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Analysis of Wastewater Surveillance and Reported COVID-19 Cases in Atlanta Public Schools

By

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Master of Public Health

Global Environmental Health

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B.S.
University of Virginia
2019

Thesis Committee Chair: Christine Moe, PhD, MS, BA

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
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Abstract

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Since the beginning of the pandemic, more than 13.9 million children under 18 years of age have been infected with SARS-CoV-2. COVID-19 has resulted in devastating loss of human life and presents an unparalleled challenge for public health as it is currently ranked among the top ten causes of death for children 5-11 years of age. School-aged children are not only susceptible to SARS-CoV-2 but can play a role in transmitting the virus through educational settings. While many wastewater-based surveillance efforts have been used to study the transmission of SARS-CoV-2 throughout college dormitories, there have been few studies employing wastewater epidemiology to analyze COVID prevalence in K-12 school sites while investigating school related factors and community factors that can influence the transmission and detection of SARS-CoV-2 in a school. Three main objectives of this study are to 1). Collect and analyze weekly wastewater samples from selected Atlanta Public Schools and compare the wastewater results from Atlanta Public schools to the weekly reported COVID-19 cases among students (August 2021-March 2022) 2). Understand why specific Atlanta Public Schools have higher SARS-CoV-2 detection rates in wastewater as compared to others, and 3). Investigate each school's catchment area and socio-demographic characteristics to identify factors that may be linked to positive wastewater outcome and COVID-19 cases in Atlanta Public Schools. Findings from the study analyses revealed that overall, 41% of wastewater samples detected the presence of SARS-CoV-2 in schools and that elementary schools were found to have the highest overall proportion of SARS-CoV-2 detection in wastewater (52%). Concordance between positive wastewater results and reported COVID-19 cases in schools shared a temporal trend consistent with the COVID-19 Omicron surge in Fulton County. Voluntary self-reporting of COVID-19 cases were likely an underestimate. Furthermore, results indicated that COVID-19 self-reported cases, staff vaccination rate, student vaccination rate, school group, and school neighborhood factors such as neighborhood population below poverty, and city council district location shared trends with positive wastewater outcomes in Atlanta Public Schools. Overall poverty rates amongst school neighborhoods were very high (24.4% to 34.9%).

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CHAPTER 1- LITERATURE REVIEW

COVID Background

Discovered in 2019, COVID-19 is a contagious respiratory illness caused by the virus SARS-CoV-2. SARS-CoV-2 is an enveloped virus with a single stranded RNA genome and four proteins consisting of spike (S), envelop (E), membrane glycoprotein (M), and nucleocapsid phosphoprotein(N) (Alsobaie, 2021; Wang, 2021). COVID-19 attacks the respiratory system causing severe acute respiratory symptoms that range from temperate symptoms to extreme health outcomes like pneumonia, respiratory failure, long term health sequelae, and multi organ degeneration. COVID-19 is transmitted mainly through respiratory droplets that originate from the mouth and nose from sneezing or coughing (COVID-19 and Your Health, 2020). Since its emergence this respiratory disease has subsequently spread throughout the world and mutated into new variants *Alpha, Beta, Gamma, Iota, Eta, Kappa Delta (B.1.617.2), and Omicron (Delta and Omicron being the most notable) (*COVID-19 and Your Health, 2020). In July 2021, the *Delta* variant was identified to be the most notable variant in the United States at the time characterized by increased transmissibility, and a reduction in neutralization by postvaccination sera and by monoclonal antibody treatments (COVID-19 and Your Health, 2020). *Omicron* (B.1.529) another variant of concern first identified in South Africa, later emerged within the United States (November 2021) and continues to be a threat. With a 70-times greater virus replication rate; it has become the overriding variant throughout the world (*HKUMed Finds Omicron SARS-CoV-2 Can Infect Faster and Better than Delta in Human Bronchus but with Less Severe Infection in Lung*, 2021). Characterized by increased transmissibility, breakthrough infections in vaccinated individuals, and unknown severity, the Omicron variant poses a high risk to the world.

Significance of COVID-19 for Children

Since the recognition of the novel coronavirus in Wuhan, China, this respiratory disease has infected about 530 million individuals and caused 6 million deaths worldwide as of . (*WHO Coronavirus (COVID-19) Dashboard*, 2021). Despite high risk for individuals aged 65 years of age and older, and those with underlying health conditions, more than 13.9 million children under 18 years of age have been infected with SARS-CoV-2 since the beginning of the pandemic. (*Children and COVID-19: State-Level Data Report*, 2022). COVID-19 has resulted in devastating loss of human life and presents an extraordinary/unparalleled challenge for public health as it is currently ranked among the top ten causes of death for children 5-11 years of age (*COVID-19 Vaccines for Children & Teens*, 2022). According to the CDC's Morbidity and Mortality Weekly Report, after the Delta variant took precedence in July 2021, the incidence of COVID-19 cases and COVID-19-related hospital visits increased for ages 0-4, 5-11, and especially for school ages 12-17 years old (Siegel, 2021). This is especially true for the Omicron variant surge which researchers have found to be the most contagious/transmissible variant infecting over 2 million children alone in the US from January 10th to January 24th 2022 (*Children and COVID-19: State-Level Data Report*, 2022).

Despite authorization of the Pfizer-BioNTech COVID-19 emergency vaccination for children ages 5 to 11, school-age children remain vulnerable to this illness. According to January 12th, 2022, Centers for Disease Control and Prevention data, only 27 percent of children in the US had obtained at least one COVID vaccination. Southern state vaccination rates for children remain at an all-time low. Within the state of Georgia, 16.1 percent of children in this age bracket had received a minimum of one shot in contrast to about 9% receiving two doses as of January 18, 2021. (*Covid Vaccination Rates among Young Children Lag in Georgia, South*, 2022). Not only does this low vaccination rate put children at higher risk of transmission, infection, and hospitalization, but also potential post-COVID long-term health conditions.

Multisystem inflammatory syndrome (MIS-C) is a severe health outcome that scientists have found to be connected to SARS-CoV-2 infection in children. It results in severe inflammation of some organs and tissues- such as the kidneys, brain, lungs, and heart (*Multisystem Inflammatory Syndrome in Children (MIS-C) and COVID-19 – Symptoms and Causes*, 2021). SARS-CoV-2 infection has also been found to increase risk of diabetes symptoms among children with diabetes. Additionally, there have been scientific findings on SARS-CoV-2 infection amongst children younger than 18 years of age and its impact on inducing higher risk of new diabetes diagnosis in children (Barrett , 2022). COVID-19 has been proven to have long-term impacts on child health.

Several factors contribute to school-age children being more vulnerable to COVID-19. Educational settings that include crowded classrooms and prolonged time spent indoors can increase transmission of SARS-CoV-2 amongst children in school. School-age children also pose an increased risk of secondary transmission to their household and community because they more often experience nonspecific symptoms, or they are asymptomatic (*Coronavirus Disease*

2019 (COVID-19), 2020). COVID-19 has also had a significant toll on children's education in that it has caused harm to students' education and mental health due to school closures and reopening’s in Georgia and throughout the United States (Tupper, 2021). COVID-19 poses a unprecedent threat to the health of children.

COVID Challenges for Child Education

COVID-19 has greatly disrupted the educational system in addition to the health of children. Since the closing of schools and the shift to online/remote learning across North America (March 2020), learning has become a greater challenge for many children. A systematic review found that younger school aged children were more adversely affected in their learning than older children ((Hammerstein, 2021). The studies involved in the systematic review focused on children in primary and secondary education, school closures due to COVID-19, and test scores as academic measures (Hammerstein, 2021). In efforts to adhere to physical distancing protocol and reduce transmission of the virus, remote learning was practiced by many individual provinces and counties across the nation during Spring 2020. The COVID-19 pandemic is a huge concern for children aged 5-12 particularly because of its impact on their foundational primary years. Ontario's early childhood learning Pedagogy explains four pivotal conditions required for successful childhood learning. The four conditions consist of belonging (creating relationships), well-being (physical, mental health), engagement (fostering curiosity), and expression (diverse communication) (Timmons et al., 2021.). Not only has the pandemic taken away the foundation and environment for children to flourish, but it has also created learning loss. In low- and middle-income countries, seventy percent of children who are ten years of age have experienced learning losses and have been found unable to read or comprehend text due to COVID-19 and disruption to education.(*COVID:19 Scale of Education Loss 'Nearly Insurmountable', Warns UNICEF*, 2022). In the United States, states such as Texas, Tennessee, North Carolina, Virginia, Maryland Ohio, Colorado, and California have indicated learning losses. In Texas, two thirds of children in the third grade tested below their grade level in math in 2021 in comparison to half of children in 2019 (*COVID:19 Scale of Education Loss 'Nearly Insurmountable', Warns UNICEF*, 2022).

In addition to the impact of school closing on child learning, children's mental health has also been drastically impacted. According to a University of Calgary meta-analysis which combines and analyzes data from 29 studies involving approximately 81,000 youth globally; anxiety and depression symptoms have increased as much as twice the rate as observed before the pandemic. Results show that 1 in 4 children throughout the globe experience clinical depression symptoms compared to 1 in 5 whom have clinical anxiety due to the pandemic (*COVID-19: Depression and Anxiety Symptoms Have Doubled in Youth, Help Needed, Warn UCalgary Clinical Psychologists*, 2022). Mental health decline has occurred not only in children, but also among children's caregivers. According to a Boston Medical Center study that sought to quantify psychosocial results of the pandemic on urban children, and social risks to families and caregivers, findings from their mid-pandemic survey showed that families and working parents faced devastating financial and social impacts. Caregivers reported higher social impacts and experience of social impacts such as food insecurity and housing instability in contrast to 16 percent before the pandemic (*Pandemic Led to a Surge in Depression and Anxiety Among Children of Color*, 2022).

Significance of Wastewater Surveillance

Wastewater –based surveillance (WBS), also referred to as “Wastewater-Based Epidemiology”, (WBE) is a tool to track prevalence of infections with human pathogens that are excreted in feces and urine. First utilized in 1948 to trace *Salmonella* Para typhi B, it has been historically utilized for detecting illicit drugs, viruses, and infectious disease pathogens ranging from poliovirus, *Vibrio cholera,* and Hepatitis A from raw wastewater (Barrett et al, 1980; Matrajt, Lillis, & Meschke, 2020; Sears et al., 1984; Tao et al., 2010). WBS for SARS-CoV-2 is currently practiced in more than three thousand sites in over fifty countries (*Summary of Global SARS-CoV-2 Wastewater Monitoring Efforts by UC Merced Researchers*, 2021). Areas in North America include Burlington, VT, New York City, NY, Ontario, OH, and Houston, TX (Public Health Ontario, 2021).

