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HEALTH SCIENCES

Schools reopening and the COVID-19 pandemic: a case study from Macaé, Rio de Janeiro, Brazil

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Abstract: Since the first reported case of COVID-19 in Brazil, the public and private educational system started to close. Up to November 2020, scientific discussions about the return of schooling activities have been rarely performed by the national scientific community and police-makers. The great delay of school returning in Brazil contrasts with successful international strategies of school reopening worldwide and seems counterintuitive with the reopening of non-essential activities. Here, important issues to be considered before and during school reopening are reviewed and discussed. COVID-19 testing is essential to avoid disease spreading, but high cost of individual RT-qPCRs impairs an extensive testing strategy for school returning. To reduce costs and increase the speed of diagnosis, we tested the efficiency of a pooled-sample PCR strategy in a cohort of the educational staff in the city of Macaé/RJ, finding five asymptomatic individuals (0,66%) among the 754 people tested. Thus, a polled-sample PCR testing strategy of the educational staff might prevent infection spreading in schools at a reasonable cost. We discuss how our test strategy could be coupled with internationally recognized safety rules to allow for a safe school return and how countries from different world regions are dealing with educational activities during COVID-19 pandemic.

Key words: COVID-19 testing strategies, educational world situation, pooled RT-qPCR, student management.

INTRODUCTION

Education is recognized as a fundamental right by the United Nations and excellence in education stands as the Sustainable Development Goal 4. In 2020, the Coronavirus disease (COVID-19) pandemic spread across the globe and, the vast majority of countries announced the temporary closure of schools and other educational institutions, impacting over 91 % of worldwide students (United Nations 2020). In Brazil, in November 2020, eight months after the first COVID-19 reported case, only a few municipalities had allowed the return of schools' classroom activities. This fact heavily contrasted with Canada, Sweden, Finland and Israel, whose school activities had already returned or had not even been interrupted (Couzin-Frankel et al. 2020) and, other European countries and the United States, where school activities had returned in August or September 2020.

However, the return of school activities among countries were not uniform and so was the COVID-19 prevention governmental policies undertaken in each of them. For instance, compared with the strong preventive measures developed all over Europe, Sweden was less prohibitive, in the early 2020, not closing schools or performing any other protective measures, such as reducing the number of students per classes, occupying open and more ventilated spaces for teaching or requiring the use of masks for staff and students. Consequently, this country presents, now-a-days, one of the highest number of deaths among European countries (14,692) (Johns Hopkins University 2020).

On the other hand, Finland that shares borders with Sweden and have similar culture and economy, implemented more restrictive orders following the World Health Organization recommendations, which led to only 1,031 deaths attributed to COVID-19 (Johns Hopkins University 2020), since the beginning of the pandemic. Even though, Finland maintained normal school class sizes, however, restricted contact among students, probably avoided viral spreading. Officials of the country related that there was no evidence of increased COVID-19 spreading after schools reopened (Couzin-Frankel et al. 2020). In May 2020, studies around the globe had already found evidence that children were not super spreaders, suggesting that return to presential school activities could take place (Munroe et al. 2020). In addition to the low probability of children being major contributors for the increase in contamination levels, other factors had encouraged the return of school's activities, for example, the drop in general life condition for people under 18 years of age, such as increase in domestic violence, mental illness and even obesity (Patrick et al. 2020).

The increase of children's time in front of the screen has raised parent and governmental concern, since extended screen exposure time has been related to an increase in obesity and other cardiovascular disease risk factors (Lissak 2018). Therefore, as long as isolation is required to avoid virus spreading with screen exposure probably reaching levels never seen before, support for physical and social activities, as well as mental health services, should be stimulated (Dunton et al. 2020, Nagata et al. 2020). National surveys conducted in USA in June 2020 measured several life conditions (health status, childcare, food security and others) of children below 18 years. The authors reported a decrease in all conditions analyzed and that police-makers should consider the unique needs of families with children (Patrick et al. 2020).

Failure to children's safeguarding has been reported in parts of the United Kingdom (U.K.) as well, where isolation measures were leading to what is called a "second pandemic" of child neglect and abuse (Munroe & Shumway 2020). The Irish Society for the Prevention of Cruelty to Children (ISPCC) reported an increase in 26% of Childline users' websites (Williams 2020). Authors have also suggested that several weeks of isolation would lead to confusion, lack of confidence and increased anxiety in children and young people of U.K. (Green 2020). Other countries, such as Germany, Canada and China also detected impacts in the quality of life and mental health in children and adolescents (Ravens-Sieberer et al. 2021, Cost et al. 2021, Jiao et al. 2020).

