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Long-term surveillance of SARS-CoV-2 in the school community from Campo Grande, Brazil

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Abstract

Background The COVID-19 pandemic has significantly impacted education systems worldwide, with Brazil being one of the countries with the longest school closures. Over a million children and teenagers have been affected, leading to increased hunger and nutritional deficiencies. This study aimed to implement long-term surveillance of SARS-CoV-2 infections in public and private schools in Campo Grande, Brazil, after returning to in-person classes.

Methods The study involved testing and genomic surveillance at 23 public and private schools in Campo Grande, Mato Grosso do Sul, Brazil, from October 18, 2021 to November 21, 2022. The participants eligible for enrollment were students aged 6–17 years and staff members from school institutions. At the time of collection, participants were asked if they had symptoms in the last two weeks. Whole-genome sequencing of SARS-CoV-2 was conducted to identify circulating variants and to compare them with those detected in the municipality. The demographic data and clinical history of the participants were described, and a logistic regression model was used to understand how the RT-qPCR results could be related to different characteristics.

Results The study included 999 participants, most of whom were women. A total of 85 tests were positive, with an overall positivity rate of 3.2%. The dynamics of case frequency were consistent with those observed in the municipality during the study period. The most common symptoms reported were cough, rhinorrhea, headache, and sore throat. Symptoms were significantly associated with SARS-CoV-2 infection. Eleven lineages were identified in school community samples, with a frequency of occurrence per period similar to that found in the sequences available for the municipality. The most prevalent lineages within the sampling period were BA.2 (59.3%) and BA.5 (29.6%).

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Conclusions Our findings demonstrate that schools can play a crucial role in epidemiological surveillance, helping trigger rapid responses to pathogens such as SARS-CoV-2. Long-term surveillance can be used to track outbreaks and assess the role of children and adults in transmission. It can also contribute to pandemic preparedness, enabling a rapid response to emergencies, such as COVID-19.

Keywords School community, School closure, Epidemiology, Genomic surveillance, Pandemic.

Background

Education systems worldwide have faced unprecedented challenges during the COVID-19 pandemic. Many nations have developed measures at schools to lower the risk of infection as part of larger plans to prevent the spread of SARS-CoV-2 [1–3]. One of the first measures taken by governments worldwide was school closures. Brazil was among the countries where schools were closed for the longest time (78 weeks), whereas the global average was approximately 38 weeks [4]. Over a million children and teenagers in Brazil were affected by the closure of their schools. On-site school closures have increased hunger and nutritional deficiencies due to the lack of school meals and increased unemployment in vulnerable pre-pandemic communities, further exacerbating pre-existing inequalities in the country [5, 6].

The reopening of schools in Brazil for in-person and hybrid learning programs began in the second half of 2020, but only a few schools returned at that time [6]. In Mato Grosso do Sul state, Brazil, in-person classes were suspended on March 17, 2020. The in-person return to school took place on July 26, 2021, for municipal schools, and on August 2, 2021, for state schools. Private schools returned to in-person classes on April 5, 2021, but with a hybrid option for the rotation of students or families who opted for the online system. Similar to other countries, strategies to prevent the transmission of SARS-CoV-2 in schools have focused on physical distancing, mask use, hygiene, staggering schedules, and cohorting [6–8]. However, an important tool for detecting active infections and potential outbreaks is testing, which was neglected in protocols for reopening Brazilian schools [6].

Surveillance of infections in school-aged children can help to understand the situation in schools, evaluate and adjust protective measures, and trigger mitigation responses [7, 9]. In addition, it can provide information that helps understand the severity of infections and possible subsequent complications, such as multisystem inflammatory syndrome in children (MIS-C) and post-acute sequelae, or “long COVID” [8, 10–12]. Other studies have demonstrated the feasibility and potential role of regular SARS-CoV-2 monitoring in schools [13–19]. With government assistance at the federal, state, and municipal levels, the surveillance of SARS-CoV-2 infections in school environments could have been implemented as one of the pillars of reopening plans. This could have reduced the time that the schools remained

closed, guaranteeing greater safety for the school community, in conjunction with preventive measures. Thus, the aim of this study was to implement long-term surveillance of SARS-CoV-2 in a representative number of public and private schools in the municipality of Campo Grande, Brazil, after all schools (private and public) returned to offering in-person classes. This study prospectively examined SARS-CoV-2 infection among children and adults in the school community, the symptoms presented, and the SARS-CoV-2 lineages circulating in this environment.

Methods

Study design and participants

Testing and genomic surveillance were conducted in public and private schools in the urban area of the municipality of Campo Grande, Mato Grosso do Sul, Brazil, from October 18, 2021, to November 21, 2022. The study was divided by school semester, totaling three surveillance periods: (i) from October 18 to December 1, 2021, the period in which all schools (private and public) already had in-person classes; (ii) from March 9 to July 4, 2022; and (iii) from August 2 to November 21, 2022 (Fig. 1). The present study is part of a larger and long-term research project, which is in accordance with the authorization of the Research Ethics Committee of Oswaldo Cruz Foundation (FIOCRUZ) of Brasília (CAAE: 47905721.9.0000.8027).

Public (municipal and state) and private schools were contacted and invited to participate in this project, and were considered eligible for enrollment of students aged between 6 and 17 years, as well as staff from school institutions, regardless of school position. In 2021, Campo Grande had 452 schools in the urban area, with 144,123 students within the age range [20]. By 2022, there were 461 schools and 145,119 students within the age range [21]. Despite the project’s authorizations, the schools had the freedom to choose whether to participate, and they could withdraw at any time. As there are schools that work at specific school levels, schools that covered the widest possible age range were prioritized to allow sampling of the entire age range covered by the study without the need to sample a large number of schools. A total of 250 schools met this condition (70 state schools, 89 municipal schools, and 91 private schools), and 23 agreed to participate in the study (14 state schools, five municipal schools and four private schools).

