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BRIEF REPORT

Persistence of Severe Acute Respiratory Syndrome Coronavirus 2 Omicron Variant in Children and Utility of Rapid Antigen Testing as an Indicator of Culturable Virus

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We screened 65 longitudinally collected nasal swab samples from 31 children aged 0–16 years who were positive for severe acute respiratory syndrome coronavirus 2 Omicron BA.1. By day 7 after onset of symptoms, 48% of children remained positive by rapid antigen test. In a sample subset, we found 100% correlation between antigen test results and virus culture.

Keywords. SARS-CoV-2 persistence in children; SARS-CoV-2 culture; SARS-CoV-2 Omicron; rapid antigen test; rapid diagnostic test.

On 20 July 2020, the Centers for Disease Control and Prevention (CDC) recommended that persons infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolate for a 10-day period, without requirements for an associated negative test for the virus [1]. This followed from studies showing that virtually all non-immunosuppressed patients with mild to moderate disease became culture negative by day 10 of infection, with virus culture providing what was regarded as the optimal laboratory marker for infectivity [2–4]. The Florida Department of Health (FDOH), in an Emergency Rule issued 6 August 2021 [5], indicated that children infected with SARS-CoV-2 could return to school sooner than 10 days if they had a negative coronavirus disease 2019 (COVID-19) test and were asymptomatic. On 14 January 2022, CDC changed its recommendation to say that children and adults infected with SARS-CoV-2 should isolate for 5 days, and, if afebrile, could then return to work/school but should wear a high-quality mask for an additional 5 days [6, 7]. The FDOH position was changed on 24 February 2022, to say that children only needed to isolate for 5 days before returning to school, without further testing, and that masking was not necessary [8]. Given the variability in recommendations, we sought to characterize SARS-CoV-2 Omicron persistence and infectivity among children with mild illness in an outpatient setting.

METHODS

Otherwise healthy children with a positive rapid antigen or reserve-transcription polymerase chain reaction (RT-PCR)–based test for SARS-CoV-2 were referred to the study's recruitment team from University of Florida Health pediatric clinics or school nurses at a local public school [9]. Following CDC definitions, day 0 of infection was defined as the day of onset of symptoms or the day a positive test for SARS-CoV-2 was obtained, whichever came first. We focused on enrolling children who were on day 5 or later in their infection, with samples collected between 20 December 2021 and 21 February 2022. In 2 instances in which multiple children from a family were enrolled, we also collected samples from SARS-CoV-2–infected parents of the participating children. The study was approved by the University of Florida Institutional Review Board with signed informed consent obtained for participants.

We obtained 65 nasal swab samples from 31 children and 8 samples from 3 parents. Two anterior nasal swab samples were obtained from each participant on each visit: 1 was immediately tested with the BinaxNOW rapid antigen test (Abbott Laboratories, Abbott Park, Illinois) and results given to participants; the second was frozen at −80°C for culture, RT-qPCR, viral load determination, and sequencing. Children who had a positive result at the time of study enrollment were asked to return for repeat testing, with testing continuing until a negative test result was obtained. If a negative rapid antigen test result was obtained before day 10 of isolation, children were given a note that permitted them to return to school, as specified in the 6 August 2021 FDOH Emergency Rule [5].

BinaxNOW testing was done following the manufacturer's instructions. As described in the Supplementary Methods, RT-qPCR was performed and a standard curve was generated