In Brazil the scenario was not different and a cross-sectioned study with an online interview conducted between April and May 2020, associated high levels of children's anxiety with absence of parents while performing social-distancing, more people living in the same house and low educational levels of the guardians (Garcia de Avila et al. 2020). Research has shown that COVID-19 school closures would also lead to substantial learning losses (reviewed by Araujo e Oliveira et al 2020) and food intake problems for the most vulnerable children, particularly the lowest-achieving students (Bacher-Hicks et al. 2021). Additionally, schools play the role of daycare facilities, thus, closed schools led to parents having to face a tradeoff between work and adequate childcare. Lastly, schools represent the largest providers of mental health services for children in many countries, including Brazil.

School return in Brazil is a major and urgent requirement and should be followed by a strategy involving symptomatic isolation, extensive SARS-CoV-2 testing and school reorganization (Couzin-Frankel et al. 2020). However, molecular tests to detect SARS-Cov-2 (e.g., real-time reverse transcription polymerase reaction, RT-qPCR) are expensive and require specialized people to conduct laboratory procedures. Furthermore, underdeveloped countries struggle with difficulties to implement a vast sampling network, with testing and isolation, as recommended by international authorities. Beyond compromising tracking and isolation measures, the low testing rates in Brazil have been associated with underestimations of COVID-19 mortality rates between 1:5 and 1:4 (Kupek 2021). The paucity of testing in Brazil was already a major concern for the international community since in the middle of 2020. During this period Brazil had tested 7,500 per million people, at least 10 times lower than the USA (74,927/million) and even lower than Portugal (95,680/million) (Medecins Sans Frontières 2020, Thornton 2020).

Due to the urgency of testing in countries with economic difficulties and the high cost of tests (from US$10,00 to hundreds of dollars) (Shmerling 2021), strategies that could lower costs or improve test usage are of great importance. Thus, the current study aimed to improve the process of real-time reverse transcription polymerase reaction (RT-qPCR) tests in asymptomatic individuals by increasing the number of tested individuals, decreasing overall test time and the cost of each test. A pooling strategy, where several nasopharyngeal swabs individually collected are combined into a single RNA extraction, allows the testing of more individuals in the same RT-qPCR reaction. The results of a pooling strategy with the educational staff of Macaé city, Rio de Janeiro, Brazil are reported, and a broad discussion has focused on the improvement and implementation of massive COVID-19 testing and its significance for school reopening is provided.

MATERIALS AND METHODS

Swab collection and PCR pooling strategy:

This study was approved by the Federal University of Rio de Janeiro, Macaé Research Ethics Committee under reference number CAAE 37357020.4.0000.5699. A total of 1,500 staff members from private and public preschool, elementary I, elementary II, high schools, as well as universities were randomly selected to participate in the study. Members of the study included all categories of school community members, such as university professors, teachers, as well as administrative and cleaning service professionals. These group of individuals comprise around 10% of the 15,000 educational staff of Macaé. Among those 1,500 invited individuals, 754 showed up for a personal interview. All of those 754 individuals were asymptomatic and were submitted to nasopharyngeal swab collection.

A pool RT-qPCR strategy was used to detect SARS-CoV-2 virus as previously described (Lim et al. 2020, Singh et al. 2020, Christoff et al. 2021) (Figure 1). Briefly, extraction of nucleic acids was performed using magnetic beads (Magmax Magnetic Kit - ThermoScientific, Massachusetts/EUA), following manufacturer's instructions. For single samples 200 µL of Dulbecco's modified Eagle's culture medium (DMEM) were employed for extraction using MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit protocol (ThermoScientific) according to the manufacturer's instructions. During the final elution step, 50 µL of RNase-free water were used to elute the purified nucleic acids. Real-time reverse transcription polymerase reaction (RT-qPCR) for the identification of SARS-CoV-2 positive samples were performed with RT-qPCR primers and probes, as previously described in the Berlin (RdRP and E targets) (Corman et al. 2020) or CDC protocols (N1 an N2 targets) (CDC 2021). The reverse transcription and amplification reaction were performed using the TaqMan™ Fast Virus 1-Step Master Mix (ThermoScientific).