WBS is nonintrusive and representative in nature in that it reflects the prevalence of a virus on a community level without invasive individual specimen collection. It also protects the anonymity of the wastewater sample population and continues to be cost effective. These factors contribute to why this surveillance approach serves as a useful monitoring system. WBS’s ability to detect pathogens excreted by symptomatic and asymptomatic infected individuals is another primary advantage of this system (Mousazadeh et al., 2021.). A promising approach to track COVID-19 burden, WBS involves the collection and analysis of wastewater samples for SARS-CoV-2 which enters wastewater via feces and urine of COVID-19 cases (Chen, Y, Chen, L, Deng, Q et al. 1., 2020). Results from a 2020 meta-analysis study revealed that approximately 51.8 percent of people with SARS-CoV-2 had detectable virus in their feces, with some studies reporting that 100 percent of COVID-19 cases had viral shedding (Van Doorn et al, 2020).WBS traditionally involves obtaining real time data from Moore swabs consisting of cotton gauze pads tied with string submerged in raw wastewater over an extended period of time and/or grab samples of wastewater collected at a specific point in time. In contrast to Moore Swabs, which serve as an uninterrupted filter that cannot be used to quantify the concentration of pathogens in a specific volume of water, grab samples essentially serve as a representative snapshot sample by capturing pathogens in the wastewater at one specific time and providing quantitative data (Sikorski & Levine, 2020). Accompanied by clinical testing, Michigan and Ohio are using wastewater surveillance to determine the presence of SARS-Cov-2 virus in communities, observe changes and trends in the pandemic, and to identify SARS-CoV–2 variants of concern (VOC) (Public Health Ontario, 2021).

WBS is not only limited to use at a municipal level; it can also be deployed at an institutional level. It has been implemented to monitor COVID-19 burden and transmission at many university and college campuses in the US. From August 2020 through February 2021, Emory University employed wastewater surveillance and weekly diagnostic antigen testing for college students in residence halls to monitor and control the transmission of SARS-CoV-2 (Wang Y et al., 2022.). Alongside contact tracing, isolation, and other transmission mitigation approaches, wastewater surveillance was deployed to detect outbreaks on campus (Wang Y et al., 2022.). Similarly, The University of Alabama, University of North Carolina-Charlotte, University of Arizona, and University of California San Diego conducted wastewater surveillance to identify and prevent COVID-19 outbreaks in student residence halls/dormitories (Gutierrez et al., 2021.).

Following the discovery of SARS-CoV-2 RNA in wastewater samples, subsequent clinical testing (consisting of antigen anterior nasal swab and RT-PCR of nasopharyngeal swabs) of individual residents was conducted (Gutierrez et al., 2021.).

Because the characteristics of school environment can facilitate SARS-CoV-2 transmission within the school and from the school to the community, school children remain a vulnerable population for COVID-19. Monitoring SARS-CoV-2 transmission and the number of COVID-19 cases in school populations remains a challenge due to lack of prioritization. The detection of SARS-CoV-2 in sewage from college dormitories and other institutional settings may serve as an early warning of outbreaks. However, there has been limited exploration of wastewater monitoring of schools. Despite evidence supporting the value of wastewater-based epidemiology for tracking COVID-19 burden, there are only a few programs and locations around the world prioritizing the use of this tool for school settings. For a few locations, there have been reports of weekly WBS efforts occurring in England (UK) amongst primary and secondary schools in Southwest England, Northwest England, and Southeast England (Gutierrez et al., 2021.). The study began in October 2020 and involved sampling twice a week for 9 weeks (total 296 samples) from 10 primary schools, 5 secondary schools, 1 post -16 school (education after 16 years of age) and 1further education school (vocational training, work-based learning) (totaling 17 schools) and testing for the detection of SARS-CoV-2 N1 and/or E gene in wastewater. Results demonstrated that SARS-CoV-2 could be detected in wastewater samples from schools. Findings also revealed that at the start of the study the proportion of wastewater samples that were positive for SAR-CoV-2 was higher for samples from primary schools compared to wastewater samples collected from secondary schools. After week 5, the positivity rate for wastewater samples from secondary schools was consistently higher than those collected from primary schools. When comparing the wastewater results to the community COVID-19 cases researchers found that the frequency of detection of the targeted genes in wastewater had a relationship with the variation in prevalence of community cases.

In response to the pandemic, schools across the nation have established prevention and control measures to mitigate the transmission of COVID-19 in schools. The county of San Diego, in partnership with UC San Diego Wertheim School of Public Health, has also employed wastewater surveillance of schools through the establishment of an evidence informed program, *Safer at School Early Alert System* (SASEA) to accompany in-person learning. The main components that make up SASEA include 1. Daily environmental sampling utilizing wastewater and surface swabs, 2. Rapid results monitored by site administrators, 3. On site diagnostic testing of staff and students upon positive wastewater outcomes, and 4. Transmission mitigation via double mask reinforcement, health communication messaging, and improved classroom ventilation (Fielding-Miller et al., 2021). Designed by UC San Diego and funded by the County of San Diego, SASEA identifies the presence of SARS-CoV-2 in childcare centers and TK-8 schools (transitional kindergarten -8th grade). Testing prevention measures include diagnostic testing, screening, surveillance testing, PCR, Antigen, NGS tests, and pooling. Through identifying infected students and staff with PCR-positive clinical samples, they are also able to prevent an outbreak event before it occurs. Daily wastewater surveillance is supplemented by other monitoring activities, such as wiping commonly touched surfaces and testing for SARS-CoV-2, routine COVID-19 diagnostic tests, diaper monitoring at childcare facilities (collecting and testing fecal samples for the presence of SARS-CoV-2 in diapers once a week), community involvement and leadership, and qualitative perspectives through the implementation of questionnaires and focus groups (*SASEA System – Safer At School Early Alert*, 2020). Upon SASEA's pilot run amongst 9 public elementary schools (2020-2021academic year) throughout San Diego, SARS-CoV-2 was identified in 374 surface samples and 133 wastewater samples (Fielding-Miller et al., 2021). SESEA results also revealed that 89 total individuals tested positive for COVID-19 onsite, there were 2 suspected occurrences of classroom transmission, and ninety-three percent of the 89 positive individuals were associated with surface samples or positive wastewater outcome (Fielding-Miller et al., 2021). Within California there have been additional developments to prevent the transmission of COVID-19 amongst children in school. In fact, Coronado Unified District, California has taken initiative in providing over one thousand free antigen tests to students and staff to detect COVID-19 in schools. With school health officials assisting with COVID test administration and processing; results come back within approximately fifteen minutes (Taketa, 2021).

Furthermore, The Houston Health Department, in collaboration with Houston Water and Houston public schools, is a leader in wastewater-based epidemiology. They have been monitoring COVID-19 prevalence through collecting, processing, and analyzing weekly wastewater samples from 51 out of 300 schools as well as other sites (wastewater treatment plants, congregate living facilities). Untreated wastewater samples are analyzed for the presence of SARS-CoV-2 at Rice University, Baylor University, and the Houston Health Department. Variant identification using SARS-CoV-2 genome sequencing is also conducted. Out of the 51 schools monitored, they work closely with eight schools by collaborating with school nurses on prevention and control guidance and sharing information on the implications and significance of the wastewater monitoring results. This collaboration helps guide implementation of vaccination clinics at schools based on geographic areas of concern.

In addition to providing weekly COVID-19 tests, Atlanta Public Schools (APS) provide daily COVID-19 screening (*Testing / Healthcheck: Student COVID-19 Screenings + Immunizations*, 2022). In partnership with Stratum Health Solutions, APS provides an online tool that helps monitor the wellness of students prior to their arrival at school. The health check survey includes questions on vaccinations, symptoms, exposures, and recent diagnosis. Until the latest CDC guidance on the mask mandate becoming optional for vaccinated individuals; APS required masks to be worn district wide by students/ staff and when in-person learning resumed in January of 2021. Contact tracing is another effort that has been enacted by APS in collaboration with the Georgia Department of Health (*Tracking and Tracing in APS*, 2022). Maintaining communication with local boards of health and the Centers for Disease Control (CDC), the Georgia Department of Public Health, and the Georgia Department of Education (GADOE), APS schools can report potential outbreaks and prevent transmission. Under non-outbreak closures (when a student/staff/faculty test positive and has exposed other individuals) and COVID-19 outbreaks in school settings, APS, along with other schools, have implemented school closures (accompanied with virtual classes) to control the spread of SARS-Cov-2 virus (*Tracking and Tracing in APS*, 2022). APS has also been proactive on cleaning and sanitizing efforts to prevent the transmission of SARS-CoV-2 amongst their schools. Some of their efforts include investing 68 million dollars to advance heating, ventilation, and air conditioning systems throughout the county, running facility wipe downs during the night, conducting deep cleans when a COVID-19 case is detected, and requiring the use of PPE (personal protective equipment) throughout school buildings. Managing building capacity while in-person learning takes place is another effort that has taken place.

Prevention and control measures to mitigate the transmission of COVID-19 in schools is not only limited to the education system; corporations are proactively making strides to address the alarming increase of SARS-CoV-2 transmission throughout schools. Concentric by Gingko launched within Gingko Bioworks, a synthetic biology company, provides on-site COVID-19 testing at magnitude to K-12 schools and companies to facilitate in-person learning. Testing essentially takes place with 4 steps: 1) Every classroom receives pooled testing together through the employment of short non-invasive nasal swabs sampling 2.) Shipping samples to the laboratory, 3.) Receiving classroom COVID-19 test results 4.) Returning to Learning (Concentric by Ginkgo, 2022). Conducting pooled COVID-19 tests for each classroom poses only minor inconvenience to in-person learning while prioritizing the health of children and staff gathering collectively to learn and teach.