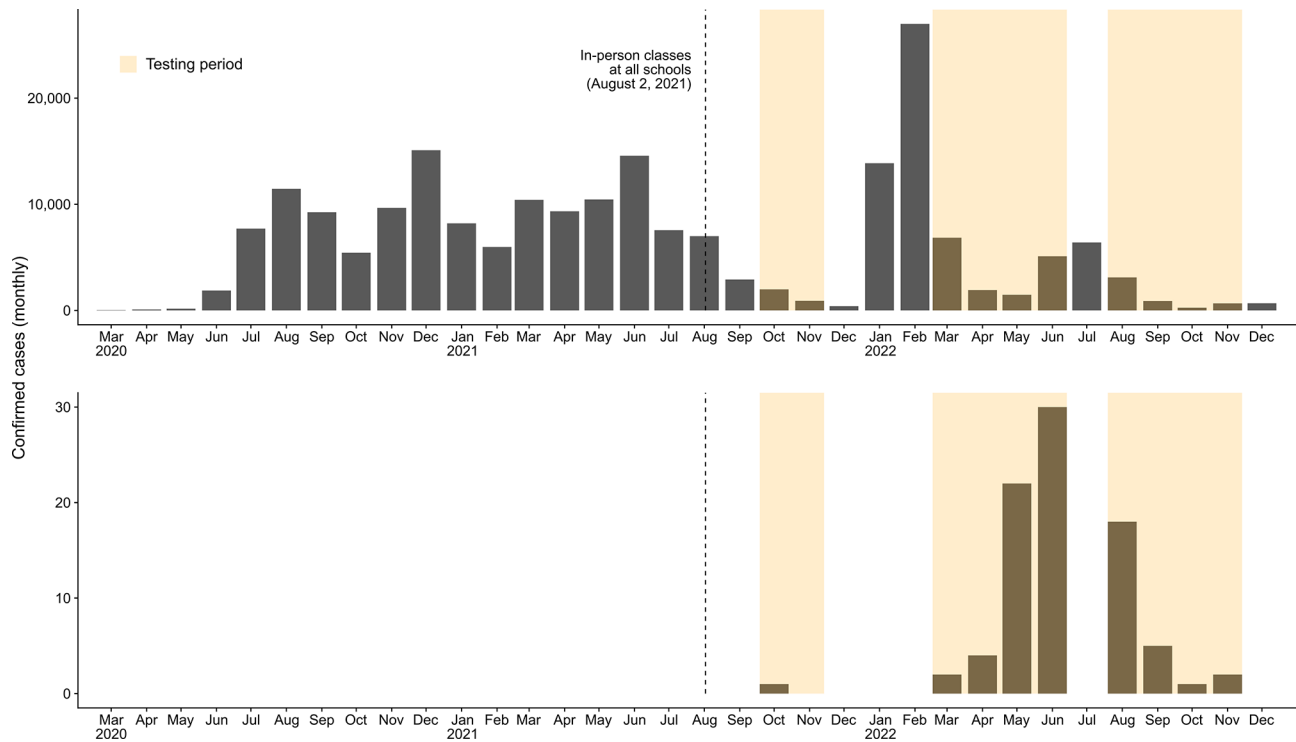


Fig. 1 Monthly confirmed COVID-19 cases (bars) for the municipality of Campo Grande (above), Brazil, and for schools in the study (below). The dashed line shows the date on which all the schools had in-person classes. Orange shading shows the testing periods

During each period, eligible participants were contacted with the help of the administrators of each school through face-to-face conversations, posters, and cell phone messages. A link was provided for online registration of school members who agreed to participate in the research. Participants needed to agree to the free and informed consent form and respond to a demographic questionnaire (Additional file 1) to be able to participate in the study. After agreeing to the free and informed consent form, the participants received an automatic copy in the email registered for contact. Participants with difficulties completing the online registration were assisted by a team member either in person or by phone. The registration of students aged 6–17 years was conducted by a legal representative. However, even with the representatives' consent, the students agreed to participate after receiving detailed information about the research and signing the free and informed assent form. A copy of the free and informed assent form was provided to be delivered to the respective representatives. The study data were collected and managed using REDCap electronic data capture tools hosted by the Oswaldo Cruz Foundation of Mato Grosso do Sul – FIOCRUZ MS [22, 23].

Contextualization of health measures in force during the study period

In Mato Grosso do Sul state, Brazil, biosafety protocols were implemented with the aim of mitigating the

transmission of SARS-CoV-2 during the return of in-person classes in 2021. These included operational procedures of hygiene and cleanliness of the school environment, furniture and pedagogical materials, measures of social distancing, waste management, guidance of family members, attendance to students, measures of maintenance of air conditioning, water tanks and drinking fountains, procedures for the entry and exit of students, and snack time and procedures to follow if a student or any other member of the school community has symptoms. Additionally, all school members were required to wear a mask, with some restrictions depending on age group. The vaccination program in Mato Grosso do Sul began in January 2021 for individuals over 12 years of age. For children aged 5 to 11 years, it started in January 2022, and for children aged 3 to 5 in July 2022. Considering the increase in the population's vaccination rate and the drop in the number of cases, the state government ceased to require the mandatory use of masks in public places, private establishments accessible to the public, and public transportation.

