



Genetic material from SARS-CoV-2 on the surface of exercise equipment

Material genético do SARS-CoV-2 em superfície de equipamentos utilizados na prática de atividades físicas

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ABSTRACT

Humanity was impacted by a Pandemic that exposed the population to contact with a highly contagious virus with an alarming lethality rate. The present study aimed to evaluate the possibility of the persistence of genetic material from SARS-CoV-2 on the surface of equipment used to practice indoor and outdoor physical activities. A sample of equipment used for the practice of physical activity was collected in five gyms and five squares and among the regulars of these environments. The RT-PCR technique was applied to detect the RNA of SARS-CoV-2 and subsequent analysis of the results. The existence of SARS-CoV-2 viral RNA particles was detected in 48.57% of the samples collected from gym equipment and 12.85% of the samples collected in squares, evidencing a lower incidence in equipment used in open spaces in all areas compared and it was found that 35.7% of the study participants tested positive for COVID-19. The positive cases for COVID-19 detected, had symptoms classified as mild to moderate and a quick recovery. The presence of genetic material in the equipment, in turn, leads us to realize the importance of proper cleaning of surfaces, as a form of prevention.

Keywords: Physical Exercise. RT-PCR test for COVID-19. Microbial Viability.

RESUMO

A humanidade foi impactada por uma Pandemia que expôs a população ao contato com um vírus de elevado contágio e com o índice de letalidade alarmante. Este estudo objetivou avaliar a possibilidade da persistência de material genético do SARS-CoV-2 na superfície dos equipamentos de estabelecimentos de prática de atividades físicas *indoor* e *outdoor*. Foram coletadas amostras de equipamentos utilizados para a prática de exercícios físicos em cinco academias, cinco praças e entre os frequentadores desses ambientes. Aplicou-se a técnica RT-PCR para a detecção do RNA do SARS-CoV-2 e posterior análise dos resultados e foi detectada a existência de partículas de RNA viral do SARS-CoV-2 em 48,57% das amostras coletadas dos equipamentos das academias e 12,85% das amostras coletadas nas praças, evidenciando uma incidência menor em equipamentos utilizados em locais abertos em todas as áreas comparadas. Além disso, constatou-se que 35,7% dos participantes do estudo testaram positivo para COVID-19. Os casos positivos para COVID-19 detectados apresentaram sintomas classificados como leves a moderados e uma recuperação rápida. A presença de material genético nos equipamentos, por sua vez, leva-nos a perceber a importância da higienização adequada das superfícies, como forma de prevenção.

Palavras-chaves: Exercício Físico. Teste RT-PCR para COVID-19. Viabilidade Microbiana.



INTRODUCTION

SARS-CoV-2, the virus responsible for the coronavirus 2019 disease (COVID-19), disseminated worldwide, and drastically affected several sectors of society, with economic, political, and social effects^{1,2}. Until May 24, 2023, there were nearly 766,895,075 confirmed COVID-19 cases, including 6,935,889 deaths, according to the WHO.

The main form of contamination by SARS-CoV-2 is through saliva droplets that travel from person to person. However, there is another form of transmission, although not the most common one: coughing fits, sneezes, or even regular conversation³ can contaminate surfaces, through which the virus can be transmitted, as indicated by previous studies^{5,6,7}.

Gyms have an intense flow of people who continuously share equipment, and, as a result, are areas with a high risk of contamination. These environments have shown events related to the super dissemination of COVID-19⁴. Checking whether viral traces of SARS-CoV-2 remain on surfaces is essential to understand the potential risk of infection, especially in areas with a high people flow, such as gyms and squares⁵. Some studies in literature show the persistence of SARS-CoV-2 on different surfaces^{5,6,7}.

According to Van Doremalen⁷, a viable form of the virus could no longer be detected on plastic surfaces from the 4th day, while another study could detect it up to the 7th day. On metal, works by Chin showed the persistence of the virus on copper, where it lasted for less than 8 hours and on stainless steel, where it lasted less than 3 days. Two other studies found 7 days or more^{8,5}.

Other studies showed that the virus survives longer in watertight materials than in porous ones, perhaps because watertight surfaces allow droplets to spread on a larger surface, increasing the size of the liquid surface area where the virus can survive. As a result, this study aimed to evaluate the likelihood that the SARS-CoV-2 genetic material could survive on the surface of exercise equipment in gyms, and the evolution of the symptoms of the disease in infected people who exercise often.