CHAPTER 2- RESEARCH OBJECTIVES AND RATIONALE

There are three main objectives for this study: The first objective of this study is to collect and analyze weekly wastewater samples from selected Atlanta Public Schools and compare the wastewater results from Atlanta Public schools to the weekly reported COVID-19 cases. Secondly, this study seeks to understand why specific Atlanta Public Schools have higher SARS-

CoV-2 detection rates in wastewater compared to others. The third objective of this study is to investigate the catchment area for each school sampled to examine socio -demographic characteristics of the surrounding community to identify factors that may contribute to positive wastewater outcomes in Atlanta Public Schools and reported COVID cases in school aged children. The rationale for doing this study involves determining school related and external variables that are significantly linked to positive wastewater outcome, COVID-19 outbreaks, and increased SARS-CoV-2 infection in Atlanta Public Schools. These results can also be used to guide school decision makers and parents on COVID-19 prevention and mitigation efforts.

METHODOLOGY

Research Design

Data utilized in this study is derived from the Atlanta Public School District and The Center for Global Safe Water, Sanitation and Hygiene (CGSW). Socio-demographic data on the Atlanta City Council districts originate from the (2019) Atlanta Regional Commission; derived from the United States Census Bureau American Community Survey 5-year estimates and Decennial Census counts (Atlanta Regional Commission, 2022).

This study compared results from weekly wastewater samples collected from selected Atlanta Public Schools to weekly reported COVID-19 diagnostic test results from staff and children from these schools. Eleven schools were identified and selected to participate in this study. Weekly wastewater samples (Moore swabs) were collected from manholes immediately downstream from the schools and then analyzed for SARS-CoV-2 RNA by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). Wastewater samples were collected from August 2021 to March 2022. For the following study, descriptive statistics were employed to summarize trends in APS reported COVID-19 cases, trends in APS positive wastewater outcome, and facilitate univariate analyses. Analyses are based on one or more independent school predictors and neighborhood demographic predictors

School and Community Characteristics

Several variables of interest were identified that may influence the likelihood of detecting SARS-CoV-2 in the school wastewater, including: *grade level of the school; vaccination rates in each school and the surrounding community; number of students and staff in each school; average nurse visits, student commute mode, weekly attendance, average classroom size, influent line catchment area wastewater results; city council district median household income, race distribution, poverty rate, education level, insurance coverage, housing value; and housing classification.* However, for the following study, school data including *student and staff vaccination rates, COVID-19 case reports, and the total number of student and staff,* were all obtained from the Atlanta Public School District ("Updates + Alerts / Self-Report+ Weekly Case Report", 2022). Atlanta city council district demographic data were imported from the American Regional Commission; United States Census Bureau American Community Survey 5-year estimates and Decennial Census counts (Atlanta Regional Commission, 2022; U.S. Census Bureau, 2022).

Outcome measures

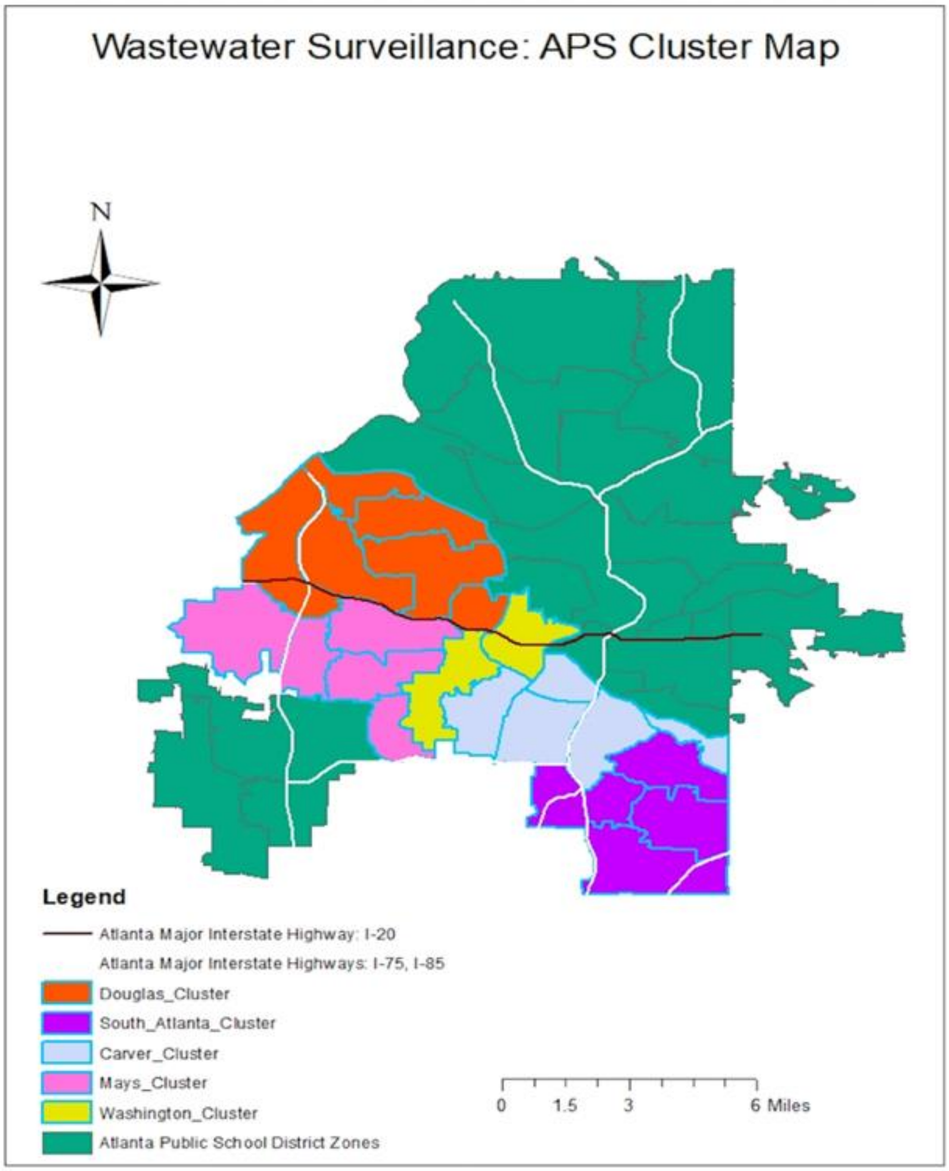
Primary outcomes of interest consist of the positive detection of SARS-CoV-2 RNA in wastewater samples and the number of self-reported cases in each of the monitored schools over time. The presence of the SARS-CoV-2 virus was determined by conducting RT-PCR on Moore swabs left in wastewater for 24 hours. The number of self-reported cases was determined by voluntary participation in COVID-19 surveillance testing under the Atlanta Public School's (APS) Comprehensive COVID-19 Testing Strategy. As a mitigation approach, APS, in partnership with public health officials, community leaders, and healthcare systems, provide weekly COVID-19 surveillance testing. COVID-19 Surveillance testing is required weekly for APS teachers/staff and optional for APS students with written parent consent (*Testing / COVID-19 Testing Strategy*, 2022). Surveillance testing is for students selected through the health screening protocols, students who have been exposed or been in contact with someone with COVID-19, with students with possible exposure, and students who are asymptomatic (*Testing / COVID-19 Testing Strategy*, 2022).

School and Community Site Selection

For this study, 11 schools (6 elementary schools and 3 middle schools, 1 high school) were identified in 5 different clusters of South and Southwest Atlanta, Georgia: 3 in the Washington Cluster (Herman J Russell, Tuskegee Airmen Global Academy, M Agnes Jones Elementary), 2 in the Carver Cluster (Finch Elementary, Perkerson Elementary), 1 in the South Atlanta Cluster (Dobbs Elementary, Kipp Vision Primary School), 1 in the Douglass Cluster (John Lewis Invictus Academy) three in the Mays Cluster (Peyton Forest Elementary School, Jean Childs Young Middle School, Benjamin E Mays). Study schools were identified from a list of all APS schools provided by the Atlanta Board of Education and Atlanta Public Schools dashboard. Google Application Programming Interfaces (API's) then located the latitude and longitude of these schools. Following this, geographical points were overlayed to OpenStreetMap using Quantum GIS (QGIS) to confirm locations. QGIS selected manholes within 200m of each school. A written code using R programming was then used to calculate the quantity of manholes upstream from each school. Lastly, manholes were checked manually on QGIS to confirm the population served (greater neighborhood, or school) by the selected manholes. *School grade (elementary and middle school), school location, manhole accessibility, and direction of wastewater flow* were inclusion criteria for the schools selected for wastewater monitoring. The presence of COVID-19 *Delta and Omicron* variants, in addition to the high incidence rates and related hospital visits for ages 0-4, and 5-11, motivated this study's interest in collecting wastewater samples from elementary schools. Public schools located in south Atlanta were prioritized to focus the study on more vulnerable populations. Furthermore, these school sites were selected based on manhole accessibility and direction of flow to catch wastewater that is directly downstream from the school buildings and not likely to be coming from other sources.