Testing

In the first and second sampling periods, each school was visited every two weeks. However, at the request of the participants regarding the interval between collections, each school was visited once a month during the third sampling period. Collection visits to each school were

conducted in the mornings and afternoons. In general, two to three schools were visited daily. All participants underwent swab collection, but they could always refuse to be tested or withdraw from the project at any time. At the time of collection, participants were asked if they had symptoms in the past two weeks, and the answers were entered into a follow-up questionnaire (Additional file 1). Eligible participants who were not enrolled in the project could register at the time of the visit without restrictions. After each collection period of the day, the samples were immediately taken to the Central Public Health Laboratory of Mato Grosso do Sul (LACEN/MS), where they were tested using real-time reverse transcription polymerase chain reaction (RT-qPCR) as a routine diagnosis for SARS-CoV-2. Nucleic acid extraction was performed using the Quick-DNA/RNA Viral Megabead kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. RT-qPCR for the detection of SARS-CoV-2 was performed using the Molecular SARS-CoV-2 (EDx) Kit (Bio-Manguinhos - FIOCRUZ, Rio de Janeiro, RJ, Brazil), following the manufacturer's instructions. The agreed deadline for releasing results for participants was 48 h, although they were usually released in less than 24 h.

After the tests were completed, the participants received a report with the results. In the case of the students, the report was sent to their legal representatives. Participants with a positive result received the research report in addition to an official report from the laboratory management platform of the Brazilian Unified Health System. This official report could be shown to healthcare professionals (doctors) for proper treatment. However, the medical treatment was not within the scope of this study. School directors/coordinators were informed so that they could act in relation to positive cases in accordance with the recommendations of the Brazilian Ministries of Health and Education, as it was not the study's role in determining such measures. In general, positive cases could remain at home for at least seven days. Participants who tested positive were not monitored when they were away but could be tested again as soon as they returned to the school environment.

SARS-CoV-2 amplification and sequencing

Samples for whole-genome sequencing were selected based on Cq values (≤ 30) to allow for high genomic coverage. The viral RNA was subjected to reverse transcription and PCR amplification using the Illumina COVIDSeq Assay (Illumina, San Diego, CA, USA), including the ARTIC v4.1 nCoV-2019 Amplicon Panel (IDT, Coralville, Iowa, USA), but following modifications to the original manufacturer's instructions, as proposed by the Fiocruz Genomic Network [24–26]. Normalized pooled amplicons of each sample were used

to prepare NGS libraries and were clustered using the MiSeq Reagent Kit V2 (300-cycles) on 2×150 cycle runs. All sequencing data were collected using an Illumina MiSeq sequencing platform.

FastQ files were generated using the Illumina pipeline in BaseSpace. Consensus sequences were generated using the Viralflow v1.0.0 workflow [27]. SARS-CoV-2 genome assembly was performed using the reference NC_045512.2, after trimming primer sequences of the ARTIC v4.1 nCoV-2019 Amplicon Panel. The quality of the consensus sequences was assessed using the Nextclade v2.14.0 [28]. Consensus sequences were initially assigned to viral lineages according to the nomenclature proposed by [29] using the Pangolin software [30] and later confirmed by phylogenetic analyses. All genomes were uploaded to the EpiCoV database in GISAID [31–33] (Additional files 2 and 3).

Data set composition and maximum likelihood phylogenetic analysis

The sequences available for the municipality of Campo Grande from October 1, 2021, to December 31, 2022, were searched in the EpiCoV database in GISAID (Additional files 2 and 3). All sequences deposited by October 14, 2023, were downloaded, regardless of quality, to compare the known lineages for the municipality with the lineages sequenced from school samples. However, only sequences with less than 10% undetermined 'N' bases were kept in the data set for phylogenetic analysis. Because of the presence of recombinants and sequences with unassigned calls by the Pangolin software, four datasets were generated to evaluate phylogenetic relationships: (i) all sequences below the 10% threshold of unidentified positions; (ii) those below the threshold of unidentified positions and no recombinants; (iii) those below the threshold of unidentified positions and no unassigned calls in Pangolin; (vi) those below the threshold of unidentified positions, no recombinants, and no unassigned calls in Pangolin. To assist in the identification of clades, the reference genomes of variants of concern (VOC), of interest (VOI), and under monitoring (VUM), provided by GISAID, were added to each dataset (Additional files 2 and 3). The sequences were aligned using MAFFT v7.520 [34, 35], inspected, and edited using AliView v1.28 [36]. The best nucleotide substitution model was measured using ModelFinder [37] and the phylogenetic analyses were performed using the IQ-TREE v2.2.2.7 [38, 39]. Branch support values were obtained using the ultrafast bootstrap approximation (UFBoot) [40, 41] and SH-aLRT branch tests [42], both with 1,000 replicates. The resulting phylogenies were visualized and edited in R software v4.3.1 [43], using the packages 'tidytree' [44], 'ggtree' [44–48] and 'treeio' [44, 49].

Statistical analysis

The characteristics of the study participants were described by summarizing the demographics and clinical history of each of them with absolute frequencies, percentages, and 25–75% interquartile range (IQR). Data on confirmed cases in the municipality of Campo Grande were obtained from the Ministry of Health, Brazil [50], for comparison with the results of tests in schools. A binomial logistic regression model with random effect for schools was used employing the R package ‘glmmTMB’ [51] to understand how the RT-qPCR results could be related to the group (staff and students) and sex (female and male) of participants, the presence of symptoms at the time of collection, and the period in which the tests were performed. Multicollinearity was checked by examining the Pearson correlation coefficient (Pearson’s r) between each pair of explanatory variables, using the R package ‘correlation’ [52, 53], and computing the variance inflation factor (VIF), with the R package ‘performance’ [54]. Tables and estimation plots were generated using the ‘jtools’ package [55]. The model was checked for normality of residuals, normality of random effects, homogeneity of variance, and residual dispersion using the packages ‘DHARMA’ [56] and ‘performance’.