Each 15 µL reaction contained 5 µL of RNA template and 10 µL of a solution containing reaction buffer, reverse transcriptase/Taq mixture and primer/probe mixture. All oligonucleotides for N1 and N2 targets were synthesized by Integrated DNA Technologies (Iowa, USA) and the E, RdRP and RNAseP synthesized by ThermoScientific. Thermal cycling conditions were 50 °C for 10 min for reverse transcription, followed by 95 °C for 2 min and then 45 cycles of 95 °C for 3 s and 58 °C for 30 s.

A series of tests with pools containing only one positive sample with low (high viral load – Cycle Thresholds-CT<23) and high CT (low viral load>32) were used to validate the technique. A positive control plasmid containing 10 copies of fragments of the human RNAse P (RP) gene, SARS-CoV-2 E and RdRP genes was used. A pool was considered for further investigation of SARS-CoV-2 presence if one or the two viral targets were amplified with a CT lower than 40. In this case, samples from the pools were processed separately for extraction and RT-qPCR procedures, being considered positive samples those that presented amplification for both targets of the virus with CT ≤40.

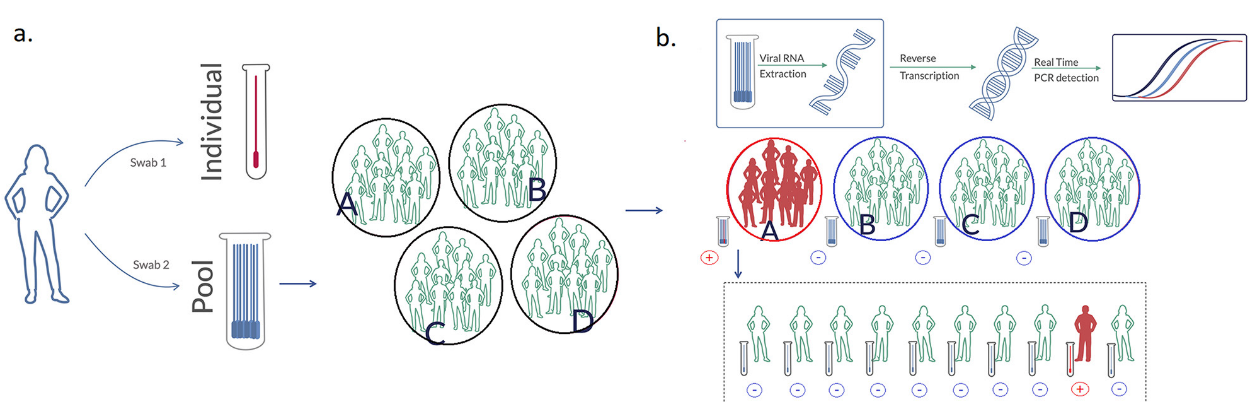

Figure 1. Pooled RT-qPCR strategy scheme, 1a. Two nasopharyngeal-swabs were collected from each patient. The first swab was pooled together with nasopharyngeal_swabs from up to 10 individuals and the second was stored separately. 1b. RT-qPCR was conducted with the pooled swabs, and whenever a pool displayed a positive result, individual swabs from each patient from that pool were analyzed to identify the infected individuals. Illustration adapted from Christoff et al. (2021).

Results and discussion

A total of 81 pools were analyzed, each one containing a mix of 10 samples/individuals. Six pools collected from the testing laboratory team were included as negative controls and later removed from further analysis. Three out of the 81 pools amplified the two different regions of the SARS-CoV-2 genome (Pool 17, 62 and 71) and one pool amplified only one viral genome target region (Pool 81) (Table I). All 37 samples from these four pools were individually extracted, while the remaining 717 samples were considered negative for the presence of SARS-CoV-2. Among these 37 samples, four amplified two regions (SARS-CoV-2 E and RdRP-P2 probes) and were considered positive. Analyses of CT values obtained from sample amplifications have been correlated with the infective potential. Lower CTs and higher viral loads occurring during pre-symptomatic or early symptomatic stages are important for virus spreading (He et al. 2020). Importantly, two asymptomatic individuals (samples 17.4 and 62.9) did show CT values of both viral targets lower than 30, strongly suggesting that they could potentially transmit COVID-19 to other educational staff members (Table II). Interestingly, the sample 81.4 amplified only one viral target with a high CT value (E = 35.5) and was submitted to another RT-qPCR test with N1 and N2 viral targets for evaluation of virus presence. The high CTs observed 38.86 (N1) and 37.23 (N2) showed that the viral amount was low in this sample but confirmed its positivity (Table II). Overall, the pooling strategy was efficient to identify SARS-CoV-2 and reduced the number of required tests from 754 to 112 (37 individual samples and 75 pools), greatly decreasing testing costs.