METHODOLOGY

STUDY FIELD AND SAMPLING

Thirty gyms were formally invited to participate in the study. Only five accepted, signing the consent form and participating in the research until its conclusion. Once the gyms that would participate were determined, we attempted to select public squares with outdoor equipment in the same neighborhoods of these gyms, in order to compare indoor and outdoor

results. When there was more than one square with equipment in the same neighborhood, the one closest to the gym was selected.

For six months, from June to November 2022, we collected data regarding the presence of symptoms in the area of the study and collected samples from the surface of shared equipment. The five gyms and five squares selected were in the neighborhoods Cidade Universitária, Bancários, Mangabeira, Bessa, Altiplano, all from the city of João Pessoa. To be included, the gyms had to sign the consent form, while the squares were selected according to their proximity to the gyms, when there was more than one. Regarding the patrons, only those aged 18 or older were considered. Were excluded gyms who did not sign the term of commitment to participate in the research, and patrons of the establishments who were younger than 18.

The gyms who volunteered and the public squares (external areas) were referred to throughout our investigation as follows: gym A, gym B, gym C, gym D, and gym E; square A, square B, square C, square D, and square E. This was done to ensure they could not be identified, as guaranteed in the consent term signed by their legal representatives.

RESEARCH ETHICS

This research was carried out in accordance with Resolution No. 466/12, by the National Council of Health, from 12/12/2012. We only started investigation after receiving the approval letter from the Research Ethics Committee at the Paraíba Federal University, with opinion 4.354.286 and Certificate of Submission for Ethical Appreciation 38778420.8.0000.5188.

COLLECTION OF BIOLOGICAL MATERIALS FROM THOSE WITH SYMPTOMS

The patrons and goers of the areas studied were instructed to undergo the RT PCR test at the Center for Tropical Medicine (NUMETROP) at the Paraíba Federal University if they presented COVID-19 symptoms. Samples were collected using sterile plastic (polypropylene) nasopharyngeal swabs, previously soaked in PBS. Later, they were stored in a 3.5 ml PBS Falcon tube, properly identified, stored in refrigerated thermal boxes, and taken to a lab.

FOLLOW UP OF POSITIVE CASES

Participants with positive COVID-19 tests were monitored for a period of up to 15 days, starting with the result of the exam. They responded daily to a follow-up form developed

by researchers, including information on the symptoms, body temperature, oximetry, medication prescribed, vaccination prior to the infection, and clinical evolution. The results were tabled and analyzed after collection was finished.

COLLECTION OF SAMPLES FROM THE EQUIPMENT

Sample collection from the contact surfaces of the equipment considered areas of 25 cm, with attempts to prioritize areas that could be exposed to respiratory droplets. Samples were collected using sterile plastic (polypropylene) swabs, previously soaked in PBS. They were stored in 3.5 ml PBS Falcon tubes, stored in refrigerated thermal boxes, and taken to a lab. In the lab, each Falcon tube with a swab was placed in a vortex for 10s. Then, the swab was softly pressed against the sides of the tube to remove the excess solution, and viral RNA was extracted from the contents of the tube.

EXTRACTION AND DETECTION OF SARS-CoV-2 GENETIC MATERIAL

SARS-CoV-2 RNA was extracted using CELCO's Total RNA Purification Kit, kept at -80° C to be analyzed the following day. RNA detection of SARS-CoV-2 was carried out using the OneStep/COVID-19 kit, which uses a chain reaction technique for real time polymerase with reverse transcription (RT-PCR). The kit allows the identification of SARS-CoV-2 using two targets: the region where ORF1ab is conserved, and the region of nucleocapsid (N) protein is conserved. The analysis was carried out using QantStudio3 and we considered as detectable all samples that amplified ORF1ab and N protein genes. For the targets ORF1ab and N gene, amplifications up to cycle threshold 40 (ct 40) are valid, according to the BIOMOL OneStep/COVID-19 kit, used in the research.

STATISTICAL ANALYSIS

Data collected was exported into Microsoft Excel and imported into SPSS, version 22.0. We carried out a descriptive analysis (absolute and relative values) and an inferential one (Chi-squared test), with a 95% confidence interval and a significance level of 5%.