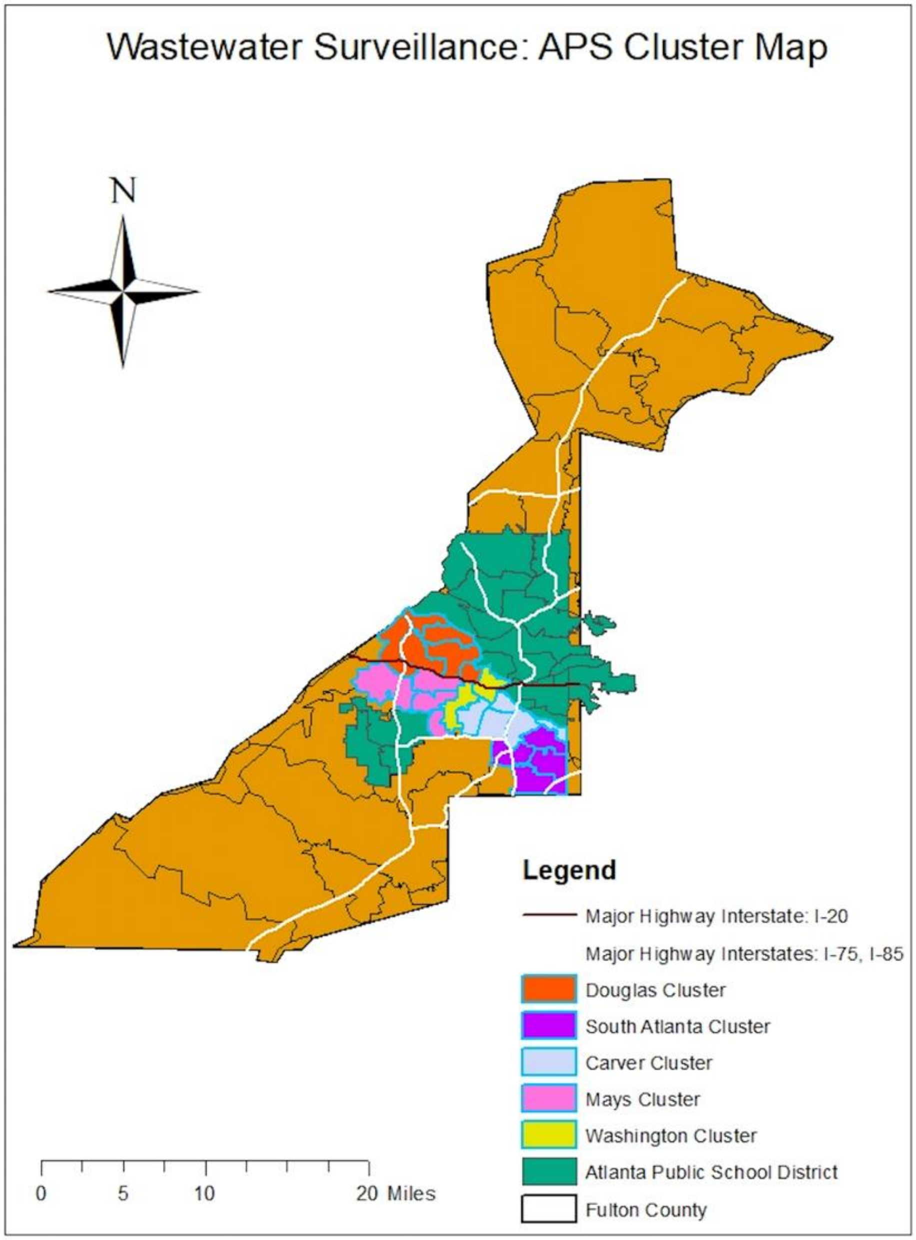
For this study, demographic data from Atlanta City Council Districts 1, 3, 4, 9, 10, 11, and 12 were transformed to provide accurate estimations for community demographic predictors. To calculate estimations from the districts, shapefiles for the APS Public School District, Atlanta City Council Districts, the City of Atlanta, and shapefiles for the 11 schools sampled were uploaded into ArcGIS. Shapefiles were then projected to an equal area projection. Following this, neighborhood polygons were split if they overlapped with multiple city council districts. For neighborhood polygons a ratio was made of the estimated population of the split block to the size of the total District. This approach was chosen when district boundaries appeared to overlap smoothly with communities surrounding our study schools. This ratio was later applied to all neighborhood predictor variables, which assumes that these variables are uniformly distributed by population of that block.

Figure 1: Wastewater surveillance neighborhoods located in South and Southeast Atlanta (below I-20) within the Atlanta Public School District and 5 Atlanta Public School clusters.



Map sourced from Atlanta Public School website

Figure 2: Wastewater surveillance neighborhoods located in South and Southeast Atlanta (below I-20) within Fulton County and Atlanta Public School District.



*Map sourced from Atlanta Public School District website

Collection of Wastewater Samples

Procedures for wastewater collection for this study followed the Moore swabs for SARS-CoV-2 detection method; described in detail in the substantive paper *A Sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level.* (Liu et al., 2021). Moore swab assembly and collection occurred over a duration of 2 days (Monday and Tuesday) throughout the Fall and Spring of the 2021-2022 academic year. If the day of swab assembly ran concurrently with a holiday and or school break, then swab assembly and collection was conducted on the next two consecutive days. Sample materials consisted of weighted fishing line (50lbs), cotton gauze, collection bags, thin rope, permanent markers, a cooler, and ice pack(s). Personal Protective Equipment included disposable gowns, N-95 mask, face shield, disposable gloves, biohazard bag (for disposable items) and steel toed shoes. Moore swab assembly (Day 1) involved Moore swabs made of cotton gauze (120 cm long by 15cm wide) tied together with fishing line. Swab placement involved the removal of the manhole covers using a manhole cover hook and tying the fishing line around a clean Moore swab. Once complete, the Moore swab was lowered into the open manhole until submerged in the wastewater stream, and the fishing line was secured to the manhole cover for retrieval. Finally, the manhole cover was returned to its initial position, leaving the submerged Moore swab for a period of 24 hours. Day 2 of Moore swab collection involved labeling collection bags with collection site (school name) and collection date. Next, the removal of the manhole cover took place; followed by retrieval of the Moore swab by drawing up the fishing line. The swab was then placed inside of the labeled collection bag. The fishing line attached to the swab and manhole cover was cut allowing for the collection bag to be sealed. Once sealed, the collection bag was placed in a cooler along with an ice pack for transport. The manhole cover was then returned to its initial closed position.

Sample Processing and Analyses

Moore swab laboratory analyses for SARS-CoV-2 detection involved four primary procedures.

1.) MOORE SWAB PROCESSING WITH STOMACHER

Procedures for Moore swab processing with the stomacher are detailed in the paper *A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level.* (Liu et al., 2021). Moore swabs were initially squeezed in a beaker to retrieve trapped liquid. After squeezing, the swab was placed in a stomacher bag and submerged in 100 mL of elution buffer consisting of 0.01% sodium polyphosphate, 0.01% Tween 80, and 0.001% antifoam Y-30 emulsion. The swab sample and diluent were then placed into the stomacher for 2 minutes. Lastly, the swab was squeezed, and all the liquid was combined. These steps were repeated once more until the eluate volume reached about 250mL.

2.) CERES NANOTRAP FOR SARS-CoV-2 CAPTURE AND CONCENTRATION

Procedures for capturing and concentrating the SARS-CoV-2 virus in wastewater samples using the automated Nanotrap method are detailed in the protocol *Nanotrap*

KingFisher Concentration/extraction & MagMax KingFisher Extraction (Sablon et al., 2022).

3.) RNA EXTRACTION

Following capture and concentration of the SARS-CoV-2 virus, designated extraction plates were prepared and aliquoted. Detailed procedures for RNA extraction are described in the protocol *Nanotrap KingFisher Concentration/extraction & MagMax KingFisher Extraction* (Sablon et al., 2022).

4.) RT-QPCR FOR SARS-COV-2 N1 GENE AMPLIFICATION AND DETECTION

The procedure for RT-qPCR amplification and detection of the SARS-Cov-2 N1 gene and the process control (BRSV) in RNA extracted from wastewater samples is described in the Center for Global Safe WASH protocol for Singleplex RT-qPCR for SARS-CoV-2 N1 and BRSV (Svezia et al., 2022).

Data Analysis

R programming was employed for all univariate descriptive analyses for this study. ARC GIS was also utilized to build Atlanta Public School Cluster Maps and facilitate neighborhood calculations. **(Figures 1 & 2**).

For the purposes of this study the dichotomous outcome variable, Wastewater Outcome (Positive or Negative detection of SARS-CoV-2), was defined to a nominal scale for all analyses. Negative wastewater results were represented by the number 0, and positive wastewater results were represented by the number 1 in R programming.

Univariate analysis was conducted for all school and neighborhood variables by wastewater outcome using descriptive statistics (frequency tables, summary statistics). Groups were compared by creating time series analyses, and tile plots to show the school variables that demonstrated temporal and spatial trends - such as positive wastewater outcome. The strength and direction of the trend was examined for each variable. School variables included in the analyses were: total number of students per school, total number of staff per school, staff vaccination rate, student vaccination rate, the total number of reported COVID-19 cases per 100 students, and school type. Neighborhood variables included in the analyses were: unemployment rate in the school neighborhood population, total population in the school neighborhood, percent of the school neighborhood classified as below the federal poverty level, neighborhood rate of children in families below the 200 federal poverty level, and the percent population in school neighborhoods residing in overcrowded owned housing units as defined by the federal poverty level(more than 1.01 persons per room).

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CHAPTER 3- RESULTS

Description of the study population

Wastewater from 11 Atlanta public schools (with approximately 7385 total students and staff) was monitored in this study. The study included six elementary schools, three middle schools, one high school, and one charter school that spanned Kindergarten through 8th grade (Kipp Vision). Included in the analyses are the most recent COVID vaccination rates reported (April 2022) by the APS. Student and staff vaccination rates from the APS were also gathered for a 4-month period (excluding Kipp Vision Primary School staff vaccination rate). The neighborhood demographic information used for these analyses were derived from seven Atlanta City Council Districts (Districts 1, 3, 4, 9, 10, 11, 12) where these 11 schools were located.

Table 1a presents information on school characteristics and demographics for each of the 11 study schools. The number of students in each sample school ranged from 358 to 1333 with a mean number of 621.2 (median number= 503). The number of staff/faculty in each sample school ranged from 30 to 98 subjects with a mean of 44.44 (median number = 37). Grade levels ranged from kindergarten to 12th grade. Overall, 63% of schools sampled were elementary schools, 27% were middle schools, and 9% were high schools. Finch Elementary School had the lowest number of student and staff with 358 students and 30 staff, while Benjamin E Mays High School had the highest number of students and staff with 1333 students and 98 staff.

Overall, the student vaccination rate from February – May 2022 ranged from 1% to 25% with a mean of 9%. (Median student vaccination rate= 7.5%) **(Figure 3a).** Staff vaccination rates over the same 4-month period ranged from 46.6% to 76.7% with a mean of 60% (Median staff vaccination rate= 56%) **(Figure3b).** Student vaccination rates from April 2022 were compared across 11 schools and ranged from 3% to 25% with a mean of 10.45% (median student vaccination = 9%) **(Figure3c).** Staff vaccination rates in ten schools in April 2022 (excluding Kip Vision Elementary School) ranged from 46.4% to 76.7% with a mean of 58.12% (SD = 6.3%, median staff vaccination rate = 55.7%) **(Figure3d).** There were considerable differences between student and staff vaccination rates. Tuskegee Airmen Global Academy elementary school had the lowest student vaccination rate of 3% in contrast to Benjamin E Mays High School with a 25% student vaccination rate. Moreover, Agnes Jones Elementary had the lowest staff vaccination rates, with 46.6%, while Finch Elementary School had the highest staff vaccination rate with 76.6%. In every school, staff vaccination rates were substantially higher than student vaccination rates (Table 1a).