Results

A total of 2,675 tests were performed on 999 participants, with three tests performed on average per school staff (IQR: 1–4) and two tests (IQR: 1–3) per student (Table 1). The majority of participants were women, and most

of them received at least one dose of the vaccine. The average age of all school staff was 43 years (IQR: 35–51 years) and that of students was 12 years (IQR: 10–15 years) (Table 1). Participation in the study was low, with an average number of 23 volunteers per school in both groups, school staff and students (staff IQR: 14–34; students IQR: 14–33) (Table 1). In total, 85 tests were positive from 83 participants, with an overall positivity rate of 3.2% (95% CI 2.5–3.9) (Table 2). Of these, 51 (60.0%) were from school staff and 34 (40.0%) were from students. The average age of all positive participants was 34 years (IQR: 13–49), 48 years (IQR: 40–56) for the school staff, and 13 years (IQR: 11–14) for the students. Of the two participants who tested positive twice, one was a female member of the school staff who tested positive 14 days after the first positive test. Because of the short time period, both results were possibly due to the same infection. She had been vaccinated and had not reported any symptoms at the time of collection. The other participant was a middle-school boy who tested positive again 104 days after the first positive test, which was possibly a case of reinfection. He was also vaccinated and reported no symptoms in either test.

Overall, the dynamics of the increase and decrease in detected cases resembled those observed in the municipality during the study period (Fig. 1). In the first testing period, there was only one positive result out of 473 tests (0.2%). The second period had the highest positivity rate, with 58 out of 1,188 (4.9%), which was significantly higher than that in the other two periods (Tables 2 and

Table 1 Study participation rates per group (staff and student), with the number of participants per category, or the average and 25–75% interquartile range (IQR) per participant

		Total		Period 1		Period 2		Period 3	
		Staff	Student	Staff	Student	Staff	Student	Staff	Student
Total participants		473	526	116	272	309	247	325	198
Sex	Female	399	305	99	168	263	131	272	119
	Male	74	221	17	104	46	116	53	79
Vaccinated	Yes	464	323	111	107	304	199	321	165
	No	6	132	5	98	3	39	3	29
	Not informed	3	71	0	67	2	9	1	4
Age (years)	Average	43	12	42	12	43	12	44	13
	25–75% IQR	35–51	10–15	35–50	10–15	35–51	10–15	37–51	11–15
RT-qPCR testing	Average	3	2	1	1	2	2	2	2
	25–75% IQR	1–4	1–3	1–2	1–1	1–3	1–3	1–3	1–3
Participants per school	Average	23	23	6	14	16	12	17	10
	25–75% IQR	14–34	14–33	3–9	5–19	12–24	7–16	12–22	6–13
Number of people in schools*	Total**	***	***	2966	18,031	4539	17,445	4539	17,445
	Average	188	887	144	902	277	872	277	872
	25–75% IQR	126–244	550–1082	94–188	592–1079	176–274	542–1092	176–274	542–1092

* It encompasses all school staff and students at schools that, regardless of whether they are enrolled in the study. The data was obtained from the Brazilian Basic Education Census of 2021 and 2022 [20, 21]

** The total number of people within the studied age range (students aged 6–17 and school staff of any age) who were linked to the schools that participated in the study

*** Number not available

Table 2 Number of tests performed (N) and positive cases (np) by characteristics of the participants, with percentages (%) and the exact 95% confidence interval (95% CI)

		Total		Period 1		Period 2		Period 3	
		N (Np)	%; 95% CI	N (Np)	%; 95% CI	N (Np)	%; 95% CI	N (Np)	%; 95% CI
Total		2675 (85)	3.2; 2.5–3.9	473 (1)	0.2; 0.0–1.2	1188 (58)	4.9; 3.7–6.3	1014 (26)	2.6; 1.7–3.7
Sex	Female	1933 (61)	3.2; 2.4–4.0	331 (1)	0.3; 0.0–1.7	830 (42)	5.1; 3.7–6.8	772 (18)	2.3; 1.4–3.7
	Male	742 (24)	3.2; 2.1–4.8	142 (0)	0.0; 0.0–2.6	358 (16)	4.5; 2.6–7.2	242 (8)	3.3; 1.4–6.4
Group	Staff	1379 (51)	3.7; 2.8–4.8	148 (0)	0.0; 0.0–2.5	601 (36)	6.0; 4.2–8.2	630 (15)	2.4; 1.3–3.9
	Student	1296 (34)	2.6; 1.8–3.6	325 (1)	0.3; 0.0–1.7	587 (22)	3.7; 2.4–5.6	384 (11)	2.9; 1.4–5.1
Age (years)	6–10	291 (3)	1.0; 0.2–3.0	76 (0)	0.0; 0.0–4.7	146 (0)	0.0; 0.0–2.5	69 (3)	4.3; 0.9–12.2
	11–14	623 (23)	3.7; 2.4–5.5	158 (1)	0.6; 0.0–3.5	278 (16)	5.8; 3.3–9.2	187 (6)	3.2; 1.2–6.9
	15–17	392 (8)	2.0; 0.9–4.0	91 (0)	0.0; 0.0–4.0	170 (6)	3.5; 1.3–7.5	131 (2)	1.5; 0.2–5.4
	≥ 18	1369 (51)	3.7; 2.8–4.9	148 (0)	0.0; 0.0–2.5	594 (36)	6.1; 4.3–8.3	627 (15)	2.4; 1.3–3.9
Self-reported symptoms	Yes	380 (32)	8.4; 5.8–11.7	5 (0)	0.0; 0.0–52.2	240 (24)	10.0; 6.5–14.5	135 (8)	5.9; 2.6–11.3
	No	2295 (53)	2.3; 1.7–3.0	468 (1)	0.2; 0.0–1.2	948 (34)	3.6; 2.5–5.0	879 (18)	2.0; 1.2–3.2

Table 3 Odds ratios (OR), 95% confidence intervals (95% CI), and p-values estimated from the binomial logistic regression model for the positive RT-qPCR result for SARS-CoV-2

Predictors	OR	95% CI	p-value
Sex [Male]	1.64	0.80–3.38	0.178
Group [Student]	0.99	0.57–1.73	0.984
Self-reported symptoms [yes]	3.03	1.90–4.81	<0.001
Period [period 1]	0.06	0.01–0.42	0.005
Period [period 3]	0.56	0.34–0.90	0.016
Group [Student] × Sex [Male]	0.53	0.19–1.44	0.212

3). An increase in the number of positives was observed each month of the second period, although March 2022 showed the highest positivity (Table 4). In the third testing period, a decrease in the number of positives was observed, with 26 out of 1,014 (2.6%), but still greater positivity than that observed in the first period (Fig. 1; Table 2). Positivity did not differ significantly between the groups and sexes, but the presence of symptoms was significantly associated with SARS-CoV-2 infection when compared with asymptomatic cases (Table 3). Among the positive results, participants reported having symptoms at the time of collection in 32 tests (37.6%) (Fig. 2).

