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Katherine Bohnert Date

Impact of Human Movement on Water and Sanitation Practices and Diarrhea Risk

By

Katherine Bohnert

Master of Public Health
Global Health

Karen Levy, PhD, MPH
Committee Chair

Matthew Freeman, PhD, MPH
Committee Member

Impact of Human Movement on Water and Sanitation Practices and Diarrhea Risk

By

Katherine Bohnert

B.S., B.A., University of Missouri – Columbia, 2013

Thesis Committee Chair: Karen Levy, PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
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2015

Abstract

Impact of Human Movement on Water and Sanitation Practices and Diarrhea Risk
By Katherine Bohnert

Background: Even though there has been a global effort to reduce diarrheal diseases, they are still the second leading cause of death among children under the age of five years old (Lanata et al. 2013). Improving water, sanitation, and hygiene (WASH) practices has been shown to reduce diarrhea; yet, these household interventions have recently been scrutinized for their effectiveness and issues with uptake/compliance. **Purpose:** In order to design more optimal WASH interventions, future research needs to investigate large-scale factors that influence the transmission of enteric diseases. The goal of the proposed research was to understand risk factors for diarrheal disease in order to provide data that could be subsequently incorporated into potential strategies for countrywide WASH interventions. This objective was achieved through five specific aims: 1) investigate changes in water and sanitation practices with short- and long-term travel, 2) compare water and sanitation conditions in urban versus rural areas, 3) identify whether water and sanitation practices are risk factors for the transmission of diarrheal disease, 4) identify whether water and sanitation practices are risk factors for transmission of rotavirus, and 5) identify whether water and sanitation practices are risk factors for transmission of parasites. **Methods:** These aims were addressed through a classic epidemiological case-control study design, matching for age. Data on human movement and water and sanitation practices were also collected. **Results:** There were significant differences with water sources between home and traveling. There were significant differences in both water and sanitation sources at home between rural and urban areas. However, these differences mostly disappeared when comparing between cases and controls. Treating water was protective against diarrheal diseases, which corroborates existing literature on the benefits of treating water. **Conclusion:** The significant results from this study suggest that water and sanitation systems can only truly be effective when used properly. Instead of proposing more countrywide strategies, more immediate efforts can be directed towards education about water and sanitation systems, specifically about water treatment methods.

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Chapter 1 – Introduction

Introduction and Rationale

Even though there has been a global effort to reduce diarrheal diseases, they are still the second leading cause of death among children under the age of five years old (Lanata et al. 2013). The World Health Organization (WHO) reports that diarrhea kills an estimated 760,000 children annually (WHO 2013). Improving water, sanitation, and hygiene (WASH) practices has been shown to reduce diarrhea (Fewtrell and Colford 2005; Clasen et al. 2007; Wolf et al. 2014). These WASH interventions typically occur at the individual or household level. Yet, these household interventions have recently been scrutinized for their effectiveness and issues with uptake/compliance (Levy et al. 2014; Brown and Clasen 2012; Enger et al. 2013) and scaling up (Schmidt & Caincross 2009; Clasen et al. 2009). In order to design more optimal WASH interventions, future research needs to investigate large-scale factors that influence the transmission of enteric diseases.

Previous research has shown that larger scale factors, like climate and travel, can be responsible for the spread and transmission of new strains of diseases (Patz et al. 1996; Gonzalez et al. 2013; Tatem et al. 2006). Human travel to and from regions that have endemic strains or pathogens can alter the epidemiological landscape for that area. For instance, the introduction of a new strain or pathogen to a naïve population can significantly affect the area. Likewise, a vulnerable host traveling to and from an endemic population could affect the regional distribution of that pathogenic strain. By understanding the distribution of pathogenic strains for an area, there could be opportunities for diarrheal disease management over large scales. Additionally, travel between urban and rural areas could affect WASH practices by potentially limiting use of safe sanitation facilities or water treatment options that otherwise would be available at home. Both rural and urban areas have their challenges with water and sanitation conditions. According to the WHO, more than three out of every five rural people lack access to basic sanitation worldwide (WHO 2006). Conversely, the water and sanitation systems in urban regions are rapidly becoming compromised due to the strain of increased migration to the urban and peri-urban areas. Overcrowding and limited access to water and sanitation systems contribute to outbreaks of infectious diseases (WHO 2006). Both urban and rural conditions present risks for diarrheal diseases.

Problem Statement

There are many broad public health implications that result from diarrheal disease. In general, diarrhea is thought of as a low disability disease. However, it is still a concern because it represents a large burden of disease, as it is so common within the population. Severe cases of enteric diseases can result in mortality, especially for children under the age of five years old. If diarrhea cannot be controlled at the individual level, it can prove problematic for those in the same household as the individual with diarrhea, given how easily many of the pathogens that cause diarrhea are transmitted under poor WASH conditions.

The basic control strategies for diarrhea occur at the individual and household or community level. Affected individuals receive treatment for diarrheal symptoms, including oral rehydration salts or antibiotics (WHO 2005). At the community level, developed water and sanitation infrastructure can contain the transmission of some enteric illnesses by protecting the water sources from contamination and preventing human contact with feces. Water and sanitation systems may be installed at the household or community level (Taylor 2013). Individuals may be infected when traveling from an urban area to a rural area where there are less sophisticated water and sanitation systems. In an ideal world, control efforts should expand to more national campaigns that systematically address water and sanitation infrastructures for the entire country in order to prevent the spread of enteric diseases. However, there is insufficient research to support that, if in reality, this investment would be feasible and effective.

Previous research has used social and behavioral data to provide insight into large-scale patterns of disease distribution. This has been seen with research in sexually transmitted diseases and tuberculosis but not with enteric diseases (Smith and Yang 2005; Kibiki et al. 2007). It is here that a knowledge gap exists. Although most existing diarrheal research looks at the individual or household for risk factors, little has been studied about large-scale factors, like travel, as a risk factor for the transmission of diarrheal diseases. Furthermore, not much is known about if and how WASH practices are compromised while traveling, which could also be a risk factor for enteric diseases.

As diarrheal disease continues to disproportionately burden children under the age of five, future research needs to examine more broadly the role that human movement between rural and urban regions plays on the transmission of pathogens in order to develop control strategies to be used at a national level.

Purpose of Study

The goal of the proposed research was to understand risk factors for diarrheal diseases in order to provide data that could be subsequently incorporated into potential strategies for countrywide WASH interventions. This objective was achieved through five specific aims, which addressed the goal by asking a series of questions about personal water and sanitation practices and travel.

Specific Aims

1. Investigate changes in water and sanitation practices with short- and long-term travel.
2. Compare water and sanitation conditions in urban versus rural areas.
3. Identify whether water and sanitation practices are risk factors for the transmission of diarrheal disease.
4. Identify whether water and sanitation practices are risk factors for transmission of rotavirus.
5. Identify whether water and sanitation practices are risk factors for transmission of parasites.

Significance Statement

The results from this research will be aggregated into a report for the Ministry of Health in Ecuador to show how the impact of travel between urban to rural areas could be a potential risk for the transmission of diarrheal disease. Furthermore, these results could provide insight into how water and sanitation conditions during travel could affect rates of diarrhea, and could potentially lead to future interventions focused on targeted water and sanitation messaging for travelers within the country of Ecuador, as well as in other countries that are experiencing a shift in the epidemiological landscape.

Definition of Terms

Diarrhea: Three or more loose, watery stools within 24 hours (WHO 2013). This definition applies for all ages.

Epidemiological landscape: The understanding of risk and geography in an attempt to map out the forward direction of disease transmission.

Human movement: Human movement in this thesis refers to travel from one of the study sites to another town or city within Ecuador. Human movement in these regions occurs mostly by bus, car, or even occasionally plane. The construction of a two-lane coastal highway in northern Ecuador has significantly increased the amount of travel for individuals who previously did not have access to these roads.

Improved sanitation: As defined by the World Health Organization, an improved sanitation source is “one that hygienically separates human excreta from human contact” (JMP). Examples of improved sanitation include flush toilet, piped sewer system, septic tank, pour flush to pit latrine, pit latrine with slab, and a composting toilet (JMP).

Improved water: As defined by the World Health Organization, an improved water source is “one that, by nature of its construction and when properly used, adequately protects the source from outside contamination, particularly faecal matter” (JMP). Examples of improved water include piped water into dwelling, piped water to yard, public tap, tubewell, protected dug well, or rainwater (JMP).

Unimproved sanitation: An unimproved sanitation source is one that allows for contamination as a result of not being protected. This includes open defecation in a field, a bucket, an uncovered pit latrine, or a hanging latrine (JMP).

Unimproved water: An unimproved water source is one that is not protected, and is thus more susceptible to fecal contamination from humans and animals, as well as unsustainable sources. This includes an unprotected spring, unprotected dug well, tanker-truck, surface water, and bottled water (JMP).

