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Using Environmental Surveillance to Detect Vaccine-Preventable Diseases in Northern Ghana

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Master of Public Health
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Abstract

Using Environmental Surveillance to Detect Vaccine-Preventable Diseases in Northern Ghana

By Enid Stephanie Swatson

Environmental Surveillance (ES) has been increasingly used for COVID-19 surveillance and can also be expanded to other pathogens. Currently, there is limited surveillance of SARS-CoV-2 in Northern Ghana where no clinical cases have been reported since the beginning of the pandemic in March 2020. We applied ES in a rural area, not only for SARS-CoV-2 but also for three other diseases, and used schools as the sampling site. Samples were collected from latrines and septic tanks in nine schools and one residential teaching college in Nanumba North District and nine schools in Mion District. Real-time quantitative polymerase chain reaction (PCR) was used to detect four target pathogens (SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera*) from a total of 114 environmental samples.

Results showed that SARS-CoV-2 (22% POS) and *Vibrio cholera* (6% POS) were the most frequently detected pathogens in the environmental samples from schools and the college. The samples from college residential halls had a higher proportion of PCR-positive tests (43.3%) compared to samples from Primary and Junior High Schools (26.2%). This is a novel and significant finding in a rural setting in Ghana that demonstrates the feasibility and value of ES for infectious disease surveillance in remote areas. We conclude that it would be helpful to expand the implementation of ES to other rural areas of Ghana and other low- and middle-income settings and use the results to better target vaccination campaigns and other prevention and control measures.

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And lastly, to my family and friends, the undercover heroes of this story, thank you for your unwavering support and prayers. You made the good days better and the hard times easier throughout this process.

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Abbreviations

AFP	Acute flaccid paralysis
BPHRU	Biomedical and Public Health Unit
CEPI	Coalition for Epidemic Preparedness Innovations
CGSW	Center for Global Safe WASH
CSIR-WRI	Council for Scientific and Industrial Research-Water Research Institute
cVDPV2	Circulating-vaccine-derived poliovirus type-2
ES	Environmental Surveillance
GHS	Ghana Health Service
GPEI	Global Polio Eradication Initiative
IDSR	Integrated Disease Surveillance and Response
LMIC	Low- and middle-income countries
MMDAs	Metropolitan, Municipal and District Assemblies
NoV	Norovirus
PCR	Polymerase Chain Reaction
TSAP	Typhoid Fever Surveillance Program in Africa
WHO	World Health Organization
WPV	Wild-type poliovirus
WWTPs	Wastewater treatment plants

Literature Review

<u>Environmental Surveillance</u>

Environmental Surveillance (ES) is a low-cost method that involves collecting wastewater or fecal sludge samples from sewage pipes, septic tanks, or latrines, performing laboratory analyses for target pathogens, then interpreting results relative to the population served by the sanitation system where the samples were collected (Andrews et al., 2020). It complements rather than replaces other infectious disease surveillance approaches based on compilation of individual diagnostic testing results. This method detects the presence of pathogens that enter the environment via human excreta (feces, urine, sputum, etc.). This approach is already used to detect and monitor poliovirus circulation in high-risk settings (*Coronavirus Disease (COVID-19): Environmental Surveillance*, n.d.-b), and monitor antimicrobial resistance (*Coronavirus Disease (COVID-19): Environmental Surveillance*, n.d.-b).

An example of ES is in Ghana, where it is used extensively to detect and monitor the circulation of polio. The purpose of ES is to provide early warning and additional evidence regarding the virus in circulation in the population, including its presence or absence, trends in concentrations, and variants of concern or interest (Environment, Climate Change and Health, 2022c). ES can help to inform decisions on interventions and help measure their effect by monitoring changes in the concentrations of the target pathogens in environmental samples which reflects changes in the number of infected people in the population.

Environmental Surveillance for Polio in Ghana

Poliomyelitis, also known as Polio, is a highly infectious viral disease that mainly affects children under five (WHO-*Poliomyelitis (Polio)*, 2019). It is a vaccine-preventable disease. Initial symptoms include fever, fatigue, headache, vomiting, and limb pain (WHO-*Poliomyelitis*, 2019). Most people infected with this virus do not experience symptoms. Polio spreads mainly through the fecal-oral route and can be transmitted from person-to-person contact (CDC-*What Is Polio*, 2023).

Since the launch of the Global Polio Eradication Initiative (GPEI), ES has been used to detect and monitor poliovirus in sewage samples (Sims & Kasprzyk-Hordern, 2020). Tremendous progress has been made in reducing the number of polio cases by more than ninety-nine percent. This is due to using ES to indicate where wild-type polio is still circulating in the population and then using this information to target vaccination campaigns (Asghar et al., 2014).

ES has been a critical strategy in Ghana's Polio Eradication efforts. Ghana has implemented an Integrated Disease Surveillance and Response (IDSR) strategy for priority diseases, conditions, and events since 2002 (Adokiya et al., 2015). In 2016, Ghana commenced its Polio Environmental Surveillance program at six sites in two regions. It has expanded to 14 sites in seven regions (*Wastewater Surveillance Programs for COVID-19 and Other Pathogens Led by African National Public Health Institutes | IANPHI | Atlanta GA*, 2022). The selection criteria included areas of high risk for polio transmission and sewer network availability. In the absence of a sewer network, acute flaccid paralysis (AFP) surveillance was used instead (*Wastewater Surveillance Programs for COVID-19 and Other Pathogens Led by African National Public Health Institutes | IANPHI | Atlanta GA*, 2022).

In 2019, circulating-vaccine-derived poliovirus type-2 (cVDPV2), a mutated strain of the original virus, began circulating in central Accra. Since then, cVDPV2 has been confirmed in 8 ES sites since the start of ES for Polio in Ghana (*Wastewater Surveillance Programs for COVID-19 and Other Pathogens Led by African National Public Health Institutes | IANPHI | Atlanta GA*, 2022). This project was mainly driven by the World Health Organization (WHO), the National Polio Laboratory, and the Ghana Health Service (GHS) (Odoom et al., 2021).

Environmental Surveillance across Africa

ES plays a valuable role in remote areas in low- and middle-income countries (LMIC), where access to diagnostic testing can be very limited. It has been used in countries like South Africa, Ghana, Nigeria, Egypt, and Kenya.

For example, Kenya has used ES to monitor polio across Nairobi (Zhou et al., 2020). This study obtained samples from the Motoine River, a latrine waste stream and two sewer conveyance lines in an urban neighborhood (Zhou et al., 2020).

In Northern Nigeria, clinical surveillance for AFP was supplemented with ES to monitor wild poliovirus (WPV) since 2011 (Hellmér et al., 2014). Its success led to the implementation of ES in other states and cities, including Lagos. Findings of ES have shown the presence of WPV, where AFP surveillance has failed to do so, indicating sufficient sensitivity (Hellmér et al., 2014).

Another example of ES is a study in South Africa that used ES to monitor the presence and circulation of various strains of Norovirus (NoV) in Vaal Dam (Mabasa et al., 2018). NoV was successfully detected, quantified, and genotyped in raw sewage and wastewater. This observation suggested the inefficient removal of NoVs during wastewater treatments (Mabasa et al., 2018). However, there is a lack of data from countries on the African continent on the prevalence and diversity of NoV circulating in the population (Mabasa et al., 2018).

South Africa has already adapted the use of ES for Polio to SARS-CoV-2. The country has created comprehensive SARS-COV-2 ES programs for the presence and concentrations of SARS-CoV-2 (Environment, Climate Change and Health, 2022b). In late 2021, ES aided in the early detection of the fourth wave of COVID-19 in Gauteng Province, South Africa (Environment, Climate Change and Health, 2022b).

SARS-CoV-2

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus (WHO, 2023). SARS-CoV-2 spreads from an infected person's mouth or nose in small liquid particles when they cough, sneeze, speak, sing, or breathe (WHO, 2023). Symptoms range from mild to moderate respiratory illness (CDC, 2023). The most common symptoms are fever, cough, fatigue, and loss of taste and smell.

COVID-19 is a vaccine-preventable disease and there are three main types of COVID-19 vaccines. The first type is the mRNA by Pfizer-BioNTech and Moderna. Pfizer-BioNTech is administered to people age 16 or older. Infants, children and teenagers ages 6 months to 15 years are also approved to receive this vaccine (Katella, 2023). The second type is the protein subunit vaccine (Novavax). People 12 or older are eligible to receive this vaccine. Novavax, Pfizer-BioNTech and Moderna all require two doses (Katella, 2023). The third type of vaccine is the viral vector by Johnson & Johnson's Janssen which requires only one dose. This vaccine is available to peoples 18 and older (Katella, 2023). COVAX was established during the pandemic to increase access to vaccines by accelerating distribution to LMICs. This was coordinated by the WHO, Gavi, the Vaccine Alliance and the Coalition for Epidemic Preparedness. Innovations (CEPI) ("COVAX Explained," 2022).

Since 2020, countries such as Italy, China, the United States, and countries in Latin America and the Caribbean have published or demonstrated proof of concept of ES for SARS-COV-2 by detecting SARS-CoV-2 in environmental samples (WHO, 2022). ES has augmented routine diagnostic testing, especially when diagnostic testing capabilities have been overwhelmed (Environment, Climate Change and Health, 2022d). ES can be used to help target vaccination campaigns to geographic regions and populations with high COVID-19 prevalence.

Since April 25th, 2020, Ghana has observed a decline in COVID-19 cases (Kenu, E. et al., 2020). This is partly due to its ES program, control measures, and protocols for managing suspected or confirmed cases. On December 2019, COVID-19 surveillance activities were initiated in Ghana; however, there is still little evidence about the operations of Ghana´s COVID-19 surveillance system since its inception (Awekeya et al., 2020).

In South Africa, a study to determine the spatial and temporal trends of SARS-CoV-2 was conducted. Wastewater samples in 23 wastewater treatment plants (WWTPs) in the City of Cape Town were collected over a six-week period. Samples were collected once a week on Mondays with sample collection occurring at a similar time each week. The result findings showed that there was an overall decrease in the amount of detected viral RNA over the 6-week study period, associated with a declining number of newly identified COVID-19 cases (Street et al., 2021c). Temporal trends in SARS-CoV-2 concentrations in wastewater were consistent with temporal trends in the number of COVID-19 cases reported in the population within the wastewater catchment area (Street et al., 2021c).

Vibrio cholera

Cholera is an acute diarrheal infection caused by the bacterium *Vibrio cholera* (WHO, 2023). Cholera is spread by eating food or drinking water contaminated by the feces of an infected person (New York Dept. of Health, 2017). It takes between 12 hours and five days for a person to show symptoms after ingesting contaminated food or water. However, most people infected with *Vibrio cholera* do not develop symptoms (WHO, 2023). Cholera surveillance is monitored by individual countries that confirm cases by identifying *Vibrio cholera* in stool samples from affected patients. Most of those who develop symptoms experience mild to moderate symptoms (WHO, 2023). In the early stages, vomiting, thirst, and profuse watery diarrhea, sometimes described as “rice-water stools,” are experienced (CDC, 2023). The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water (WHO, 2023).

In places experiencing a humanitarian crisis, the disruption of water and sanitation systems, or the displacement of populations to inadequate and overcrowded camps – can increase the risk of cholera transmission (WHO, 2023). There is a lack of data on cholera due to multiple other enteric disease outbreaks occurring simultaneously that strain the overall response capacity of public health authorities to conduct cholera diagnostic testing and surveillance (Cholera – Global Situation, 16 December 2022 - World, 2022). In Uganda, cholera is a major public health problem in areas surrounding the African Great Lake basin. A prospective study conducted between February 2016 and February 2019 looked at 28 surface water source sites and tested each of them for the presence of *V. cholerae* species using PCR. From the 322 water samples tested, only 10.8% were positive (Bwire et al., 2018). This finding showed that *Vibrio cholera* can be detected in environmental samples. However, it is not an example of ES.

There are currently three WHO pre-qualified oral cholera vaccines (Dukoral, Shanchol, Euvichol) which all require two doses (WHO, 2023). Dukoral can be administered to individuals over the age of two years and is almost mostly used by travelers. Shanchol and Euvichol provide individuals with protection for three years and is mainly used in mass vaccination champaigns (WHO, 2023).