Overall, for each school the median number of reported COVID-19 cases per 100 students from August 2021 through March 2022 ranged from 0 to 0.002544 cases/per 100 students with a mean of 0.002650. (**Table 1b)** Finch Elementary School had the highest total COVID-19 prevalence rate with 0.22901 reported cases per 100 students, while John Lewis Invictus had the lowest COVID-19 prevalence rate with 0.005842 reported cases per 100 students. Benjamin E Mays High School followed John Lewis Invictus as having the second lowest total number of reported COVID cases per 100 students of 0.007687.

Table 1a.:Description of Study Population by School

School	Cluster	School Group	# Of Students	# Of Staff	Student Vaccination Rate (%)***	Staff Vaccination Rate(%)***	Total Reported COVID-19 cases /Per 100 Students****	Total Proportion of Positive Wastewater Outcome (%)*****	Grade	Ethnicity (%)
Tuskegee Airmen Global Academy	Washington	ES	582	42	3	63.8	0.009615	70	K-5	BL:97% HS: 1% Two or More: 1%
Peyton Forest Elementary	Mays	ES	427	35	4	61.9	0.019481	20	K-5	BL: 90% HS:9%
Agnes Jones Elementary	Washington	ES	628	49	8	46.6	0.011817	10	K-5	BL:97% HS: 1% PI:1% W:1%
Dobbs Elementary	South Atlanta	ES	428	34	7	65.1	0.012987	70	K-5	BL:92% HS: 6% PI:1% W:1%
Finch Elementary	Carver	ES	358	35	8	76.7	0.022901	50	K-5	BL:97% HS: 1% Two or More: 1%
Perkerson Elementary	Carver	ES	367	37	9	53.2	0.017327	20	K-5	BL:98% HS: 1% Two or More: 1%
Kipp Vision Primary School	South Atlanta	ES**	503	30	9	NA*	0.011257	70	K-8	BL:96% HS: 4%
John Lewis Invictus Academy	Douglass	MS	962	65	11	50.8	0.005842	20	6-8	BL:94% HS: 5% Two or More: 1%
Jean Child Young Middle School	Mays	MS	812	58	17	55.5	0.011494	20	6-8	BL:90% HS: 8% Two or More: 1%
Herman J. Russell West End Academy	Washington	MS	985	52	14	55.9	0.009643	10	6-8	A:1% BL:96% HS:2% W: 1%
Benjamin E Mays	Mays	HS	1333	98	25	51.7	0.007687	10	9-12	BL:91% HS: 8% Two or More: 1%

*Kipp Primary Vision School is a Charter school. All other schools are public neighborhood schools.

**Kipp Primary Vision School is an Elementary and Middle School. For the following analyses, this schools is defined as an Elementary School.

