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ORIGINAL ARTICLE

Pediatric SARS-CoV-2 Seroprevalence in Arkansas Over the First Year of the COVID-19 Pandemic

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Background. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) seroprevalence studies largely focus on adults, but little is known about spread in children. We determined SARS-CoV-2 seroprevalence in children and adolescents from Arkansas over the first year of the coronavirus disease of 2019 (COVID-19) pandemic.

Methods. We tested remnant serum samples from children ages 1-18 years who visited Arkansas hospitals or clinics for non-COVID-19-related reasons from April 2020 through April 2021 for SARS-CoV-2 antibodies. We used univariable and multivariable regression models to determine the association between seropositivity and participant characteristics.

Results. Among 2357 participants, seroprevalence rose from 7.9% in April/May 2020 (95% CI, 4.9-10.9) to 25.0% in April 2021 (95% CI, 21.5-28.5). Hispanic and black children had a higher association with antibody positivity than non-Hispanic and white children, respectively, in multiple sampling periods.

Conclusions. By spring 2021, most children in Arkansas were not infected with SARS-CoV-2. With the emergence of SARS-CoV-2 variants, recognition of long-term effects of COVID-19, and the lack of an authorized pediatric SARS-CoV-2 vaccine at the time, these results highlight the importance of including children in SARS-CoV-2 public health, clinical care, and research strategies.

Key words. antibody; child; children; coronavirus; COVID-19; epidemiology; ethnic disparity; racial disparity; SARS-CoV-2; serology; serum.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in late 2019 and spread globally to cause the coronavirus disease of 2019 (COVID-19) pandemic [1, 2]. SARS-CoV-2 has infected nearly 200 million and killed more than 4 million people worldwide, causing massive disruptions to daily life and untold economic losses [3]. SARS-CoV-2 was first detected in the United States in early 2020 and has caused nearly 800 000 deaths [4–6]. Children were thought to be important for driving SARS-CoV-2 transmission [7, 8]. In March 2020, schools nationwide closed for in-person instruction and classes moved online to curtail SARS-CoV-2 spread [7, 8]. However, pediatric infection rates, and thus the potential level of natural immunity in children, are not known [9, 10]. Because SARS-CoV-2 vaccines for those 12 years and below were not approved until late 2021, knowing infection rates in children is important information for school officials as they consider risks and protective measures to limit infection.

Children typically experience less severe COVID-19 and may be more likely to have asymptomatic infections, allowing them to unknowingly spread SARS-CoV-2 [11]. With asymptomatic infections estimated as high as 50%, estimating true infection rates remains a challenge [12, 13]. Although nucleic acid testing can identify active SARS-CoV-2 cases, most infections clear within 2 weeks and leave asymptomatic cases undocumented [12–14]. Antibodies generated against previous infections can last for months to years [10, 15]. The presence of SARS-CoV-2 antibodies in the blood can indicate that a person was infected at some point in the pandemic. Serological studies can capture asymptomatic and symptomatic cases, and more accurately estimate how many people were infected with SARS-CoV-2 in the absence of known vaccination status [10, 15].

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Herein, we present the results of a seroprevalence study of children ages 1-18 years of age who visited hospitals or regional clinics in Arkansas for non-COVID-19-related reasons over the first year of the COVID-19 pandemic.