Symptoms were reported in 25 (49.0%) positive tests by school staff and in seven (20.6%) by students (Fig. 2). The most commonly reported symptoms were cough, rhinorrhea, headache, and sore throat among both students and school staff (Fig. 2).

As of October 31, 2023, 741 sequences were deposited in the EpiCoV database in GISAID from samples collected between October 2021 and December 2022 in the municipality of Campo Grande. Of these, 714 (96.4%) were from samples unrelated to the present study, but with 106 sequences having more than 10% undetermined 'N' bases. A total of 60 lineages/sublineages were identified from the 671 sequences, as it was not possible to determine the lineages/sublineages for 43 sequences (Fig. 3; Table 5; Additional file 3). Three recombinants were identified: XAG and XM, recombinants of Omicron BA.1/BA.2, and XBB, recombinants of two lineages of Omicron BA.2 (Fig. 3; Table 5). Pangolin unassigned calls occurred in 74 sequences, including the recombinants XAG and XM. In this study, 27 samples from schools were sequenced, covering the period from April to November 2022. Eleven lineages were identified, with a

Table 4 Number of tests performed (N) and positive cases (np) per month, with percentages (%) and the exact 95% confidence interval (95% CI)

Year	Month	Total		Staff		Student	
		N (Np)	%; 95% CI	N (Np)	%; 95% CI	N (Np)	%; 95% CI
2021	October	173 (1)	0.6; 0.0–3.2	46 (0)	0.0; 0.0–7.7	127 (1)	0.8; 0.0–4.3
	November	300 (0)	0.0; 0.0–1.2	102 (0)	0.0; 0.0–3.6	198 (0)	0.0; 0.0–1.8
2022	March	23 (2)	8.7; 1.1–28.0	10 (2)	20.0; 2.5–55.6	13 (0)	0.0; 0.0–24.7
	April	187 (4)	2.1; 0.6–5.4	57 (0)	0.0; 0.0–6.3	130 (4)	3.1; 0.8–7.7
	May	395 (22)	5.6; 3.5–8.3	195 (12)	6.2; 3.2–10.5	200 (10)	5.0; 2.4–9.0
	June	555 (30)	5.4; 3.7–7.6	325 (22)	6.8; 4.3–10.1	230 (8)	3.5; 1.5–6.7
	July	28 (0)	0.0; 0.0–12.3	14 (0)	0.0; 0.0–23.2	14 (0)	0.0; 0.0–23.2
	August	331 (18)	5.4; 3.3–8.5	220 (12)	5.5; 2.8–9.3	111 (6)	5.4; 2.0–11.4
	September	289 (5)	1.7; 0.6–4.0	174 (1)	0.6; 0.0–3.2	115 (4)	3.5; 1.0–8.7
	October	222 (1)	0.5; 0.0–2.5	127 (1)	0.8; 0.0–4.3	95 (0)	0.0; 0.0–3.8
	November	172 (2)	1.2; 0.1–4.1	109 (1)	0.9; 0.0–5.0	63 (1)	1.6; 0.0–8.5