RESULTS

TEST OF THE SYMPTOMS

112 persons who practice physical activities and had symptoms indicative of COVID-19 went to the Center of Tropical Medicine (NUMETROP) at Paraíba Federal University to be tested for the SARS-CoV-2 genetic materials. They were directed by the participating gyms and by the professors who were monitoring the regular activity of the squares that were included in the research. 35.71% (40/112) of them had positive results and were monitored for fifteen consecutive days starting with the result of their exam, to check the evolution of the symptoms, hospitalization cases, medication given to them, and the general evolution of their state.

During collection, from June to November 2022, we evaluated their temperature, oximetry, registered the symptoms reported by the participant of the research, whether there were any comorbidities, such as asthma, which could lead to more severe symptoms. Regarding oximetry, only two participants presented values below 85%. One of them was a patron of one of the gyms, while the other exercised outdoors. The other 98.12% (110/112) of people tested presented normal oximetry values. From all individuals monitored, only one participant required hospital care and hospitalization. During monitoring, this participant mentioned having asthma. Among the symptoms reported, stood out: headaches, runny nose, and pain in the body and throat. Regarding temperature, 12.5% (14/112) of participants presented fever at the time of collection for the test.

Table 1 shows the results of the tests carried out in the participants of the research. It shows that 35.7% (40/112) presented a detectable result for SARS-CoV-2.

Table 1. Results for the COVID-19 tests. João Pessoa, PB, Brazil.

Variables	n (%)	Valid sample
Detectable	39 (35.71%)	112
Not detectable	72 (64.28%)	

Source: Research data.

The search for patients with symptoms counted on the participation of representatives from the five gyms and squares, who spontaneously decided to undergo the test. Most positive cases were in patrons of the gyms, as opposed to participants who exercised outdoors. It is not possible to determine how the transmission took place in these cases, but it can be suggested that the probability of infection in external environments would be significantly inferior than in closed spaces (Figure 1).

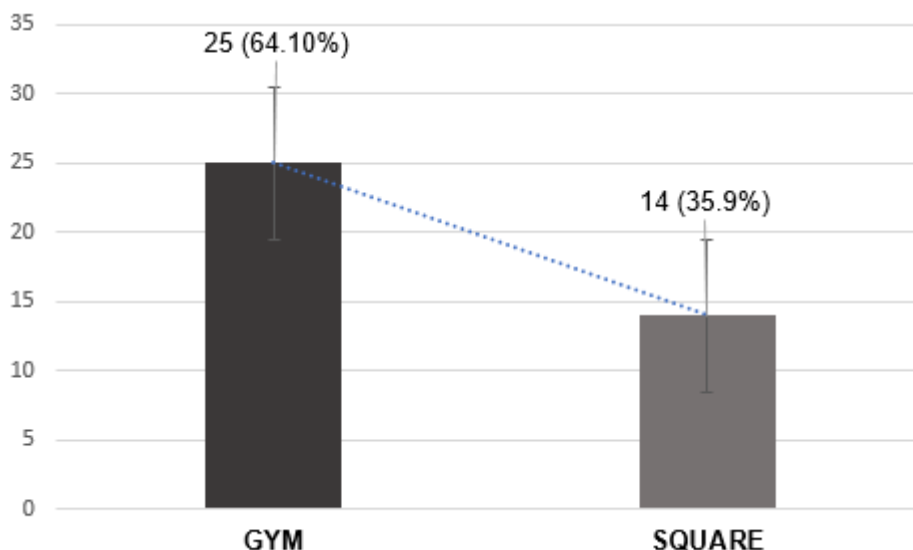


Figure 1 - Relative distribution of positive cases of participants who exercise indoor or outdoor.

A comparison between the different closed spaces showed that gyms B and C had the highest numbers of infected (Figure 2). These were in the neighborhoods Cidade Universitária and Mangabeira, respectively, both middle class neighborhoods.

