfaces from facilities with large amounts of pedestrian traffic (7). Cycle threshold (Ct) values of qPCR positive samples ranged between 34 and 40 (3, 5, 6), which could indicate either a partially degraded RNA or a low viral load. Disinfection using recommended chemicals—particularly ethanol, chlorine-based products or hydrogen peroxide (8)—significantly reduce viral presence on surfaces, which led to the negative qPCR results obtained in previously published papers (9, 10). Recently, concerns have been raised about the futility of disinfecting surfaces in public, non-hospital places (11). In our study, the sampling of public places was always done after 6 p.m. and cleaning and disinfection of surfaces was carried out after the end of sampling. Although disinfection was carried out several times a day in common days, its omission during the experiment should have reduced the degradation of the virus and its RNA and thus increased the probability of virus capture. Despite omitting cleaning and disinfectant use, no positive sample was found. It

could therefore be assumed, that the effect of disinfection on spreading the virus could be excluded in our study.

Sampling in the hospital COVID-19 unit revealed positive samples in 40% of surfaces (10 positive from 25). All patients in the rooms were in advanced stage of recovery from SARS-CoV-2 and were qPCR negative, except for one patient (Table 2). Swabs from the end stage of infections are not infrequently negative. Nevertheless, it should be noted that the patients' hands and mouths were swabbed, instead of performing a nasopharyngeal swab, which means that the results could potentially manifest as qPCR-negative results. An air sampling, performed in Room 1, revealed completely negative results. In the study by Ma et al. (12), measurement of exhaled breath condensate in SARS-CoV-2 patients showed a breath emission rate between 10^5 and 10^7 virus particles per hour. However, sampling of air in the rooms of these patients showed significantly lower concentrations of SARS-CoV-2 (10^3 virus particles per hour) and positivity was proven in only one air sample of 26 (3.8%) (12). Positive air sample status varies through different studies from zero (9, 13) to more than 50% positivity (14, 15). Even if viral RNA is shown to be in the air, it says nothing about viability of SARS-CoV-2. The measuring of viable viral particles is usually not carried out or is unsuccessful (16). Nevertheless, demonstration of virions' ability to grow in a plaque assay has been proven. Lednicky et al. (17) found between 6 and 74 TCID₅₀ units of SARS-CoV-2 viral particles per liter of air sampling in COVID-19 rooms in a hospital. Negative results of air sampled in hospitals can be explained by low virus emission, its inactivation by disinfectants, and its dilution or removal by fresh air flow as a common practice in hospital wards (12). Even then, the negative results of the air sample in our case was most probably caused by the patient's favorable condition and qPCR negativity. Any or all of the above-mentioned factors could further support this negative status.

Likewise in air, data on positivity of surface samples taken in hospital rooms with SARS-CoV-2 patients varies from higher (15, 16, 18) to very low or zero incidence (10, 19, 20). After disinfection procedures, which are subject to stricter requirements in hospitals as opposed to non-hospital facilities, the positivity of environmental samples is significantly reduced (18) or completely eliminated (9, 10, 21). In our case, SARS-CoV-2 was detected in all rooms examined, including staff rooms. Except in the room of Patient No. 2, samples from the floor (including shoe soles) were always positive despite regular cleaning and disinfection of rooms. Redmond et al. (18) also described higher contamination in floor samples compared to other high-touch surfaces. The floor is a relatively common SARS-CoV-2 contaminated surfaces in a hospital, primarily in patients' rooms (9, 16, 22). Similar to our case, viral RNA was also found in rooms other than the patients' such as the diagnostic room or the staff room (9, 12). Floor contamination may be related to subsequent contamination of shoes, which could then serve as transmission vectors of the virus (22). The reason for viral positivity on the floor is not fully clear. It could be explained by gravity and air flow causing virus particles on condensate to settle on the ground. Movement of medical staff around the ward can contribute to spreading the virus throughout the floor, including rooms without patients (22). Infrequent cleaning and

the use of detergents instead of disinfectants could also play a certain role (18). The potential impact of contaminated cleaning equipment, such as the mop or floor rag, must also be taken into consideration.

The average viral load found in hospital surface swabs ranged from 1 to 56 particles per qPCR reaction (corresponding to 30–1,680 particles per swab). Similar results were obtained by Ma et al. (12) and Guo et al. (22), who estimated surface-borne viral load in hospitals to range from 10^1 to 10^3 viruses/cm² and from 10^3 to 10^4 viral particles per swab. The comparison with other similar studies is however complicated due to expressing the results in Ct values (15, 16, 18) instead of counting to average viral particles. Survival of SARS-CoV-2 virus on various surfaces for hours up to days was proven by *in vitro* studies (1, 23). However, high viral titres were used in the initial inoculum (10^4 – 10^7) in those studies, which does not usually reflect the real conditions. Despite evidence of SARS-CoV-2 on the surface, their growth capacity is low (17) and their ability to induce a cytopathic effect is null (24).

We are aware of the limitations of the study, which mainly lie in the one-time sampling. However, the aim of the survey was not to investigate the viability of the virus, but the real risk of potential infection of customers occurring in high concentrations in a given public place. Potential positivity in the case of sampling other public places, such as public transport, could not be refuted. However, both post-office and shopping center belong to very crowded places and moreover they enabled us to examine air comparing to, e.g., public transport.

In addition, the use of disinfectant, which was normally used several times a day, was omitted on the day of sampling in order to reduce the likelihood of degradation of the virus and its RNA and thus increase the likelihood of a positive detection. Nevertheless, no positive sample was detected at the post office or shopping center. Although negative data from both public places were submitted to the authorities, a complete lockdown of the entire country was declared 2 days after second sampling in the shopping center at the end of February. Based on our data, and previously published papers regarding low survival of the virus in the environment, its limited ability to grow in cultures, and low risk of virus transmission from fomites, we believe that closing the stores was not necessary and did not have considerable effect on minimizing the spread of the virus. In addition, compliance with anti-epidemiological regulations such as wearing masks, hand sanitization, controlling the limited number of customers per store, etc., would have been relatively easy to implement and readily observed, especially in the small local stores that were most affected by the lockdown.

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CONCLUSIONS

Despite the high incidence of newly-identified SARS-CoV-2 positive cases during the sampling in all three locations, none of the surface samples collected neither at the central post office nor in the shopping mall were positive. These findings could be probably attributed to the obligatory wearing of masks indoors, mandatory disinfection of indoor surfaces, and limited numbers of customers per sales area in the Czech Republic at the time of sampling. As expected, SARS-CoV-2 RNA was found in swabs from a local hospital. Except for patients' rooms, positive swabs were found in all rooms tested, including the staff area, despite regular cleaning and disinfection. It may be speculated that movement of staff through the unit, ineffective cleaning or disinfection procedures, or contaminated cleaning tools are potential vectors of virus transmission throughout the hospital ward.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RD: conceptualization, investigation, methodology, resources, writing—original draft preparation, and writing—review and editing. PK: conceptualization, funding acquisition, investigation, methodology, resources, visualization, writing—original draft preparation, and writing—review and editing. OŠ: conceptualization, funding acquisition, methodology, resources, supervision, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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