Similar numbers have also been found by Singh et al. (2020), during the emerging COVID-19 outbreak in India, where 545 nasopharyngeal and oropharyngeal samples were used in 109 pools. Considering that each test costs at least $10 Dollars (Shmerling 2021), the Indian study saved approximately US$4,360.00 (545 versus 109 samples). Likewise, in the present study, around US$6,420.00 or R$ 32,100.00 was saved (754 versus 112 samples), with the total cost of processed samples being only 15% the cost of all samples processed separately (US$1,120.00 compared with US$7,540.00).

Massive testing plans generate fundamental information for the development of adequate plans and policies not only in the education, but also in economy, tourism, transport and, obviously, in health. A country COVID-19 testing

Table I. SARS-CoV-2 pool RT-qPCR analysis. Positive or undetermined pools. 81 pools containing 6 to 10 samples each were analyzed. Pools 17, 62 and 71 were positive and pool 81 was initially considered undetermined (-). The 37 samples were individually processed, and the positive results are described in Table 1B. Pool 81 was only considered positive after N1 and N2 genes were targeted by RT-qPCR.

Pool CT	Primer E	Primer P2	Primer RP	Individual extraction	Primer E	Primer P2	Primer RP	Primer N1	Primer N2	Primer RP
	24.67	26.99	23.88	Sample 17.4	23.7	26.45	27.98			
Pool 62	28.37	29.39	27.44	Sample 62.9	26.65	28.39	27.2			
Pool 71	32.07	32.91	25.8	Sample 71.2	36.06	36.42	28.48			
				Sample 71.8	30.94	32.06	30.91			
Pool 81	-	34.67	26.18	Sample 81.4	35.55	-	32.61	38.86	37.23	32.23
Pool 17										

database is available online (Hasell et al. 2020) showing graphics and map information frequently updated. Part of this data, from selected countries are summarized in Table III. Unfortunately, in South America Brazil has contributed with this database only during short periods, mainly between August and September 2020, and since then no information has been updated.

This lack of information is also observed in Guyana, Suriname, French Guyana and Venezuela. On the other hand, Chile and Uruguay have tested more extensively in South America. Chile has performed regular testing, with increasing numbers reaching around 2 - 3 tests per 1000 people, while Uruguay started testing later, but reached higher numbers, ~ 6 tests per 1000 people in June 2021 (Hasell et al. 2020). Contrastingly, at the end of 2020, most of the developed countries in the Northern hemisphere had performed over 2 tests per 1000 people, with countries like Denmark achieving 17 tests per 1000 people and the United Kingdom and France having over 10 tests per 1000 people during that period (Hasell et al. 2020).

Most SARS-CoV-2 identification performed abroad are PCR or rapid antigen tests associated with PCRs. Since PCRs are the gold standard method COVID-19 detection and are costly and labor intensive (Shmerling 2021, Hasell et al. 2020), any improvement in the number of people tested by PCR, as performed in the present study, are of great interest. Here, five positive individuals were identified among the 754 people analyzed, thus, a rate of 660 positives per 100.000 inhabitants was observed. This value is at least 30-130 times higher than the rates previously suggested to be the safest for school returning activities (5-20 PCR positive per 100.000 inhabitants) (CDC 2021b).

Analyses of previous reopening experiences might provide the key to foster future activities. For instance, in the Republic of Ireland schools reopened in late August 2020 and a massive testing experiment was conducted with 15,533 adults and children being tested. Of those, 399 tested positive, a rate of 2.6% (White et al. 2021), concluding that this rate was low enough, supporting policies of schools' reopening. Therefore, the 0.66% of positivity identified in our study should not raise major concerns, although a well-planned testing strategy should be applied.