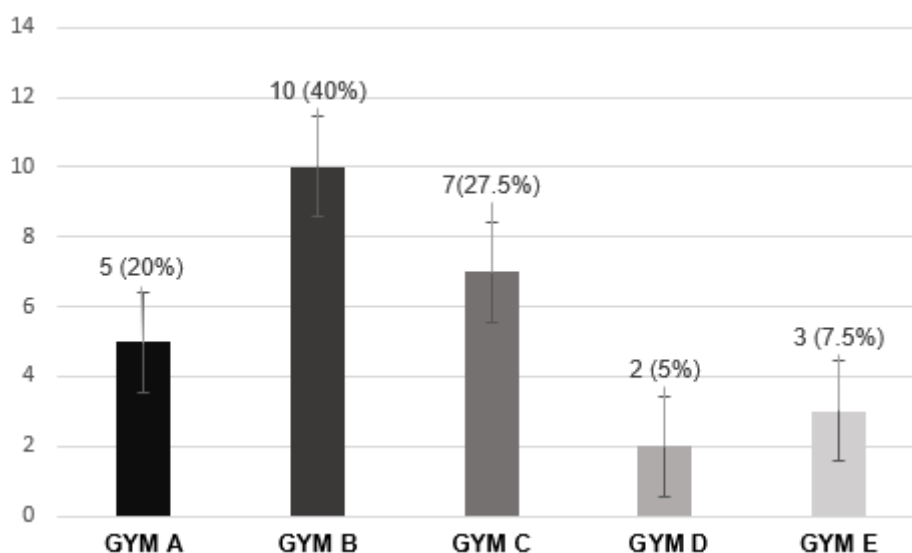


Figure 2 - Relative distribution of positive cases in participants who exercised in gyms.

MONITORING OF DETECTABLE CASES

The positive cases were monitored for 15 days after the result of the exam of each participant. One participant refused the monitoring, meaning that 39 were effectively monitored in the period. The oximetry of the patients monitored remained normal, except for two of them, whose levels were considered below the ideal (95%, which would indicate good blood

oxygenation). The two participants in question presented oximetries of 72% (indoor gym patron) and 75% (outdoor square goer), respectively.

During the follow up of positive cases, we found that 35.89% (14/39) of participants who were monitored presented mild to moderate fever, from 37.8°C to 37.9°C, which persisted for three days. The others presented normal temperatures. Other symptoms included pain in the body and throat, headache, runny nose, coughs, and diarrhea.

Regarding the types of medication used by the patients with positive results in the test, 97.5% (39/40) reported using analgesics to relieve the pain in the body and head, while 15% (6/40) used corticosteroids, and 15% (6/40) used antibiotics prescribed by their physicians. Only one of the individuals monitored did not use any medication.

Table 2 details the other information found during the follow up of detectable SARS-CoV-2 cases. Noteworthy results include the short duration of symptoms, the low number of COVID-19 hospitalizations, and the few days of symptom persistence, which were mild and moderate, and receded quite satisfactorily. It is worth noting that one participant did not accept being monitored.

Table 2. Frequency of information about the monitoring of detectable COVID-19 cases who sought testing (N=39).

Variables	Gym n (%)	Outdoor n (%)
Preexisting disease		
Yes	4 (10.25%)	3 (7.7%)
No	21 (53.85%)	11 (28.20%)
Hospitalization		
Yes	1 (2.57%)	0 (0.0)
No	24 (61.53%)	14 (34.9%)
Disease progression		
Mild symptoms	18 (46.15%)	11 (28.20%)
Moderate symptoms	7 (17.95%)	3 (7.7%)
Duration of symptoms		
Up to four days	14 (35.9%)	5 (12.82%)
Up to five days	10 (25.64%)	9 (23.07%)
Up to 15 days	1 (2.57% of total cases) hospitalization case	0 (0.0)

Source: Research data.

EQUIPMENT ANALYSIS

We found SARS-CoV-2 viral RNA particles in 48.57% of the samples collected from equipment from the five gyms, and in 12.85% of the samples collected from equipment in the squares, showing that outdoor equipment presented a lower incidence of viral DNA, with Ct values from 20 to 33.5 in the samples.

It was also found that, before cleansing, all gyms presented equipment with detectable COVID-19 results, though with different incidences - except for gym C, where there was no positive sample. Among gyms that present a higher level of contaminated equipment, gyms A (n=14; 100.0%) and D (n=9/14; 64.26%) After cleansing, the rate of contaminated equipment found was lower. Gym C used the product VULCAN HOSPITALAR PHM to disinfect equipment. The other gyms used the Clarax Cleaner, whose recommended dilution is 1:64. Gym A, in turn, was diluting it in a proportion that was 15 times the recommended amount, according to local observations. This inadequate level of dilution may have influenced the high level of infected equipment, including after sanitation for collection, since the positive results persisted in 9 of the 14 pieces of equipment in the gym (Table 3).

Table 3. Frequency of SARS-CoV-2 detection in gym equipment, before and after sanitation. João Pessoa, PB, Brazil.