Chapter 2 – Literature Review

Background on Diarrheal Disease

Morbidity and Mortality of Diarrhea

Reducing the diarrheal disease burden has been a prerogative for both government and non-profit agencies alike. It has captured the attention of academia and has been the subject of many cutting edge technologies. Yet in light of all this, diarrheal diseases still claim the lives of approximately 1.2 million people and they annually affect more than 2.8 billion people worldwide (WHO 2013). Figure 1 below illustrates the global mortality from diarrhea in children under the age of 5 years old (Croxen et al. 2013).

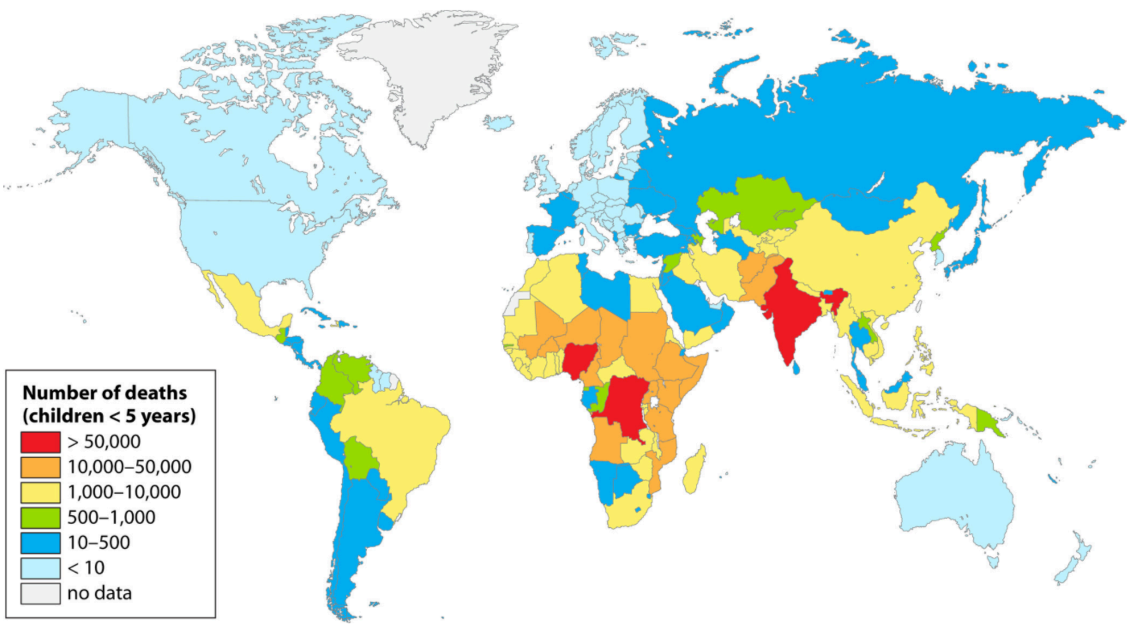


Figure 1: The global mortality from diarrhea in children under the age of 5 years old in 2010. Map Source: Croxen et al. 2013

Regions where diarrheal diseases present a more significant burden include Africa, South Asia, and parts of Latin America (Lamberti et al. 2012). However, diarrheal diseases are not limited to only impoverished areas but they also can play a large role in morbidity in developed or urban regions.

Definition of Diarrhea

Before quantifying the morbidity and mortality of diarrheal diseases, it is important to understand the standard definition of diarrhea. According to the World Health Organization, diarrhea is considered when a patient has three or more loose, watery stools within 24 hours (WHO 1988). This case definition applies to all ages and can have many characteristics. These characteristics, including mucous and bloody stools, can indicate either the severity of the infection or at times the pathogen responsible for the infection.

Etiology of Diarrhea

There are several known causes for diarrheal diseases or gastroenteritis. Bacteria, parasites, and viruses alike can result in diarrheal disease (WHO 1988). These different etiologic agents can affect the severity of the disease. Some of these pathogens are mostly associated with morbidity but some of the most prevalent pathogens also are associated with mortality. Numerous studies have attempted to qualify and quantify the pathogens most likely responsible for the staggering rates of diarrhea worldwide, including Rotavirus, *Escherichia coli*, and *Giardia* spp. (Levine 1987; Kotloff et al. 2013; Croxen et al. 2013; Benmessaoud et al. 2015).

Rotavirus is the leading cause of severe enteric diseases, even though it was recently discovered, in 1973 (Tate et al. 2012). Rotavirus causes an estimated 450,000 deaths each year in children under the age of five years old, in addition to hospitalizing millions of patients worldwide (Bines and Kirkwood 2015). In 2006, the first safe rotavirus vaccine was introduced and by 2009, the WHO recommended that all children should receive the vaccine to protect against rotavirus infection (Bines and Kirkwood 2015). Although the vaccine has made great improvements in reducing rotavirus disease burden in developed countries, diarrhea due to rotavirus persists in developing countries. Rotavirus, though, is not the only pathogen contributing globally to high rates of diarrhea.

Bacteria, specifically *Escherichia coli*, are another major contributor to the global burden of diarrheal disease. The Global Enteric Multicenter Study (GEMS) examined the etiology of diarrheal disease in children in sub-Saharan Africa and South Asia. The three year long, prospective case-control study identified enterotoxigenic *Escherichia coli* (ETEC) and typical enteropathogenic *E. coli* (EPEC) as two pathogens that severely infected the population of interest – children aged 0-59 months (Kotloff et al. 2013). Other studies have shown that Shiga toxin-producing *E. coli* (STEC) and ETEC are more common agents in adults for diarrheal disease than children (Croxen et al. 2013). All of these pathogens range in severity of disease, from more mild, chronic cases of diarrhea caused by EPEC to more severe, acute cases of diarrhea caused by ETEC and STEC. The distinction between these different pathotypes of *E. coli* has affected how the disease should be treated and/or controlled.

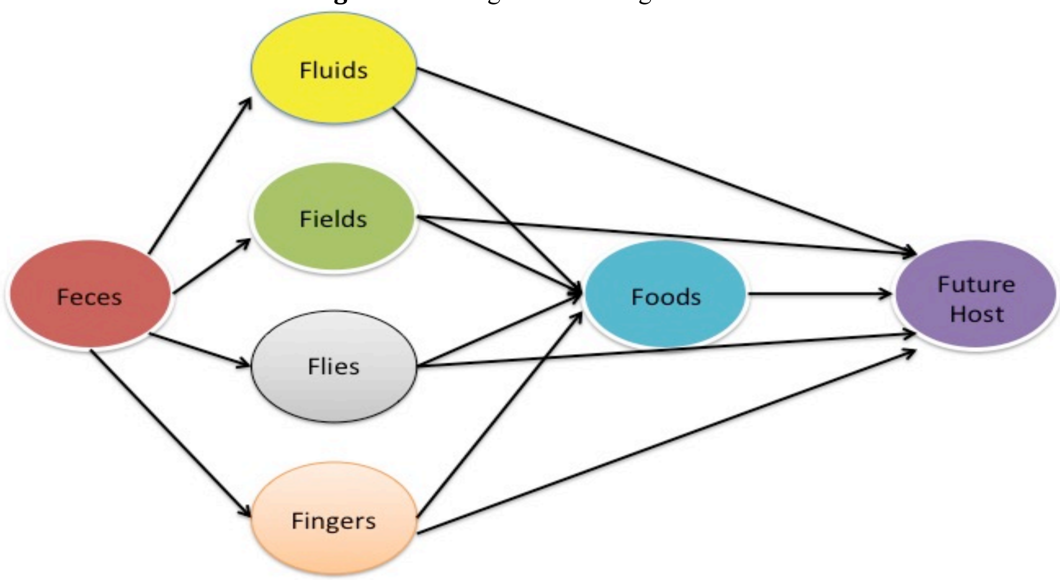
In addition to rotavirus and *E. coli*, parasites also have been studied as key players in causing diarrheal disease. Compared to rotavirus and diarrheagenic *E. coli*, diarrhea caused by parasites, including *Giardia* spp. and *Entamoeba histolytica*, is less severe and doesn't require as many hospitalizations among those infected (Benmessaoud et al. 2015). However, there are some parasites that are associated with more severe diarrheal episodes. According to the GEMS study, *Crytosporidium* is a significant risk factor for moderate to severe diarrhea and was the second most prevalent pathogen in infants (Kotloff et al. 2013).

There are many etiologic agents for diarrheal diseases, making it difficult to prioritize the importance of these different organisms. Perhaps of more importance to reducing the burden of diarrheal diseases is the route of transmission of these organisms. Many of these pathogens are transmitted in similar patterns and it is through transmission that diarrhea can be controlled.

Transmission of Diarrhea

Most diarrhea-causing pathogens are fecal-orally transmitted, as demonstrated in Figure 2. This figure, adapted from that of Wagner and Lanoix, portrays how pathogens in feces can potentially infect a future host (Wagner and Lanoix 1958). This transmission route is especially

Figure 2: F-Diagram of Pathogen Transmission



important with children under the age of five years old, as much of the transmission happens within the household environment (Mattioli et al. 2015). Each of the transmission pathways represents an area in which diarrheal disease could be interrupted. For example, if infected feces were contained in an improved sanitation system (as opposed to spread in a field as a result of open defecation), they would not contaminate the produce grown in that field, which is then consumed by a different individual. Once consumed, that individual can be subsequently infected from the contaminated produce. Without implementing interventions, such as improved sanitation and water interventions or behavioral changes, the transmission of diarrhea persists; therefore, creating a health impact worldwide.