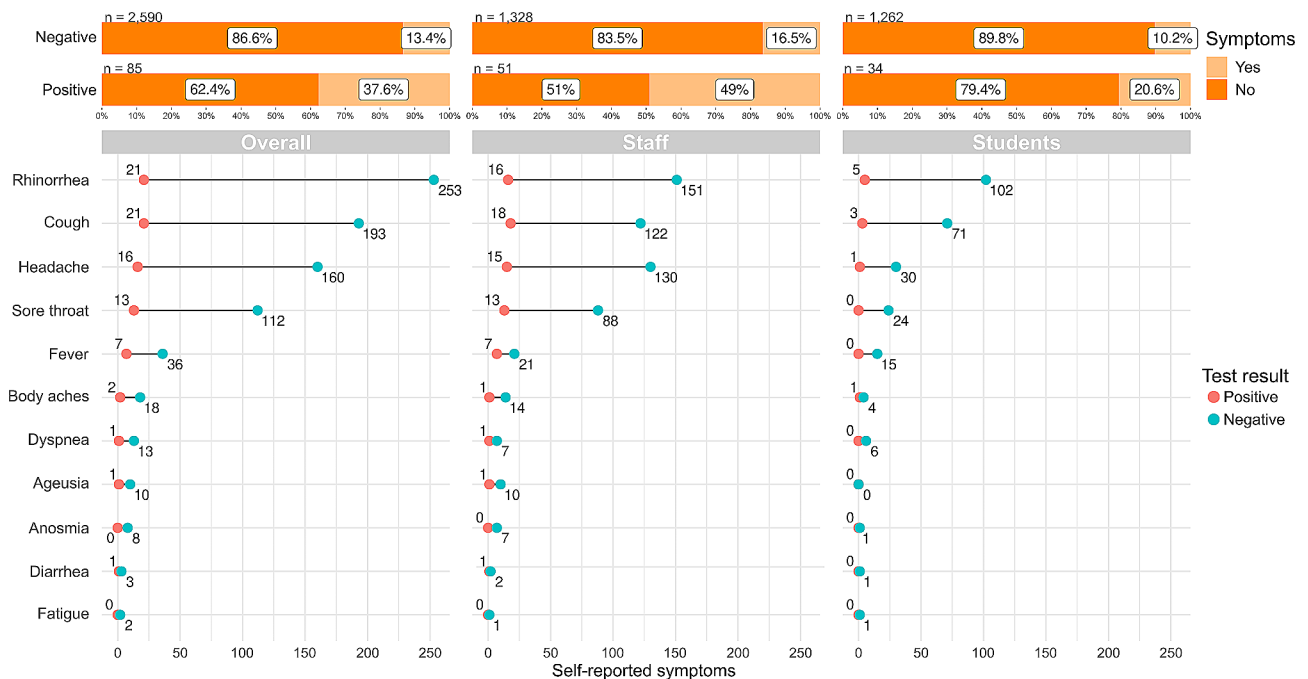


Fig. 2 Self-reported symptoms by testing for all participants, school staff and students. Above is the percentage of tests with symptoms for each test result (positive and negative). Below is the frequency of each symptom reported at the time of collection, by test result (positive and negative)

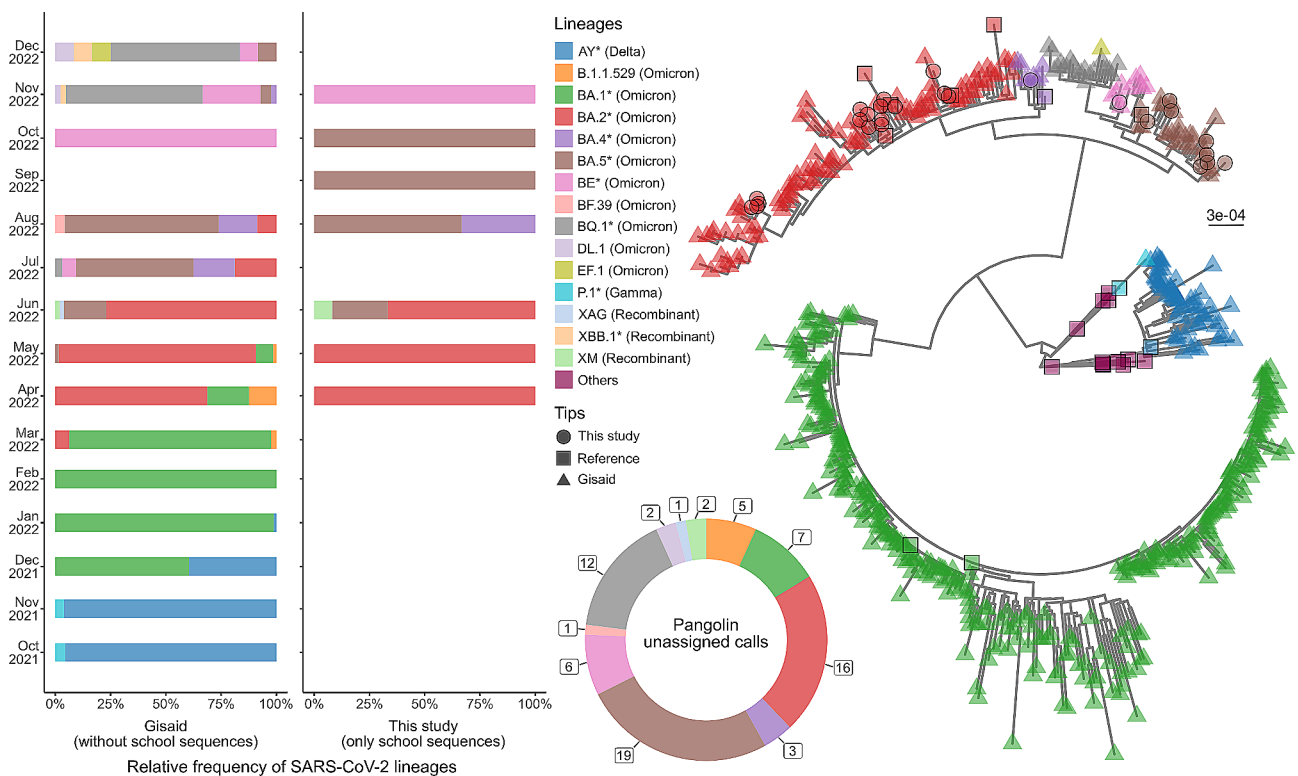


Fig. 3 SARS-CoV-2 lineages and phylogenetic tree. Bar charts show the relative frequency of lineages sequenced during the sampling months of the study. Donut chart shows lineages with unassigned calls in Pangolin. The phylogenetic tree was reconstructed with the data set (vi) with sequences below the threshold of unidentified positions, no recombinants, and no unassigned calls in Pangolin (Model: GTR+F+I+R2). Phylogenetic reconstructions with the other data sets are in the Additional file 4. Reference tips are the reference genomes of the variants provided by GISAID. For detailed information on lineages and tips, see Additional file 3

Table 5 Number of sequences and pangolin unassigned calls per SARS-CoV-2 lineage, available on GISAID (without school sequences) and sequenced in the present study (only school sequences)