***Student and Staff vaccination rates included in this table are from April 2022.

**** The Total Reported COVID-19 cases /Per 100 Students included this table are from August 2021-March 2022.

***** The Total Proportion of Positive Wastewater Outcome included in this table are from August 2021-March 2022.

Figure 3a. Student Vaccination Rates, by School, February-May 2022

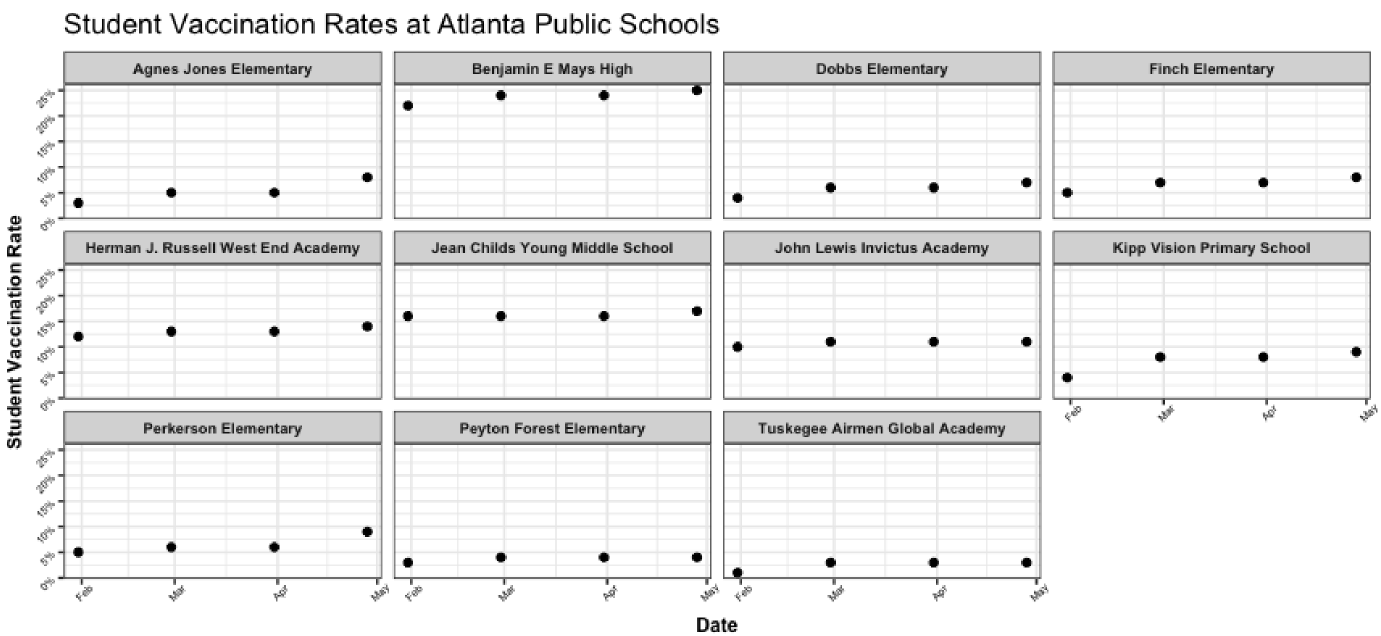


Figure 3b. Staff Vaccination Rates, by School, February-May 2022

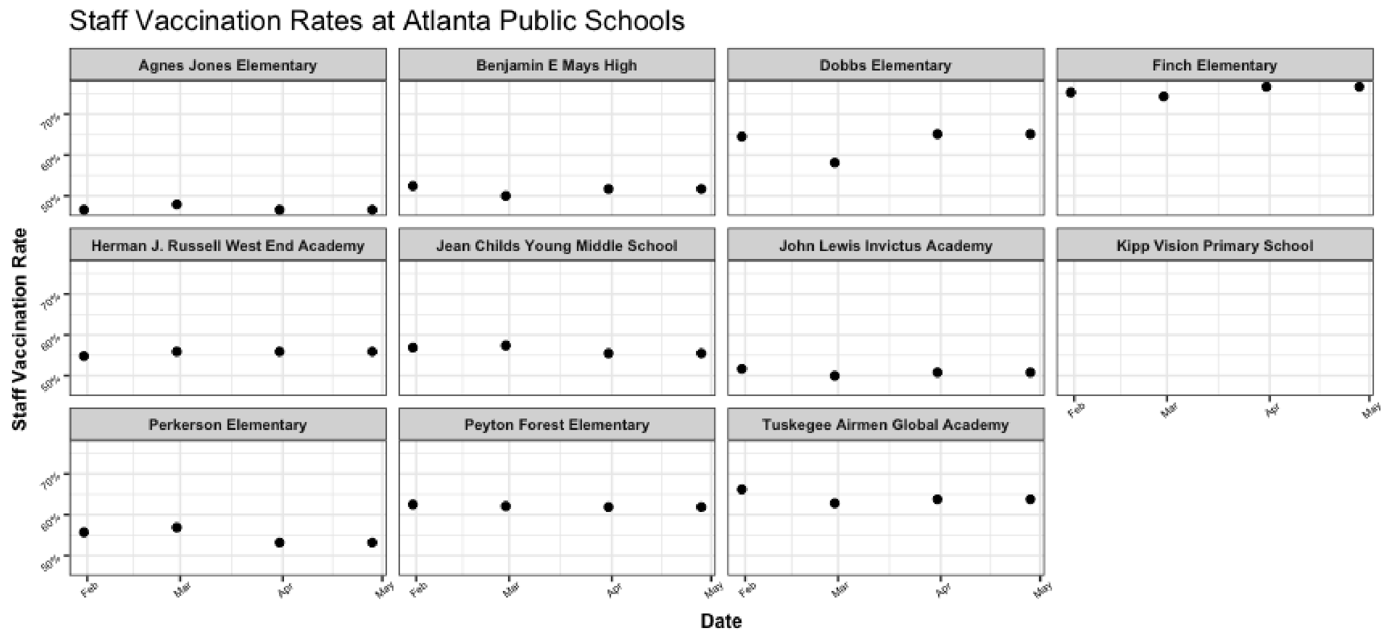


Figure 3c : Student Vaccination Rates by School, April 2022

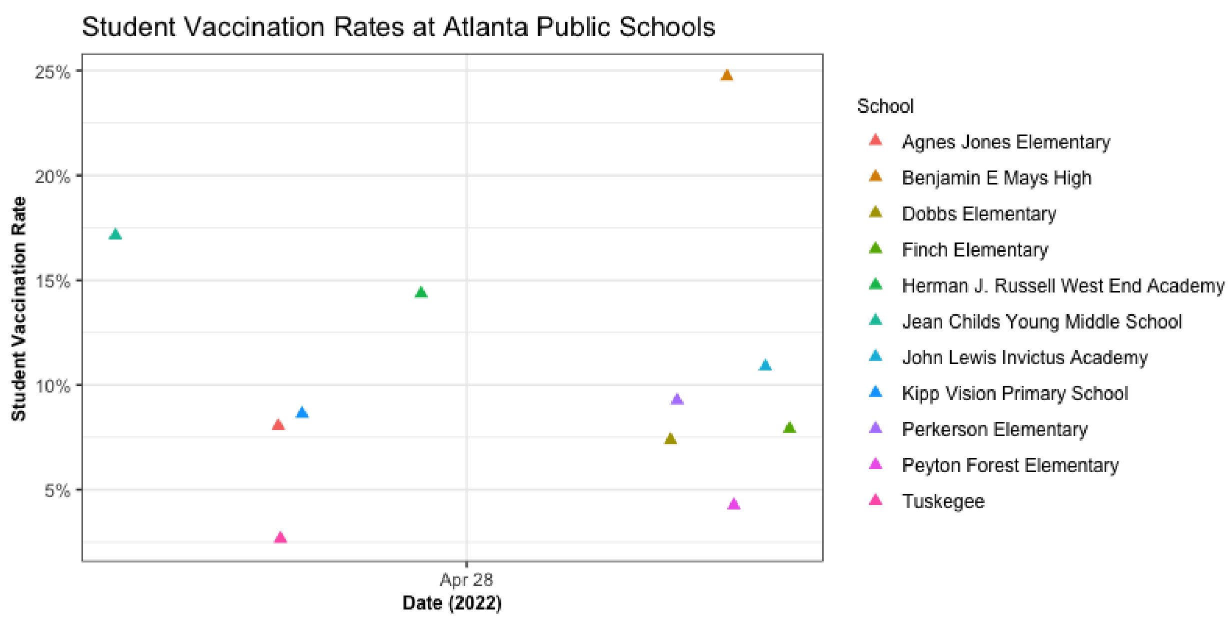


Figure 3d: Staff Vaccination Rates by School, April 2022

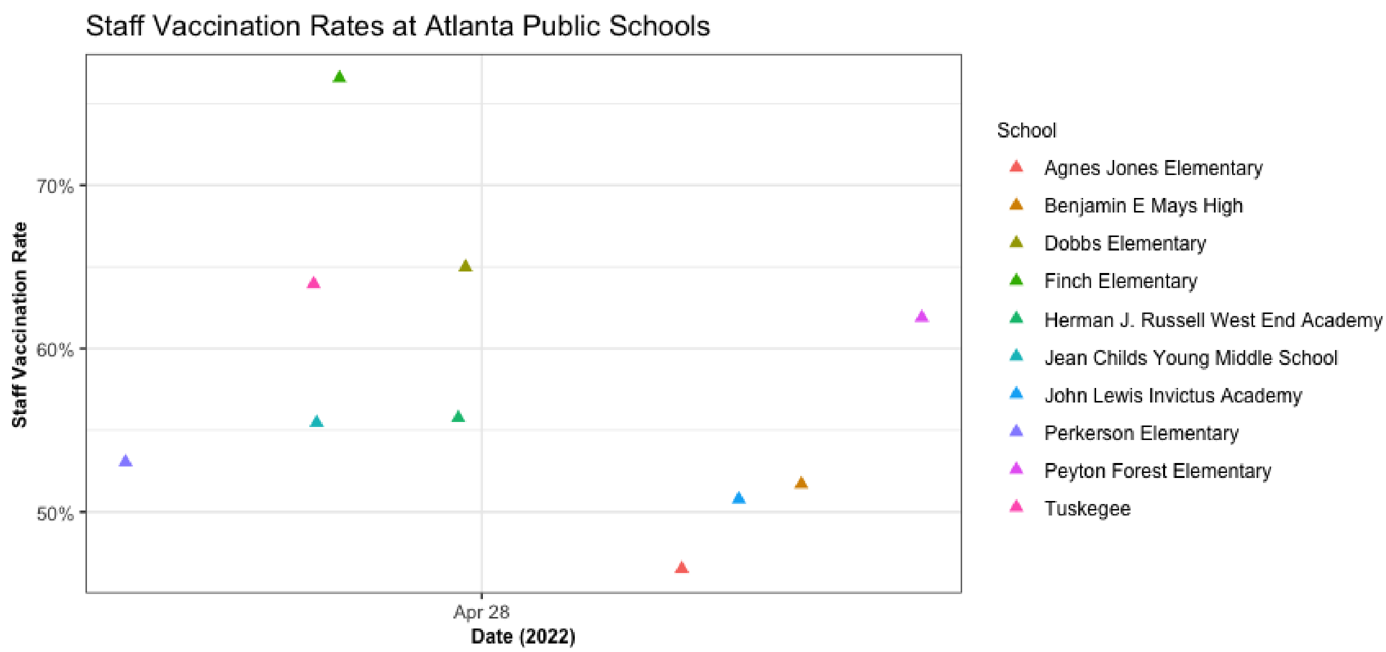


Table 1b. Summary Statistics by School Variable

Student Vaccination Rates (April 2022)						
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**	**SD**
3.00	7.50	9.00	10.45	12.50	25.00	NA
Staff Vaccination Rates (April 2022)						
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**	**SD**
46.60	52.08	55.70	58.12	63.33	76.60	6.3

Staff Vaccination Rate Feb-May 2022					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
46.60	53.20	55.90	59.02	65.10	76.70
Student Vaccination Rate Feb-May 2022					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
1	4.75	7.500	9.227	12.250	25
Reported COVID-19 Cases Per 100 Students (August 2021-March 2022)					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
0.00000	0.00000	0.00115	0.00260	0.00379	0.02290
Total # of Students					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
358.0	428.0	503.0	621.2	628.0	1333
Total # of Staff					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
30	34	37	44.44	49.00	98.00

Description of the School Neighborhood

Information on the catchment neighborhoods of the study schools was derived from data on seven Atlanta City Council Districts where the schools were located. The total population in each school neighborhood ranged from 9,662 to 37,729 with a mean population of 18,944. (Median Total population= 22,555) (**Table2a)**. Kipp Vision Primary School and Dobbs Elementary School were in District 1 and had the lowest total population of 9,662. In contrast, John Lewis Invictus Academy neighborhood spanned Districts 10 and 11 and had the highest total population. Unemployment rates in APS neighborhoods ranged from 4% to 11.8% with a mean of 5.5%. (Median unemployment rate = 5.6%) **(Table 2b).** Neighborhoods surrounding Dobb Elementary School, Kipp Vision Elementary, and John Lewis Invictus had the lowest unemployment rate (4%). In contrast, the neighborhood around Benjamin E Mays High School, located in District 10 and 11, had the highest unemployment rate of 11.8%. Neighborhood rates of uninsured populations (no health insurance) varied greatly with a range of 5.6% to 16.4% and a mean of 13.2% (median=13.4%) (**Figure 2b).** The neighborhood surrounding Perkerson Elementary had the lowest proportion of uninsured population (5.6%) in contrast to the Finch Elementary neighborhood with the highest proportion of uninsured population (16.4%). The proportion of the neighborhood population classified as below the federal poverty level were quite high for all neighborhoods, ranging from 24% to as high as 35% with a mean of 30.7% (median rate below poverty = 28.4%) **(Figure 2b)** . John Lewis Invictus Academy had the lowest proportion of the neighborhood population below poverty at 24.4%, while Agnes Jones Elementary had the highest proportion of the neighborhood population below poverty at 34.9%.

Proportions of overcrowded housing units as defined by the federal census (more than 1.01 persons -per- room) in each school neighborhood ranged from 0.7% to 2% with a mean of 0.8% (median overcrowded = 0.8%) (**Figure 2b).** Neighborhoods surroundings Tuskegee Airmen Global Academy, Herman J. Russell West End Academy, and Agnes Jones Elementary had the lowest proportion of overcrowded housing units of 0.7% compared to John Lewis Invictus located in Districts 3,4,9, and 10 with 2.0% of overcrowded housing units. The proportion of children in families below 200 percent federal poverty level in school neighborhoods were relatively high ranging from 13% to 19% with a mean of 16% (median = 16.6%)(**Figure 2b).** The neighborhood around John Lewis Invictus Academy had the lowest population rate of children in families below the 200 percent poverty level. In contrast, neighborhoods of Perkerson Elementary located in District 12 had the highest proportion of 19 % children in families below the 200% poverty level. **Table 2a** presents a descriptive table of neighborhood characteristics and demographics for each of the study schools.

Table 2a: Description of demographic characteristics by School Neighborhood

ATL City Council District	School	% Without Health Insurance*	% Population below Poverty**	%Unemployed	%Children in Families below 200 poverty level***	%Overcrowded units ****	#Total Population	Total Proportion of Positive Wastewater Outcome*****	Neighborhood Ratios
District 4,10,11,12	Tuskegee Airmen Global Academy	15.8	33.5	5.6	14.4	0.7	22,555	70	50:100; 4:100; 6:100; 2:100
District 10,11	Peyton Forest Elementary	13.4	27.5	5.5	18.7	0.8	28,556	20	63:100;10:100
District 3,4	Agnes Jones Elementary	15.4	34.9	5.5	13.3	0.7	13,704	10	12:100; 33:100
District 1	Dobbs Elementary	11.6	28.4	4.0	16.6	0.9	9,662	70	25:100
District 4,12	Finch Elementary	16.4	34.7	5.7	14.8	0.8	10,469	50	8:100; 20:100
District 12	Perkerson Elementary	5.6	34.0	6.0	19.0	1	10,273	20	30:100
District 1	Kipp Vision Primary School	11.6	28.4	4.0	16.6	0.9	9,662	70	25:100
District 3,4,9,10	John Lewis Invictus Academy	12.5	24.4	4.0	13	2	37,729	20	18:100; 4:100; 52:100; 23:100
District 10,11	Jean Child Young Middle School	13.4	27.5	5.5	18.7	0.8	28,556	20	63:100;10:100
District 4,10,11,12	Herman J. Russell West End	15.8	33.5	5.6	14.4	0.7	22,555	10	50:100; 4:100; 6:100; 2:100
District 10,11	Benjamin E Mays	13.4	27.5	11.8	18.7	0.8	31,624	10	64:100; 10:100

*% Without health insurance/uninsured is defined as the % of civilian non-institutionalized population age 19 and over, with no health insurance. The 2019 estimates are derived from 2014-19 American Community Survey.
** % of population below poverty is defined as the % of population living below U.S federal poverty threshold. The 2019 estimate is derived from 2014-19 American Community Survey.
*** % Children in Families below 200 poverty level is defined per U.S federal threshold. The 2019 estimate is derived from 2014-19 American Community Survey.
****% Overcrowded units is defined per federal definitions- the % of population residing in overcrowded owned housing units with more than 1.01 persons per room. The 2020 estimate is derived from 2016-2020 American Community Survey.
*****The Total Proportion of Positive Wastewater Outcome included in this table are from August 2021-March 2022.