Place of practice	Result	
	Detectable n (%)	Not detectable n (%)
Gym A		
Before sanitation	14 (100 %)	0 (0.0%)
After sanitation	9 (64.26%)	5 (35.74%)
Gym B		
Before sanitation	4 (28.56%)	10 (71.44%)
After sanitation	2 (14.28%)	12 (85.72%)
Gym C		
Before sanitation	1 (7.14%)	13 (92.85%)
After sanitation	0 (0.0%)	14 (100%)
Gym D		
Before sanitation	9 (64.26%)	5 (35.74%)
After sanitation	4 (28.56%)	10 (71.44%)
Gym E		
Before sanitation	7 (50.0%)	7 (50.0%)
After sanitation	2 (14.28%)	12 (85.72%)

Source: Research data.

The incidence of contaminated equipment in the squares (outdoor environment) was significantly lower than that of gym equipment. In three of five investigated squares, we found

SARS-CoV-2 genetic material, but at lower levels than we found in the closed environments (gyms).

Square A, with 28.57% of infected equipment (n=4) presented the largest level. After sanitation, all equipment presented a non-detectable result (Table 4). Sanitation of these external environments was carried out with an alcohol 70% solution. Ethanol is also recommended by the WHO to disinfect surfaces³.

Table 4. Frequency of SARS-CoV-2 detection in the equipment available in public squares, before and after sanitation. João Pessoa, PB, Brazil.

Place of practice	Result	
	Detectable n (%)	Not detectable n (%)
Square A		
Before sanitation	4 (28.57%)	10 (71.43%)
After sanitation	0 (0.0)	14 (100.0)
Square B		
Before sanitation	2 (14.28%)	12 (85.72%)
After sanitation	0 (0.0)	14 (100.0)
Square C		
Before sanitation	0 (0.0)	14 (100.0)
After sanitation	0 (0.0)	14 (100.0)
Square D		
Before sanitation	1 (7.14)	13 (92.86)
After sanitation	0 (0.0)	14 (100.0)
Square E		
Before sanitation	2 (14.28)	12 (85.72)
After sanitation	0 (0.0)	14 (100.0)

Source: Research data.

Figure 3 shows a comparison of the detection of SARS-CoV-2 in the equipment of squares and gyms studied. The gyms presented more contaminated equipment when compared to the squares, before sanitation.

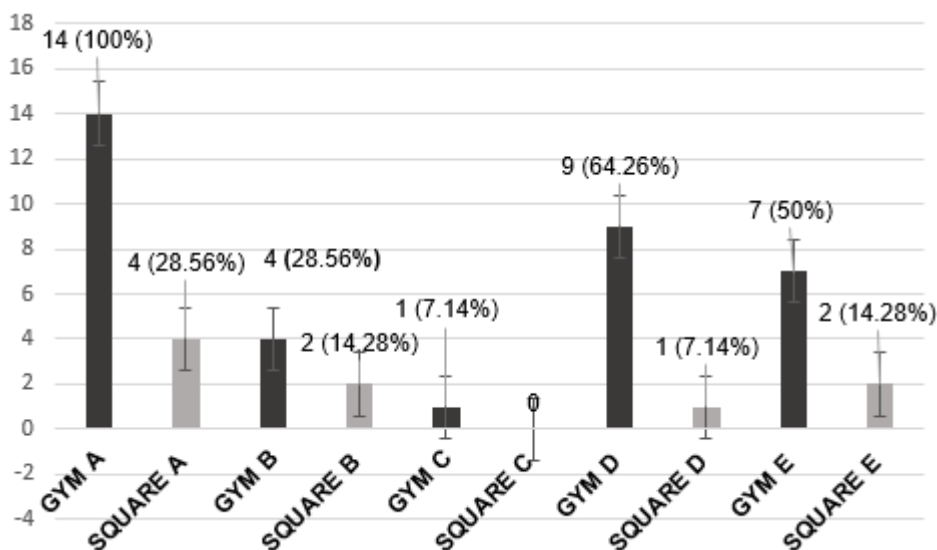


Figure 3 - Comparison of the detection levels of SARS-CoV-2 on the equipment of gyms and squares of João Pessoa, PB, Brazil.

* The total N was 140 pieces of equipment analyzed, 70 in the squares and 70 in the gyms (14 per location)

DISCUSSION

A comparison between the number of detectable cases in indoor and outdoor exercise practitioners, we found that there were more positive cases indoor, showing the influence of the environment on the survival of viral cases. A study by Guo (2020) identified the SARS-CoV-2 RNA on different surfaces and environments in closed spaces, such as hospitals, showing an effective dynamic of viral dissemination in these places¹⁰.