Rotavirus

Rotaviruses are a double-stranded RNA virus of the genus Reoviridae (WHO, 2023). The most common symptoms of rotavirus are vomiting, fever, abdominal pain, and severe dehydrating diarrhea in children under five years old (WHO, 2023; CDC, 2023). Nearly half the diarrhea hospitalizations of Ghanaian children under five are caused by rotavirus (PATH, 2013). The fecal-oral route is the primary transmission mode, usually through direct contact between people or indirectly via contaminated fomites (WHO, 2023). Sentinel surveillance, which is the monitoring of rate of occurrence of specific conditions to assess the stability or change in health levels of a population. It has been widely used worldwide to estimate the prevalence of circulation and hospitalizations for Rotavirus (*Surveillance Guide for Vaccine-Preventable Diseases in the WHO South-East Asia Region*, 2017). Rotavirus is a vaccine-preventable disease with four vaccines available internationally on the market. These four oral live, attenuated vaccines (Rotarix, RotaTeq, Rotavac and RotaSiil) are not highly effective in low-middle income countries due to possible concurrence of other enteric infections (WHO, 2023).

Salmonella Typhi

Salmonella Typhi is a gram-negative bacterium that causes typhoid fever (WHO-Typhoid, 2018). It is commonly transmitted through drinking water or food contaminated with the feces of an infected person (Information for Healthcare Professionals | Typhoid Fever | *CDC*, 2023). *Salmonella* Typhi is a systemic infection that affects multiple organs in the body. Severe cases of typhoid can lead to serious complications or even death (WHO-Typhoid, 2018). The Typhoid Fever Surveillance Program in Africa (TSAP) collected blood culture data between 2010 and 2014 and generated typhoid incidence rates in Sub-Saharan African countries, including Ghana. During this time, stool samples and oropharyngeal swabs were also taken and sent to laboratories run by TSAP for confirmation. South Africa, Tanzania, Ethiopia and Burkina Faso have all integrated the TSAP into their surveillance infrastructure. Detection of *S*. Typhi in environmental samples has recently been used to investigate community transmission and risk factors for typhoid fever in Democratic Republic of Congo and Nigeria (Cb et al., 2021). *Salmonella* Typhi is a vaccine-preventable disease with several types of vaccines on the international market. The first type, Ty21, is an oral vaccine which is available as a capsule or a liquid suspension. The other two are Vi polysaccharide-containing vaccines which are administered subcutaneously or intramuscularly (*Typhoid Fever*, WHO, 2023 n.d.).

Introduction

Infectious Disease Surveillance

Infectious diseases are caused by microorganisms such as bacteria, viruses, fungi, or parasites (WHO EMRO| Infectious Diseases| Health Topics, n.d). The rapid spread of infectious diseases across borders due to globalization continuously threatens global public health. These diseases are spread from person-to person, through contact with animals or by consuming food or water that is contaminated (WHO EMRO| Infectious Diseases| Health Topics, n.d). Symptoms can range in severity from mild to life-threatening and can have a significant impact on public health. LMICs are often disproportionately affected by infectious diseases due to various factors such as inadequate healthcare systems, limited access to clean water and sanitation, and economic hardships (Mosisa, D. et.al, 2021).

Therefore, infectious disease surveillance is crucial for monitoring trends in highly infectious diseases and is a foundation of public health and public health response. It has the capability to estimate the current burden of infectious disease within a community, to evaluate spatial and temporal trends of diseases, and to provide early detection of outbreaks (Murray J, Cohen AL., 2016). This makes it a powerful epidemiological tool to inform response planning and helping to reduce the impact of transmission. Surveillance approaches vary based on the need and depend on a variety of factors such as the available resources, the setting and the pathogens of interests (Holmes KK, Bertozzi S, Bloom BR, et al., 2017).

Generally, active and passive surveillance are the two main categories of surveillance. Passive surveillance is an inexpensive strategy where a reporting system is established, and reports are received from hospitals, clinics and public health institutions concerning diseases and illnesses. However, a disadvantage is that a lack of reporting can lead to underestimation of the true disease burden due to incompleteness in reporting data and variability in quality amongst providers (Sims N, Kasprzyk-Hordern B., 2020).

The World Health Organization defines active surveillance as the collection of case study information as a continuous pre-organized process (WHO, 2023). Simply put, this surveillance system requires health departments and public health workers to actively request and pursue information about diseases and health conditions (Murray J, et.al, 2016). An advantage of active surveillance is that it can validate passive surveillance reports, ensure complete reporting of events, and supplement epidemiologic investigations.

Environmental Surveillance

Environmental surveillance (ES) involves the systematic collection of environmental samples that are contaminated with human feces, laboratory analysis for target pathogens, and subsequent interpretation of the results (Wang Y, Moe CL, 2020). It is a powerful tool used to detect the presence and circulation of pathogens. Unlike clinical surveillance which mostly identifies symptomatic cases, ES is able to anonymously and noninvasively measure asymptomatic and undocumented cases within a population through pathogen detection in human sewage samples (Hovi T et al., 2012). This is a very useful method in LMIC countries where clinical testing of individuals may be limited or delayed due to cost and availability of testing facilities. However, with ES early warnings of increasing or decreasing trends for pathogens and information about variants of concern or interest in a community are more readily provided. ES can help inform public health authorities and guide implementation of necessary control measures.

WHO guidelines warn that the following circumstances can make ES difficult: 1) if a formal sewer network is not present, or 2) if the routes of wastewater are not well known. In these settings, selecting appropriate sampling locations will be difficult and may not be representative of the source population (WHO, 2003). This is a disadvantage that in theory limits the use of ES in many villages in LMIC where there are informal sewer networks or only on-site sanitation systems.

The limited access and capacity for clinical diagnostic testing and the ability to do infectious disease surveillance in rural communities in LMIC makes it difficult for public health authorities to understand the true burden of certain infectious diseases and how to respond effectively. In Ghana, the reported prevalence of COVID in various regions has been influenced by the state of the healthcare infrastructure, the ability to conduct regular diagnostic testing, and the capacity of healthcare workers (Kavanagh et al., 2020; Martinez-Alvarez et al., 2020).

A recent successful proof-of-concept study of ES for COVID-19 in low-resource settings in Greater Accra, Ghana showed that SARS-CoV-2 RNA could be detected in ES samples taken from sewage and public toilets. During the time of this study, there were reported COVID-19 cases (Yakubu H., Center for Global Safe WASH, 2023). This finding demonstrated the role ES can play in identifying the presence of SARS-CoV-2 infections in low-income urban settings with limited sewerage infrastructure. The success of this study has led to the development of an integrated an ES system to help public health authorities make critical decisions.

In the study described here, this same ES approach was applied to institutional settings in two districts in the Northern Region of Ghana to answer the following questions:

Research Questions

There have been no reported COVID-19 cases in the Northern Region of Ghana since the beginning of the pandemic in 2020. Our research questions are:

1) Can we apply ES to detect possible COVID-19 cases in Northern Ghana?
2) Can we apply ES to detect other vaccine-preventable diseases in Northern Ghana?

Objectives

This thesis has two overall objectives:

1. Develop a strategy to monitor SARS-CoV-2, *Salmonella* Typhi, Rotavirus, and *Vibrio cholera* using ES in low-income semi-rural settings.
2. Conduct institutional-level ES in Primary, Junior High, Senior High schools and College to determine the feasibility and value of ES as a tool to track SARS-CoV-2, *Salmonella* Typhi, Rotavirus, and *Vibrio cholera* infection among children and adolescents.

    2a: Identify whether or not genetic markers for the four target organisms were detected in wastewater samples from septic tanks and latrines at multiple schools in two districts in Northern Ghana.

    2b: Examine spatial and temporal patterns of pathogen detection at the study schools.

This project was a partnership between the Ghana Health Service (GHS) and the Center of Global Safe WASH (CGSW) at Emory University and was funded by a grant from the Rockefeller Foundation. Ghana Health Service (GHS) is the agency responsible for the implementation of the health policy and administration of health services for the government of Ghana.

Laboratory analyses for this study were conducted by the Council for Scientific and Industrial Research-Water Research Institute (CSIR-WRI) which is mandated to conduct research on water and related resources in Ghana. It is the leading institution in wastewater assessment and monitoring of pathogens over the past 20 years. The Biomedical and Public Health Unit (BPHRU) of the institute is one of the accredited labs in Ghana to conduct COVID-19 RT-PCR diagnostic testing. Thanks in part to a recent investment by the Bill & Melinda Gates Foundation, CSIR-WRI has state-of-the-art equipment, facilities, and human resources to successfully conduct this expanded ES program. The Microbiology lab and BPHRU conducted the environmental sampling and laboratory analyses in the recent proof-of-concept study of ES for COVID-19 in Accra.

Training, Research and Networking for Development (TREND) is a national NGO with over 25 years of experience providing services to support WASH services delivery within Ghana and sub-Saharan Africa. Capacity areas include community sensitization, community management, process facilitation, WASH capacity building, mapping and social research, among others. TREND supported the recent proof-of-concept study through extensive stakeholder engagement, community entry, and study area mapping.

Study Sites

Map of Ghana highlighting the Northern Region

Figure 1. The Northern Region is the second largest region in Ghana. It is about 26.5 thousand square kilometers and has an estimated population of 2,310,939 (*Northern (Region, Ghana) - Population Statistics, Charts, Map and Location*, n.d.). This region is mostly made up of households involved in the agriculture and fishing sectors.

<u>Location of Bimbila and Chamba within Nanumba North District</u>

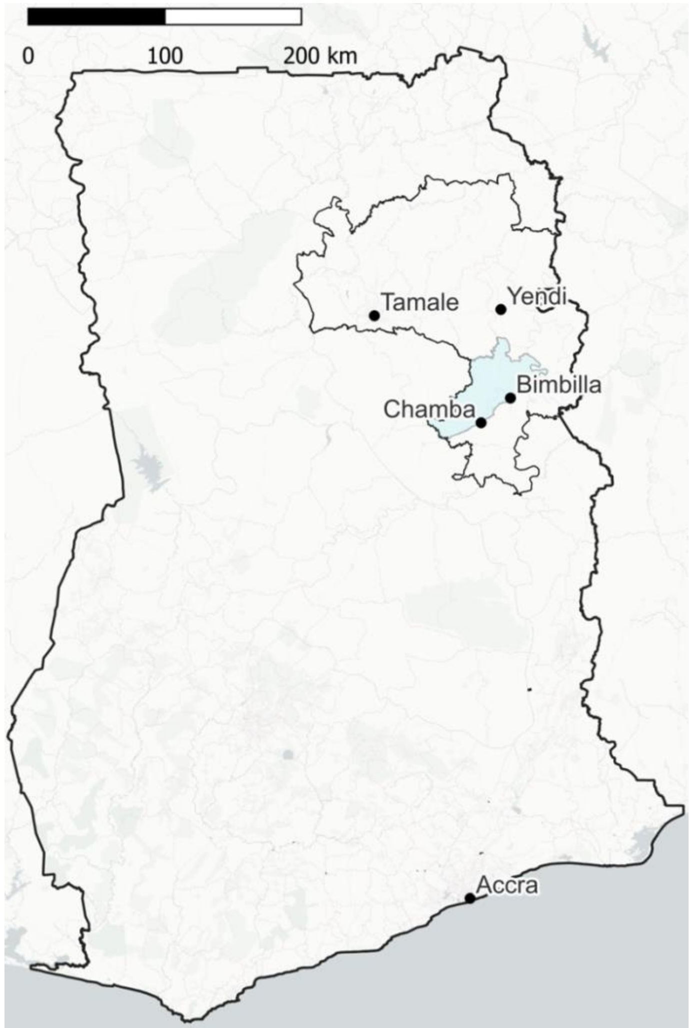


Figure 2. Within the Northern Region is Nanumba North District Municipality.

The Nanumba North Municipal is one of the 261 Metropolitan, Municipal and District Assemblies (MMDAs) in Ghana, and forms part of the 16 MMDAs in the Northern Region. The district capital of Nanumba North is Bimbila. According to a 2021 population and housing census, the population of the Municipality is 188,680 (*Ghana Districts: A Repository of All Local Assemblies in Ghana*, n.d.). It shares boundaries with five other districts, Mion District being one. The Nanumba North District is predominantly rural. Chamba, the second largest community is about 28.8km west of Bimbila and has an estimated population more than 4,826(*2021 Composite Budget- Northern Region | Ministry of Finance | Ghana*, n.d.). The District has an agrarian economy, with the agriculture, fishing, and farming sectors employing a large proportion of the population (*2021 Composite Budget- Northern Region | Ministry of Finance | Ghana*, n.d.).