Table 2b. Summary Statistics of School Neighborhood Variables

Total Population 2019					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
9662	9968	22555	18944	25556	37729
% Of Population Unemployed 2018*****					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
4	4	5.6	5.513	5.600	11.8
% Of Population Without Health Insurance*					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
5.60	11.60	13.40	13.22	15.80	16.40
% Of Population Below Poverty 2019**					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
24.40	27.95	28.40	30.74	33.50	34.90
% Of Population with Overcrowded Housing Units *****					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
0.7000	0.7000	0.8000	0.8862	0.9000	2.0000
% Of Children in Families Below 200 Poverty ***					
Min.	**1st Qu.**	**Median**	**Mean**	**3RD Qu.**	**Max.**
13	14.40	16.60	16.05	18.70	19.00

*% Without health insurance/uninsured is defined as the % of civilian non-institutionalized population age 19 and over, with no health insurance. The 2019 estimates are derived from 2014-19 American Community Survey.
** % of population below poverty is defined as the % of population living below U.S federal poverty threshold. The 2019 estimate is derived from 2014-19 American Community Survey.
*** % Children in Families below 200 poverty level is defined per U.S federal threshold. The 2019 estimate is derived from 2014-19 American Community Survey.
****% Overcrowded units is defined per federal definitions- the % of population residing in overcrowded owned housing units with more than 1.01 persons per room. The 2020 estimate is derived from 2016-2020 American Community Survey.
*****% 2019 Estimates from population unemployed is derived from 2014-2019 American Community Survey.

Prevalence of Positive SARS-CoV-2 Wastewater Outcome

Of the one hundred and seventy Moore swab samples collected at the 11 study schools between August 2021 and March 2022, the overall proportion with a positive wastewater outcome (SARS-CoV-2 N1 detection) was 41% (69/170). After stratification by school grade, the overall proportion of SARS-CoV-2 prevalence indicated by wastewater surveillance was the lowest in high schools with 10%, followed by middle schools with 16%. In contrast, elementary schools had the highest overall percent of wastewater samples that were positive for SARS-CoV-2 (52%). **Figure 4** shows the distribution of the total proportion of positive and negative wastewater samples by school group.

After stratification by school, overall wastewater results indicated that Dobbs Elementary and Tuskegee Airmen Global Academy had the highest percent of wastewater samples with SARS-CoV-2 detection (70%) compared to the other study schools. 50% of Finch Elementary's wastewater samples were positive, and 20% of Peyton Forest Elementary, Perkerson Elementary, John Lewis Invictus Academy, and Jean Childs Young Middle School's wastewater samples tested positive for SARS-CoV-2. Agnes Jones Elementary, Herman J Russell West End Academy, and Benjamin E Mays High School all had the lowest percent of wastewater samples with SARS-CoV-2 detection (10%) **(Table 2a).**

Relationship between reported COVID cases and wastewater results

Figures 5 & 6 illustrate the trends in COVID-19 prevalence and how the weekly reported COVID-19 cases and wastewater outcome results compare to each other. From Figure 6, COVID-19 case reports for each week appear to vary, between school and week.

However, wastewater outcome appears to have a temporal trend lacking variation as the weeks lead up to week 49 and end of the year (December 2021). This trend corresponds to the time APS schools and students were gearing up for the Winter Break. Despite wastewater outcome indicating increasingly positive results from week 36 and onward, COVID-19 case reports and wastewater outcome results are discordant. Discordance between case reports and positive wastewater outcome is most schools during week 05-2022 up until the last week of data included in this study (week 10-2022). 36% of the schools (4:11) had discordant results during week 5-2022. Peyton Forest Elementary and Herman J. Russell reported 2 COVID-19 cases despite wastewater results indicating no presence of SARS- CoV-2. Additionally, Benjamin E. Mays reported 1 COVID-19 case, with negative wastewater results. From week 36- 2021 to week 46-2021, Dobbs elementary school case reports and wastewater outcomes are concordant. From week 36-2021 to week 05-2022, Tuskegee Airmen Global Academy also has discordant results. COVID-19 cases were reported, but the wastewater outcome did not indicate the presence of SARS-CoV-2. During week 50-2021 Herman J. Russell West End Academy reported concurrent COVID-19 case reports and wastewater outcome. It had a total 10 reported cases and positive wastewater results. Temporally this corresponds to final weeks of the 2021 fall semester 1, the start of the winter holiday, and the Omicron surge illustrated by positive wastewater results.

Furthermore, week 02-2022 indicates an overall rise in case reports. Wastewater results were not collected during this week.

Figure 4: Percent of Wastewater Samples with SARS-CoV-2 Detection by School Grade.

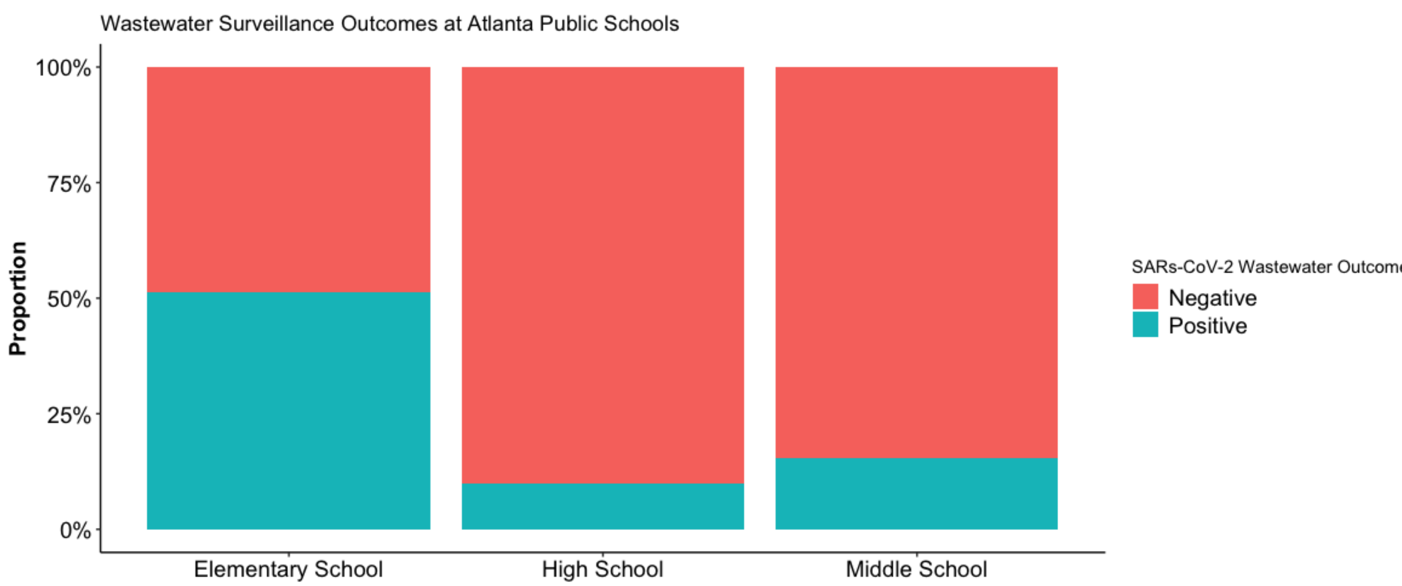
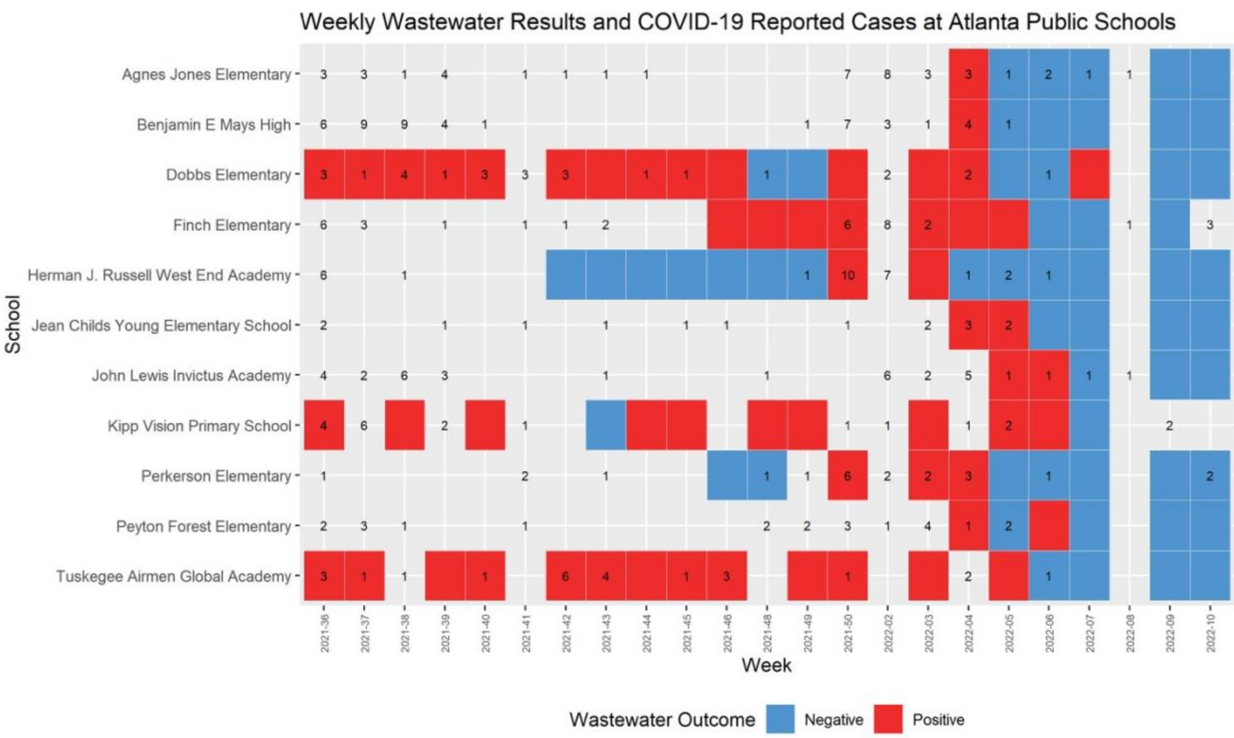


Figure 5. Reported COVID -19 cases/per 100 Students and Wastewater Outcome, by School, August 2021-March 2022

Figure courtesy of Yuke Wang, Emory University

Figure 6: Weekly Wastewater Results and COVID-19 Reported Cases by School, August 2021-March 2022



*Colored box without a number indicates week of non-reported COVID-19 cases.