METHODS

Human Specimens

All human specimens were obtained with oversight from the UAMS Institutional Review Board (IRB), and waiver of consent and Health Insurance Portability and Accountability Act (HIPAA) applied. Remnants of serum samples collected for routine, non-COVID-19-related clinical laboratory tests were obtained from Arkansas Children's Hospital (ACH, Little Rock, AR), Arkansas Children's Northwest (Springdale, AR), and UAMS Family Medical Centers (Ft. Smith, AR, and Pine Bluff, AR). Samples were collected across 5 time periods (waves): wave 1, April 2 to May 6, 2020 (n = 316); wave 2, June 6 to August 10, 2020 (n = 299); wave 3, September 8 to October 17, 2020 (n = 583); wave 4, November 7 to December 17, 2020 (n = 570); and wave 5, April 5 to April 28, 2021 (n = 589). Collection waves were selected roughly corresponding to (1) early pandemic, (2) 2020 summer surge, (3) return to in-person school, (4) beginning of holiday surge, and (5) end of school-year/beginning of vaccine availability, which was only approved for 16- to 18-year-old persons at this time and accounted for a minimal number of samples collected. Collection periods were based on time to accumulate approximately 300-500 samples per wave. Samples were de-identified prior to testing. Inclusion criteria were ages 1-18 years and Arkansas resident. Samples were only collected from outpatient visits. During the collection period, outpatient visits were not available to individuals exhibiting COVID-19 symptoms. Samples were excluded with the following International Classification of Diseases-10 (ICD-10) and Current Procedural Technology (CPT) codes: immunodeficiency (primary immune deficiency, D80-D89), transplant recipient (codes beginning with Z94), and cancer (C00-D49). Samples from patients receiving chemotherapy (before 2 months), steroids (before 30 days), and/or intravenous immunoglobulin (before 6 months) were excluded. Clinical and demographic variables were stored in a secure REDCap database [16, 17] and included age, sex, race/ethnicity, zip code, and county of residence. Metropolitan status was determined by cross-referencing zip codes with the Federal Office of Rural Health Policy (FORHP) data files identifying non-metropolitan counties and rural census tracts [18].

Protein Production and Purification

HEK293T cells were cultured in Dulbecco's modified Eagle media (DMEM; Gibco) supplemented with 10% heat-inactivated calf serum (CS, VWR), 2 mM L-glutamine (Invitrogen), and 100 U/mL penicillin/100 µg/mL streptomycin (Invitrogen). Briefly, 15 cm dishes seeded with 9×10^6 cells on the preceding day were transfected with 20 µg of pCAGGS-SARS-CoV-2 Wuhan-Hu-1 receptor-binding domain (RBD)-C-terminal 6-His tag (BEI Resources), pCAGGS SARS-CoV-2 Wuhan-Hu-1 ectodomain Spike glycoprotein gene-C-terminal 6-His tag, or pCMV3 2019-nCoV nucleoprotein-C-terminal 6-His tag (Sino Biological) using polyethylenimine (PEI) at a 1:3 ratio. DNA:PEI mixtures were incubated at RT for 10 mins and added to cells with the media volume reduced to 5 mL/dish. After 4-6 hours, the media volume was raised to 25 mL. SARS-CoV-2 RBD and Spike ectodomain proteins were purified as described [19]. Nucleoprotein (N) was isolated as described above under denaturing conditions [20]. Protein concentration was measured by DC Protein Assay (BioRad). Purified proteins were confirmed by Coomassie and Western blot using antigen-specific antibodies and stored at −80°C.

Enzyme-Linked Immunosorbent Assays (ELISAs)

Serum was inactivated at 56°C for 1 hour and initially screened by ELISA specific for the RBD of the SARS-CoV-2 S protein [21–27]. Commercial anti-RBD (Sino Biologicals; 1:2500 dilution) and SARS-CoV-2-reverse transcription-polymerase chain reaction (RT-PCR)-positive patient sera (1:50 dilution) served as positive controls. Purified human immunoglobulin G (IgG) (Sigma; 1:2500 dilution) and pre-COVID-19 patient sera (1:50 dilution) served as negative controls. Serum immunoglobulin M (IgM) and IgG were detected using horse-radish peroxidase-conjugated anti-human IgM + IgG (Jackson ImmunoResearch; diluted 1:5000 in phosphate-buffered saline + 0.01% Tween-20 [PBS-T] + 1% milk) and SureBlue TMB 1-Component Peroxidase Substrate (SeraCare; 75 µL). After 5 minutes, the reaction was terminated using TMB Stop Solution (SeraCare; 75 µL). The optical density at 450 nm (OD_{450}) was measured using a FluoStar Omega plate reader (BMG Labtech). The final OD_{450} was calculated by subtracting the mean OD_{450} of blank wells from the mean OD_{450} of duplicate samples. The statistical cutoff for RBD binding was defined as the mean OD_{450} + 3 standard deviations of pre-COVID-19 sera [27].