Lineage	GISAID		Schools	
	Total (%)	Unassigned (%)	Total (%)	Unassigned (%)
AY*	66 (9.2%)	0 (0.0%)	-	-
B.1.1.529	5 (0.7%)	5 (6.8%)	-	-
BA.1*	360 (50.4%)	7 (9.6%)	-	-
BA.2*	122 (17.1%)	16 (21.9%)	16 (59.3%)	0 (0.0%)
BA.4*	11 (1.5%)	3 (4.1%)	1 (3.7%)	0 (0.0%)
BA.5*	47 (6.6%)	19 (26.0%)	8 (29.6%)	0 (0.0%)
BE*	16 (2.2%)	6 (8.2%)	1 (3.7%)	0 (0.0%)
BF.39	1 (0.1%)	1 (1.4%)	-	-
BQ.1*	34 (4.8%)	12 (16.4%)	-	-
DL.1	2 (0.3%)	2 (2.7%)	-	-
EF.1	1 (0.1%)	0 (0.0%)	-	-
P.1*	2 (0.3%)	0 (0.0%)	-	-
XAG	1 (0.1%)	1 (1.4%)	-	-
XBB.1*	2 (0.3%)	0 (0.0%)	-	-
XM	1 (0.1%)	1 (1.4%)	1 (3.7%)	1 (100%)
Unidentified	43 (6.0%)	0 (0.0%)	-	-

frequency of occurrence per period similar to that found in the sequences available in the GISAID (Fig. 3). The most prevalent lineages within the sampled period were BA.2 (59.3%) and BA.5 (29.6%) (Fig. 3; Table 5; Additional file 4). Pangolin unassigned calls occurred only in the recombinant XM sequence. Phylogenetic analysis recovered all the school sequences in the respective clades of their lineage (Fig. 3; Table 5; Additional file 4).

Discussion

This study demonstrated the potential benefits of long-term surveillance in school communities. Even with a small participation, compared to the number of students and professionals in schools (Table 1), the observed pattern of increase or decrease in detected cases was similar to that observed in the municipality, although on a much smaller scale (Fig. 1), with the highest positivity in the period between March and June 2022 (Tables 2 and 3). The SARS-CoV-2 lineages also corresponded to those obtained per period in the municipality, with the sequenced samples demonstrating the predominance of BA.2 and BA.5 omicron lineages (Fig. 3; Table 5). Although further studies are still essential, especially to assess the cost-effectiveness of implementing such measures, it was possible to demonstrate the viability of surveillance tests combined with genomic surveillance within schools, allowing the detection and isolation of positive cases, identification of virus circulation dynamics, and monitoring of variants.

Population-based studies conducted in other states in Brazil, as well as in other countries, showed that reopening schools was not a factor that significantly increased the number of COVID-19 cases [19, 57–64]. Additionally,

studies have shown that the transmission of COVID-19 in school environments is related to the level of community transmission, although with a lower incidence rate [59, 64]. During the present study, when cases in the municipality increased, a similar pattern was observed in the results of tests carried out in schools, and the same was observed with the reduction in cases (Fig. 1). After the return to in-person classes in all schools (private and public) in the municipality in 2021, there was no apparent increase in the number of registered cases compared to the previous months, while only one case was detected in the schools studied (Fig. 1). The increase in cases observed in January 2022 was caused by the introduction of the Omicron variant in Brazil (Figs. 1 and 4), which was first recorded in November 2021, in addition to a possible relaxation of preventive measures owing to the significant drop in the number of cases in the previous months [65, 66].

Although the presence of symptoms has been associated as a good predictor of SARS-CoV-2 infection (Table 3), many cases without reported symptoms were found, especially among students (with a potential case of reinfection) (Fig. 2). Among the school staff, 51% of the positive cases did not report any symptoms at the time of testing, compared to 74% among students (Fig. 2). Other studies have shown that children and young adults may experience a milder form of the disease than adults, usually with an expressive percentage of asymptomatic cases [15, 67, 68]. However, there is no definitive answer as to how asymptomatic and pre-symptomatic transmission contribute to the transmission of SARS-CoV-2 [19, 69, 70]. Despite these uncertainties, surveillance testing could potentially help provide clearer answers regarding

the role of children in the transmission of SARS-CoV-2, in addition to identifying active cases and potential outbreaks [19, 69].

The sequencing results of this study also demonstrate the potential for long-term surveillance in schools. It was possible to identify the predominant lineages in this period, the replacement of BA.2 by the BA.5 Omicron lineage, and the co-circulation of different lineages (Fig. 3). Likewise, until November 16, 2023, the only known lineage of samples collected in September 2022 came from the present study (Fig. 3). To date, genomic surveillance of SARS-CoV-2 has not been specifically conducted in schools in Brazil. SARS-CoV-2 sequencing allows monitoring of potentially more virulent, transmissible, or emerging variants [17, 71–74]. Recently, public health systems have prioritized pathogen genome surveillance, particularly since the COVID-19 pandemic. There is increasing international understanding of the role of genomic surveillance in public health as an important tool for pandemic (or epidemic) preparedness proposed by the WHO [75, 76]. Therefore, school environments can be considered important targets for genomic surveillance because of their potential role in the community spread of infectious diseases, considering the high mixing rates among school-age children [77, 78]. In addition, genomic surveillance in schools has gained attention as a tool during the COVID-19 pandemic, with studies focusing on wastewater surveillance demonstrating its application and effectiveness [79, 80].

Our study has some limitations. Participation was voluntary and not all urban schools agreed to participate in the study. Some schools that participated in 2021 did not continue in 2022, while others only joined from 2022 onwards. Student involvement was lower than anticipated despite efforts to raise awareness of the significance of the study in the school community. Because the parents completed the children's registration, the sample size of this group was low owing to the difficulty in getting in touch with the parents. Part of the reason for low participation can be attributed to the refusal to undergo swab collection for testing, not only in children and adolescents but also in adults. Some potential participants even described refusing due to the discomfort of swab collection, especially when considering the possibility of repeating it periodically throughout the study. In addition, it was observed throughout the study that people's perception of risk may be a factor that influenced their acceptance of participating in the study. When people had someone close to them who tested positive for SARS-CoV-2 or there was news about an increase in cases in the country, there was a greater demand to participate in the study, especially among adults. Since participation was voluntary, it is assumed that the sampling resulted in a selection bias toward those who were more

cautious and concerned about the pandemic. Therefore, the results should be interpreted with caution because generalizations can be misleading. Finally, it is worth mentioning the possibility of false-negative results in RT-qPCR testing. Considering that sampling was periodic, the time of collection for each participant may have influenced the sensitivity in detecting SARS-CoV-2 owing to the variation in viral load throughout the infection [81].