Health Impacts of Diarrhea

Diarrhea can result in numerous adverse health effects. Discomfort, severe dehydration, and loss of productivity are a few of the more common effects of acute diarrheal disease. More severe cases can result in bloody stool, hospitalization, and even death. Diarrheal diseases can be treated with medication and other remedies. Oral rehydration salts is one of the primary means of managing diarrhea, as well as antibiotics, and zinc treatments (Di John and Levine 1988; Guandalini 2011; WHO 2004). The treatment regimes can vary based on severity and age.

For children under the age of five years old, diarrheal diseases can have more significant health impacts. Many research studies have examined the role that enteric pathogens have played in malnutrition of children aged 0-59 months. The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) study is examining the link between malnutrition and high incidence of diarrheal disease in eight countries (Acosta et al. 2014). The foundation of this research project is that diarrheal disease at an early age not only can disrupt the absorptive capacity of the intestine but that it can also increase inflammation, ultimately resulting in micronutrient deficiencies and chronic immune stimulation (Acosta et al. 2014). This consequently leads to malnutrition and a heightened susceptibility to infectious diseases. Therefore, diarrhea promotes a cycle that results in more enteric illnesses, malnutrition, and in severe cases, mortality. These deleterious health impacts are driving researchers to better understand not just the etiologic agents but also risk factors in order to further prevent the spread of transmission.

Epidemiological Risk Factors for Diarrhea

Water and Sanitation Risk Factors

The link between water and sanitation infrastructure and diarrhea risk was hypothesized over a century ago (Kolsky 1993). More currently, studies have demonstrated how inadequate infrastructure can lead to an increase of pathogens in the environment, which can subsequently infect individuals through flies or contaminated soil. (Nmorsi et al. 2007; Rosas et al. 1997). In the event of extreme weather conditions, these pathogens can be further distributed in the community (Auld et al. 2004). For example, flooding of an unprotected latrine disperses pathogens, which may be found in fecal material, into the environment. This, in turn, can contaminate unprotected water sources or even produce grown in fields. The advent of improved water and sanitation infrastructure has reduced the transmission of pathogens but if the systems are not well maintained, even they can present a risk of exposure to pathogens. Therefore, proper usage is just as important as the infrastructure itself in order to eliminate diarrhea risk from water and sanitation systems (Hunter et al. 2009).

Travel as a Risk Factor

Another risk factor for the spread of diarrheal disease is human travel. The increase in human contact from traveling can result in the transmission of pathogens from one community to another. The construction of a coastal highway where previously there had been no roads is one example of how pathogen transmission can be affected by migration (Eisenberg et al. 2006). In this study by Eisenberg and colleagues, a causal link was drawn between road proximity, social connectedness outside of the community, introduction of pathogenic strains, and an increase in diarrhea rates (Eisenberg et al. 2006). Short-term travel can be a risk factor by connecting communities that otherwise were not previously connected, allowing for the introduction of new pathogenic strains into a susceptible community from an endemic area. This is further complicated by the short-term travel of an asymptomatic host who unknowingly introduces a pathogen into a vulnerable, naïve community.

Travel between urban and rural areas can also present as a risk factor for the spread of infectious diseases. Both short-term and long-term travel between these areas can contribute to the distribution of pathogens. Some studies have examined how travel between urban and rural areas affects the transmission for sexually transmitted diseases, respiratory diseases, vector-borne diseases, and even cholera (Coffee et al. 2005; Ali and Keil 2006; Rogers and Packer 1993; Schaetti et al. 2013). Travel from urban to rural regions poses a risk for the distribution of some infectious diseases because of underdeveloped water and sanitation infrastructure. However, there is also a risk of traveling to some larger, peri-urban cities. The rate of migration to these areas has compromised existing resources, including water supply and improved sanitation systems. With a lack of these resources, pathogens are not contained in this environment and subsequently transmitted to the population, as well as anyone from rural areas that are traveling to this region. Therefore, there are risks associated with travel to both urban and rural regions. This is especially pertinent to contagious fecal-oral pathogens found in both urban and rural settings, like rotavirus and *E. coli*, which can heighten the risk for diarrhea. Consequently, intervention efforts need to not only control diarrhea within an infected community but also prevent the distribution of pathogens into other communities.

Control Efforts for Diarrhea

In order to address the many routes of pathogen transmission and risk factors involved with diarrheal disease, there are a number of approaches to either prevent diarrheal disease from spreading or to lessen the burden of the infection. Interventions range from the construction of improved water and sanitation infrastructure to education on personal hygiene to treatment of water sources (Fewtrell et al. 2005; Clasen et al. 2007; Wolf et al. 2014). Some of these interventions have advantages and disadvantages with implementation, accessibility, and sustainability. Additionally, all of the interventions come at a cost – not just to implement but also to maintain the intervention. As a result, success rates for these interventions sometimes have been mixed.

According to a recent review, there are significant reductions in diarrheal diseases through these water and sanitation interventions, though to varying effects (Wolf et al. 2014). Wolf and colleagues determined that for water at the household-level, the best intervention was a point-of-use filter with safe water storage and that for the community level, high quality piped water was most effective (Wolf et al. 2014). However, there were accounts of when water quality was contaminated through improper handling and storage (Wolf et al. 2014). As for sanitation, sewage systems were more effective than individual sanitation structures (although there were a paucity of sanitation studies in the review) but compromised sewage systems could have effects for communities downstream (Wolf et al. 2014).

These results from improved water and improved sanitation interventions suggest that while they reduce pathogen transmission, there is always the possibility of reintroducing pathogens in low- to middle-income countries. Therefore, new and innovative strategies are needed to reduce the diarrheal disease burden. Future interventions should consider cultural sensitivities, inhibition of exposure pathways, and the ability to scale up to a national level in order to be most effective in their goals to combat diarrheal disease.

Research Gaps and the Relevance

Diarrhea is difficult to study, given the numerous etiologic agents, transmission routes, and risk factors involved. Failed water and sanitation interventions and the intricacy of pathogen transmission often make diarrheal diseases problematic to understand and to control. Furthermore, the epidemiological landscape is shifting in developing areas of the world, as evidenced by increased human movement between rural and urban areas. In order to develop a control strategy that addresses the aforementioned problems with diarrhea, there needs to be more research that combines social, epidemiological, and molecular data towards understanding transmission processes. Furthermore, there are no existing studies that explore how water and sanitation practices during travel can impact diarrheal risk. What role does travel play in exposing individuals to diarrheal disease pathogens via household and community-level water and sanitation systems of different quality than the systems that they use at home? The data collected from this research project seeks to answer this question.

By contributing to the existing knowledge of diarrhea and risk factors, new evidence-based strategies can be developed to explore potential water and sanitation interventions at a national level, as opposed to the current data on individual and household interventions. For example, if urban sites are a source of pathogens for rural areas, a countrywide intervention could lead to a more cost-effective approach to controlling diarrheal disease by focusing on urban communities as targets for the interventions. This shift in strategy could ultimately lead to the reduction in diarrheal disease burden by preventing morbidity and mortality in both urban and rural settings.

Chapter 3 – Methodology

Introduction

The primary purpose of this case-control study was to explore human travel between urban and rural areas as a risk factor for diarrheal disease and carriage of *E. coli*. The secondary purpose of this case-control study was to examine how water and sanitation practices while traveling can be a risk factor for diarrheal disease, parasites, and rotavirus. Data were collected using a mixed-methods approach with surveys and laboratory assays. The study team in Ecuador collected data from March 2014 to February 2015 from hospitals and clinics in four sites – Borbón, villages surrounding Borbón, Esmeraldas, and Quito. These sites represent a rural-urban gradient, with Quito representing a large urban region (population: 1,622,000), Esmeraldas a mid-size urban area (population: 145,000), Borbón a small town (population: approximately 5,000), and the villages surrounding Borbón rural villages (maximum population of approximately 500 in any given town) (Central Intelligence Agency 2014). The methodology for this data collection is outlined in the following sections.

Figure 3: Map of study regions as indicated by stars.

Study Region

The primary study region is located in northwestern Ecuador, along the coast, in the Province of Esmeraldas. There are 125 villages in the Province, as well as three rivers that converge at the periphery of Borbón, one of the study sites indicated in Figure 3. In Borbón, data were collected at the Borbón Hospital.

To examine diarrhea in rural areas, the study team procured data in several of the villages surrounding Borbón. The data in these rural villages were collected via home visits.

Data were also collected in the city of Esmeraldas, the capital of the Province of Esmeraldas. In Esmeraldas, the study team gathered data at the Hospital Delfina Torres Concha, as well as at a Subcentro de Salud (a health clinic).

Quito, the capital of Ecuador, was also selected as a study site that fell outside of the Province of Esmeraldas. In Quito, the study team worked at Hospital Enrique Garcés (commonly known as Hospital del Sur) and at a Subcentro de Salud.