Location of Sambu and Sang within Mion District

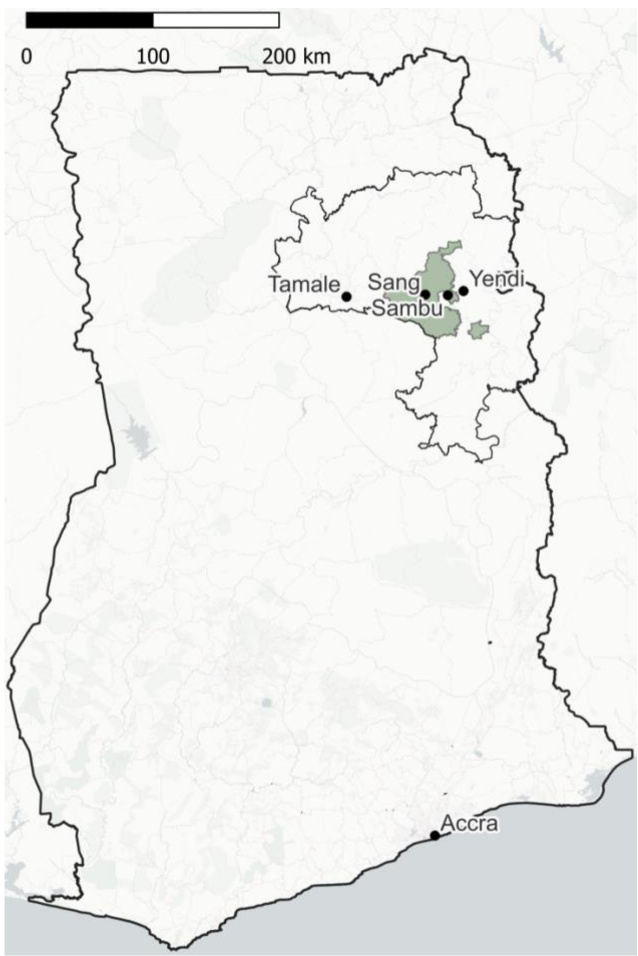


Figure 3. Mion District Assembly is located 537 km from Accra and covers an area of 2714.1 square kilometers. The Mion District is one of the 261 Metropolitan, Municipal and District

Assemblies (MMDAs) in Ghana, and forms part of the 16 MMDAs in the Northern Region. The capital of Mion is Sang. According to a 2021 population and housing census, the population of the District is 94,930 (*Ghana Districts: A Repository of All Local Assemblies in Ghana*, n.d.-b). Like Nanumba North, this district is rural, and majority of the workforce here are engaged in agricultural, fishing, and farming activities.

Methods

Site Selection and Sample Collection

Sampling sites were chosen to capture a larger population and not individual households, therefore places like schools were ideal. Wastewater and fecal sludge samples were collected from septic tanks and latrines at schools in four communities on a weekly basis. The communities were selected based on their history of no reported cases of COVID since the beginning of the pandemic. Nanumba and Mion District were chosen based on their semi-rural setting. Samples were collected between September - November 2022.

Laboratory analysis

Solid Fecal Sludge Samples

Samples of fecal sludge solids were processed using the protocol described by Boehm *et al*. 2021.

Liquid Wastewater Samples

Concentration of Microorganisms

Nanotrap Magnetic Virus Particles and Nanotrap Enhancement Reagent 1, Ceres Nanosciences, Inc.; SKU# 44202 and Nanotrap® Enhancement Reagent 1 (ER1): Ceres

Nanosciences, Inc.; SKU# 10111 were used to capture and concentrate the target pathogens from 10mL wastewater samples as described by Cavallo *et al.*, 2022 [https://www.protocols.io/view/manual-nanotrap-concentration-and-rna-extraction-f-8epv5961ng1b/v1].

Total Nucleic Acid Extraction Procedure

Total nucleic acid was extracted from solid and liquid wastewater samples using the Applied Biosystems™ MagMAX™ Microbiome Lysis Solution (ThermoFisher Cat# A42361) and Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher) following the manufacturer's instructions and as described in Cavallo *et al.,* 2022.

PCR Protocols

Genetic markers for the target pathogens were detected by Realtime RT-PCR (SARS-CoV-2 and rotavirus) and Realtime PCR (*S*. Typhi and *V. cholera*) using primers and probes listed in Table 1. The primers and probes for SARS-CoV-2 detection are described in Svezia *et al.*, 2022 [https://www.protocols.io/view/singleplex-qpcr-for-sars-cov-2-n1-and-brsv-bp2l61kqkvqe/v1].

Table 1. Triplex Realtime PCR Primer and Probe Sequence for each target organism

Target/Name	Component	Sequence
Rotavirus (JVK)	Forward Primer	5’-CAGTGGTTGATGCTCAAGATGGA-3’
	Reverse Primer	5’-TCATTGTAATCATATTGAATACCCA-3’
	Probe (FAM)	ACAACTGCAGCTTCAAAAGAAGWGT (5' FAM/ZEN/3' IBFQ)
Salmonella Typhi (tviB)	Forward Primer	5’-TGT GGT AAA GGA ACT CGG TAA A-3’
	Reverse Primer	5’-GAC TTC CGA TAC CGG GAT AAT G-3’
	Probe (HEX)	TGGATGCCGAAGAGGTAAGACGAGA (5’ HEX/ZEN/3’ IBFQ)
Vibrio cholera(hlyA)	Forward Primer	5’-CGCTTTATTGTTCGATGCGTTA-3’
	Reverse Primer	5’-ACTCGGTTATCGTCAGTTTGG-3’
	Probe (Cy5)	AATCTTGGGCAATCGC (5’ Cy5/ZEN/3’ IBFQ)

Study Location 1: Nanumba North District

In Nanumba North District, wastewater and fecal sludge samples were collected from five schools in Bimbila and four schools in Chamba and a residential college (Table 2). Students in the Primary schools are between six and twelve years old. In the Junior High school, students are between the age range of 13-15 years and in the Senior High school, students are between the age of 16-18 years. The Primary and Junior High Schools are day schools. The Senior High Schools and the E.P College of Education are residential campuses.

Table 2. Nanumba North District School Sampling Sites

Category	Bimbila Cluster 1 Basic Schools		Age range (years)
	Jilo M/A Primary School "A"	Boys 276 Girls 215	6-12
	Jilo M/A Junior High School "A"	Boys 68 Girls 74	13-15
Schools	**Bimbila Cluster 2 Basic Schools**		
	Our Lady of Peace Primary School	Boys 455 Girls 445	6-12
	Our Lady of Peace Junior High School	Boys 240 Girls 228	13-15
	Bimbila Senior High School	Boys 445 Girls 596	16-18
	Evangelical Presbyterian College of Education		
College	Main Female Hall Annex Female Hall	250 students 158 students	16-18
	Galevo	Not available	16-18
	Nkrumah Hall	Not available	16-18
	Dassana Hall	Not available	16-18
	Adjanku Hall	Not available	16-18
	Chamba Cluster 1 Basic Schools		
	Chamba Primary School “A”	Boys 133 Girls 99	
	Chamba Primary School “B”	Not available	6-12
	Chamba Junior High “A”	Not available	

	Chamba Junior High "B"	Boys 111 Girls 73	13-15

Table 3. Sample collection sites in Bimbila and Chamba

Bimbila Town		
Sites	Number of sites	Number of Samples collected
Latrines of Primary and Junior schools	4	16
Septic tank in Bimbila Senior High School	2	5
Latrine in Bimbila Senior High School	2	6
Latrines of residential halls in E.P. College of Education	4	16
Septic tanks of residential halls in E.P. College of Education	2	9
Chamba Town		
Latrines of primary and junior schools	4	16

Study Location 2: Mion District

In Mion District, wastewater and fecal sludge samples were collected from three schools in Sambu and seven schools in Sang (Table 4). Students in the Primary schools are between six and twelve years old. In the Junior High school, students are between the age range of 13-15 years and in the Senior High school, students are between the age of 16-18 years. The Primary, Junior High and Senior High Schools are day schools.

Table 4. Mion District School Sampling Site Population Figures

School	Sambu Town	Age range (years)
Sambu Islamic Primary school	Boys 312 Girls 220	6-12
Sambu Girls Model Junior High School	Girls 45	13-15
Sambu Islamic Junior High School	Boys 82 Girls 17	13-15
	Sang Town	
Sang Girls Model Junior High School	Girls 113	
Sang Islamic Primary School	Boys 403 Girls 298	6-12
Sang Islamic Junior High School	Boys 154 Girls 44	13-15
Sang Zakaria Junior High School	Boys 115 Girls 72	13-15
Sang St. Anthony Primary School	Boys 144 Girls 127	6-12
Sang Zakaria Primary School	Boys 260 Girls 234	6-12
Sang Community Senior High Day School	Not available	16-18

Table 5. Sample collection sites in Sambu and Sang

Sites	Number of sites	Number of Samples collected
Latrines of Primary and Junior Schools	3	22
Latrines of Primary and Junior Schools	5	20
Septic tanks of residential Schools	1	4

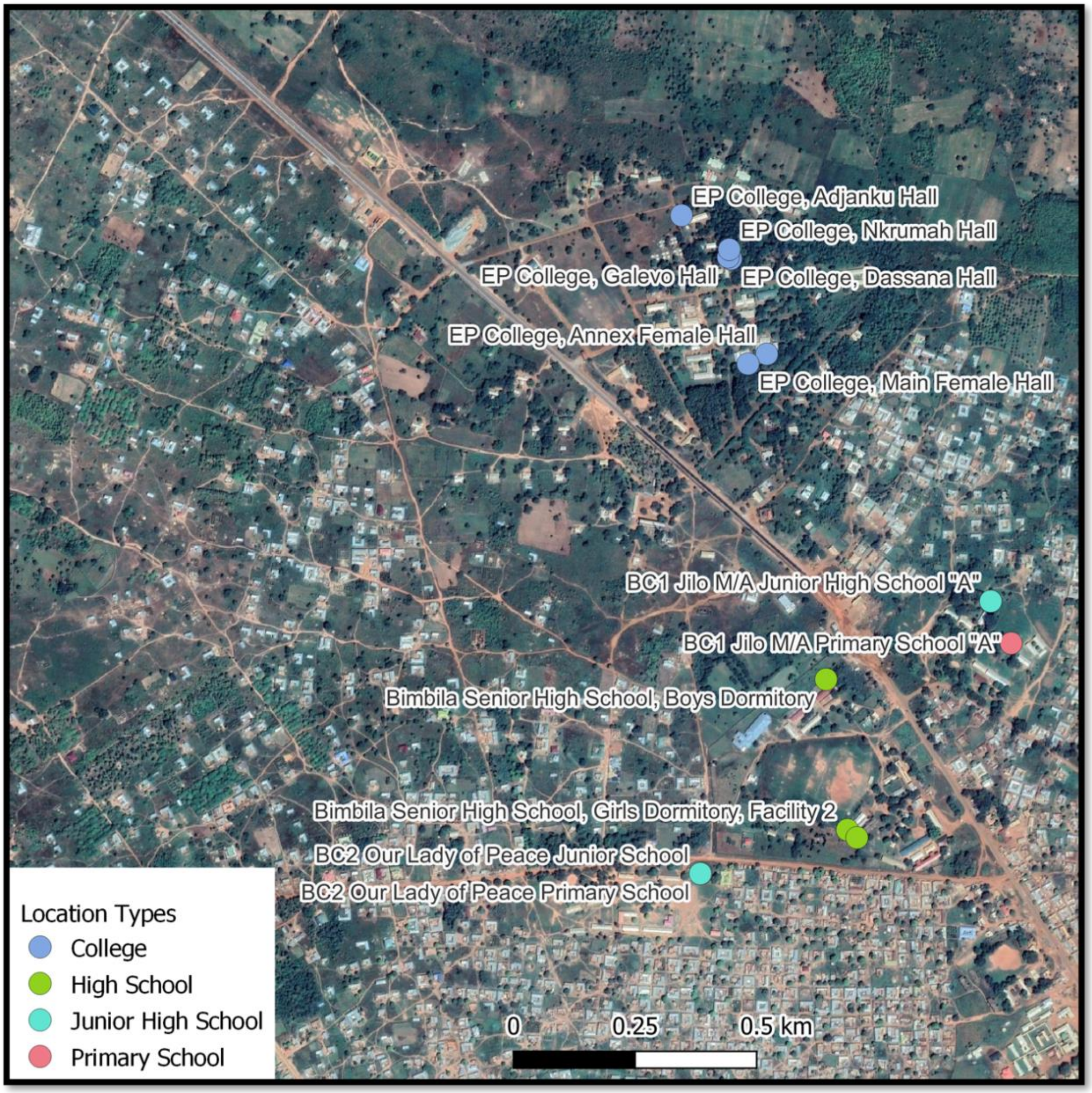


Figure 4. Bimbila Sample Points in Nanumba North District (Primary Schools, Junior High Schools, and residential halls).

An aerial view of the six different sampling points in Bimbila is shown in Figure 4. There were two primary schools (Bimbila Cluster 1 Jilo M/A Primary School “A”, Bimbila Cluster 1

Our Lady of Peace Primary School), six Residential Halls from the Evangelical Presbyterian College of Education, two Junior High Schools (Bimbila Cluster 2 Jilo M/A Junior School "A" and Bimbila Cluster 2 Our Lady of Peace Junior School).