In turn, public places, which are more concerned with the use of natural ventilation and landscaping, help controlling airborne infectious agents¹¹. In most cases, ventilation will quickly disperse aerosols. Therefore, adequate natural ventilation reduces the use of air-conditioning, increasing the rates of air exchange. In closed spaces, on the other hand, there are recommendations for periodical maintenance of ventilation systems¹².

In turn, a comparison between gyms showed that gyms B and C, in lower middle class neighborhoods, presented the higher levels of infection, corroborating the study by DEMENECH¹³, according to whom social and economic inequality can have an important role in the impact of COVID-19 in the Brazilian territory, regarding hygiene habits and resistance to the social distancing measures implemented¹⁴.

Regarding the positive cases, followed up for 15 days starting with the results of the exams of each participant, we found that most of them had mild to moderate symptoms, which disappeared in four to 5 days, on average. The fact that participants showed predominantly mild

symptoms can be related with the vaccination, which was already available. However, physical activity is believed to modulate positively the ability of the immune system to deal with infections. Active individuals seem to have better immunity to protect themselves against the symptoms of infection or reduce their gravity¹⁵. According to Walsh¹⁶, the benefits of physical activities include the reduction of viral infections. To Lira¹⁷, exercising brings benefits to pulmonary function, especially by improving its functioning dynamics, in addition to improving the strength of respiratory muscles¹⁸.

Metabolic, physical, immunological, and inflammatory changes caused by exercise improve one's functional capacity¹⁹, improving the efficiency of the cardiorespiratory system²⁰. Frequent exercise is known to have a positive effect on the cardiorespiratory system, improving clinical recovery in case of viral diseases¹⁹.

Outdoor exercise equipment showed lower levels of SARS-CoV-2 when compared to indoor equipment. Public places, which are more concerned with the use of natural ventilation and landscaping, help controlling airborne infectious agents¹¹. In most cases, ventilation will quickly disperse viral aerosols. Therefore, adequate natural ventilation reduces the use of air-conditioning, increasing the rates of air exchange. In closed spaces, on the other hand, there are recommendations for periodical maintenance of ventilation systems¹².

Environmental issues can be a strong indicator of a reduction in the detection of SARS-CoV-2 genetic materials on public square equipment, as this is an outdoors environment¹³. The influence of environmental factors, such as temperature and relative humidity (RH) was investigated by Biryukov et al., showing that the virus persistence was lower as these variables increased⁹. An investigation by Chin⁸ found the same behavior, with viral viability decreasing as temperature increased.

RNA detection on the surface of equipment suggests there was no adequate sanitation⁵, since studies have shown that SARS-CoV-2 becomes inactive after contact with chlorine in a 1:49 and 1:99 proportion, ethanol (70%), povidone-iodine (7.5%), hand-soap solution (50%), and others⁸.

Studies by Kampf¹⁵ showed that human coronaviruses can continue to present a risk of infection for nearly nine days. Therefore, the contamination of shared equipment in gyms should be seen as a potential source of infection. Studies published by Chin⁸ found that SARS-CoV-2 continues to be viable on metal, leather, and plastic surfaces for nearly 4 days, at 22⁰⁰C, showing that these infectious particles can be recovered from these surfaces, which, in this study, were positive for the presence of SARS-CoV-2 genetic material.

The fact that gyms presented more positive cases than outdoor spaces shows the relevance of evaluating the presence of the virus in the environment¹⁶, on objects and surfaces, which is essential to understand the risk of infection in the population. Furthermore, the

information found can be used by health managers as tools to control the dissemination of infectious agents, and for the implementation of measures of environmental disinfection⁵.

CONCLUSION

Gyms are mostly closed spaces, considered to be of high risk for the transmission of SARS-CoV-2. Although we attempted to detect the presence of the viral RNA of SARS-CoV-2 on the equipment, we could not determine viral viability for COVID-19 transmission. However, it is important to call attention to the risk of infection and the importance of correctly sanitizing this equipment before and after use. Comparing indoor (gyms) and outdoor (public squares) exercise equipment regarding the presence of SARS-CoV-2 genetic material shows that the genetic material of the virus is less incident on outdoor equipment, and, as a result, the importance of future studies that evaluate the importance of the environment in the control and dissemination of diseases.

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