As the Republic of Ireland and other countries in Europe have already restarted school activities, reports from Norway shows that reopening kindergarten and schools has also a limited effect in the spread of COVID-19 (Rypdal et al. 2021). Additionally, in France, researchers have concluded that, despite the reopening of

Table II. SARS-CoV-2 individual samples positive for SARS-CoV-2. Out of 37 samples analyzed five were positive for SARS-CoV-2. One pool displayed two positive samples 71.2 and 71.8 (Pool 71). Sample 81.4 was only considered positive after N1 and N2 genes were targeted by RT-qPCR. "-" are undetermined values.

Individual extraction	Primer E	Primer P2	Primer RP	Primer N1	Primer N2	Primer RP
Sample 17.4	23.7	26.45	27.98			
Sample 62.9	26.65	28.39	27.2			
Sample 71.2	36.06	36.42	28.48			
Sample 71.8	30.94	32.06	30.91			
Sample 81.4	35.55	-	32.61	38.86	37.23	32.23

schools, the transmission among children and adolescents was lower than observed among adults (Gras-Le Guen et al. 2021). In Germany, researchers used the summer break to test if schools' returning would have an impact in the increase of SARS-CoV-2 cases in the country and showed that there was no evidence of a positive effect of school reopening on the increase in case numbers (Isphording et al. 2021). However, it was highlighted that reopening should be conducted under strict hygiene measures, quarantine and containment measures (Isphording et al. 2021).

Conversely, other European countries present studies, showing contagious outbreaks in schools and related increases in SARS-CoV-2 transmission. In Italy, researchers observed that subjects older than 10 years old contributed to the increase in viral transmission and found evidence that even children under 10 years old could also contribute to the spread of SARS-CoV-2 (Sebastiani & Palu 2020, Sebastiani & Palu 2021). In England 969 schools reported outbreaks of SARS-CoV-2, comprising 2% of primary and 10% of secondary schools in the country, with higher rates of positivity among the teaching staff compared with students, particularly in primary schools (Aiano et al. 2021a).

These differences between schools reopening and increase in transmission rates observed among European countries was already reported by Stage et al. (2021), who compared growth rates in daily hospitalizations or confirmed cases in four countries, concluding that school reopening could contribute to an increase in spreading rates if community transmission is relatively high. In contrast, countries where transmissions are generally low, schools reopening is feasible. More recently, these differences have also been observed even inside a country, for instance, Rozhnova et al. (2021) proposed a model for evaluation of school and non-school related measures to control transmissions in Netherlands, with analyses suggesting that the impact of measures to reduce school-based contacts is dependent on the capacity to reduce non-school-based contacts.

As in Europe, these differences were also observed within the USA and Canada, with studies reaching the same conclusions (Doyle et

Table III. COVID-19 data from selected countries.

	Total deaths	Deaths per million/pop	Cases per million/pop	Test per thousand/pop	Test per confirmed case
Brazil	472,861	2,209.70	87,863.10	253.18	-
Peru	186,073	5,577.83	61,951.84	136.11	12.0
Uruguay	4,583	1,315.01	107,107.52	820.00	6.0
USA	596,667	1,792.25	101,547.78	1,425.19	49.0
Finland	959	172.84	17,404.96	1,001.92	141.7
Sweden	14,523	1,429.41	107,368.40	-	19.8
UK	127,922	1,875.49	72,353.96	2,888.12	159.1
Ireland	4,941	991.59	55,116.65	1,132.54	44.6
Japan	13,534	107.37	6,406.18	126.62	33.2
South Korea	1,973	38.46	3,148.63	206.49	47.0

Numbers correspond to the period from March 2020 until early June 2021, retrieved from https://ourworldindata.org/coronavirus.

al. 2021, Espanã et al. 2021a, Phillips et al. 2021). For example, in Florida, 60% of COVID-19 cases in school-aged children were found to be not school-related, concluding that the introduction of SARS-CoV-2 into schools is dependent on levels of community transmission and adherence to mitigation measures in schools (Doyle et al. 2021). Analyses conducted in Indiana, USA reached similar conclusions regarding mitigation measures, especially regarding face mask adherence and schools operation capacity. It was observed that number of infection and deaths in the state, are consistent with high levels of school's capacity (80-95%) and intermedial levels of face mask adherence (40-70%), this scenario have more than 40 and 9 times that the number of infections and deaths, respectively, if schools would have operated remotely (Espanã et al. 2021a). These relationship between school's capacity and transmission rates were also observed by Phillips et al. (2021) in Canada, where after reopening schools the test positivity rate in schoolchildren were two times higher compared with other ages. Based on these results schools were closed again, and it was proposed that switching to smaller class sizes and grouping siblings might provide conditions for schools reopening.