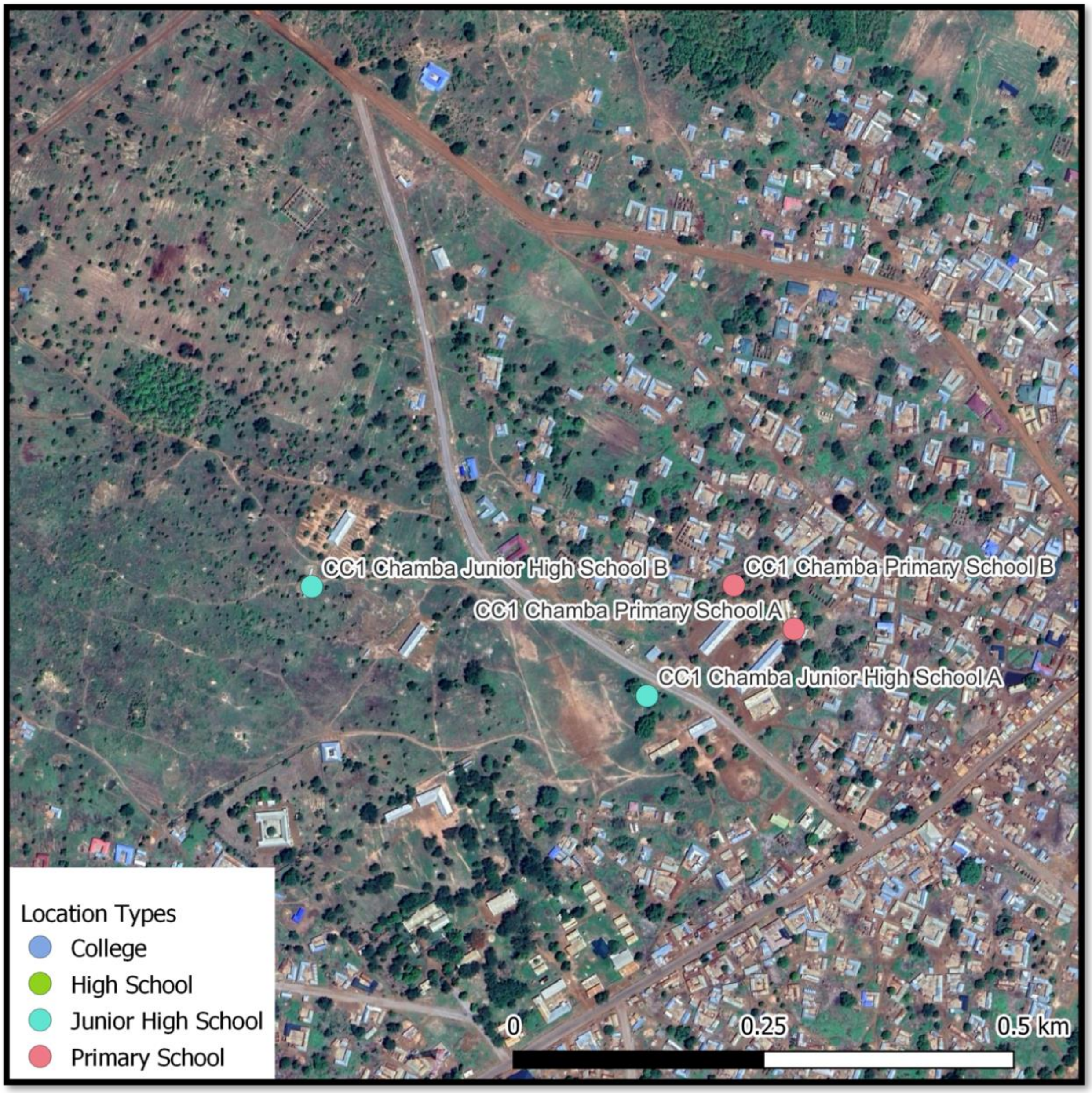


Figure 5. Chamba Sample Points in Nanumba North District (Primary Schools, Junior High Schools).

An aerial view of the four different sampling points in Bimbila is shown in Figure 5. There were two primary schools (Chamba Cluster 1 Primary School A, Chamba Cluster 1 Primary School B) and two junior schools (Chamba Cluster 1 Junior School A, Chamba Cluster 1 Junior School B).

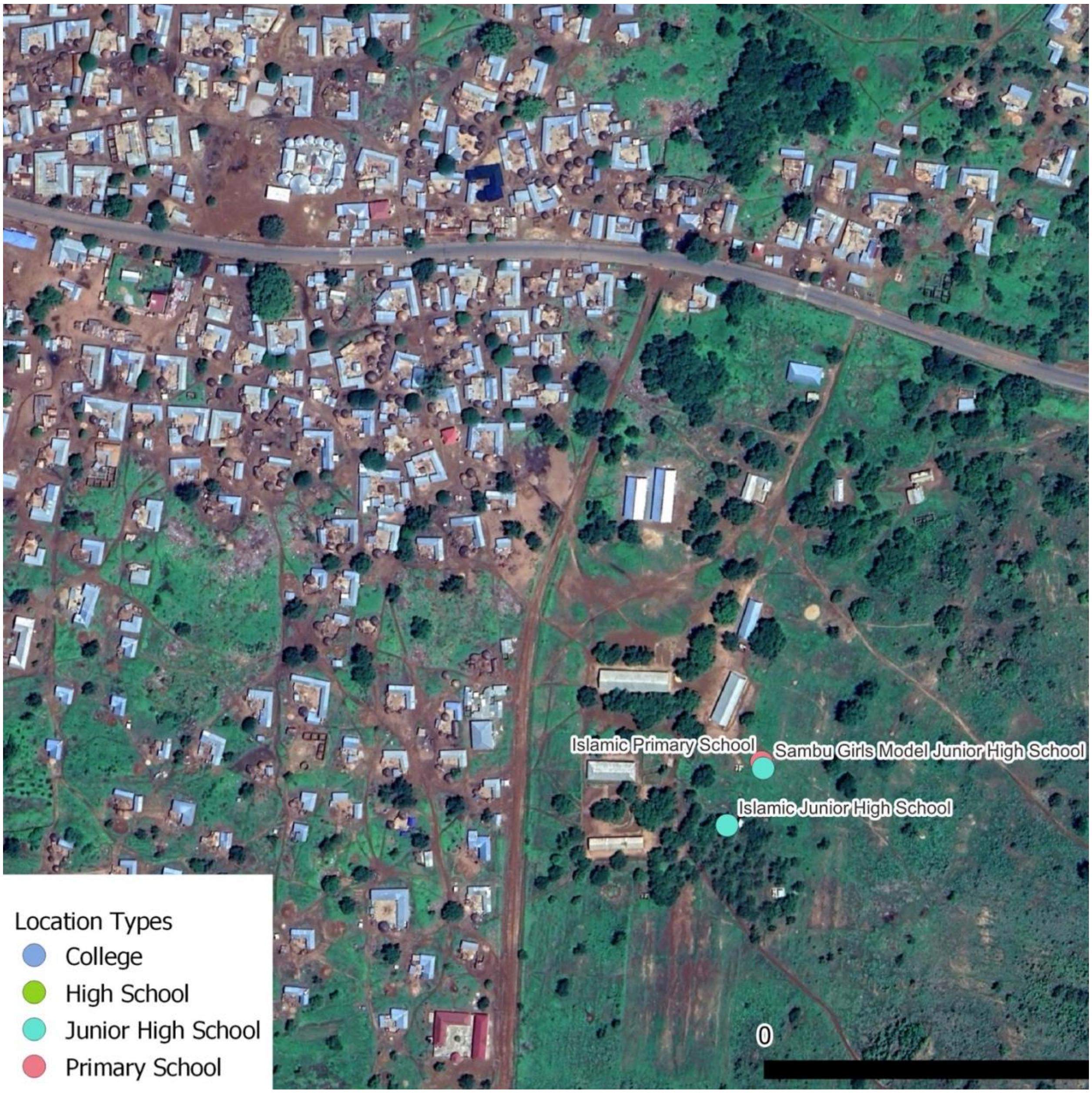


Figure 6. Sambu Sample Points in Mion District (Primary Schools, Junior High Schools).

An aerial view of the three different sampling points in Mion is shown in Figure 6. There were two primary schools and one Junior High School.

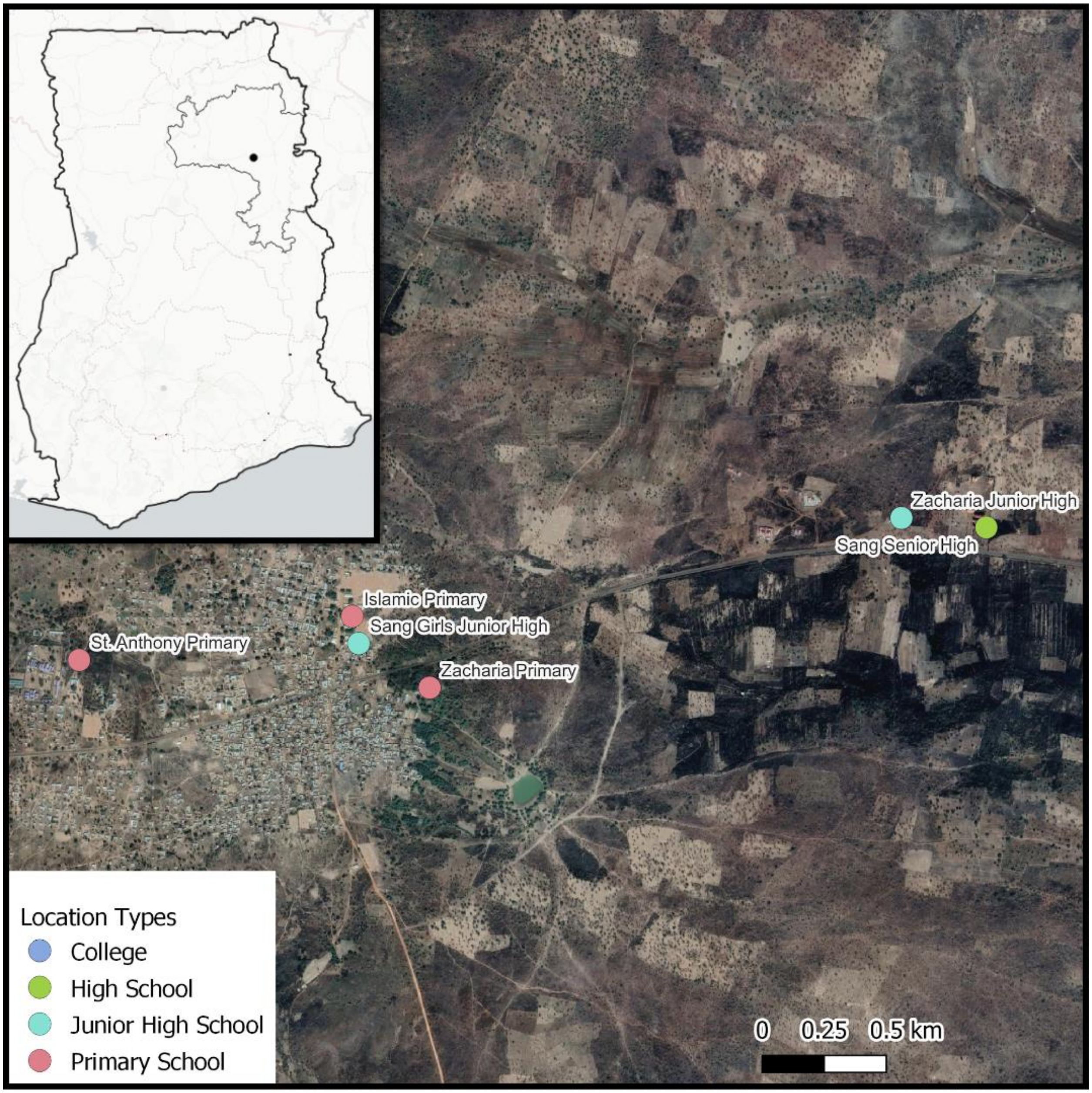


Figure 7. Sang Sample Points in Mion District (Primary Schools, Junior High Schools).

An aerial view of the six different sampling points in Mion is shown in Figure 7. There were three Primary Schools two Junior Schools and one Senior High School.

Data analysis

The laboratory data was compiled in MS Excel which was imported into RStudio and analyzed by creating summary statistics for each of the four target organisms (SARS-CoV-2, *Salmonella* Typhi, *Vibrio cholera* and Rotavirus) in the Nanumba North Municipality District and the Mion District.

The dataset contained the following types of data:

Dichotomous data: The PCR results were categorized as “positive” or “negative” and indicated the presence or absence of pathogen detection from a sample. A bar graph was used to summarize the percent of positive samples in Nanumba and Mion District respectively.