Both the city of Esmeraldas and Quito (Figure 3) were chosen as sites because as larger cities, they receive visitors from the Borbón area. They also share cultural overlap with Borbón, making them ideal locations to explore pathogenic strain distribution between Borbón, a more rural area, and densely populated urban cities. All of these hospitals and clinics in the study region reported that at least half of the patients who visited each month presented with infectious diseases.

Research Design

This research project employed a classic epidemiological case-control study in each of the four study sites to evaluate risk factors for diarrhea. A common protocol was shared between each of the study sites. The research team administered a survey to all of the cases and controls, asking questions about travel, socioeconomic status, and water and sanitation practices. Additionally, the research team collected stool samples from all of the participants and processed them for *E. coli*, parasites (all sites except Quito), and rotavirus (details below). All contact with human subjects was approved by the Emory University IRB and the Ethical Committee of the Universidad San Francisco de Quito.

Population and Sample

The participants in the study were patients, both males and females, and of all ages, reporting to the hospital and clinic recruitment sites. The one exception to this was participants that lived in the villages near Borbón. Those individuals were recruited in conjunction with regular Ministry of Health field visits to the villages

Physicians, other medical personnel, and study staff identified cases, defined as patients who presented with acute diarrheal disease or gastroenteritis. Controls were originally defined as just those patients who presented with skin disorders but not enough controls with these criteria were identified during data collection. Therefore, the inclusion criteria were broadened to any patient who suffered from an infection or disorder other than acute diarrhea. Controls were matched by age to cases according to the following criteria: cases that were 0-24 months of age were matched with controls that were within +/- 6 months of that age range, cases that were 25-60 months of age were matched with controls that were within +/- 12 months of that age range, cases that were 61-180 months of age were matched with controls that were within +/- 24 months of age, and any case over the age of 181 months was matched with any control over the age of 15 years.

We applied the following exclusion criteria to enrollment in the study: patient lived outside of the study region; patient had not lived in the study region for longer than six months; consumption of antibiotics within the last seven days. In addition, controls were excluded if they had experienced diarrhea within the last seven days. The inclusion and exclusion criteria are demonstrated in Figure 4.

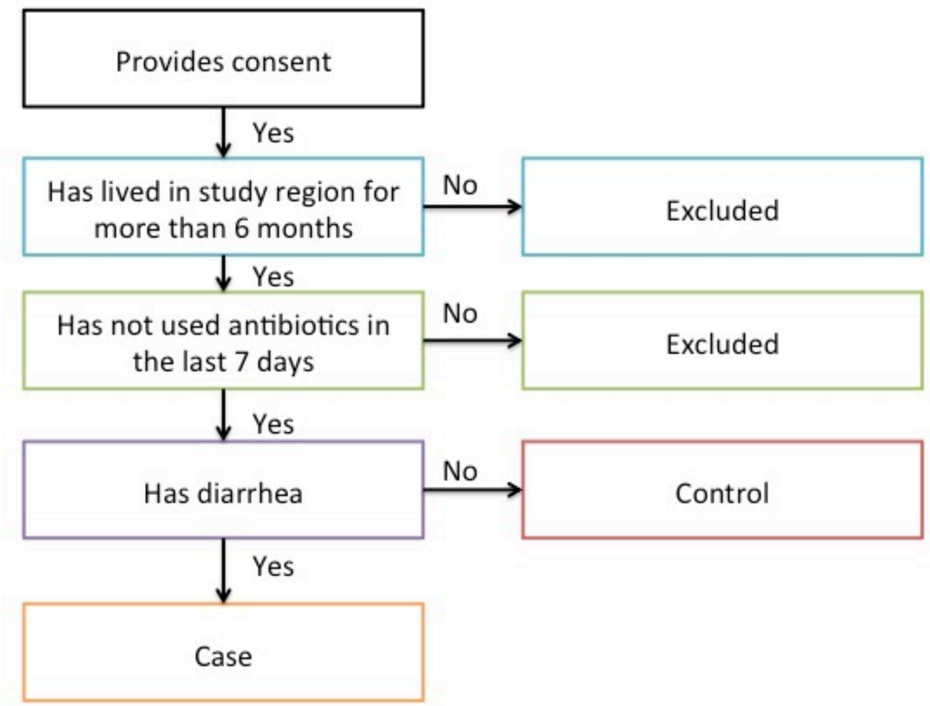


Figure 4: A diagram displaying the inclusion and exclusion criteria.

Instruments

The Principal Investigator, with the help of the research team, developed a survey instrument in English, which was then translated to Spanish and translated again to English to ensure the Spanish meaning of the questions and responses. The survey instrument was divided into four primary categories: demographics, health symptoms, travel, and household characteristics (full survey in Appendix 1).

The demographics category asked questions about age, gender, race, and for participants who were under the age of three, if they attended a nursery and if they breastfed. The symptoms category for controls asked about their reason for visiting the hospital or clinic. The symptoms category for cases asked duration of diarrhea, characteristics of the stool, and perceptions of the cause(s) of the diarrhea. The travel category asked about travel within the past week, as well as travel in the past year. For those who responded that they had recently traveled, they were asked about what types of water and sanitation systems they used while traveling. The survey also asked about destination and duration of both recent travel and travel within the last year. Lastly, the household characteristics asked about water and sanitation systems within the household, animal contact in the last week, education level of the participant, and other socioeconomic factors.

In addition to surveys, the research team collected stool samples from every case and control within 24 hours of administering consent. All of the laboratory analysis occurred in either a field laboratory or at a microbiology laboratory at the Universidad San Francisco de Quito, a collaborating institution of the study. The research team ordered their laboratory supplies from the microbiology laboratory at Universidad San Francisco de Quito in order to carry out the processes on site.

Procedures

Before data collection could begin, the PI met with the leaders and head epidemiologists at the hospitals and clinics to discuss the purpose of the study and to seek permission to carry out the research project in those settings. The PI hired a research team to facilitate the study at each site, as well as established a relationship with the laboratory at Universidad San Francisco de Quito for part of the processing of stool samples. There were approximately two research team members per study site conducting the research.

At each site, patients who presented with diarrhea were referred to the research team by a healthcare professional. A majority of the patients enrolled in the study were identified in the waiting area of the emergency room. When a patient was referred to a team member, that team member read the patient a brief description of the project and then the informed consent document. If the patient consented, s/he was given an identification number that was subsequently used to label the survey and stool specimen.

The research team administered the surveys to the participants upon receiving informed consent, using Open Data Kit (ODK) Collect mobile data collection software. Research team members each had their own mobile devices, which they used to read off the survey questions and record the participants' responses. Survey data were sent directly to a Google Appspot server. The data were managed and aggregated on the online server and then extracted as an Excel file for analysis.

After administering the survey, the research team provided the patient with a container to collect a stool sample. The team member asked the patient to return the sample to the hospital or clinic laboratory within 24 hours or otherwise the sample could not be processed. The patient was also advised on how to properly collect the stool sample to avoid any possible contamination with the toilet seat, toilet water, or hands. If a participant was in diapers, the team member provided additional instructions to assist the parent or guardian in properly handing the stool specimen.

Once the patient returned the stool sample to the laboratory, the research team processed the sample for *E. coli*, rotavirus, and parasites. A portion of the stool was plated directly onto MacConkey and XLD agars. Then, another aliquot of stool was placed in a test tube. The presence of rotavirus antigens was determined by RIDA Quick Rotavirus test (r-biopharm, Darmstadt, Germany), according to the manufacturer's instructions. Two crioconservation tubes were filled with stool samples and stored in liquid nitrogen. The tubes will be used for future microbial community analyses. The remainder of the stool was preserved with a formaldehyde solution and sent to the laboratory in Quito to be processed for the presence of select parasites that are prevalent in that region. All of the results from these exams were given to the participant and if the patient was positive for a pathogen, the research team referred the patient to the doctor to discuss treatment options.

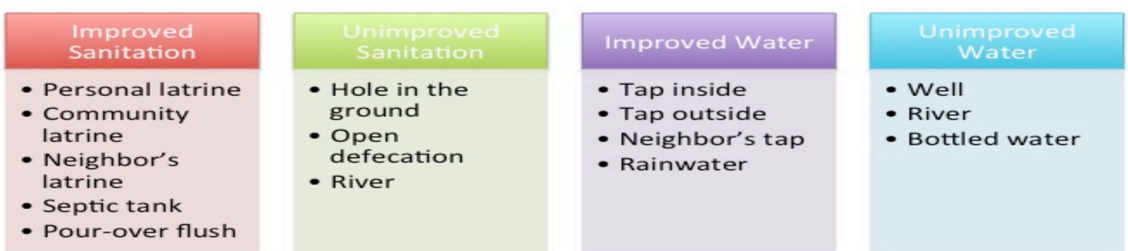
Data Analysis

The survey data were imported into SAS software, 9.3 (SAS Institute Inc., Cary NC). The data were analyzed individually by each site and then merged into one dataset, where the remainder of the analysis was conducted. For all of the 'non-response"/"don't know" responses, the response was coded as missing.