Confirmation of RBD-positive specimens was performed using a Four-Antigen Confirmation Test (FACT) ELISA. An additional 5% of negative sera was randomly selected and tested in parallel. Plates were coated with 2 µg/mL RBD, spike, nucleoprotein, or bovine serum albumin (BSA; Sigma Aldrich), and FACT ELISA was performed as above. Confirmed positive samples were defined by (1) a mean signal for any viral antigen over 0.6 OD_{450}, (2) BSA-subtracted viral antigen value >0.3 OD_{450}, and (3) and at least 2 positive antigens (RBD+/Spike+, RBD+/N+, Spike+/N+, or RBD+/Spike+/N+). The 0.3 OD_{450} cutoff was selected based on the highest reading of pre-COVID sera. The FACT antigen distribution for samples that scored positive in each wave is presented in Supplementary Table 1. The assay sensitivity and specificity were 94.6% and 100%, respectively, based on 37 pre-COVID-19 and 19 RT-PCR-confirmed SARS-CoV-2-positive sera.

Statistical Analyses

The descriptive analysis was conducted for the study population demographics by wave. SARS-CoV-2 antibody positivity rates were reported with 95% confidence intervals (CIs) using exact binomial distributions. The 2019 US Census Bureau Arkansas state population estimates (age ≤ 18) were used to calculate the standardized positivity rates [28]. The age- and sex-standardized positivity rates were reported for each wave. Univariable and multivariable analyses were performed to examine the relationship between variables and the SARS-CoV-2 antibody positivity rate for each wave. Relative risk was estimated by modified Poisson regressions with robust error variance as the measure to characterize association effects [29]. For multivariable analyses, factors being considered were age group, sex, race, and ethnicity. The statistical significance level was set at .05. We conducted all analyses using SAS version 9.4 (SAS Institute).

RESULTS

Enrollment and Demographic Representation

We collected 2357 total remnant pediatric serum samples across 5 collection periods (waves) (Figure 1). Samples were collected from 74 of 75 counties in Arkansas (Supplementary Figure 1). Table 1 presents the demographic characteristics of the study. The 1-4 years age group had the fewest samples (n = 317, 13.5%), while the 10-14 years group had the most samples (n = 780, 33.2%). Females represented 54.7% (n = 1286), whereas males represented 45.3% (n = 1064) of total samples analyzed. The racial distribution of the study population was 58.0% white (n = 1312), 22.3% black (n = 504), and 19.8% (n = 448) from other races. With respect to ethnicity, 84.9% (n = 1825) were non-Hispanic and 15.1% were Hispanic (n = 325). Most samples were collected from children living in urban (n = 1534, 75.4%) compared with rural areas (n = 500, 24.6%). Obesity was the most common co-morbidity reported (n = 328, 14.0%), followed by asthma (n = 233, 10.0%), diabetes mellitus (n = 112, 4.8%), and hypertension (n = 109, 4.6%) (Supplementary Table 2).

Estimates of Seroprevalence

Table 2 presents the unadjusted SARS-CoV-2 seroprevalence rates by age, sex, and race/ethnicity. The raw antibody positivity rate for wave 1 was 7.9% (95% CI, 4.9-10.9), which increased in wave 2 to 9.4% (95% CI, 6.0-12.7) and wave 3 to 16.5% (95% CI, 13.4-19.5), followed by a slight decrease to 13.9% (95% CI, 11.0-16.7) in wave 4. The seroprevalence rate was the highest in wave 5 at 25.0% (95% CI, 21.5-28.5). The overall trend was statistically significant ($P < .0001$ using the Cochran-Armitage trend test). When standardized to match Arkansas population in the distribution of age and sex [28], seroprevalence rates followed a similar trend to the non-adjusted rates, increasing over wave 1 (8.6%; 95% CI, 4.9-11.6), wave 2 (9.5%; 95% CI, 5.8-13.2), and wave 3 (17.3%; 95% CI, 13.6-21.0), with a decrease in wave 4 (13.1%; 95% CI, 10.0-16.2) and a peak in wave 5 (23.4%; 95% CI, 19.4-2.74) (Figure 2). The 1- to 4-year-old age group had the highest seroprevalence rates in wave 1 (10.7%), wave 2 (15.2%), and wave 3 (20.8%), but the lowest in wave 4 (7.9%) and wave 5 (16.0%). The 15- to 18-year-old group had the highest percentage of reactive specimens in wave 4 (14.7%) and 10 to 14 year-olds were the highest in wave 5 (29.1%). There were no statistically significant differences between age groups within each wave. No statistically significant difference was observed between males and females.