Continuous data: The PCR data were also reported as Ct values. The average of duplicate assay wells is reported. Box and whisker plots were used to compare the strength of the PCR signal across the four target pathogens. The strength of the Ct value is a surrogate for pathogen concentration in the environmental sample. Estimates of pathogen concentration in genome copies per liter of wastewater were not available at the time of this data analysis.

Results

Study Location 1. Nanumba North District

A total of 68 environmental samples were collected from septic tanks and latrines at nine schools plus the E.P. College of Education in the Nanumba North District from September 27th to October 20th. Each school was sampled typically four times during the study period. All samples were analyzed for four target pathogens by PCR, and the results are presented in Figure 8.

<u>Results from nine study schools located in Bimbila and Chamba</u>

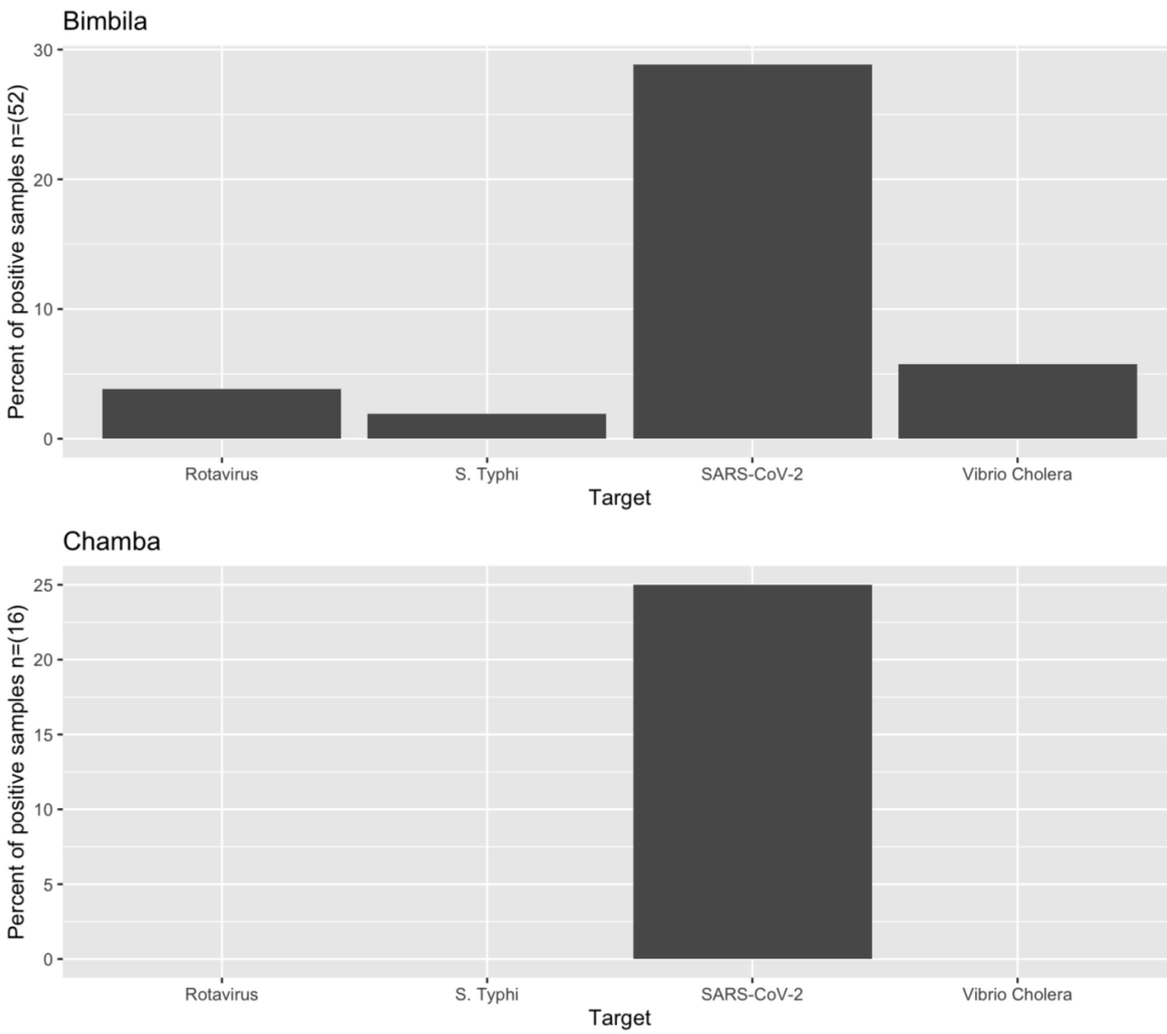


Figure 8. Percent of environmental samples that were positive for each target organism (SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera*) in Bimbila (N=52) and Chamba (N=16).

The percentages of PCR-positive environmental samples for each target organism (SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera*) across schools in Bimbila and Chamba are shown in Figure 8. SARS-CoV-2 was the most frequently detected target organism (28.8%) in the environmental samples from Bimbila schools. The second most commonly detected target organism (5.7%) in the environmental samples was *Vibrio cholera*, followed by Rotavirus (3.8%) and *Salmonella* Typhi (1.9%).

In Chamba, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera* were not detected in any of the 16 environmental samples. However, 25% of the environmental samples were positive for SARS-CoV-2 (Figure 8).

Table 6. Detection of target pathogens in environmental samples (n=27) from Bimbila Cluster 1& Cluster 2 Basic Schools and the Bimbila Senior High School, September 27th to October 20th, 2022.

School	Number of Samples	% of samples that were PCR-positive			
		Rotavirus	*Salmonella* Typhi	SARS-CoV-2	*Vibrio cholera*
Bimbila Cluster 1 Basic Schools					
Jilo M/A Primary School "A"	4	0	25	25	0
Jilo M/A Junior High School "A"	4	0	0	50	0
Bimbila Cluster 2 Basic Schools					
Our Lady of Peace Primary School	4	0	0	25	0
Our Lady of Peace Junior High School	4	0	0	0	0
Bimbila Senior High School					
Bimbila Senior High School, Boys Dormitory	3	0	0	66	0
Bimbila Senior High School, Girls Dormitory, Facility 1	3	0	0	0	33
Bimbila Senior High School, Girls Dormitory, Facility 2	5	0	0	0	40

The detection of SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera* in Bimbila schools is shown by school and sampling site in Table 6. SARS-CoV-2 was more frequently detected in all schools compared to Rotavirus, *Salmonella* Typhi, and *Vibrio cholera.* The second most frequently detected pathogen was *Vibrio cholera* (73%) in the Bimbila Senior High School, Girls Dormitory, Facility 1 (33%) and the Girls Dormitory, Facility 2 (40%) respectively. There was a higher percentage of PCR-positive samples from the Senior High School compared to the Primary and Junior Schools in Bimbila. However, all the environmental samples from the Primary, Junior, and Senior High School were negative for Rotavirus.

Table 7. Detection of target pathogens in environmental samples from Female and Male Residential Halls at the E.P College of Education (n=25) in Bimbila, September 27th to October 20th, 2022.

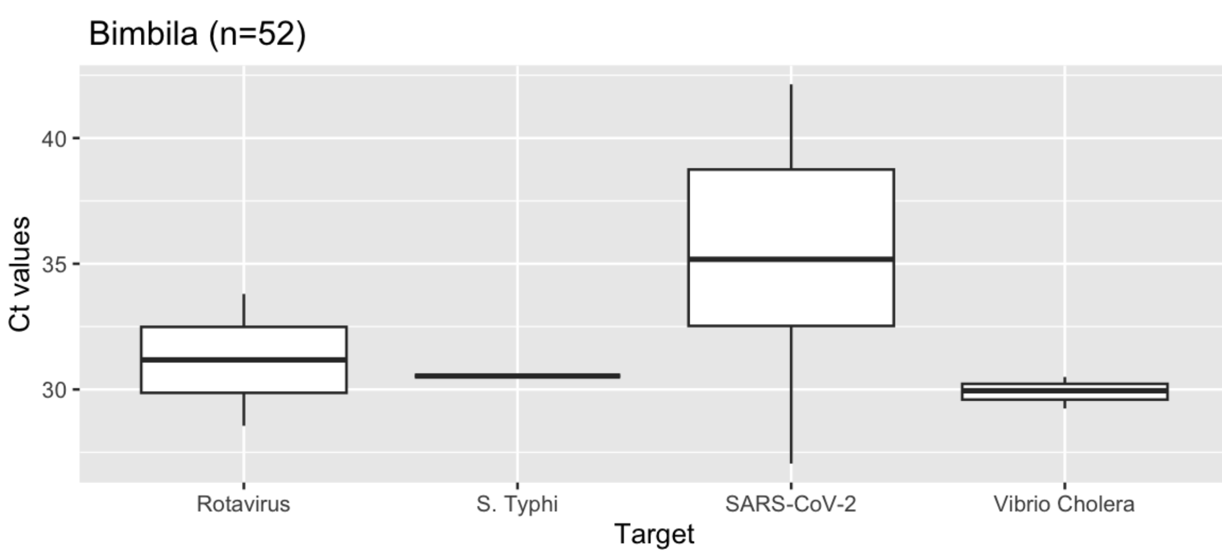
College Residential Halls	Number of Samples	% of samples that were PCR-positive			
		Rotavirus	*Salmonella* Typhi	SARS-CoV-2	*Vibrio cholera*
E.P College of Education – Female Halls					
Annex Hall	5	20	0	40	0
Main Hall	4	25	0	50	0
E.P College of Education – Male Halls					
Adjanku Hall	4	0	0	0	0
Dassana Hall	4	0	0	25	0
Galevo Hall	4	0	0	50	0
Nkrumah Hall	4	0	0	50	0

The detection of SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera* in Residential Halls at the E.P College of Education is shown in Table 7. The most frequently detected pathogen in the Female and Male Halls was SARS-CoV-2. All the environmental samples from the residential halls at the College were negative for *Salmonella* Typhi and *Vibrio cholera*. In the Male Halls, all the environmental samples were negative for Rotavirus. However, 45% of environmental samples from the Female Halls – Annex Hall (20%) and Main Hall (25%) were positive for Rotavirus.

Table 8. Detection of target pathogens in environmental samples (n=16) from Chamba Cluster 1 Basic Schools, September 27th to October 20th, 2022.

School	Number of Samples	% of samples that were PCR-positive			
		Rotavirus	*Salmonella* Typhi	SARS-CoV-2	*Vibrio cholera*
Chamba Cluster 1 Basic Schools:					
Chamba Primary School “A”	4	0	0	0	0
Chamba Junior High School "A"	4	0	0	0	0
Chamba Primary School “B”	4	0	0	25	0
Chamba Junior High School “B”	4	0	0	75	0

The detection of SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera* in Chamba schools is shown by school and sampling site in Table 8. SARS-CoV-2 was the only pathogen detected in Chamba schools. In Chamba Primary School “B”, 25% of environmental samples were PCR-positive. In Chamba Junior High School “B”,75% of environmental samples were PCR-positive. However, environmental samples from all schools in Chamba were negative for Rotavirus, *Salmonella* Typhi, SARS-CoV-2, and *Vibrio cholera*.



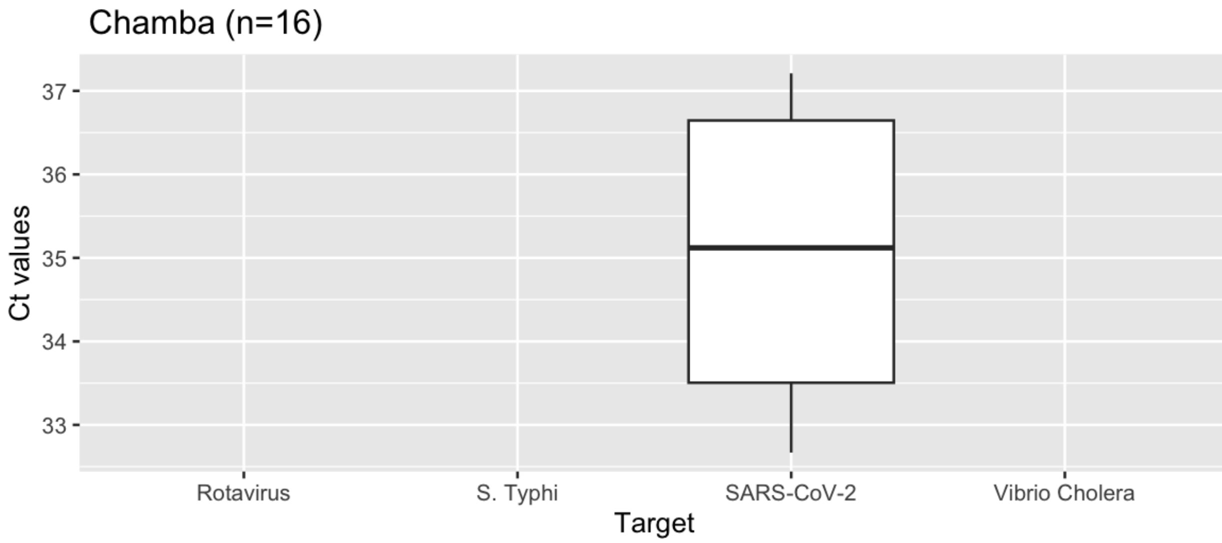


Figure 9. Distribution of Ct values for four pathogen targets in 68 environmental samples from ten schools in Bimbila and Chamba, October 26th to November 16th, 2022.