All of the variables in the dataset were reviewed and the number and percentage for each variable were recorded in a summary table. This was done for each site, as well as by cases and controls within each site. A Chi-square test of association was done for all of the variables to determine if there were any differences between cases and controls for each site. A statistically significant difference was considered as a p-value of <0.05.

For demographics, the age variable was converted into the following categories: 0-24 months, 24-60 months, 60-180 months, and 180+ months. In the water and sanitation practices section, water and sanitation variables were categorized as improved or unimproved sources, according to the World Health Organization's (WHO) guidelines. It is important to note that one exception was made to the standard guidelines. According to the WHO, bottled water is considered an unimproved source of water globally because of its lack of sustainability. For this study region though, given the circumstances, bottled water was considered an improved source of water. The categorization of the variables is depicted below in Figure 5. The newly created categorical variables (improved sanitation, unimproved sanitation, improved water, and unimproved water) were used in the remainder of the analyses.

Figure 5: Categorization of water and sanitation responses according to WHO guidelines.



For Specific Aim 1, a Chi-square test of association was used to determine if there were differences between water and sanitation practices at home versus while traveling. The null hypothesis stated that there were no significant differences between water and sanitation practices at home versus water and sanitation practices while traveling.

For Specific Aim 2, water and sanitation variables at home were compared across rural and urban sites using a Chi-Square test of association. The null hypothesis stated that there were no differences between home water and sanitation practices in rural sites versus urban sites.

For Specific Aim 3, logistic regression was used to relate diarrheal disease status to a set of water and sanitation predictors. The outcome variable was diarrheal disease, coded as case=1 and control=0. The predictor variables were improved sanitation used while traveling, improved sanitation used at home, improved source of water while traveling, improved source of water at home, treated water while traveling, and treated water at home.

For Specific Aim 4, logistic regression was used to relate rotavirus to a set of water and sanitation predictors. The outcome variable was rotavirus, coded as positive=1 and negative=0. The predictor variables were improved sanitation used while traveling, improved sanitation used at home, improved source of water while traveling, improved source of water at home, treated water while traveling, and treated water at home.

For Specific Aim 5, logistic regression was used to relate parasitic infection to a set of water and sanitation predictors. The outcome variable was parasitic infection, coded as positive=1 and negative=0. The predictor variables were improved sanitation used while traveling, improved sanitation used at home, improved source of water while traveling, improved source of water at home, treated water while traveling, and treated water at home. For Specific Aims 3-5, the predictive odds ratio (POR) was reported for each model.

Ethical Considerations

The research protocol and instruments were submitted to Emory University, Universidad San Francisco de Quito, and the Ministerio de Salud Pública del Ecuador/Dirreción de Inteligencia de la Salud, where the project was approved. Before collecting data, the researcher read in Spanish an informed consent document and written consent was received from every participant. Upon receiving written consent, each participant was assigned a unique identification number to link the survey data and laboratory results to the participant without identifying s/he by name. If a participant was under the age of six years old, the parent or guardian granted consent on behalf of the participant. If a participant was between six and ten years old, an assent form was read and the participant gave verbal consent. If a participant was between eleven and sixteen years old, an assent form was read and the participant gave written consent. If the participant was seventeen years old, the assent form was read to both the participant and his/her legal guardian and the participant provided written consent. All consent forms were stored in a secure location at each study site.

Chapter 4 – Results

A total of 720 participants were enrolled in the study from all four sites. For the rural communities, Borbón, Esmeraldas and Quito there were 104, 108, 237, and 271 patients, respectively. The descriptive statistics for demographics, medical history, water and sanitation history, and travel questions are displayed in Tables 1 and 2. The numbers and percentages are shown for each variable by site in Table 1 and the same variables are then divided by cases and controls within each site in Table 2. Additionally, those who did not meet the inclusion criteria were excluded from the summary statistics in Table 2. Two individuals did not consent to participation in the study. Ten individuals in Esmeraldas were excluded because they lived in a city other than Esmeraldas. One participant in Esmeraldas and five in Quito were excluded because they had not lived in that study site for more than six months. Six controls, three in Esmeraldas and three in Quito, were excluded because they reported having diarrhea within the last seven days. Lastly, 25 participants were excluded because they reported using antibiotics within the last days. Fourteen of these controls were recruited from Esmeraldas, ten from Quito, and one from Borbón. After determining there was a significant difference between age categories in Quito, all 45 of the observations from the Quito hospital were also excluded because only adults were being enrolled in the study. After excluding all of these individuals, the total sample size dropped to 673 from the original 720 participants.

Table 1: Descriptive Statistics for All Sites, *n*=720. Reported *p*-values test the null hypothesis that there is no difference between sites.

*=p<0.05, **=p<0.01, ***=p<0.001

Characteristics	*N* 720	Rural Villages *n*=104	Borbón *n*=108	Esmeraldas *n*=237	Quito	*p*-value
		n (%)	*n* (%)	*n* (%)	*n* (%)	
Cases	380	64 (61.5)	58 (53.7)	119 (50.2)	139 (51.3)	0.250
Demographics						
Age						0.002**
0-2 years	268	29 (27.9)	49 (45.4)	90 (38)	100 (36.9)	
2-5 years	126	33 (31.7)	15 (13.9)	39 (16.5)	39 (14.4)	
5-15 years	136	18 (17.3)	23 (21.3)	49 (20.7)	46 (17)	
15+ years	190	24 (23.1)	21 (19.4)	59 (24.9)	86 (31.7)	
Male	386	52 (50)	67 (62)	133 (56.1)	134 (49.4)	0.110
Race Reported	697	104 (100)	108 (100)	222 (93.7)	263 (97)	0.002
Black	195	33 (31.7)	66 (61.1)	93 (41.9)	3 (1.1)	
Indigenous	14	4 (3.8)	5 (4.6)	0 (0)	5 (1.9)	
Manaba	30	9 (8.7)	9 (8.3)	8 (3.6)	4 (1.5)	
Mixed	419	29 (27.9)	28 (25.9)	119 (53.6)	243 (92.4)	
White	9	0 (0)	0 (0)	1 (0.5)	8 (3)	<0.001***
Other	30	29 (27.9)	0 (0)	1 (0.5)	0 (0)	
Socioeconomic Status						
Reported SES Data	698	104 (100)	108 (100)	223 (94.1)	263 (97)	0.003**
Receives assistance	135	46 (44.2)	30 (27.8)	44 (19.7)	15 (5.7)	<0.001***
Employment	361	21 (20.2)	40 (37)	97 (43.5)	203 (77.2)	<0.001***
Loaned house	66	10 (9.6)	6 (5.6)	25 (11.2)	25 (9.5)	<0.001***
Owned house	418	88 (84.6)	84 (77.8)	160 (71.7)	86 (32.7)	
Rented house	214	6 (5.8)	18 (16.7)	38 (17)	152 (57.8)	
Some Education	690	102 (98.1)	107 (99.1)	219 (92.4)	262 (96.7)	0.012*
Elementary	76	26 (25)	5 (4.6)	15 (6.3)	30 (11.1)	<0.001***
High School	399	67 (64.4)	85 (78.7)	117 (49.4)	130 (48)	
University	215	9 (8.7)	17 (15.7)	87 (36.7)	102 (37.6)	
Reported Nursery Use	340	52 (50)	59 (54.6)	107 (45.1)	122 (45)	
Nursery in the past month	70	17 (32.7)	19 (32.2)	19 (17.8)	15 (12.3)	0.002**
Reported Contact with Animals	340	41 (39.4)	51 (47.2)	101 (42.6)	147 (54.2)	0.020*
Broiler chickens	31	11 (26.8)	10 (19.6)	3 (3.0)	7 (4.8)	<.0001***