Children from "other" races (ie, not identifying as white or black) had the highest seroprevalence rate in waves 1, 2, 3, and 5. The peak for this group was in wave 5 at 41.0%. The antibody reactivity rate in black children was lower compared with white children in waves 1 and 2, but higher in waves 3, 4, and 5, although none of the differences were statistically significant. The highest seroprevalence rates for white (17.0%) and black (28.4%) children were observed in wave 5. With respect to ethnicity, Hispanic children had higher seroprevalence rates than non-Hispanic children in each wave, reaching a maximum of 40.5% in wave 5. Differences between Hispanic and non-Hispanic children were statistically significant in waves 3, 4, and 5. It is notable that within the "other" racial category, no single race (Asian, American Indian or Alaska Native, Native

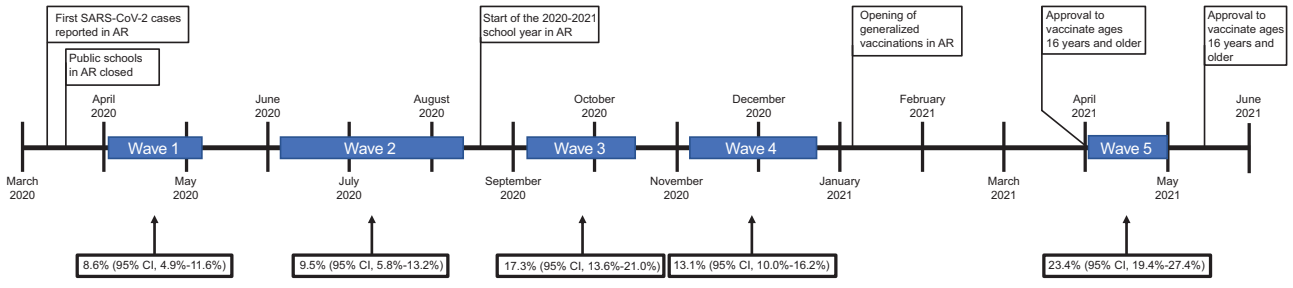

Figure 1. Timeline of Arkansas COVID-19 milestones and sample collection waves. The schematic shows the timeline for relevant study-related events. The sample collection periods (waves) are indicated by blue boxes. The age- and sex-standardized seroprevalence rate for each wave is indicated as the percent (95% CI). CI, confidence interval; COVID-19, coronavirus disease of 2019.

Table 1. Sample Population Demographics for SARS-CoV-2 Antibody Testing in Arkansas From April 2, 2020, to April 28, 2021

Characteristic	Number (%)[a]					
	Wave 1 (n = 316)	Wave 2 (n = 299)	Wave 3 (n = 583)	Wave 4 (n = 570)	Wave 5 (n = 589)	Overall (N = 2357
Collection Dates	April 2 to May 6, 2020	June 6 to August 10, 2020	September 8 to October 17, 2020	November 7 to December 17, 2020	April 5 to April 28, 2021	April 2, 2020 to April 28, 2021
Age category						
1-4 years	56 (17.7)	33 (11)	77 (13.2)	76 (13.5)	75 (12.8)	317 (13.5)
5-9 years	61 (19.3)	75 (25.1)	112 (19.2)	114 (20.2)	142 (24.2)	504 (21.5)
10-14 years	90 (28.5)	98 (32.8)	200 (34.3)	204 (36.1)	188 (32.0)	780 (33.2)
15-18 years	109 (34.5)	93 (31.1)	194 (33.3)	171 (30.3)	182 (31.0)	749 (31.9)
Sex						
Female	161 (50.9)	155 (51.8)	318 (54.6)	320 (56.6)	332 (56.6)	1286 (54.7)
Male	155 (49.1)	144 (48.2)	265 (45.5)	245 (43.4)	255 (43.4)	1064 (45.3)
Race						
White	180 (57.3)	158 (54.1)	344 (61.3)	307 (56.5)	323 (58.3)	1312 (58.0)
Black	90 (28.7)	53 (18.2)	111 (19.8)	141 (26.0)	109 (19.7)	504 (22.3)
Other	44 (14.0)	81 (27.7)	106 (18.9)	95 (17.5)	122 (22.0)	448 (19.8)
Ethnicity						
Hispanic	28 (9.4)	65 (23.1)	91 (16.8)	52 (10.3)	89 (17.0)	325 (15.1)
Non-Hispanic	270 (90.6)	216 (76.9)	450 (83.2)	455 (89.7)	434 (83.0)	1825 (84.9)
Metropolitan status						
Urban	Not Reported	250 (83.6)	448 (76.8)	405 (71.7)	431 (73.4)	1534 (75.4)
Rural	Not Reported	49 (16.4)	135 (23.2)	160 (28.3)	156 (26.6)	500 (24.6)

SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

[a]Percentages calculated from non-missing values for all periods. Missing values in wave 1 included race (n = 2), ethnicity (n = 18), and metropolitan status (n = 316). Missing values in wave 2 included race (n = 7) and ethnicity (n = 18). Missing values in wave 3 included race (n = 22) and ethnicity (n = 42). Missing values in wave 4 included age category (n = 5), sex (n = 5), race (n = 27), ethnicity (n = 63), and metropolitan status (n = 5). Missing values in wave 5 included age category (n = 4), sex (n = 2), race (n = 35), ethnicity (n = 66), and metropolitan status (n = 2).

Table 2. Age-Specific, Sex-Specific, Race/Ethnicity-Specific SARS-CoV-2 Seroprevalence Estimates in Arkansas From April 2, 2020, to April 28, 2021

	Persons Reactive, % (95% CI) (No.)				
Characteristic[a]	Wave 1 (n = 316)	Wave 2 (n = 299)	Wave 3 (n = 583)	Wave 4 (n = 570)	Wave 5 (n = 589)
Collection Dates	April 2 to May 6, 2020	June 6 to August 10, 2020	September 8 to October 17, 2020	November 7 to December 17, 2020	April 5 to April 28, 2021
Age category					
1-4 years	10.7 (2.4-19.1) (6)	15.2 (2.2-28.1) (5)	20.8 (11.5-30.0) (16)	7.9 (1.7-14.1) (6)	16.0 (7.5-24.5) (12)
5-9 years	4.9 (0.0-10.5) (3)	8.0 (1.7, 14.3) (6)	18.8 (11.4-26.1) (21)	13.2 (6.9-19.5) (15)	19.0 (12.5,-25.5) (27)
10-14 years	8.9 (2.9-14.9) (8)	11.2 (4.9-17.6) (11)	14.5 (9.6-19.4) (29)	14.7 (9.8-19.6) (30)	28.7 (22.2-35.3) (54)
15-18 years	7.3 (1.4-12.3) (8)	6.5 (1.4-11.5) (6)	15.5 (10.3-20.6) (30)	15.8 (10.3-21.3) (27)	29.1 (22.5-35.8) (53)
Sex					
Female	8.7 (4.3-13.1) (14)	9.0 (4.5-13.6) (14)	17.9 (13.7-22.2) (57)	14.4 (10.5-18.2) (46)	26.5(21.7-31.3) (88)
Male	7.1 (3.0-11.2) (11)	9.7 (4.8-14.6) (14)	14.7 (10.4-19.0) (39)	13.1 (8.8-17.3) (32)	22.8 (17.6-27.9) (58)
Race					
White	8.9 (4.7-13.1) (16)	8.9 (4.4-13.3) (14)	12.2 (8.7-15.7) (42)	12.4 (8.7-16.1) (38)	17.0 (12.9-21.1) (55)
Black	5.6 (0.7-10.4) (5)	3.8 (0.0-9.1) (2)	18.0 (10.8-25.3) (20)	16.3 (10.1-22.5) (23)	28.4 (19.8-37.0) (31)
Other	9.1 (0.2-17.9) (4)	14.8 (6.9-22.7) (12)	28.3 (19.6-37.0) (30)	15.8 (8.3-23.3) (15)	41.0 (32.1-49.8) (50)
Ethnicity					
Hispanic	10.7 (0.0-22.9) (3)	13.9 (5.2-22.5) (9)	29.7 (20.1-39.2) (27)	28.9 (16.1-41.6) (15)	40.5 (30.1-50.8) (36)
Non-Hispanic	8.1 (4.9-11.4) (22)	8.8 (5.0-12.6) (19)	14.7 (11.4-17.9) (66)	12.5 (9.5-15.6) (57)	20.7 (16.9-24.6) (90)
Total	7.9 (4.9-10.9) (25)	9.4 (6.0-12.7) (28)	16.5 (13.4-19.5) (96)	13.9 (11.0-16.7) (79)	25.0 (21.5-28.5) (147)

CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

[a]Standardized positivity rates were calculated based on the 2019 US Census Bureau Arkansas state population estimates (age ≤ 18).