A comparison of the distribution and variability of Ct values for all four target pathogens (SARS-CoV-2, Rotavirus, *Vibrio cholera,* and *Salmonella* Typhi) detected in school samples from Bimbila and Chamba is shown in Figure 9. In Bimbila, the strongest PCR signal was for *Vibrio cholera* which has the lowest median Ct value (30). SARS-CoV-2 had the greatest variability in Ct values of all the four pathogens and the highest median Ct value of 38. It also showed a normal distribution of Ct values. In Chamba, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera* were not detected from samples collected from either septic tanks or latrines. For SARS-CoV-2, the upper quartile Ct value was 36.5, and the lower quartile was 33.5.

<u>*Study Location 2. Mion District*</u>

A total of46 environmental samples was collected from septic tanks and latrines at nine schools in Mion District from September 27th to October 20th. Each school was sampled between four to eight times during the study period. All samples were analyzed for four target pathogens by PCR, and the results are in Figure 10.

<u>Results from nine study schools located in Sambu and Sang</u>

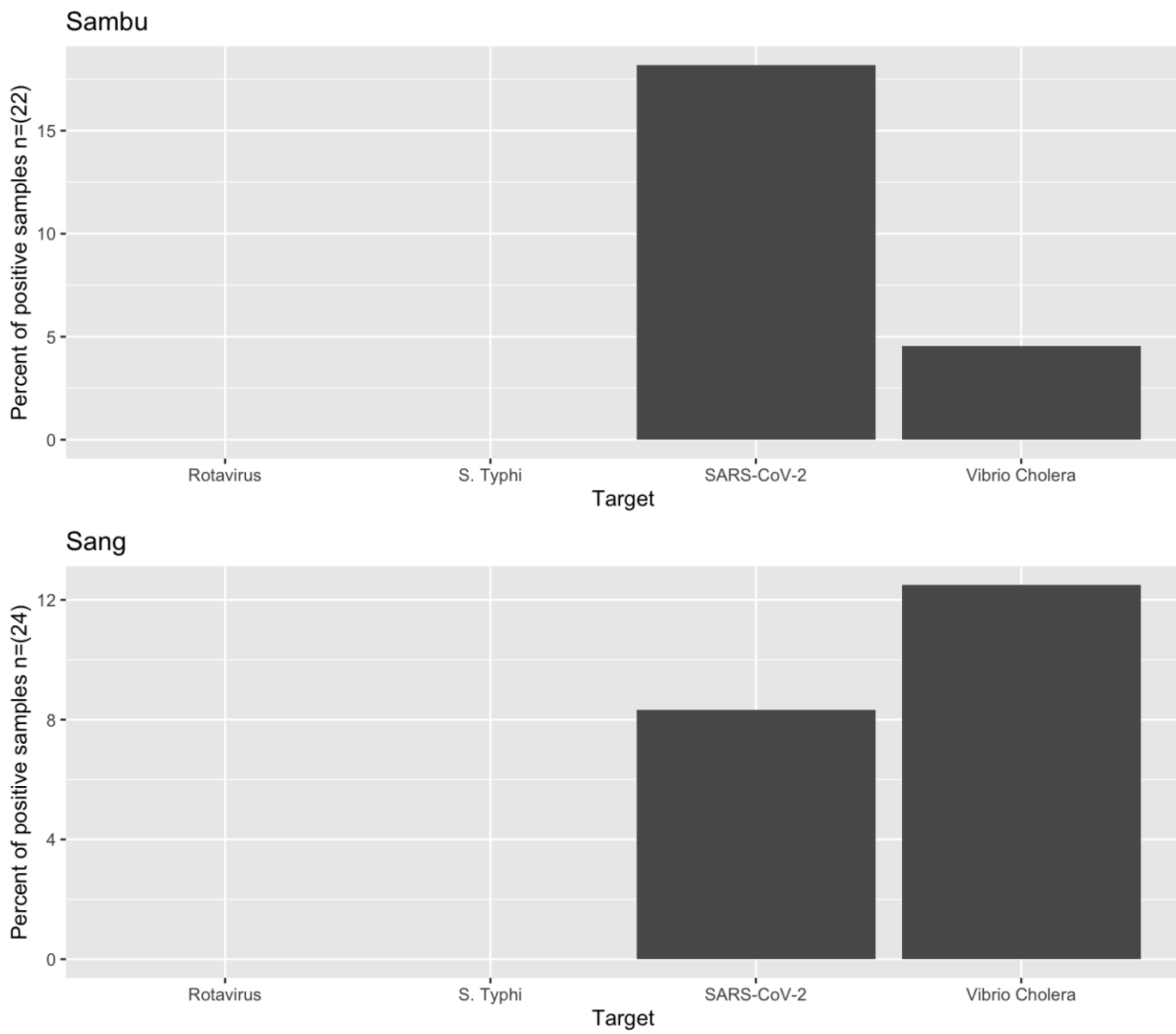


Figure 10. Percent of environmental samples that were positive for each target organism (SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera*) in Sambu (N=22) and Sang (N=24).

The percentage of PCR-positive environmental samples for each target organisms (SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera)* across schools in Sambu and Sang is shown in Figure 10. All environmental samples from Sambu and Sang schools were collected from latrines. In Sambu, Rotavirus and *Salmonella* Typhi were not detected in any of the 22 samples from the three study schools. SARS-CoV-2 was the most frequently detected target organism (18%) in the environmental samples from Sambu schools. The second most commonly detected target organism in the environmental samples was *Vibrio cholera* (4.5%).

In Sang, Rotavirus, and *Salmonella* Typhi were not detected in any of the 24 samples from the six study schools. However, *Vibrio cholera* (12.5%) was the most frequently detected target organism followed by SARS-CoV-2 (8.3%) in the environmental samples from schools.

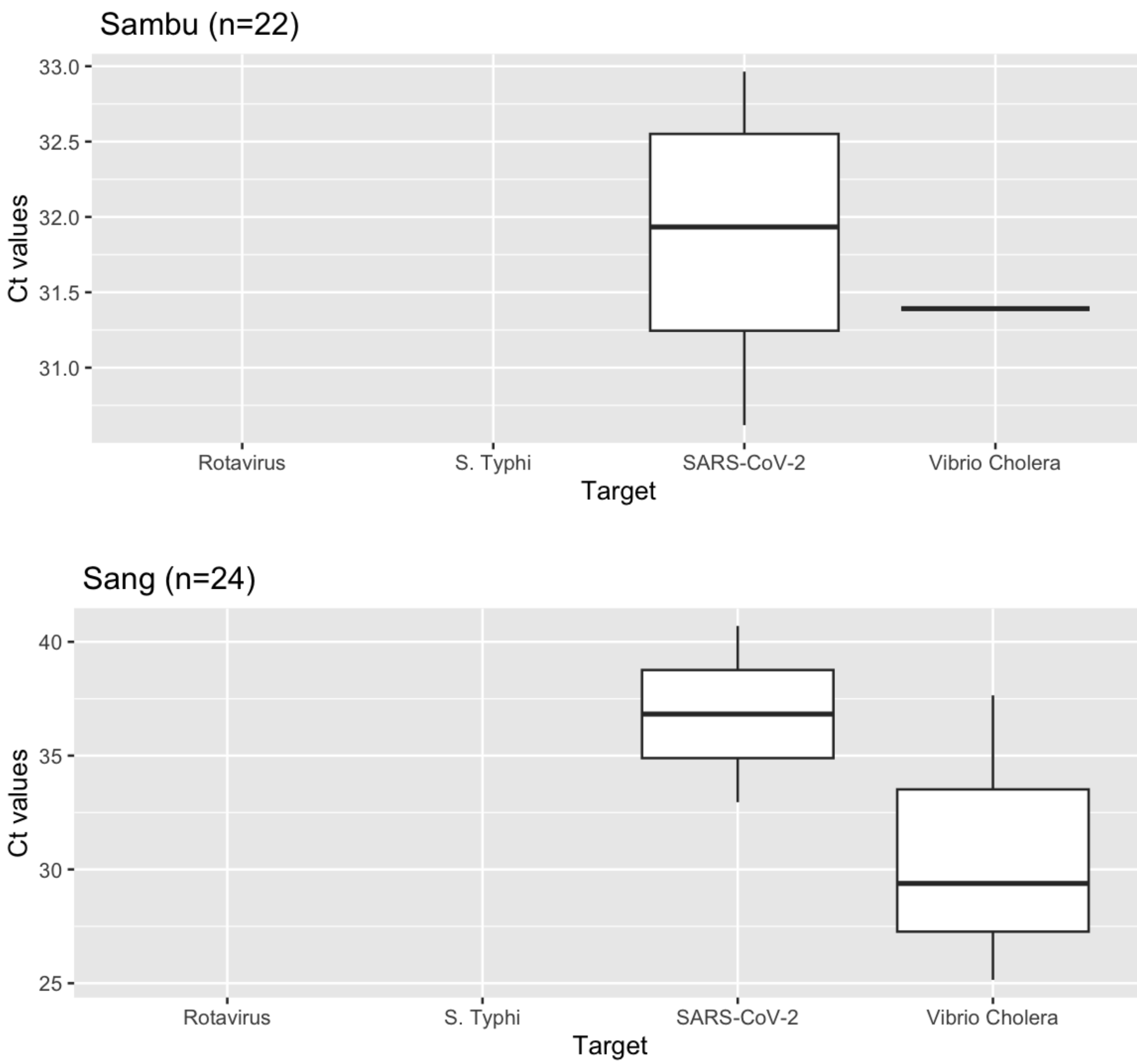


Figure 11. Distribution of Ct values for four pathogen targets in 46 environmental samples from nine schools in Sambu and Sang, October 25th to November 18th, 2022.

A comparison of the distribution and variability of Ct values for all four pathogens (SARS-CoV-2, Rotavirus, *Vibrio cholera*, and *Salmonella* Typhi) in the environmental samples from the study schools in Sambu and Sang is shown in Figure 11. In Sambu, Rotavirus and *Salmonella* Typhi were not detected from samples collected from school latrines. The Ct values for SARS-CoV-2 have the greatest variability and the highest Ct value (median 31.9). The Ct values for SARS-CoV-2 also appear to have a normal distribution with an upper quartile of 32.5 and a lower quartile of 31.3. *Vibrio cholera* has the lowest Ct value (median 31.4). In Sang, Rotavirus and *Salmonella* Typhi were also not detected from collected samples from latrines across schools. *Vibrio cholera* has the greatest variability in Ct values, between 28 and 33. The median Ct value (29) for *Vibrio cholera* was lower than the median Ct value for SARS-CoV-2. The Ct values for SARS-CoV-2 appear to have a normal distribution with an upper quartile of 38 and a lower quartile of 35.

Table 9. Detection of target pathogens in environmental samples (n=22) from Islamic Primary and Junior School and Sambu Model Junior High School, October 25th to November 18th,2022.

School	Number of Samples	% of samples that were PCR-positive			
		Rotavirus	*Salmonella* Typhi	SARS-CoV-2	*Vibrio cholera*
Sambu Primary and Junior Schools					
Islamic Primary School	8	0	0	25	12.5
Islamic Junior High School	8	0	0	0	0
Sambu Junior High School					
Sambu Girls Model Junior High School	6	0	0	33	0

The detection of SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera* in 22 samples from three Sambu schools is shown in Table 9. SARS-CoV-2 was the most frequently detected pathogen in all schools (58%). *Vibrio cholera* was the second most frequently detected pathogen

(12.5%) which was only detected in the Islamic Primary School. All the environmental samples collected from all three schools in Sambu were negative for Rotavirus and *S.* Typhi.

Table 10. Detection of target pathogens in environmental samples from Sang Primary, Junior School and Senior High School (n=24) in Sang, September 27th to October 20th, 2022.