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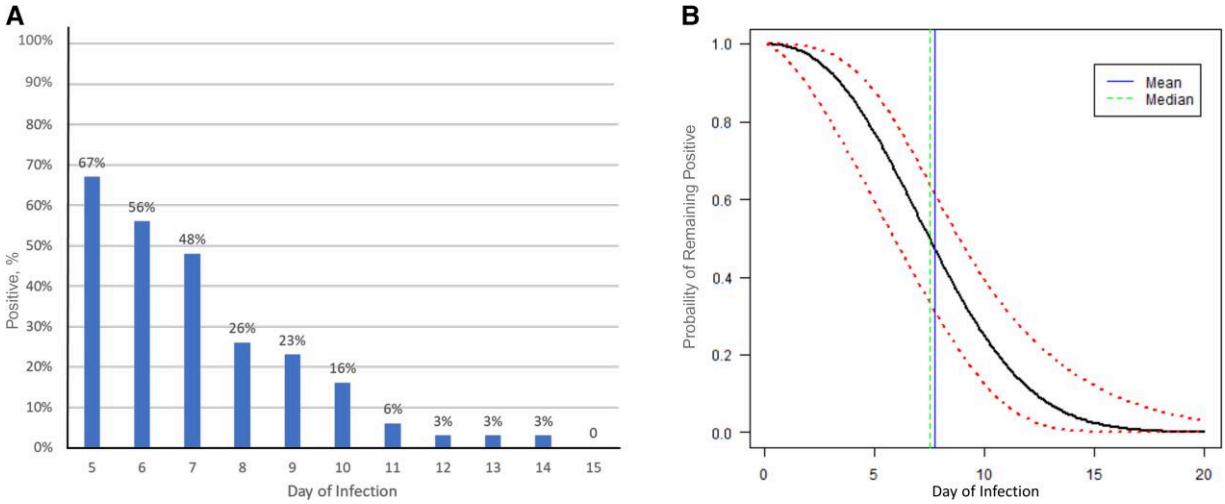

Figure 1. *A*, Percentage of children with positive rapid antigen test, by day of infection. *B*, Model-predicted probability of remaining test-positive since day 0 of infection. This probability curve (black solid line) is simply the survival function for the fitted Weibull model, and the 95% confidence bands (red dashed lines) are derived using the delta method. Mean (blue solid line) and median (green dashed line) times from symptom onset to turning negative are shown as vertical lines.

using N1 quantitative standards 10-fold diluted to determine viral copies. Sequences were aligned with those from other local cases to determine relatedness with community spread using ViralMSA and the MN908947 reference sequence [10]. A maximum likelihood phylogenetic tree was reconstructed using IQ-TREE with the best-fitting nucleotide substitution model according to the Bayesian Information Criterion and 1000 bootstrap replicates [11].

We used a variety of cell lines for virus culture, including LLC-MK2 and Vero E6 cells and A549 cells expressing angiotensin-converting enzyme 2 (ACE2), HEK 293 T cells expressing human ACE2, and VeroE6 with high endogenous ACE2 [12–14]; see Supplementary Methods and Supplementary Tables 1–4 for details. Cells were observed daily for 1 month before being scored negative for virus isolation. When virus-induced cytopathic effects were evident, the presence of SARS-CoV-2 was determined by RT-PCR [15, 16]. Isolation of SARS-CoV-2 at or after 6 days post–inoculation of cells was most effective in VeroE6-ACE2 and HEKT293-ACE2 cells (10^4 to 10^7 genome equivalents/μL of purified viral RNA [vRNA]), with marginally lower virus yields in LLC-MK2 and VeroE6 cells, and generally low yields in A549-ACE2 cells (10^1 to 10^2 genome equivalents/μL of purified vRNA).

RESULTS

Median age of the 31 children enrolled was 7 years (range, 0–16 years), including 10 children aged <5 years; two-thirds were boys. Results of the rapid antigen test were negative for 10 children at the time they enrolled in our study, which was on day 4 of infection or later; these children were not tested further. We obtained at least 1 positive rapid antigen test after study enrollment for the remaining 21 children, with a subsequent negative test for 14 children, collected a median of 2 days after their last positive test. Including the 10 children who had had a negative test at the time of enrollment and assuming that children would be positive on all days before their last positive test, 67% would have been positive for SARS-CoV-2 on day 5, with 48% still positive by rapid antigen on day 7 (Figure 1*A*). We fitted a parametric survival model to estimate the percentage remaining positive over time, accounting for interval-censoring and right-censoring of the exact transition times from positive to negative (Supplementary Methods). The mean and median durations of remaining positive were estimated to be 7.74 (95% confidence interval [CI], 6.54–9.17) days and 7.51 (95% CI, 6.24–9.04) days, respectively, and the interquartile range was 5.23–9.99 (Figure 1*B*).