Asian countries have recently suffered from different pandemics (SARS, 2003; MERS, 2015), which might help to properly deal with COVID-19 pandemic. Japan and South Korea have reported the first COVID-19 cases in January 2020. A similar pattern of disease spreading with a relative low number of confirmed COVID-19 cases and deaths was observed. However, while South Korea implemented an aggressive and proactive testing/tracing/treatment protocol, Japan was more cautious and adopted a more self-restraint based politics, testing only selected symptomatic individuals (Moon et al. 2021, Karaco et al. 2021, Seong et al. 2021).

Regarding educational facilities, schools' closure began early March 2020 in Japan, and safety protocols were established for reopening in June 2020. A study conducted one month after the beginning of school reopening found low rates of infection with transmission route being related to the reopening of schools (Wada et al. 2020). In South Korea, educational authorities delayed the beginning of school activities after the winter break. Classroom activities were reestablished grade by grade from May 20 to June 8 (Byun & Slavin 2020). As in Japan, a study analyzed the route transmission of pediatric infections of COVID-19 in South Korea and found low rates of infections related to school activities (2%, three children among the 127 cases analyzed) (Kim et al. 2020). However, another study in South Korea showed that transmission was increasing among adolescents even with high acceptance of face mask use. A lower adherence to social distance in adolescents was associated with higher COVID-19 spreading and raised concerns about school activities of this group (Park & Oh 2021).

If the safety of reopening schools in developed countries has been questioned with subsequent reopening and closing events, the scenario is even worse in the underdeveloped ones. Up to April 2021, India had attempted to re-open schools several times, but after a sharp increases in COVID-19 cases among teachers and students, schools were closed (Sharma & Joshi 2021). A similar scenario was observed in Pakistan, where reopening and closing of schools have been happening since 2020, stressing the importance of carefully planning schools reopening and strictly monitoring if preventive measures are actually being conducted (Pradhan & Nanii 2021). The great difficulties of Pakistan schools to provide the basic structure for the appropriate sanitation and hygiene, a situation shared by most underdeveloped countries of the Southern hemisphere, have been also associated with unsuccessful attempts of reopening in african countries (Wada & Oloruntoba 2021, Mathebula & Runhare 2021, Dzinamarira & Musuka 2021, Ofori et al. 2022, Brand et al. 2021). The need for an overall improvement in handwash facilities was also observed in Nigeria (Wada 2021), where the lack of soap in hand-wash stations and even the unavailability of these stations in 40% of female toilets visited in schools showed that basic hygiene protocols should be established before reopening schools (Mathebula 2021, Dzinamarira & Musuka 2021, Ofori et al. 2021).

Other factors to be considered during the evaluation of schools' reopening are the emergence of new virus variants with higher transmission capacity, as well as the advance in vaccinations programs worldwide. How these factors interact with one another might lead to the development of new models based on the different safety measures adopted. In Kenya a model was developed to explain the causes of the first three waves of infection observed and has predicted the upcoming of a fourth wave, if new variants arrive in the country or if significant immunity waning occurs in individuals previously exposed to the SARS-CoV-2 (Brand et al. 2021).

Models of pandemic development are interesting, particularly in Latin America, where approximately 70% of the countries kept schools closed until the end of 2020 (Fernandez-Guzman et al. 2021).

In Colombia, with respect to schools' capacity, researchers proposed a model based mainly on differences of social contact, assuming a high adherence of face mask use, and concluded that with 35% of classroom capacity and 75% adherence of face mask use, reopening schools will have a small impact in the number of deaths caused by COVID-19 (Espanã et al. 2021b). Likewise, in Mexico, Munõz-Naval (2021) argued for a "real-world" strategy that would be more realistic for under-developed countries, where operational costs associated with a safe reopening of schools are not possible. Divisions of students in "bubbles" of interaction with restricted interactions among classes and fewer students was proposed as a realistic strategy.

In Brazil, two studies proposed models that describe the pandemic behavior from the worst to the best possible scenarios (Cruz et al. 2021, Massad et al. 2021). One alerted for the possibility of health care system's collapse if all schools reopened all at once with full capacity (Cruz et al. 2021), but demonstrated that a controlled reopening with several stages, carefully increasing the number of students per class, could keep infections rates under control if 50% of isolation inside school is maintained and if the general population follows all sanitary rules.