School	Number of Samples	% of samples that were PCR-positive			
		Rotavirus	*Salmonella* Typhi	SARS-CoV-2	*Vibrio cholera*
Sang Primary and Junior Schools					
Zacharia Primary School	4	0	0	0	0
Zacharia Junior High School	4	0	0	25	75
Islamic Primary School	4	0	0	0	0
St. Anthony Primary School	4	0	0	25	0
Sang Girls Model Junior High School	4	0	0	0	0
Sang Senior High School					
Sang Community Day Senior High School	4	0	0	0	0

The detection of SARS-CoV-2, Rotavirus, *Salmonella* Typhi, and *Vibrio cholera* in 24 environmental samples collected from six Sang schools is shown in Table 10. *Vibrio cholera*, which was detected in the Zacharia Junior High School, was the most frequently detected pathogen (75%), followed by SARS-CoV-2 (50%) which was detected in St. Anthony Primary School and the Zacharia Junior School. However, all the environmental samples collected from Sang Community Day Senior High School and Sang Girls Model Junior High School were negative for all pathogens.

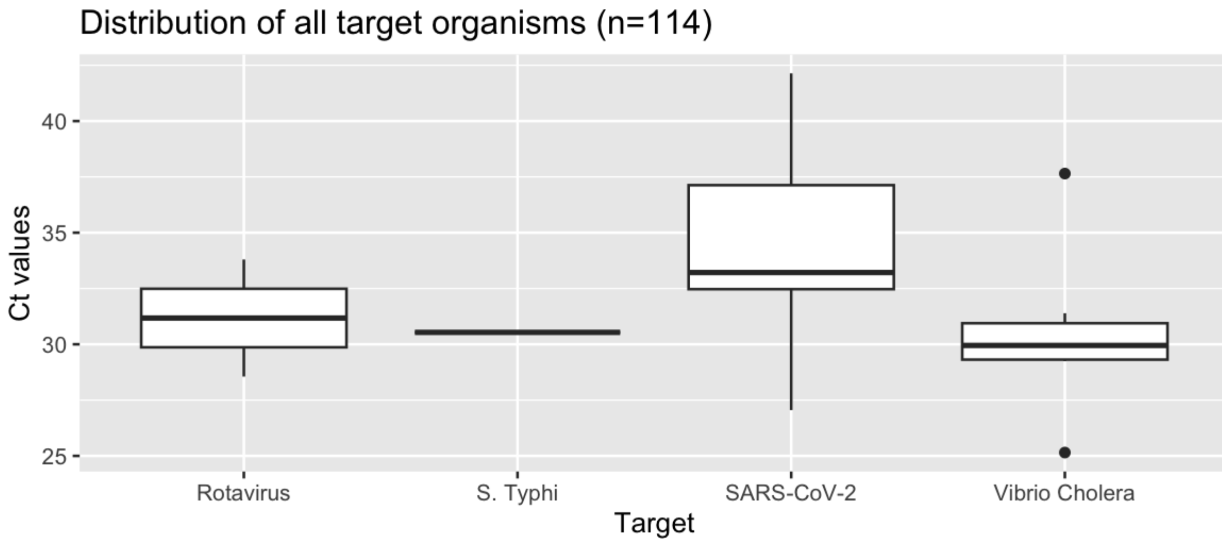


Figure 12. Distribution of Ct values for all target organisms from Nanumba North and Mion District in 114 environmental samples, September 27th to November 18th, 2022.

The distribution of the Ct values for all four target organisms in the 114 environmental samples from Nanumba North and Mion District is shown in Figure 12. The target organism with the highest concentration is *Vibrio cholera* which has the lowest median Ct value compared to SARS-CoV-2, which has the highest median Ct value.

The distribution of the Ct values for the four targets greatly varies by pathogen across the environmental samples. Comparing SARS-CoV-2 to *Vibrio cholera*, SARS-CoV-2 has more variability in the PCR signal indicating that the distribution of data points is much more spread out. Most of the Ct values for *Vibrio cholera* are around 30-33. However, for the SARS-CoV-2 data, there was a wider range of Ct values. Some samples had a Ct value of 33 (the 25th percentile), and some samples were around 37 (the 75th percentile). Some environmental samples had a very weak positive signal near the limit of detection of the assay.

Table 11. Detection of target pathogens in environmental samples from Bimbila, Chamba, Sambu, and Sang (n=114), September 27th to November 18th, 2022.

Town	Number of Samples	% of samples that were PCR-positive			
		Rotavirus	*Salmonella* Typhi	SARS-CoV-2	*Vibrio cholera*
Bimbila	52	3.8	1.9	28.8	5.7
Chamba	16	0	0	25	0
Sambu	22	0	0	18	4.5
Sang	24	0	0	8.3	12.5
Total	114	1.8	0.8	21.9	6.1

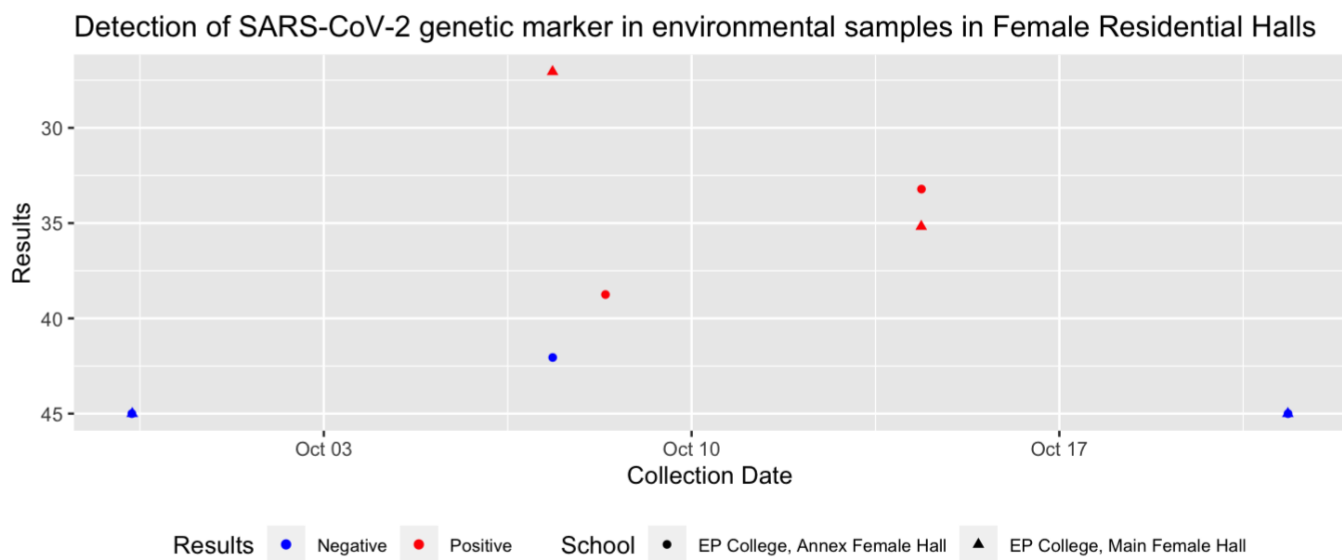


Figure 13. Detection of SARS-CoV-2 in Female Residential Halls at the E.P College of Education in Bimbila, September 29th to October 21st.

The detection of SARS-CoV-2 in environmental samples from the Annex Female Hall and Main Female Hall at the E.P College of Education in Bimbila is shown in Figure 13. Samples from both residential halls were collected from septic tanks. Between October 7th and October 14th, there were four PCR-positive environmental samples for SARS-CoV-2 and one PCR-negative environmental sample. Two of the positive samples were from the septic tank in the Main Female Hall and the other two positive samples were from septic tanks in the Annex Female Hall. There was only one negative environmental sample on October 9th in the Annex

Female Hall. On September 29th and October 21st there were two negative environmental sample in the Annex and Main Female Hall.

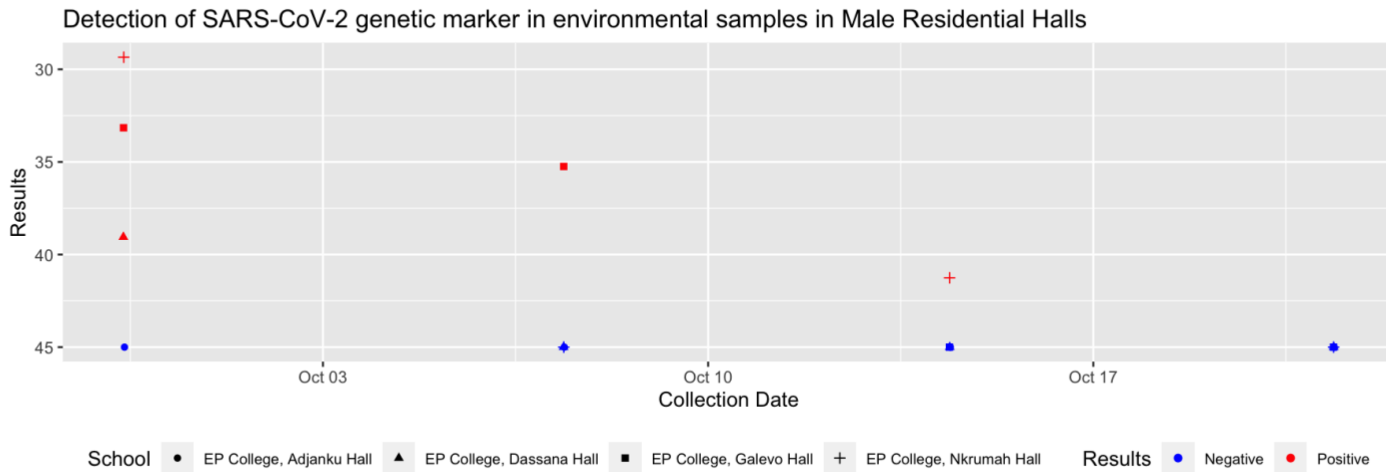


Figure 14. Detection of SARS-CoV-2 in Male Residential Halls at the E.P College of Education in Bimbila, September 29th to October 21st, 2022.

The detection of SARS-CoV-2 in environmental samples from Male Residential Halls at the E.P College of Education in Bimbila is shown in Figure 14. Samples from all Male Residential Halls were collected from latrines. On September 29th, there were three PCR-positive environmental samples from Dassana Hall, Galevo Hall and Nkrumah Hall, and one negative environmental sample from Adjanku Hall. On October 7th, there were three negative environmental samples from Adjanku Hall, Dassana Hall and Nkrumah Hall. On the 14th, there were also three negative environmental samples for Adjanku Hall, Dassana Hall and Galevo Hall. Between October 7thand October14th, there were two PCR-positive environmental samples.

One from Galevo Hall and the other from Nkrumah Hall. There were also two negative environmental samples from Galevo Hall and Nkrumah Hall on October 21^{st}.

Discussion

Study Location #1: Nanumba North District

The two main objectives of this thesis project were to: 1) develop a strategy to monitor SARS-CoV-2, *Salmonella* Typhi, Rotavirus, and *Vibrio cholera* using wastewater surveillance in low-income semi-rural settings; and 2) conduct institutional-level environmental surveillance in primary, junior high and secondary schools in the Nanumba North District and Mion District to determine the value of environmental surveillance as a tool to track SARS-Cov-2, *Salmonella* Typhi, Rotavirus, and *Vibrio cholera* circulation among adolescents. The percent of positive detections by pathogen and location was analyzed by examining the strength of the PCR, which was a surrogate for concentration.

All four target pathogens were detected in Bimbila; however, SARS-CoV-2 and *Vibrio cholera* were the most frequently detected organism, (Figure 8) indicating infection in the population represented by the wastewater samples collected from septic tanks and latrines across schools. Only SARS-CoV-2 was detected in Chamba, indicating infection within the population. There was no detection of Rotavirus, *Vibrio cholera*, and *Salmonella* Typhi in environmental samples. This finding may indicate that there could be genetic markers of Rotavirus, *Vibrio cholera,* and *Salmonella* Typhi in the wastewater, but the concentration was so low that it could not be detected. An alternate explanation is that there was no Rotavirus, *Vibrio cholera*, or *Salmonella* Typhi present in these environmental samples. This would mean that these pathogens are not in circulation within the population at all. For example, the only people who are going to be excreting *Vibrio cholera* in their feces are individuals who are infected. This would be the case for the other pathogens (Rotavirus, *Salmonella* Typhi) as well. In the residential Halls in the Bimbila Senior High School, SARS-CoV-2 was detected in the Boys' Dormitory. This finding indicates that this pathogen is circulating within the dorms.

The residential halls at the E.P. College of Education in Bimbila had higher percentages of SARS-CoV-2 PCR- positive environmental samples compared to the Bimbila Primary and Junior Schools (see Table 6 and Table 7). This finding indicates infection of the most frequently detected pathogen, SARS-CoV-2, in the college population. This observation could be due to simply having a high population density in the residence halls, which could increase the risk of transmission of any contagious disease. In addition to risks of person-to-person transmission in this setting, there is the sharing of common spaces like bedrooms, bathrooms, and toilets which could facilitate airborne transmission and fomite transmission. Unlike the Primary and Junior school students who return to their households every day after school, students in the College live with each other and are in close proximity to other students more regularly.