Virus cultures were performed for the first 15 samples collected from children and the 8 samples from parents; these samples were collected on days 3–16 of infection. SARS-CoV-2 grew in cultures of 16 of these 23 samples (11 from children, 5 from adults), all of which were also positive by rapid antigen testing. No growth was detected in 7 cultures (4 samples from children, 3 from adults), all of which were negative by rapid antigen testing. The correlation between culture and rapid antigen testing results was significant for both children and adults ($P < .0001$ for children, $P = .02$ for adults, Fisher exact test).

A total of 40 positive rapid antigen test results were obtained for children enrolled in the study; all were positive by RT-qPCR. Among 25 samples with a negative rapid antigen test result, 9 were RT-qPCR positive, with a median viral load of $\log_{10}$ 3.45 copies/mL (range, $\log_{10}$ 3.08–4.23 copies/mL). Four of these 9 samples were cultured and were

Table 1. Results of Severe Acute Respiratory Syndrome Coronavirus 2 Rapid Antigen Testing, Cell Culture, Cycle Threshold, Viral Load, and Symptoms, by Days of Infection in Otherwise Healthy Children Aged <5 Years

Day of Infection	Rapid Antigen Testing	Cell Culture	Ct Value	Viral Load, Log_{10} Copies/mL	Symptoms
6	Pos	Pos	22.12	8.5	None
8	Pos	Pos	24.04	7.6	None
10	Pos	Pos	31.15	5.8	None
11	Pos	Pos	30.48	5.5	None
12	Pos	Pos	33.95	4.7	None
13	Pos	Pos	35.20	4.6	None
14	Pos	Pos	33.61	4.2	None
16	Neg	Neg	38.10	3.3	None

Abbreviations: Ct, cycle threshold; Neg, negative; Pos, positive.

culture-negative. For children for whom serial samples were available, all showed a consistent pattern of decreasing viral load across time. We did not see an age-related difference in viral load after correcting for day of illness, nor was viral load or duration of infection correlated with vaccination status (42% of children were vaccinated) [17, 18]. Representative results from 1 child for whom 8 serial samples were available are shown in Table 1: This was an otherwise healthy child aged <5 years who was febrile (38.3°C) with mild upper respiratory symptoms for 1 day when first diagnosed, with no further symptoms.

All SARS-CoV-2–positive samples were sequenced, and all were Omicron BA.1; sequence data have been submitted to GISAID (Supplementary Methods). Phylogenetic analysis revealed minimal variability across the sample set, consistent with spread of a single clade within the community. While approximately one-third of children in the sample attended a single public school, we did not see any evidence of increased clustering among children from that school. We did identify tighter clustering within families (including the families for which we had sequence data for both children and parents), as might be expected if transmission was occurring primarily within families rather than within community settings.

DISCUSSION

While the numbers of participants and samples analyzed were low, we found an exact correlation between results from the BinaxNOW rapid antigen test and results of cell culture, generally accepted as the best marker for virus infectivity [2–4, 17, 18]. We had samples that were negative by rapid test and positive by RT-qPCR; however, viral loads in these instances were low, and it is unclear that children from whom these samples were obtained would have been infectious, particularly in light of the negative cultures obtained. We emphasize that these findings are specific for SARS-CoV-2 Omicron BA.1, and it will require epidemiologic studies with this and other variants to fully assess infectivity.

In this study, close to half of infected children remained positive for SARS-CoV-2 (and likely were infectious) for at least 2 days after they would have returned to school under the 14 January 2022 CDC guidelines. While transmission would be reduced by wearing a mask as recommended by CDC, FDOH specifically recommended against the use of masks in its 24 February 2022 statement [8]. There is clear value, from a social and educational standpoint, in minimizing the isolation period of children infected with SARS-CoV-2. At the same time, from a public health standpoint, there is a need to minimize the risk that infected children will continue to transmit the virus after their return to school. Consideration should be given to lengthening the recommendation for 5 days of isolation before return to school, potentially in combination with requirements for a negative rapid test result.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. Z. M. L. and J. J. S. report other financial or nonfinancial interests from the Alachua County Health Department for contact tracing for COVID-19. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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