*No colored box indicates school holiday; school break or week wastewater samples were not collected.

Figure courtesy of Stephen Hilton, Emory University

CHAPTER 4- DISCUSSION AND CONCLUSION

This study *1).Collected and analyzed weekly wastewater samples from selected Atlanta Public Schools and compared the wastewater results from Atlanta Public schools to weekly reported COVID-19 cases. 2). Examined why specific Atlanta Public Schools have higher positivity rates as observed by others. 3).Investigated catchment areas for schools sampled to examine characteristics of school communities and significant variables that influence positive wastewater outcome in Atlanta Public Schools and in school aged children.*

Major Findings:

1) *Overall, the prevalence of positive wastewater samples from the schools was 41% positive.*
    a. *Wastewater results varied over time*
        i. The time-period with the most frequent positive wastewater results was in Dec 2021-Jan 2022 which coincided with the Omicron surge in Fulton County ("COVID-19 Epidemiology Reports", 2022)
    b. *Wastewater results varied by school*
        i. Elementary schools had higher proportion of positive samples (52%) compared to middle schools (16%) and the high school (10%). (Figure 5) Most of our study schools were elementary schools, and most of the

wastewater samples in this study were collected from elementary schools (55%).

ii. *W*hen analyzing the proportion of positive wastewater outcomes by school -Dobb Elementary, Tuskegee Airmen Global Academy, and Kipp Vision Primary School all have the three highest proportions of positive wastewater outcome (70%,70% ,50%). Kipp Vision Primary School and Dobb Elementary are located within the same district strengthening the consideration that neighborhood characteristics may influence the COVID-19 burden in the community and this is reflected in the wastewater outcomes for these schools.

c. Vaccination rate varied by school

i. The elementary schools had lower student vaccination rates (3% to 9%) than the middle schools (11% to 14%) and high school (25%).

ii. Out of the 3 schools with the highest student vaccination rates (Benjamin E Mays, Jean Childs Young Middle School, Herman J. Russell West End) 2 had the lowest proportion prevalence of positive wastewater(Benjamin E Mays, Herman J. Russell West End).

iii. Out of the 3 schools with the highest student vaccination rates (Benjamin E Mays, Jean Childs Young Middle School, Herman J. Russell West End) 2 had the lowest total COVID-19 report case rate (Benjamin E Mays, Herman J. Russell West End).

iv. Of 3 schools with the highest staff vaccination rate (Finch Elementary, Dobbs Elementary, Tuskegee Airmen Global Academy) all 3 of them had the highest proportion prevalence of positive wastewater outcome.

2) *This study used data on self-reported COVID cases that were registered with the Atlanta Public Schools and available on the Atlanta Public School website. The school-specific overall prevalence rates ranged from 0.007676 to 0.022901 per 100 students.*
   a. *Voluntary COVID-19 self-reporting of cases from schools were likely greatly underestimated.*
   b. *Temporal variability in reported cases per 100 students illustrated no uniform trends across all schools (Figure 5)*
3) *Comparison of wastewater results to COVID-19 reported cases*
   a. Results from the following descriptive statistics indicate that prior to large discordance in negative wastewater results and positive COVID-19 case reports (early January) a trend is observed with the academic year **(Figures 5 & 6)** . The large surge in reported COVID-19 cases/per 100 students and positive wastewater outcome from mid to late December also illustrates a pattern with final weeks of the 2021 fall semester 1 and the start of the winter break for students and staff. Furthermore, the rise in the trend of reported COVID-19 case reports during week 02-2022 coincide with the second week of the start of semester 2 and the return of students and staff to schools.
   b. Data from figure 6 suggests that the metric that was more frequently positive and more likely to be the measure with greater sensitivity are positive wastewater samples. Taking into consideration what results reveal about likely

underestimated self-reported COVID-19 cases and neighborhood context (low-income, low-resourced communities) strengthen the belief that wastewater samples are the more accurate metric to determine the presence of SARS-CoV-2 in schools.

c. Dobbs Elementary School had one of the highest COVID-19 self-reported cases/per 100 student and total proportion of positive wastewater outcome when stratified by school. Kipp Primary Vision had one of the highest proportions of positive wastewater outcome.

d. John Lewis Invictus had lowest total COVID-19 reported cases/per 100 students and one of the lower proportion prevalence of positive wastewater outcome

4) School/neighborhood context

a. Demographic characteristics of the neighborhoods where the study schools were located indicate that these were vulnerable, low-income communities

i. Overall poverty rates amongst all schools are very high, with little variability. They ranged from(24.4% to 34.9%). In contrast unemployment rates amongst schools are low(4.0% to 11.8%). The inverse correlation between these two variables and COVID-19 reflects the disproportionate toll COVID-19 has taken on the working poor. This is suggestive that children in these neighborhoods are from families with limited time and resources.

ii. The relationship between COVID-19 self-reported cases, wastewater outcome, and demographic characteristics in this study suggest that the working poor and their children are more susceptible to be exposed with

SARS-CoV-2. Additionally, with limited resources for COVID-19 testing, diagnoses, and reporting of SARS-CoV-2 infection it is likely that the true COVID- 19 burden in our school neighborhoods are under-ascertained and underestimated. Low vaccination rates amongst students in all the school sites support the underestimation of COVID-19 prevalence in these neighborhoods.

iii. Perkerson Elementary had the second largest neighborhood population below poverty , the second lowest school neighborhood population (10,273) and third lowest staff vaccination rate(53.2%) despite having the third highest COVID-19 reported case rate (0.017).

iv. Finch Elementary had the highest neighborhood population below poverty in comparison to having the highest staff vaccination rate (76.7%) and one of the highest proportions of positive wastewater outcome (50%).

v. In contrast Tuskegee Airmen Global Academy had one of the highest percent of population below poverty (33.5%), highest staff vaccination rates (63.8%), lowest student vaccination rates(3%) and largest proportion of positive wastewater outcome (70%).

LIMITATIONS AND RECOMMENDATIONS

Sample Size:

The study population included 11 schools totaling 7385 subjects. Only 13% (11:87) of APS schools were monitored in this study. In future research studies, the sample size should be increased to include at least 50% of APS schools. The smaller sample size for the analyses reported here could undermine the external validity of the study results through lack of generalizability to the whole APS population.

Estimates of School Neighborhood Demographic Characteristics :

Atlanta City Council District demographics from the Atlanta Regional Commission Census Bureau were used to calculate an estimate for the school neighborhood that was weighted by how much of the school neighborhood geographic area fell into the different City Council Districts.

Despite better overlay unto school neighborhood boundaries in comparison to employing census blocks; estimations of these variable have the possibility of producing more uncertainty as the districts are larger than school neighborhood polygons; assuming that the neighborhood characteristics are uniformly distributed across the geographic area. If time is not a constraint, we recommend trying to use neighborhood demographic data Census tracts and Census block individual level data to gather socio-demographic characteristics in future research designs. Future directions for incorporating SES data from the Atlanta Regional Commission and Census Bureau could also include employment of *K-means clustering*. K-means clustering is essentially a method employed through use of a Euclidean distance measure to create centroids (clusters) and/or aggregate data points based on how alike, close, or similar they are to one another. This method would aid in forming school neighborhoods and nuanced SES status based on similar observations and values in the dataset.

Data Variability and Statistical Analyses:

In addition to summarizing temporal trends related to wastewater outcome, a bivariate statistical analysis should be conducted to further analyze the relationship between school variables, neighborhood variables, and positive wastewater outcome. Future directions of this study can entail bivariate analyses such as a Pearson product- moment correlation and/or a multiple logistic regression. This will enable analyses of significance, correlation, and odds of detecting positive wastewater results and examining the effect of school factors such as the average classroom size, average number of nurses visits per week, average weekly attendance for students and staff, and average student commute mode. Similarly, examining the effect of additional neighborhood characteristics may explain some of the spatial variability in the school wastewater results.

Wastewater Outcome Bias

Wastewater surveillance at schools assumes that the students and staff are using the school toilets and the SARS-CoV-2 excreted by COVID cases in the school population is captured in the school wastewater. However, the frequency with which elementary school children utilize the restroom due to scheduled restroom breaks may be different than students in middle and high School grades. This difference in toilet use during the school day may introduce information bias or sampling bias which would affect the generalizability of the results from elementary schools to older school populations. Wastewater surveillance may be a less reliable approach for estimating COVID burden in middle schools and high schools.

Predictor Variable : Self- Reported COVID-19 cases:

Self-reported COVID-19 cases from each school sampled are determined by voluntary participation in COVID-19 surveillance testing under the Atlanta Public School's (APS) Comprehensive COVID-19 Testing Strategy. Optional participation in this program and voluntary reporting of COVID cases likely resulted in underestimation of weekly COVID-19 cases among the students in the study schools. Under-reporting of COVID cases may have affected our ability to detect an association between the weekly reported COVID cases and the weekly school wastewater results.

CONCLUSION

Overall results from this study suggest that *COVID-19 reported cases/per 100 students*, staff vaccination rate, *student vaccination rate*, and *School group/grade, and neighborhood characteristic including percent of population below poverty c*ould influence or share trends with positive wastewater outcome.

In the Fielding-Miller study (2021), the trend in wastewater positivity corresponded to COVID outbreaks confirmed by the County of San Diego Health and Service Agency. In this study with APS schools, high reported COVID-19 cases, and positive wastewater outcomes throughout the mid-end of December (Figure 6) correspond to the surge in Omicron infection.

In the Castro-Gutierrez study (2021) in the UK, the findings revealed that the positivity rate of SARS-CoV-2 in elementary schools was much higher compared to middle and high schools. These findings are consistent with this study in that the overall proportion of positive wastewater in elementary schools are higher than observed in middle and high schools (52%). Overall, 41% (69:170) of wastewater samples collected indicated the presence of SARS-COV-2. 45% of positive wastewater results also corresponded to COVID-19 case reports during the same week. Like many studies based on wastewater epidemiology, this study supports the fact that SARS-CoV-2 RNA can be detected in school wastewater samples using wastewater surveillance, even when COVID-19 cases are not being reported in vulnerable and low-income communities.

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