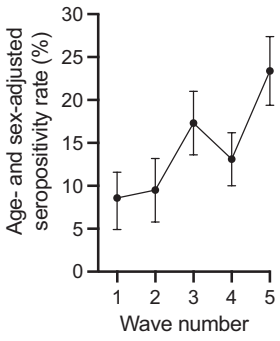

Figure 2. Age- and sex-standardized seroprevalence rates by wave. Error bars indicate 95% CI. CI, confidence interval.

Hawaiian or other Pacific Islander) was sufficiently represented for further analysis. Many that selected the "other" racial category also identified as being of Hispanic ethnicity.

There was no significant marginal association between age, sex, or metropolitan status and the likelihood of testing positive for SARS-CoV-2 antibodies (Supplementary Table 3). Hispanic children showed a higher relative risk of testing positive compared with white children in wave 4 (risk ratio [RR] 4.23; 95% CI, 1.88-9.48) (Table 3). Black children (RR 1.69; 95% CI, 1.13-2.54) and children from other races (RR 2.31; 95% CI, 1.39-3.84) had a higher risk of antibody reactivity compared with white children in wave 5 (Table 3).

Children with asthma (unadjusted RR 4.83; 95% CI, 1.96-11.87; $P = .0006$) or diabetes (unadjusted RR 4.17; 95% CI, 1.49-11.67; $P = .007$) had higher risk of having antibodies against SARS-CoV-2 than children who did not have asthma or diabetes in wave 1 (Supplementary Table 4). However, this difference was not observed in the remaining waves. PCR testing was performed for 702 of the 2357 total nasal or nasopharyngeal specimens, with 37 positive PCR tests reported (Supplementary Table 5). A positive RT-PCR test was significantly associated with antibody positivity in waves 2 through 5 (Supplementary Table 6).

DISCUSSION

Our results demonstrate that by the end of April 2021, approximately 25% of children in Arkansas had SARS-CoV-2-specific antibodies. The seroprevalence was much higher than the total number of confirmed cases which on April 28, 2021, was 11% for the total population of Arkansas (335 288 positive cases according to the Arkansas Department of Health, population of 3 011 524 according to 2019 census data). This finding strongly suggests that those children had been infected with SARS-CoV-2 and are likely to have at least some natural immunity. Conversely, our findings indicate that most children in Arkansas likely have not been infected with SARS-CoV-2 and remain susceptible to infection. Although COVID-19 was less severe in children than adults early in the pandemic, the emergence of the SARS-CoV-2 delta variant in May 2021 dramatically increased infection and hospitalization rates, including among those below 18 years of age [11, 30, 31]. Developing multisystem inflammatory syndrome in children (MIS-C), a severe inflammatory disorder that results from a current or recent SARS-CoV-2 infection, is also a risk for those below 18 years [32–34]. Increased SARS-CoV-2 transmission rates combined with a highly susceptible pediatric population led us to predict that SARS-CoV-2 would spread rapidly in schools and daycares as in-person learning resumed, which was indeed the case. More children infected with SARS-CoV-2 led to an increase in the number of severe COVID-19 and MIS-C cases, and a rise in pediatric deaths [35, 36].

The first SARS-CoV-2 infections in Arkansas were reported in March 2020 (Figure 1) [37]. Arkansas schools suspended in-person learning on March 15, 2020, and many activities where children congregate during the summer were closed. We found that the seroprevalence rate in children increased modestly between spring and summer, suggesting that these protective measures effectively limited SARS-CoV-2 spread among children in Arkansas. The larger increase in seroprevalence for September/October (wave 3) corresponded with the start of the 2020-2021 school year. However, masks and social distancing measures were in place, and many Arkansas students began the school year as remote learners. These actions likely reduced SARS-CoV-2 spread in schools, as reported outbreaks were relatively rare. Despite a dramatic increase in SARS-CoV-2 cases in Arkansas during November and December, the seroprevalence rate in children dropped during wave 4 [38]. The decrease suggests that preventative measures in schools were effective at limiting SARS-CoV-2 spread among children during this time. However, seroprevalence increased sharply in wave 5 to approximately 25%. Waves 4 and 5 were approximately 3 months apart and included the winter holidays, when children were outside the controlled school setting, and the corresponding nationwide surge in COVID-19 cases. Our data confirm that the surge also impacted the pediatric population, correlating with a large increase in the number of SARS-CoV-2-exposed children.