Similarly, Massad et al. (2021), adopted a conservative level of children's infection transmissibility (10%) and showed that with schools operating in full capacity, the cases caused by infected children among school staff and relatives would vary between 2 to 85 cases. Both studies agree with the idea that to reopen schools in Brazil, community transmissions must be controlled and preventive measures must be adopted by schools. However, both studies point out that even in the most optimistic scenario, infections rates should rise, and thus reopening schools should be associated with the advance of vaccination programs (Cruz et al. 2021, Massad et al. 2021).

In Brazil since the beginning of the pandemic, early 2020, the Federal government did not adopt any official restrictive measures regarding people mobility or test/tracing programs, and only municipal and state governments carried out preventive procedures, independently. While by mid-May 2020 Uruguay and Paraguay had their epidemic scenario relatively under control, Brazil and Peru presented the highest numbers of confirmed cases and deaths due to COVID-19 in South America (Gonzalez-Bustamante 2021). Besides the serious health conditions observed in Peru, the country had adopted several measures to prevent and control the disease such as: the obligatory use of face mask, lockdown periods and massive testing program, nevertheless, the epidemic scenario in Peru is one of the worst in the continent. Some factors that contribute to this health condition was the massive publishing of fake news related to the pandemic (Alvarez-Risco et al. 2020) and the segmentation of healthcare system. Both factors contributed to leaves 15% of population without any medical support and other socio-economic issue of Peruvian residences, leading to more circulation of people to essential trade such markets and banks, that leads to more frequent crowding, and could improve and facilitate the spread of the virus (Pescarini et al. 2020).

Contribution of asymptomatic and pre-symptomatic transmissions in the spread of SARS-CoV-2 infection is another issue without a clear answer. In a revision on the subject Savvides & Siegel (2020) showed that most studies have supported asymptomatic and presymptomatic transmissions, however, the authors do not fully endorse this conclusion, since some inadequacies among studies were reported. Another review on asymptomatic SARS-CoV-2 infection suggested that asymptomatic transmission may account to approximately 40-45% of SARS-CoV-2 infections, having the potential to transmit the virus for longer periods (>14 days), thus, it is imperative that testing programs include asymptomatic individuals as well (Oran & Topol 2020). Similar conclusions were pointed out by Vermund & Ptizer (2021), with discussion of asymptomatic transmission and its implication for school reopening, also highlighting that even though COVID10 related deaths are far less frequent among children, transmission may occur among teachers, school workers and parents.

On the other hand, Gaythorpe et al. (2021), in their systematic review on the children's role in the COVID-19 pandemic, identified a lower number of asymptomatic children infected (21%) than the observed by Oran (2020), who did not identify any study that was actually designed to evaluate transmissibility among children. The reopening of schools might, in fact, provide conditions to implement test procedures and study protocols that will allow us to circumvent these questions and get clearer answers regarding the role of children in the community COVID-19 spread (Gaythorpe et al. 2021).

All studies presented so far provided evidence that testing procedures are not only paramount to get a clearer answer regarding the mechanisms of virus transmission, but also to define measures/protocols to implement safety in schools' activities when they reopen. Testing procedures are necessary to follow changes in virus spread and to isolate infected people. Additionally, the identification of asymptomatic individuals should reduce the possibility of COVID-19 outbreaks and protect school staff and parents. To implement these testing regimes, actions as the one proposed by the present study are extremely important and provide conditions for middle to low-income countries to advance in RT-qPCR tests using the same pooled sample protocol, thus, reducing costs but maintaining the same quality of results.

In an observed frequency of 1% positivity rates, a 10-sample pooling strategy would require 10 pools and 10 individual samples, needing only 20 RNA extractions and RT-qPCR amplifications, instead of 100 individually processed samples. Since the nucleic extraction procedure is the most laborious and most expensive step of SARS-CoV-2 identification, the pooling strategy described here can decrease at least 60% of costs and increase the processing capacity in at least five times. Overall, the combination of RT-qPCR pooling with the newly available rapid antigen tests (CDC 2021c) can overcome the difficulties of testing a large set of individuals from schools and other educational establishments. It has been widely recognized that a higher frequency of tests is important to avoid community spreading of SARS-CoV-2 (Mina et al. 2020), particularly with isolation and immediate testing of putatively infected individuals.