Characteristics	***N***	**Rural Villages**	**Borbón**	**Esmeraldas**	**Quito**	***p*-value**
Cat	141	19 (46.3)	32 (62.8)	52 (51.5)	38 (25.9)	<.0001***
Cow	5	1 (2.4)	2 (3.9)	1 (1.0)	1 (0.7)	0.254
Dog	257	26 (63.4)	34 (66.7)	68 (67.3)	129 (87.8)	<.0001***
Pig	23	8 (19.5)	11 (21.6)	1 (1.0)	3 (2.0)	<.0001***
Poultry	38	11 (26.8)	12 (23.5)	3 (2.97)	12 (8.2)	<.0001***
Rat	1	0 (0)	0 (0)	0 (0)	1 (0.7)	>0.99
Other	8	2 (4.9)	0 (0)	2 (2.0)	4 (2.7)	0.473
Medical						
Rotavirus Results Known	574	91 (87.5)	85 (78.7)	198 (83.5)	200 (73.8)	
Positive for rotavirus	44	9 (9.9)	7 (8.2)	13 (6.6)	15 (7.5)	0.800
Had full course of vaccine	7	1 (11.1)	1 (14.3)	1 (7.7)	4 (26.7)	0.695
Rotavirus Vaccine Known	128	12 (11.5)	15 (13.9)	34 (14.3)	67 (24.7)	
1st dose of rotavirus vaccine	112	11 (91.7)	14 (93.3)	29 (85.3)	58 (86.6)	0.949
2nd dose of rotavirus vaccine	98	10 (90.9)	9 (64.3)	26 (89.7)	53 (91.4)	0.076
Parasite Results Known	187	6 (5.8)	20 (18.5)	160 (67.5)	1 (0.4)	
Positive for parasites	40	0 (0)	7 (35)	33 (20.6)	0 (0)	0.289
Reported Medicine Use	687	104 (100)	107 (99.1)	213 (89.9)	263 (97)	
Used medicine in past 7 days	191	1 (1)	4 (3.7)	78 (36.6)	108 (41.1)	<0.001***
Reported Breastfeeding Practices	338	50 (48.1)	59 (54.6)	107 (45.1)	122 (45)	
Never breastfed	4	0 (0)	0 (0)	1 (0.9)	3 (2.5)	<0.001***
Finished breastfeeding	175	31 (62)	39 (66.1)	66 (61.7)	39 (32)	
Complementary feeding	109	15 (30)	18 (30.5)	24 (22.4)	52 (42.6)	
Exclusive breastfeeding	50	4 (8)	2 (3.4)	16 (15)	28 (23)	
Under 1 year	47	2 (50.0)	2 (100)	15 (93.8)	28 (100)	0.008**
Under 6 months	36	1 (25.0)	1 (50.0)	10 (62.5)	24 (85.7)	0.026*
Water and Sanitation Practices						
Reported Sanitation Practices at Home	693	104 (100)	108 (100)	218 (92)	263 (97)	
"Improved Sanitation"	627	61 (59.8)	87 (80.6)	217 (99.5)	262 (99.6)	<0.001***
Community latrine	9	7 (6.7)	1 (0.9)	1 (0.5)	0 (0)	<0.001***
Diaper	175	21 (20.2)	33 (30.6)	65 (29.8)	56 (21.3)	0.050*
Hole in ground	51	31 (29.8)	19 (17.6)	1 (0.5)	0 (0)	<0.001***
Open defecation	14	11 (10.6)	2 (1.9)	0 (0)	1 (0.4)	<0.001***
Personal latrine	33	9 (8.7)	5 (4.6)	9 (4.1)	10 (3.8)	0.240
Pour-over flush	294	0 (0)	1 (0.9)	98 (45)	195 (74.1)	<0.001***
River	2	2 (1.9)	0 (0)	0 (0)	0 (0)	0.022*
Septic tank	117	24 (23.1)	47 (43.5)	45 (20.6)	1 (0.4)	<0.001***

Characteristics	***N***	**Rural Villages**	**Borbón**	**Esmeraldas**	**Quito**	***p*-value**
Reported Sanitation Practices While Traveling	55	2 (1.9)	8 (7.4)	16 (6.8)	29 (10.7)	
"Improved Sanitation"	52	1 (50)	8 (100)	14 (87.5)	29 (100)	0.154
Community latrine	2	1 (50)	0 (0)	0 (0)	1 (3.4)	0.091
Diaper	14	0 (0)	4 (50)	6 (37.5)	4 (13.8)	0.077
Hole in ground	1	1 (50)	0 (0)	0 (0)	0 (0)	0.036*
Open defecation	2	0 (0)	0 (0)	2 (12.5)	0 (0)	0.172
Personal latrine	3	0 (0)	2 (25)	0 (0)	1 (3.4)	0.143
Pour-over flush	26	0 (0)	0 (0)	4 (25)	22 (75.9)	<0.001***
Septic tank	7	0 (0)	2 (25)	4 (25)	1 (3.4)	0.083
Reported Water Source at Home	696	104 (100)	108 (100)	221 (93.2)	263 (97)	
"Improved Water Source"	670	93 (89.4)	95 (88)	220 (99.5)	262 (99.6)	<0.001***
Bottled water	212	22 (21.2)	49 (45.4)	79 (35.7)	62 (23.6)	<0.001***
Neighbor's tap	5	4 (3.8)	1 (0.9)	0 (0)	0 (0)	<0.001***
Rainwater	57	51 (49)	6 (5.6)	0 (0)	0 (0)	<0.001***
River	13	8 (7.7)	3 (2.8)	1 (0.5)	1 (0.4)	<0.001***
Tap inside	374	9 (8.7)	33 (30.6)	112 (50.7)	220 (83.7)	<0.001***
Tap outside	46	7 (6.7)	6 (5.6)	32 (14.5)	1 (0.4)	<0.001***
Well	15	5 (4.8)	10 (9.3)	0 (0)	0 (0)	<0.001***
Treat Water at Home	302	23 (22.1)	23 (21.3)	87 (39.4)	169 (64.3)	<0.001***
Reported Water Source While Traveling	96	41 (39.4)	10 (9.3)	16 (6.8)	29 (10.7)	
"Improved Water Source"	58	5 (100)	10 (100)	15 (93.8)	28 (96.6)	>0.99
Bottled water	40	5 (100)	10 (100)	11 (68.8)	14 (48.3)	0.005**
Rainwater	1	0 (0)	0 (0)	1 (6.3)	0 (0)	0.517
River	2	0 (0)	0 (0)	1 (6.3)	1 (3.4)	>0.99
Tap inside	14	0 (0)	0 (0)	2 (12.5)	12 (41.4)	0.016*
Tap outside	3	0 (0)	0 (0)	1 (6.3)	2 (6.9)	>0.99
Treat Water While Traveling	15	0 (0)	0 (0)	4 (25)	11 (37.9)	0.059
Travel History						
Reported Travel Practices	698	104 (100)	108 (100)	223 (94.1)	263 (97)	
Traveled in the Past Week	61	5 (4.8)	10 (9.3)	16 (6.8)	30 (11.1)	0.160
Traveled in the Past Year	427	102 (100)	108 (100)	75 (33.6)	142 (54)	<0.001***
Exclusion Criteria						
Wrong city		0 (0)	0 (0)	10 (4.2)	0 (0)	0.000
Has not lived in the area for at least 6 months	6	0 (0)	0 (0)	1 (0.5)	5 (2.1)	0.226
Control had diarrhea	6	0 (0)	0 (0)	3 (2.6)	3 (2.3)	0.774
Antibiotics in past 7 days	25	0 (0)	1 (0.9)	14 (6.2)	10 (3.8)	0.009**

Table 2: Descriptive Statistics for All Sites, by Cases and Controls n=673. Reported *p*-values test the null hypothesis that there is no difference between sites.