It is well appreciated that underrepresented racial and ethnic groups are disproportionately affected by the COVID-19 pandemic [39]. We found that Hispanic children were more likely to have SARS-CoV-2 antibodies compared with non-Hispanic children in all waves. Similarly, black children were more likely to have antibodies than white children in wave 5. The increase in SARS-CoV-2 antibodies in Hispanic and black children corresponded to an increase in antibody level in Hispanic and black adults during the same timeframe (REF-Kennedy [40]). Higher antibody prevalence in Hispanic and black children may reflect multiple socioeconomic factors, including income inequality, economic instability, work circumstances, and housing [41]. Reports indicate that Hispanic and black parents may be less likely to hold jobs that allow them to work remotely,

Table 3. Age- and Sex-Adjusted Association With SARS-CoV-2 Antibody Positivity by Time Period

	Adjusted RR (95% CI)				
Characteristic	Wave 1 (n = 316)	Wave 2 (n = 299)	Wave 3 (n = 583)	Wave 4 (n = 570)	Wave 5 (n = 589)
Collection Dates	April 2 to May 6, 2020	June 6 to August 10, 2020	September 8 to October 17, 2020	November 7 to December 17, 2020	April 5 to April 28, 2021
Age category					
1-4 years	1.56 (0.52-4.70)	1.88 (0.64-5.54)	1.34 (0.76-2.35)	0.71 (0.30-1.70)	0.56 (0.31-1.02)
5-9 years	0.65 (0.18-2.36)	1.00 (0.34-2.96)	1.34 (0.81-2.22)	1.00 (0.55-1.84)	0.72 (0.46-1.11)
10-14 years	1.20 (0.44-3.25)	1.58 (0.61-4.07)	0.91 (0.56-1.47)	0.99 (0.60-1.64)	0.80 (0.57-1.13)
15-18 years	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
Sex					
Female	1.37 (0.61-3.08)	1.00 (0.50-1.98)	1.12 (0.77-1.65)	1.15 (0.74-1.77)	1.04 (0.77-1.41)
Male	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
Race					
Black	0.60 (0.23, 1.58)	0.49 (0.11, 2.12)	1.60 (0.99, 2.61)	1.48 (0.90, 2.44)	**1.69 (1.13, 2.54)**[b]
Other	1.15 (0.21, 6.24)	1.94 (0.77, 4.86)	1.64 (0.79, 3.43)	0.54 (0.24, 1.22)	**2.31 (1.39, 3.84)**[b]
White	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)[a]	1.0 (Ref)	1.0 (Ref)[a]
Ethnicity					
Hispanic	1.09 (0.16, 7.35)	0.91 (0.36, 2.28)	1.62 (0.77, 3.42)	**4.23 (1.88, 9.48)**[a]	1.09 (0.66, 1.83)
Non-Hispanic	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)

Statistically significant values are shown in bold text.

CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

[a]Indicates Wald statistics for type 3 *P*-value = .0005 for ethnicity.

[b]Indicates Wald statistics for type 3 *P*-value = .002 for race.

which increases their potential exposure to SARS-CoV-2 in the workplace and limits their ability to utilize remote learning or in-home childcare to decrease their children's SARS-CoV-2 exposure [42, 43]. Residing with parents with a higher risk of workplace SARS-CoV-2 exposure combined with increased school/daycare attendance could drive infection rates in minority children. Our results underscore that the pandemic exacerbated existing racial and ethnic disparities.