Conclusions

In conclusion, this study showed that the school community might be an important instrument in epidemiological surveillance, which can act as a public health component to trigger a rapid response. Long-term surveillance in schools could be interesting not only for SARS-CoV-2 but also for other pathogens. This was reinforced by the presence of negative tests, in which the participants reported symptoms, which is a possible indication of the circulation of other pathogens. Therefore, it can be used to track outbreaks and transmissible variants and to evaluate the role of children and adults in the transmission of these pathogens. Surveillance in schools can also be seen as part of pandemic (or epidemic) preparedness, allowing a rapid response to emergencies, such as the COVID-19 pandemic. In conjunction with health education on the importance of vaccines and hygiene care, surveillance in schools can avoid or at least reduce the time that schools would need to remain closed, thus reducing the impact of adverse effects on children's well-being and their potential to exacerbate inequality. Finally, it is important to highlight that additional studies are needed to compare the results and evaluate the cost-effectiveness of implementing measures, such as those employed in this study, especially when considering the public health system.

Abbreviations

CI	Confidence intervals
COVID-19	Coronavirus disease 2019
FIOCRUZ	Oswaldo Cruz Foundation
FIOCRUZ MS	Oswaldo Cruz Foundation of Mato Grosso do Sul
IQR	Interquartile range
LACEN/MS	Central Public Health Laboratory of Mato Grosso do Sul
MIS-C	Multisystem Inflammatory Syndrome in Children
RT-qPCR	Real-time reverse transcription polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SH-aLRT	Approximate likelihood ratio test and Shimodaira–Hasegawa branch support
UFBboot	Ultrafast bootstrap approximation branch support
VIF	Variance inflation factor
VOC	Variant of concern
VOI	Variant of interest
VUM	Variant under monitoring
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12889-024-19555-x>.

Supplementary Material 1: Additional file 1: Questionnaires. Registration questionnaire and participant follow-up questionnaire. The names of the schools were omitted to ensure the privacy of participants and school institutions

Supplementary Material 2: Additional file 2: GISAID Supplemental Tables. Supplemental Tables provided by GISAID containing sequence information used in this study, along with EPI_SET ID and the corresponding DOI

Supplementary Material 3: Additional file 3: Sequence summary information. Table with summarized information on the sequences used in the present study. Virus name, standardized name of the sequence in GISAID; Accession ID, distinctly formatted sequence accession identifiers assigned to individual sequence records submitted to GISAID; Location, location where the sequence sample was collected (Continent / Country / State / Municipality); Collection date: date on which the sequence sample was collected; Origin: information whether the sequence was generated in the present study, whether it was obtained from GISAID, or whether it was a reference sequence for SARS-CoV-2 variants; Phylogeny: Information if the sequence was used in phylogenetic analyses; Lineage group: SARS-CoV-2 lineage group defined in the present study; Lineage: lineage identified by the Pango lineage system (Pangolin); WHO name: lineages classified using Greek letters (such as Omicron) by the World Health Organization (WHO); Scorpio unassigned: Indicates whether the quality of the sequence is sufficient for confident lineage classification; N-Content%: Percentage of Ns present in the sequence

Supplementary Material 4: Additional file 4: Phylogenetic trees of the four datasets. Supplemental Figure 1. The phylogenetic tree reconstructed with the data set with all sequences below the threshold of unidentified positions (dataset i in Material and Methods). Supplemental Figure 2. The phylogenetic tree reconstructed with the data set with sequences below the threshold of unidentified positions and no recombinants (dataset ii in Material and Methods). Supplemental Figure 3. The phylogenetic tree reconstructed with the data set with sequences below the threshold of unidentified positions and no unassigned calls in Pangolin (dataset iii in Material and Methods). Supplemental Figure 4. The phylogenetic tree reconstructed with the data set with sequences below the threshold of unidentified positions, no recombinants, and no unassigned calls in Pangolin (dataset vi in Material and Methods)

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Author contributions

DMCA: Conceptualization, methodology, investigation, data curation, formal analysis, software, writing - original draft, writing - review & editing. CMS: Methodology, investigation, data curation, formal analysis, writing - original draft, writing - review & editing. JMT: Methodology, investigation, data curation, writing - original draft, writing - review & editing. CS: Methodology,

investigation, data curation, writing - original draft, writing - review & editing. CAV: Investigation, data curation, writing - original draft, writing - review & editing. RMSM: Investigation, writing - review & editing. RR: Investigation, writing - review & editing. GEBM: Investigation, writing - review & editing. ECF: Investigation, writing - review & editing. FMLM: Conceptualization, investigation, writing - original draft, writing - review & editing. ECFBS: Investigation, writing - review & editing. TFO: Resources, data curation, software, writing - review & editing. EFL: Data curation, writing - review & editing. UVA: Investigation, writing - review & editing. GGCL: Investigation, writing - review & editing. LHFD: Resources, writing - review & editing. MCSUZ: Resources, writing - review & editing. CCMG: Resources, funding acquisition, writing - review & editing. JFG: Conceptualization, resources, funding acquisition, project administration, writing - review & editing. ZCF: Conceptualization, methodology, investigation, resources, data curation, funding acquisition, project administration, supervision, writing - original draft, writing - review & editing. All authors read and approved the final manuscript.

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Data availability

The dataset containing participant information is available from the corresponding author upon reasonable request. All sequence data generated or analyzed during this study are included in this published article and its additional files.

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of the Oswaldo Cruz Foundation (FIOCRUZ) of Brasília (CAAE: 47905721.9.0000.8027). Adult participants and legal representatives of children and adolescents agreed to participate in the study and signed the free and informed consent form. In addition, even with representatives' consent, the children and adolescents agreed to participate and signed the free and informed assent form.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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