In June 2020, the Public Health England (PHE) initiated a prospective national surveillance of SARS-CoV-2 in primary schools (sKIDs) andAiano et al. (2021b) used this opportunity to evaluate the feasibility and acceptability of this test regime on children, school staff and parents, observing that all groups were supportive of regular tests for SARS-CoV-2, with nose-swab and oral fluids been the most accepted test by all groups. A new version of "sKIDs" was published in May 2021 (Ladhani et al. 2021), with the surveillance of SARS-CoV-2 in English primary schools being still taken place and can be used as a guide for the implementation of testing regimes in other countries.

Lastly, although a proper testing strategy, as the one described here, could help to foster schools' return, this is only one among several adaptations that should be performed in the working environment and praxis of an educational system. Besides keeping social distance, the proper use of protective masks inside and outside classrooms and a routine of hygiene procedures, such as alcohol disinfection and frequent hand washing, are essential to prevent SARS-CoV-2 spreading in the school environment. Additionally, emphasis should be given to the most vulnerable risk groups of the educational staff. For instance, staff working in more than one educational unit, displaying advanced age, comorbidities and daily traveling in public transportation should be followed with care to avoid disease spreading and further deaths. Regarding all the damages that children's well-being has suffered through the COVID-19 pandemic (Tan 2021, Buonsenso et al. 2021), UNICEF proposed a six-point plan called "Averting a lost COVID generation" that focus in mitigating the impact in children's health, economic, social status, which might guide governmental policies to overcome these impacts (UNICEF 2020).

At the beginning of 2020, as the pandemic of COVID-19 rise all over the world, schools' closures were one of the first sanitation measures taken by most countries. However, schools reopening were not homogenous as their closures, with each country showing different pandemic stage. Thus, reopening happened at several moments along 2020/2021, in high-middle-low-income countries, with different outcomes and results from each scenario, making hard to trace down and find patterns that could link schools' reopening with major increases in COVID-19 cases in the general community or with the maintenance of infected rates already observed in the country.

Regarding the children, they do not seem to be super-spreaders as thought at the beginning of the pandemic, but their infection capacity should not be neglected, since asymptomatic cases may also, together with the symptomatic ones, contribute to the spread of the virus. However, more studies are needed to confirm the real role of children in the community outbreaks of COVID-19.

Questions regarding the effect of closing/reopening of schools during the COVID19 pandemic still remains, but it is clear that they have a severe impact in the general well-being of children mainly in middle to low-income countries. Appropriate adaptation of teaching spaces, decreasing number of students per classes and strictly following sanitary rules, with the use of face masks and restriction of certain activities that have potential of increasing virus spreads (e.g., collective sports), should allow the reopening of schools, since it is an essential activity. As vaccination programs are extended to all ages, monitoring testing surveillance should be implemented even in asymptomatic persons, identifying and isolating cases in early periods of infection, making COVID-19 outbreaks in schools harder to happen, and turning schools into a safe place for all people involved in this fundamental activity.

CONCLUSIONS

School's returning is an essential step to overcome the effects of the pandemic over children's development. Presential returning should be preceded by a school's adaptation plan together with a proper testing strategy, followed by contact and isolation of COVID-19 positive individuals. Data suggest that staff members living in other municipalities, where virus circulation is unknown, might represent critical virus reservoirs. Special attention should be given to school staff displaying comorbidities and advanced age. Overall, elementary school returning is necessary and if safety measures are undertaken, this return can be feasible and with minor risk of community COVID-19 spreading.

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All the authors participated in different stages of sampling, bench procedures, samples analyses, results discussions, and elaboration of the manuscript. Allan Pierre Bonetti Pozzobon conducted laboratorial procedures, generated tables and figures and wrote final version of the manuscript. Natália M. Feitosa conceptualized, designed and coordinated the study from the beginning, Cintia Monteiro de Barros designed the study, wrote a preliminary version of the paper, initiated the submission and revised the manuscript. Renata Coutinho dos Santos designed the study and conducted the first paper draft with editors. Carla Zilberberg, José L. Nepomuceno-Silva and Ana C. Petry designed the study, revised the English language and the general manuscript. Rodrigo Nunes da Fonseca participated in the conception, formal analysis, preparation of the original draft, writing, review and final editing of the manuscript.