We found that the percentage of SARS-CoV-2-seropositive children who did not have N-specific antibodies increased over the course of the study, reaching a peak of 40.1% in wave 5 (Supplementary Table 1). Because the current vaccine formulations deliver the Spike protein [44], an S- and RBD-positive/N-negative antibody profile is consistent with a vaccine response [45]. However, the majority of samples for this study were collected before the start of vaccinations (Table 1 and Figure 1). Consequently, vaccine responses are not likely to be a confounding factor in our analysis (see later). N-specific antibody levels decline more rapidly than antibodies against the S protein [46]. Thus, the gradual increase in RBD/S-positive and N-negative specimens may reflect the fact that samples collected in later waves have a higher potential for more time to elapse between infection and sample collection than in earlier waves.

Our data also highlight the importance of vaccinating children against SARS-CoV-2. General vaccinations in Arkansas began in January 2021 for those above 18 years and were expanded to 16 years and older on March 30, 2021 [47]. The CDC's Advisory Committee on Immunization Practices recommended the Pfizer-BioNTech vaccine for 12 to 15 year olds on May 10, 2021 [47]. Wave 5 samples were collected in April 2021, after the expansion of vaccinations to those 16 years and up but prior to inclusion of 12 to 15 year olds. Only 8 of 145 total 16 to 18 year olds in our cohort reported being vaccinated during wave 5, and no subjects reported full vaccination at least 2 weeks prior to sample collection. Thus, our data reflect the pediatric seroprevalence rate in Arkansas prior to widespread vaccination efforts in children and adolescents. As authorization to vaccinate children younger than 12 years did not occur until late 2021, our findings indicate that most children in Arkansas remained susceptible to SARS-CoV-2 infection entering the 2021-2022 school year. Importantly, we found that the seroprevalence rate in younger children was lower than for older children and adolescents, which strongly emphasizes the ongoing risk of infection for a vulnerable part of the population that cannot yet be vaccinated. This is a critical consideration for policy makers as more infectious variants emerge that exhibit increased infection and likelihood of causing severe disease in younger portions of the population.

Limitations

Remnant serum samples may not provide an accurate representation of the Arkansas population. The strengths and limitations of convenience samples are described elsewhere [48]. As our study samples were collected from health clinics, our sampling method may favor patients who were more ill or more willing to seek health care. It is possible that our seroprevalence rates are overestimated due to potential cross-reactivity between SARS-CoV-2 antigens and seasonal coronaviruses, especially in wave 1, resulting in uninfected individuals, particularly children and adolescents, with antibodies that cross-react with the SARS-CoV-2 Spike protein [49]. Furthermore, we measured a combination of IgM and IgG antibody isotypes, as opposed to testing solely for IgG [50]. Although studies indicate that IgG and IgM responses to SARS-CoV-2 can develop simultaneously, some individuals develop detectable IgM and IgG antibodies at different times [51, 52]. Consequently, we may identify individuals with IgM and/or IgG, whereas other assays may only identify specimens with IgG. IgM also can be more nonspecific than IgG [53]. Together, these limitations may cause an overestimation of antibody reactivity. However, the inclusion of multiple SARS-CoV-2 antigens and the BSA control serves to limit the potential for detecting nonspecific reactions in our assay.

CONCLUSIONS

The analysis of remnant samples collected from children and adolescents in Arkansas demonstrates a steady increase in SARS-CoV-2 infection during the first 8 months of the pandemic, followed by a more rapid increase to approximately 25% by the end of April 2021. This finding is notable, as it was more than twice the number of confirmed SARS-CoV-2 diagnoses in the state on the final collection date. No obvious comorbidities were identified for seropositivity in children. Racial and ethnic disparities exist, with Hispanic and black children being at increased risk for SARS-CoV-2 infection compared with white children. We conclude that SARS-CoV-2 infections in children were more common than previously recognized. With the emergence of SARS-CoV-2 variants, recognition of long-term effects of SARS-CoV-2 even after mild or asymptomatic infections, and the previous lack of an authorized pediatric SARS-CoV-2 vaccine, these results highlight the importance of including children in SARS-CoV-2 public health, clinical care, and research strategies. When vaccines are unavailable, it is important that protective measures are enacted, particularly in settings where children are grouped together, to limit the risk of infection, super-spreader events, severe disease, long-term sequelae, and death.

Supplementary Data

Supplementary materials are available at the *Journal of the Pediatric Infectious Diseases Society* online (http://jpids.oxfordjournals.org).

Notes

Disclaimer. The views expressed in this paper are not necessarily those of the Arkansas Department of Health.

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