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Short Communication

Monitoring of SARS-CoV-2 circulation using saliva testing in school children in Rome, Italy

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ABSTRACT

Objectives: To describe the trend of SARS-CoV-2 RNA in saliva samples from children attending nine schools in Rome in the local surveillance unit RM3 during the period of September 2021-March 2022, in parallel with the trend of SARS-CoV-2 RNA observed in nasopharyngeal swabs (NPSs) from the population in the same catchment area that was routinely tested at our laboratory in the same period.
Methods: Saliva samples were collected using the Copan LolliSponge™ device and analyzed by Aptima® SARS-CoV-2 Assay on the Panther® System. NPSs were tested using either Aptima® SARS-CoV-2 Assay or Alinity m SARS-CoV-2 Assay.
Results: The percentage of positivity in the two populations was different; of the 2222 saliva samples from students, 0.99% had positive results, whereas the percentage was higher (33.43%) in the 8994 NPSs representing the population from local surveillance unit RM3. Interestingly, the trend of SARS-CoV-2 RNA in saliva samples from students was consistent with that observed in NPSs from the population in same catchment area, although with peaks slightly anticipated.
Conclusion: Overall, screening of saliva in the schools represents a good system to monitor SARS-CoV-2 circulation in the population, allowing early detection and quick isolation of students who are asymptomatic with positive test results and thus prevention of virus transmission.

Background

Since October 2020, the identification of suspected cases and contact tracing in the Lazio region have been supported by active surveillance initiatives in schools through antigen tests performed mainly on saliva (Bordi *et al.*, 2021; Iwasaki *et al.*, 2020). For the school year 2021-2022, the National Plan implemented the "Plan for monitoring of the circulation of SARS-CoV-2 in primary and lower secondary schools". The Laboratory of Virology of the National Institute for Infectious Diseases "L. Spallanzani" was involved in the screening of saliva samples collected from students belonging to the local surveillance unit (ASL) RM3 of Rome. The use of saliva as an alternative sample for SARS-CoV-2 RNA detection has been the focus of numerous discussions; scientific evidence has emerged indicating that molecular tests performed on saliva have diagnostic sensitivity and specificity comparable to those observed with nasopharyngeal swab (NPS) (Al Suwaidi *et al.*, 2021; Bordi *et al.*, 2020; Butler-Laporte *et al.*, 2021), suggesting that saliva represents the first-choice sample in community mass-screening programs (Edward *et al.*, 2021; Jurkutat *et al.*, 2022; Ruggiero *et al.*, 2020; Vogel *et al.*, 2022).

In this study, we describe in parallel the trend of SARS-CoV-2 RNA in saliva samples collected during the period of September 2021-March 2022 from children attending nine schools in ASL RM3, according to the National Plan for screening in the school setting, and the trend of SARS-CoV-2 RNA observed in NPSs from people living in the same catchment area and routinely tested in the same observation period according to the Italian screening Plan for SARS-CoV-2 in the setting of the general population.

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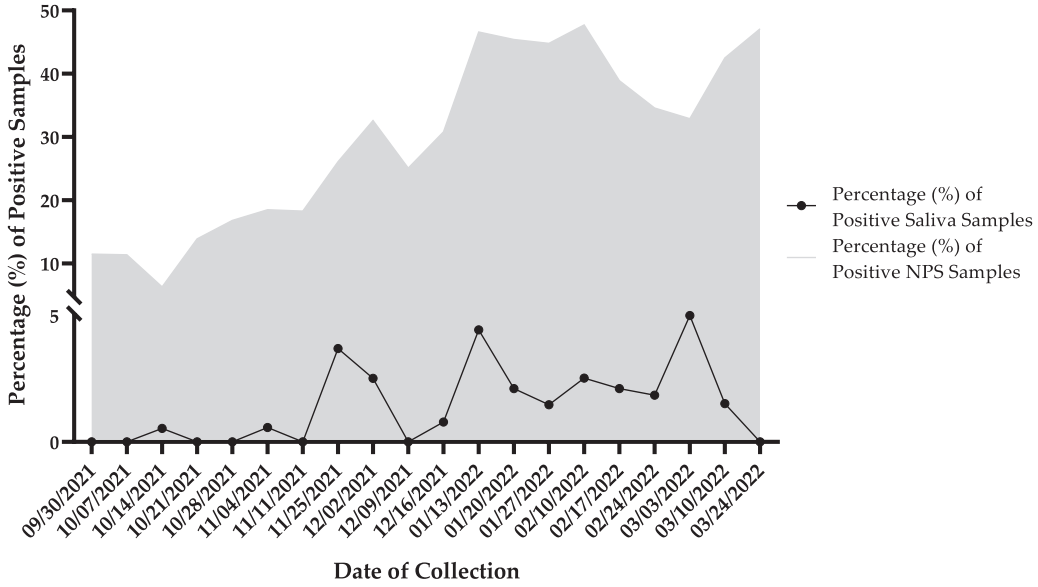

Figure 1. Percentage of samples with positive SARS-CoV-2 test results during the school year 2021-2022. The black line with the dots represents the percentage of saliva samples with positive SARS-CoV-2 test results from children coming from nine schools in the local surveillance unit RM3; the gray area represents the percentage of NPSs with positive SARS-CoV-2 test results analyzed in our laboratory and coming from the same local surveillance unit (RM3).
NPSs, nasopharyngeal swabs.