*=*p*<0.05, **=*p*<0.01, ***=*p*<0.001

	Total			Rural Villages			Borbón			Esmeraldas			Quito		
Characteristic	**Control**	**Case**	***p*-value**	**Control**	**Case**	***p*-value**	**Control**	**Case**	***p*-value**	**Control**	**Case**	***p*-value**	**Control**	**Case**	***p*-value**
	321	352		40	64		49	58		109	100		123	130	
	n (%)	*n* (%)		*n* (%)	*n* (%)		*n* (%)	*n* (%)		*n* (%)	*n* (%)		*n* (%)	*n* (%)	
Demographics															
Age															
0-2 years	116 (36.1)	126 (35.8)		9 (22.5)	20 (31.3)		22 (44.9)	26 (44.8)		38 (34.9)	35 (35)		47 (38.2)	45 (34.6)	
2-5 years	56 (17.4)	65 (18.5)		13 (32.5)	20 (31.3)		6 (12.2)	9 (15.5)		20 (18.3)	16 (16)		17 (13.8)	20 (15.4)	
5-15 years	66 (20.6)	65 (18.5)		7 (17.5)	11 (17.2)		12 (24.5)	11 (19)		24 (22)	23 (23)		23 (18.7)	20 (15.4)	
15+ years	83 (25.9)	96 (27.3)	0.890	11 (27.5)	13 (20.3)	0.740	9 (18.4)	12 (20.7)	0.880	27 (24.8)	26 (26)	0.970	36 (29.3)	45 (34.6)	0.730
Male	158 (49.2)	197 (56)	0.080	19 (47.5)	33 (51.6)	0.690	29 (59.2)	37 (63.8)	0.630	54 (49.5)	59 (59)	0.170	56 (45.5)	68 (52.3)	0.280
Race Reported	320 (99.7)	352 (100)	0.477	40 (100)	64 (100)	0.340	49 (100)	58 (100)	0.290	108 (99.1)	100 (100)	>0.99	123 (100)	130 (100)	--
Black	98 (30.6)	90 (25.6)		16 (40)	17 (26.6)		32 (65.3)	33 (56.9)		49 (45.4)	38 (38)		1 (0.8)	2 (1.5)	
Indigenous	3 (0.9)	11 (3.1)		0 (0)	4 (6.3)		1 (2)	4 (6.9)		0 (0)	0 (0)		2 (1.6)	3 (2.3)	
Manaba	15 (4.7)	14 (4)		4 (10)	5 (7.8)		4 (8.2)	5 (8.6)		5 (4.6)	2 (2)		2 (1.6)	2 (1.5)	
Mixed	192 (60)	210 (59.7)		12 (30)	17 (26.6)		12 (24.5)	16 (27.6)		53 (49.1)	59 (59)		115 (93.5)	118 (90.8)	
White	4 (1.3)	5 (1.4)		0(0)	0(0)		0 (0)	0 (0)		1 (0.9)	0 (0)		3 (2.4)	5 (3.8)	
Other	8 (2.5)	22 (6.3)	0.050*	8 (20)	21 (32.8)	0.250	0 (0)	0 (0)	0.691	0 (0)	1 (1)	0.276	0 (0)	0 (0)	0.951
Socioeconomic Status															
Receives government assistance	62 (19.3)	70 (19.9)	0.850	17 (42.5)	29 (45.3)	0.780	13 (26.5)	16 (27.6)	0.900	22 (20.2)	20 (20)	0.970	10 (8.1)	5 (3.8)	0.150
Employment	166 (51.7)	185 (52.6)	0.830	6 (15)	15 (23.4)	0.300	20 (40.8)	20 (34.5)	0.500	48 (44)	46 (46)	0.780	92 (74.8)	104 (80)	0.320
Loaned house	28 (8.7)	35 (9.9)		2 (5)	8 (12.5)		0 (0)	6 (10.3)		13 (11.9)	9 (9)		13 (10.6)	12 (9.2)	
Owned house	200 (62.3)	208 (59.1)		36 (90)	52 (81.3)		41 (83.7)	42 (72.4)		82 (75.2)	70 (70)		41 (33.3)	44 (33.8)	
Rented house	93 (29)	109 (31)	0.680	2 (5)	4 (6.3)	0.489	8 (16.3)	10 (17.2)	0.052	14 (12.8)	21 (21)	0.260	69 (56.1)	74 (56.9)	0.940
Some Education	318 (99.1)	347 (98.6)	0.727	40 (100)	62 (96.9)	0.522	49 (100)	57 (98.3)	>0.99	106 (97.2)	99 (99)	0.623	123 (100)	129 (99.2)	>0.99
Elementary	30 (9.3)	40 (11.4)		9 (22.5)	17 (26.6)		1 (2)	3 (5.2)		5 (4.6)	9 (9)		15 (12.2)	11 (8.5)	
High school	180 (56.1)	208 (59.1)		29 (72.5)	38 (59.4)		40 (81.6)	45 (77.6)		56 (51.4)	56 (56)		55 (44.7)	69 (53.1)	
University	108 (33.6)	99 (28.1)	0.402	2 (5)	7 (10.9)	0.473	8 (16.3)	9 (15.5)	0.813	45 (41.3)	34 (34)	0.369	53 (43.1)	49 (37.7)	0.350
Reported Nursery Use	154 (48)	172 (48.9)		16 (40)	36 (56.3)		26 (53.1)	32 (55.2)		53 (48.6)	45 (45)		59 (48)	59 (45.4)	
Nursery in past month	31 (20.1)	32 (18.6)	0.730	7 (43.8)	10 (27.8)	0.260	11 (42.3)	8 (25)	0.160	7 (13.2)	7 (15.6)	0.740	6 (10.2)	7 (11.9)	0.770
Reported Animal Contact History	154 (48)	172 (48.9)		21 (52.5)	20 (31.3)		18 (36.7)	33 (56.9)		45 (41.3)	49 (49)		70 (56.9)	70 (53.8)	

Characteristic	Total			Rural Villages			Borbón			Esmeraldas			Quito		
	Control	Case	*p*-value	Control	Case	*p*-value	Control	Case	*p*-value	Control	Case	*p*-value	Control	Case	*p*-value
Broiler chickens	20 (13.0)	11 (6.4)	0.043*	5 (23.8)	6 (30.0)	0.655	7 (38.9)	3 (9.1)	0.023*	3 (6.7)	0 (0)	0.106	5 (7.1)	2 (2.9)	0.441
Cat	57 (37.0)	80 (46.5)	0.083	5 (23.8)	14 (70.0	0.003**	10 (55.6)	22 (66.7)	0.433	23 (51.1)	27 (55.1)	0.699	19 (27.1)	17 (24.3	0.699
Cow	4 (2.6)	1 (0.6)	0.193	1 (4.8)	0 (0)	>0.99	1 (5.6)	1 (3.0)	>0.99	1 (2.2)	0 (0)	0.479	1 (1.4)	0 (0)	>0.99
Dog	120 (77.9)	127 (73.8)	0.390	14 (66.7)	12 (60)	0.658	12 (66.7)	22 (66.7)	>0.99	30 (66.7)	33 (67.4)	0.944	64 (91.4)	60 (85.7)	0.438
Pig	13 (8.4)	10 (5.81)	0.355	4 (19.1)	4 (20)	>0.99	7 (38.9)	4 (12.1)	0.037*	1 (2.2)	0 (0)	0.479	1 (1.4)	2 (2.9)	>0.99
Poultry	24 (15.6)	14 (8.1)	0.037*	5 (23.8)	6 (30.0)	0.655	8 (44.4)	4 (12.1)	0.015*	3 (6.7)	0 (0)	0.106	8 (11.4)	4 (5.7)	0.227
Rat	0 (0)	1 (0.6)	0.058	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	1 (1.4)	>0.99
Other	5 (3.3)	2 (1.2)	0.262	2 (9.5)	0 (0)	0.488	0 (0)	0 (0)	--	1 (2.2)	1 (2.0)	>0.99	2 (2.9)	1 (1.4)	>0.99
Medical															
Rotavirus Results Known	282 (87.9)	271 (77)		39 (97.5)	52 (81.3)		46 (93.9)	38 (65.5)		98 (89.9)	88 (88)		99 (80.5)	93 (71.5)	
Positive for rotavirus	1 (0.4)	41 (15.1)	<0.001***	0 (0)	9 (17.3)	0.009**	0 (0)	7 (18.4)	0.003**	1 (1)	11 (12.5)	0.001***	0 (0)	14 (15.1)	<0.001**
Had full course	0 (0)	7 (17.07)	>0.99	0 (0)	1 (11.11)	--	0 (0)	1 (14.3)	--	0 (0)	1 (9.1)	>0.99	0 (0)	4 (28.6)	--
Rotavirus Vaccine Known	63 (19.6)	55 (15.6)		3 (7.5)	9 (14.1)		9 (18.4)	5 (8.6)		19 (17.4)	10 (10)		32 (26)	31 (23.8)	
1st dose of rotavirus vaccine	52 (82.5)	51 (92.7)	0.098	3 (100)	8 (88.9)	>0.99	8 (88.9)	5 (100)	>0.99	16 (84.2)	9 (90.0)	>0.99	25 (78.1)	29 (93.6)	0.148
2nd dose of vaccine	47 (90.4)	42 (82.4)	0.234	3 (100)	7 (87.5)	>0.99	5 (62.5)	3 (60.0)	>0.99	15 (93.8)	7 (77.8)	0.530	24 (96.0)	25 (86.2)	0.358
Parasite Results Known	91 (28.3)	84 (23.9)		1 (2.5)	5 (7.8)		10 (20.4)	10 (17.2)		80 (73.4)	68 (68)		0 (0)	1 (0.8)	
Positive for parasites	25 (27.5)	13 (15.5)	0.050*	0 (0)	0 (0)	0.190	5 (50)	2 (20)	0.350	20 (25)	11 (16.2)	0.190	0 (0)	0 (0)	--
Reported Medicine Use	314 (97.8)	348 (98.9)		40 (100)	64 (100)		48 (98)	58 (100)		103 (94.5)	96 (96)		123 (100)	130 (100)	
Used medicine in past 7 days	57 (18.2)	109 (31.3)	<0.001***	1 (2.5)	0 (0)	0.385	1 (2.1)	2 (3.4)	>0.99	23 (22.3)	41 (42.7)	0.002**	32 (26)	66 (50.8)	<0.001**
Reported Breastfeeding Practices	153 (47.7)	171 (48.6)		15 (37.5)	35 (54.7)		26 (53.1)	32 (55.2)		53 (48.6)	45 (45)		59 (48)	59 (45.4)	
Finished breastfeeding	77 (50.3)	90 (52.6)		10 (66.7)	21 (60)		17 (65.4)	22 (68.8)		33 (62.3)	27 (60)		17 (28.8)	20 (33.9)	
Complementary feeding	43 (28.1)	62 (36.3)		4 (26.7)	11 (31.4)		8 (30.8)	9 (28.1)		10 (18.9)	13 (28.9)		21 (35.6)	29 (49.2)	
Exclusive breastfeeding	30 (19.6)	18 (10.5)	0.050*	1 (6.7)	3 (8.6)	>0.99	1 (3.8)	1 (3.1)	>0.99	9 (17)	5 (11.1)	0.501	19 (32.2)	9 (15.3)	0.121
Under 1 year	30 (100)	15 (83.3)	0.047*	1 (100)	1 (33.3)	>0.99	1 (100)	1 (100)	--	9 (100)	4 (80)	0.357	19 (100)	9 (100)	--
Under 6 months	23 (76.7)	12 (66.7)	0.513	0 (0)	1 (33.3)	>0.99	0 (0)	1 (100)	>0.99	5 (55.6)	4 (80)	0.580	18 (94.7)	6 (66.7)	0.084
Water and Sanitation															
Reported Home Sanitation Practices	321 (100)	347 (98.6)		40 (100)	64 (100)		49 (100)	58 (100)		109 (100)	95 (95)		123 (100)	130 (100)	
"Improved Sanitation"	296 (92.5)	306 (88.4)	0.080	26 (66.7)	35 (55.6)	0.270	39 (79.6)	47 (81)	0.850	109 (100)	94 (98.9)	0.466	122 (99.2)	130 (100)	0.486
Community latrine	7 (2.2)	2 (0.6)	0.096	5 (12.5)	2 (3.1)	0.104	1 (2)	0 (0)	0.458	1 (0.9)	0 (0)	>0.99	0 (0)	0 (0)	--
Diaper	86 (26.8)	79 (22.8)	0.230	6 (15)	15 (23.4)	0.300	19 (38.8)	13 (22.4)	0.070	33 (30.3)	24 (25.3)	0.430	28 (22.8)	27 (20.8)	0.700
Hole in ground	19 (5.9)	32 (9.2)	0.110	11 (27.5)	20 (31.3)	0.680	8 (16.3)	11 (19)	0.720	0 (0)	1 (1.1)	0.466	0 (0)	0 (0)	--
Open defecation	6 (1.9)	8 (2.3)	0.690	3 (7.5)	8 (12.5)	0.524	2 (4.1)	0 (0)	0.207	0 (0)	0 (0)	--	1 (0.8)	0 (0)	0.486
Personal latrine	13 (4)	19 (5.5)	0.390	4 (10)	5 (7.8)	0.730	0 (0)	5 (8.6)	0.061	3 (2.8)	6 (6.3)	0.309	6 (4.9)	3 (2.3)	0.323
Pour-over flush	138 (43)	142 (40.9)	0.590			0.400	0 (0)	1 (1.7)	>0.99	50 (45.9)	42 (44.2)	0.810	88 (71.5)	99 (76.2)	0.400
River	1 (0.3)	1 (0.3)	>0.99	1 (2.5)	1 (1.6)	>0.99	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	0 (0)	--
Septic tank	53 (16.5)	64 (18.4)	0.510	11 (27.5)	13 (20.3)	0.400	19 (38.8)	28 (48.3)	0.320	23 (21.1)	22 (23.2)	0.720	0 (0)	1 (0.8)	>0.99