An unexpected finding was the detection of Rotavirus in the Girls' residential Halls of the E.P. College of Education in Bimbila. PCR-positive tests for Rotavirus was detected in 50% of environmental samples. This is surprising because Rotavirus infections are common in children under five. One explanation for this finding could be a potential food-borne outbreak at the college. In 2000, there was a food-borne outbreak of Rotavirus among students at a university in the District of Columbia. Students who became ill reported eating chicken salad and tuna sandwiches from a dining hall on campus (*Foodborne Outbreak of Group a Rotavirus Gastroenteritis Among College Students --- District of Columbia, March--April 2000*, 2000).

Study Location #2: Mion District

Within the Mion district, SARS-CoV-2 was the most frequently detected pathogen in Sambu and *Vibrio cholera* was the most frequently detected pathogen in Sang, which indicates infection within the population of these two communities (Figure 10). In Sambu, *Vibrio cholera* was the second most frequently detected pathogen (4.5%) indicating that this pathogen is also circulating within the population. Again, there was no detection of some pathogens in environmental samples in Sambu (Rotavirus and *Salmonella* Typhi) and Sang (Rotavirus, *Salmonella* Typhi, and *Vibrio cholera*).

SARS-CoV-2 was detected in the Primary, and Junior High Schools in Sambu (Table 9). This finding is indicative of infection within the population. In Sang, 75% of *Vibrio cholera* PCR- positive environmental samples were found in the Zacharia Junior High School.

A high proportion of PCR-positive samples for *Vibrio cholera* suggests that the risk of transmission among students in the Junior High School is high. There was no detection of any of the four target pathogens (Rotavirus, *Salmonella* Typhi, SARS-CoV-2, and *Vibrio Cholera*) in environmental samples in the Primary Schools and the Senior High School in Sang (Table 10) schools, which may imply that the concentrations of the genetic markers of Rotavirus, *Vibrio cholera*, SARS-CoV-2, and *Salmonella* Typhi were so low that they could not be detected. An alternate explanation is that there was no Rotavirus, *Vibrio cholera*, SARS-CoV-2, or *Salmonella* Typhi present in the environmental samples at all because these infections were not present in the populations at these schools.

This ES study was conducted in Primary, Junior, and Secondary schools in Ghana. Residential halls at one local teacher college were also included in this study. Therefore, our study involved populations between the age range of six to twenty-one years. Populations aged twenty-two and older were not included. This is the first time this kind of data has been collected among this age group in Ghana. The age group of children in primary (6-12) and junior schools (13-15) makes this study unique, considering that this age group would not necessarily be represented in a testing program. Results from the study indicate that infections from several important pathogens may be going unrecognized.

There has not been a study examining wastewater monitoring for multiple pathogens in an LMIC setting before, therefore comparing our results to other studies is not possible. However, ES studies of a single pathogen, like poliovirus and *V. cholera*, have been conducted in Ghana before. One study used ES to detect the circulation of vaccine-derived poliovirus type 2 (cVDPV2) in the absence of clinical cases at Agbogbloshie in the Greater Accra Region. Sewage samples from four sites and stool samples from healthy children were collected from August 27th to August 30th, 2019 for laboratory diagnosis. Samples were collected from a partially opened drain within the Agbogbloshie market area (Odoom et al., 2021b). The results showed that ES confirmed cVDPV2 in 75% of sewage samples collected from surveillance sites. Stool samples from 40 healthy children yielded non-polio enteroviruses, and 75% of sewage samples contained cVDPV2 (Odoom et al., 2021b). Another study from 2020 in South Africa used ES to monitor COVID-19 infection within communities in the KwaZulu-Natal province. Four wastewater treatment plants (WWTPSs) in the province were selected and 21 grab samples of raw sewage were collected over approximately four months (Pillay et al., 2021). Results showed that there was a correlation between high viral loads in wastewater and high peaks in clinical cases of COVID-19 within the Province (Pillay et al., 2021).

There are a couple of similarities between the results from the polio ES study in Greater Accra and the South African ES study of COVD-19 in KwaZulu-Natal province compared to our multi-pathogen study in Northern Ghana. The first similarity is that the target pathogen (Poliovirus) in the Greater Accra study was detected in sewage samples in the absence of reported clinical cases of AFP. This finding demonstrates that ES provides valuable information on the pathogens circulating in the population even if the infectious disease surveillance systems for clinical cases are weak. Another similarity is that ES is also detecting asymptomatic cases. In our study schools, there may have been asymptomatic infections of SARS-CoV-2 and *Vibrio cholera* that may not have been recognized or reported. The main difference between the polio study in Greater Accra, the COVD-19 South African study and our study of multi-pathogens in Northern Ghana is that we looked at four pathogens (Rotavirus, *Salmonella* Typhi, SARS-CoV-2, *Vibrio cholera*) while the other two studies looked at just one pathogen. Additionally, our multi-pathogen study was conducted in a rural area whereas the Polio study in Greater Accra and the COVD-19 study in South Africa were conducted in urban areas. A strength of our Northern Ghana study is that we looked at a rural area where there were no reported cases of COVID-19. This approach demonstrated the value of ES for multiple diseases in this type of setting. Secondly, conducting our Northern Ghana study in a rural setting demonstrated the value and feasibility of collecting samples from schools in an area without formal sanitation systems and sewage.

Limitations

There are a number of limitations that need to be acknowledged, the most important being that the wastewater sample collection in this study was for a short period of time.

Therefore, it is difficult to fully understand why there are significant differences in detecting some pathogens. To address this concern, a potential next step could be extending the sample collection dates over a longer period of time to get a better understanding of transmission within the schools and possibly identify trends, if any. Secondly, there is limited surveillance data available for these districts, and we do not have any information on cases of the four target pathogens. Another limitation is that our study relied on sending samples to Accra for PCR analysis because there was no lab to process samples in Nanumba North or Mion. This demonstrates some potential sustainability and cost challenges for using ES in remote regions in Ghana.

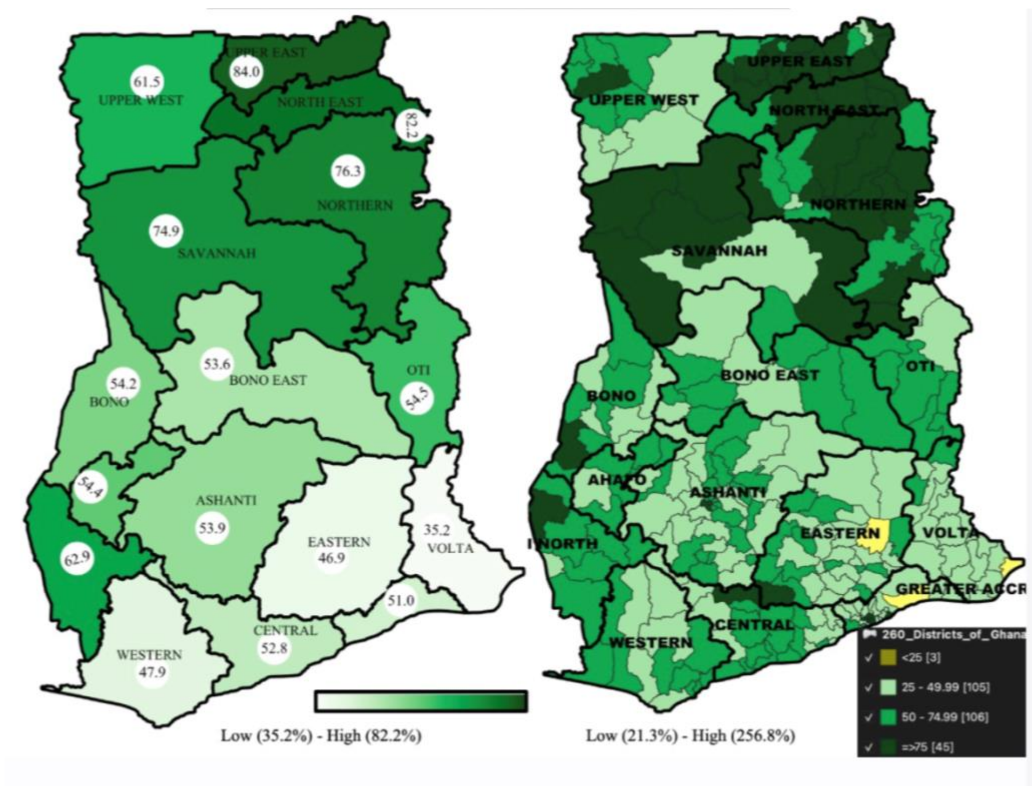


Figure 15. Proportion (%) of persons fully vaccinated by Region and District (*COVID-19 Updates | Ghana*, n.d.).

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Health Data from the Ghana Health Service shows that about 76.3% of people in the Northern region have been fully vaccinated (*COVID-19 Updates | Ghana*, n.d.). Comparing this data to the results of PCR-positive detection of SARS-CoV-2 in environmental samples suggests that although most people are vaccinated, SARS-CoV-2 still persists within the population, especially Bimbila, located in the Nanumba North District. As of March 31st, 2023, the number of active COVID-19 cases reported in Northern Ghana (9) is low compared to the Greater Accra Region (19) (*COVID-19 Updates | Ghana*, n.d.). However, it is the highest number of cases compared to other regions of Ghana, which have between one to seven reported active cases.

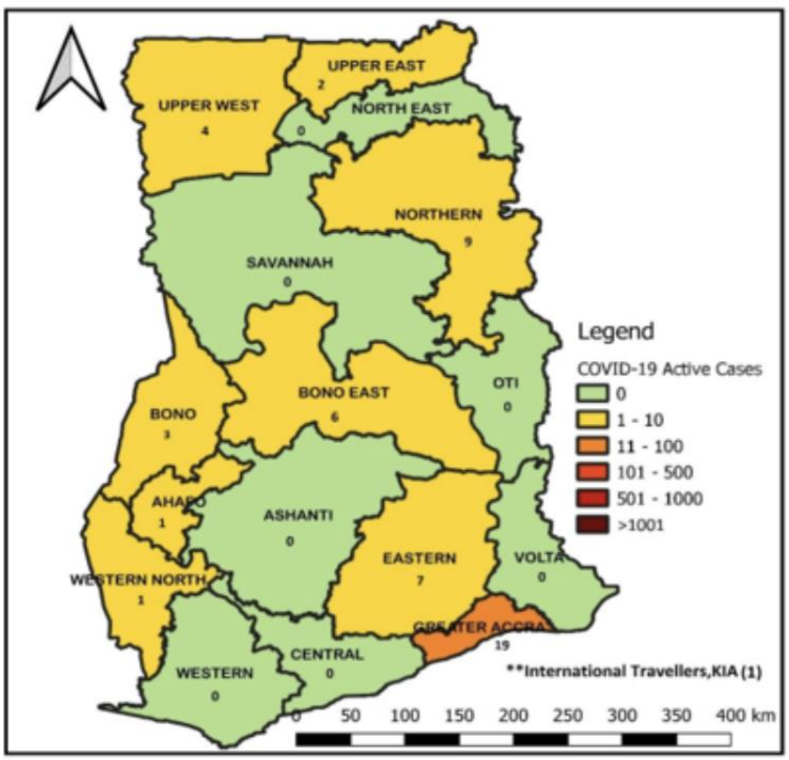

Figure 16. Active COVID-19 cases in Ghana by region, March 31st, 2023(*COVID-19 Updates | Ghana*, n.d.).

Conclusions

All four target pathogens (Rotavirus, *Salmonella* Typhi, SARS-CoV-2, and *Vibrio cholera*) were detected in one or more wastewater samples from 18 schools and one college in the Nanumba North and Mion Districts in Northern Ghana. High proportions of PCR-positive environmental samples for SARS-CoV-2 confirm the circulation of the virus within the population, although vaccination rates are high within the region. The importance of ES as a tool to detect other vaccine-preventable diseases is steadily gaining recognition in Ghana, especially because of the COVID-19 pandemic and the success of its use in detecting poliovirus. Our multi-pathogen ES study has demonstrated that there really is value in using ES to help detect circulation of a pathogen in a population even when there are no reported clinical cases. This finding is important because it could help inform public health response by helping to guide control measures especially in rural areas where clinical diagnostic testing may be limited. Public health officials can also rely on ES to help guide their response and better target vaccination campaigns and other risk mitigation strategies for these infectious diseases. Cholera was the second most detected pathogen in our study. This finding suggests that there is a need for more robust surveillance for cholera by Ghana Health Service.

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