Methods

Saliva samples from students who joined the National Plan for screening by filling in the informed consent (with no other selection criterion) were collected nearly every week by dedicated personnel from ASL RM3 using the Copan LolliSponge™ devices (Copan Italia s.p.a., Brescia, Italy). Samples were centrifuged (450 *g* for 60 s) to extract saliva from the sponge; 100 μl of saliva was then diluted with 400 μl of Minimum Essential Medium, transferred into the Specimen Lysis Tube, and individually analyzed using Aptima® SARS-CoV-2 Assay on the Panther® System (Hologic Inc., San Diego, California). NPSs from a population randomly selected based on the catchment area (ASL RM3) were tested using either Aptima® SARS-CoV-2 Assay or Alinity m SARS-CoV-2 Assay (Abbott s.r.l., Rome, Italy).

Statistical analysis (percentage and min-max 95% confidence interval [CI]) was performed using GraphPad Prism 9.0.2 (GraphPad Software, La Jolla, California).

Results

From September 30, 2021 to March 24, 2022, a total of 2222 saliva samples from schoolchildren (1177 females, 1045 males, median age 10 y, age range 6-14 y) were analyzed; of these, 22 (0.99%, min-max 95% CI 0.64-1.66) had positive test results. Notably, 171 saliva samples were not tested because insufficient volume was obtained after centrifugation; these were excluded from the analysis. Regarding the trend of SARS-CoV-2 RNA in saliva samples, the percentage of positivity remained <0.6% until mid-November, peaked at 3.7% on November 25, and decreased thereafter. A further increase was observed on January 13 (4.4%); percentage of positivity subsequently decreased to 2.0%, remained quite stable until March 3 (when the peak of 5.0% was achieved), and decreased to 0.0% on March 24 (Figure 1). Notably, at the end of March the number of students tested was drastically reduced (about 50% fewer); this could partially account for the 0% observed.

Of the 8994 NPSs from the population in same catchment area, 3007 (min-max 95% CI 89.65-211.0) had positive results, showing a higher prevalence (33.43%) than that observed in the schools. Interestingly, there was an increase with time in the percentage of NPS positive tests until December 2, when a peak of 32.6% was observed. The peak was followed by a decrease; a further increase (>44.0%) was revealed between January 13 and February 10. Percentage of positivity then progressively decreased to 32.9% on March 3 and increased again to 47.1% on March 24 (Figure 1).

Conclusions

Our results show that, at least in the considered period, the circulation of SARS-CoV-2 in primary and lower secondary schools in Rome ASL RM3 was low (0.99% positive tests), suggesting a relatively low risk of becoming infected at school; this finding is in agreement with similar studies (Jurkutat *et al.*, 2022; Vogel *et al.*, 2022). The gap in the percentages of positivity between saliva and NPS tests is attributable to the fact that they are from different populations. Nevertheless, the trend of SARS-CoV-2 RNA in saliva, which is representative of a smaller population of schoolchildren, was consistent (although with peaks slightly anticipated) with that observed in NPSs, which is representative of a wider population living in the same geographic area. Results also confirm that saliva represents an excellent sample for screening programs–especially for children–because of its stability and its easy and painless collection.

Overall, molecular screening on saliva from children in the schools represents a good system to monitor SARS-CoV-2 circulation in the population, allowing early detection and quick isolation of students who are asymptomatic with positive test results and preventing virus transmission, especially in a high-prevalence catchment area.

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Ethical approval statement

The study was conducted within the "National Plan for monitoring of the circulation of SARS-CoV-2 in primary and lower secondary schools", according to which informed consent has been compiled and signed by the parents of the students; the data for biological samples were collected for diagnostic purposes and were used only after their complete anonymization.

Author contributions

LB organized workflow, conceptualized the study, analyzed data, and wrote the manuscript; GS analyzed data and contributed to the writing of the manuscript; Covid-Saliva Laboratory team performed acceptance procedures of the samples and diagnostic assays; RG contributed to the workflow's organization; FV supervised the activities; and FM critically read the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interests

The authors have no competing interests to declare.

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