Characteristic	Total Control	Total Case	Total *p*-value	Rural Villages Control	Rural Villages Case	Rural Villages *p*-value	Borbón Control	Borbón Case	Borbón *p*-value	Esmeraldas Control	Esmeraldas Case	Esmeraldas *p*-value	Quito Control	Quito Case	Quito *p*-value
Reported Travel Sanitation Practices	27 (8.4)	26 (7.4)		1 (2.5)	1 (1.6)		5 (10.2)	3 (5.2)		5 (4.6)	10 (10)		16 (13)	12 (9.2)	
"Improved Sanitation"	27 (100)	23 (88.5)	0.111	1 (100)	0 (0)	>0.99	5 (100)	3 (100)	--	5 (100)	8 (80)	0.524	16 (100)	12 (100)	--
Community latrine	2 (7.4)	0 (0)	0.491	1 (100)	0 (0)	>0.99	0 (0)	0 (0)	--	0 (0)	0 (0)	--	1 (6.3)	0 (0)	>0.99
Diaper	8 (29.6)	5 (19.2)	0.380	0 (0)	0 (0)	--	3 (60)	1 (33.3)	>0.99	2 (40)	3 (30)	>0.99	3 (18.8)	1 (8.3)	0.613
Hole	0 (0)	1 (3.8)	0.491	0 (0)	1 (100)	>0.99	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	0 (0)	--
Open defecation	0 (0)	2 (7.7)	0.236	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	2 (20)	0.524	0 (0)	0 (0)	--
Personal latrine	1 (3.7)	2 (7.7)	0.610	0 (0)	0 (0)	--	0 (0)	2 (66.7)	0.107	0 (0)	0 (0)	--	1 (6.3)	0 (0)	>0.99
Pour-over flush	12 (44.4)	14 (53.8)	0.490	0 (0)	0 (0)	--	0 (0)	0 (0)	--	1 (20)	3 (30)	>0.99	11 (68.8)	11 (91.7)	0.139
Septic tank	4 (14.8)	2 (7.7)	0.669	0 (0)	0 (0)	--	2 (40)	0 (0)	0.464	2 (40)	2 (20)	0.560	0 (0)	0 (0)	--
Reported Home Water Practices	321 (100)	351 (99.7)		40 (100)	64 (100)		49 (100)	58 (100)		109 (100)	99 (99)		123 (100)	130 (100)	
"Improved Water Source"	311 (96.9)	335 (95.4)	0.330	38 (95)	55 (85.9)	0.197	43 (87.8)	51 (87.9)	0.980	108 (99.1)	99 (100)	>0.99	122 (99.2)	130 (100)	0.300
Bottled water	97 (30.2)	106 (30.2)	1.000	11 (27.5)	11 (17.2)	0.210	20 (40.8)	29 (50)	0.340	37 (33.9)	35 (35.4)	0.830	29 (23.6)	31 (23.8)	0.486
Neighbor's tap	2 (0.6)	3 (0.9)	>0.99	1 (2.5)	3 (4.7)	>0.99	1 (2)	0 (0)	0.458	0 (0)	0 (0)	--	0 (0)	0 (0)	--
Rainwater	24 (7.5)	33 (9.4)	0.370	21 (52.5)	30 (46.9)	0.580	3 (6.1)	3 (5.2)	>0.99	0 (0)	0 (0)	--	0 (0)	0 (0)	--
River	4 (1.2)	9 (2.6)	0.220	2 (5)	6 (9.4)	0.708	0 (0)	3 (5.2)	0.248	1 (0.9)	0 (0)	>0.99	1 (0.8)	0 (0)	0.486
Tap inside	184 (57.3)	175 (49.9)	0.050*	3 (7.5)	6 (9.4)	>0.99	17 (34.7)	15 (25.9)	0.320	60 (55)	46 (46.5)	0.220	104 (84.6)	108 (83.1)	0.750
Tap outside	16 (5)	30 (8.5)	0.070	2 (5)	5 (7.8)	0.705	2 (4.1)	4 (6.9)	0.685	12 (11)	20 (20.2)	0.070	0 (0)	1 (0.8)	>0.99
Well	7 (2.2)	8 (2.3)	0.930	1 (2.5)	4 (6.3)	0.647	6 (12.2)	4 (6.9)	0.507	0 (0)	0 (0)	--	0 (0)	0 (0)	--
Treated water at home	159 (49.5)	135 (38.5)	0.004**	7 (17.5)	16 (25)	0.370	13 (26.5)	10 (17.2)	0.240	53 (48.6)	32 (32.3)	0.020*	86 (69.9)	77 (59.2)	0.080
Reported Travel Water Practices	42 (13.1)	52 (14.8)		16 (40)	25 (39.1)		5 (10.2)	5 (8.6)		5 (4.6)	10 (10)		16 (13)	12 (9.2)	
"Improved Water Source"	26 (92.9)	30 (100)	0.229	2 (100)	3 (100)	--	5 (100)	5 (100)	--	4 (80)	10 (100)	0.333	15 (93.8)	12 (100)	>0.99
Bottled water	16 (57.1)	23 (76.7)	0.110	2 (100)	3 (100)	--	5 (100)	5 (100)	--	2 (40)	8 (80)	0.251	7 (43.8)	7 (58.3)	0.450
Rainwater	0 (0)	1 (3.3)	>0.99	0 (0)	0 (0)	--	0 (0)	0 (0)	--	0 (0)	1 (10)	>0.99	0 (0)	0 (0)	--
River	2 (7.1)	0 (0)	0.229	0 (0)	0 (0)	--	0 (0)	0 (0)	--	1 (20)	0 (0)	0.333	1 (6.3)	0 (0)	>0.99
Tap inside	8 (28.6)	6 (20)	0.450	0 (0)	0 (0)	--	0 (0)	0 (0)	--	1 (20)	1 (10)	>0.99	7 (43.8)	5 (41.7)	0.910
Tap outside	2 (7.1)	0 (0)	0.229	0 (0)	0 (0)	--	0 (0)	0 (0)	--	1 (20)	0 (0)	0.333	1 (6.3)	0 (0)	>0.99
Treated water while traveling	10 (35.7)	5 (16.7)	0.100	0 (0)	0 (0)	--	0 (0)	0 (0)	--	3 (60)	1 (10)	0.077	7 (43.8)	4 (33.3)	0.705
Travel History															
Reported Travel History	321 (100)	352 (100)		40 (100)	64 (100)		49 (100)	58 (100)		109 (100)	100 (100)		123 (100)	130 (100)	
Travel in the past week	28 (8.7)	31 (8.8)	0.970	2 (5)	3 (4.7)	>0.99	5 (10.2)	5 (8.6)	>0.99	5 (4.6)	10 (10)	0.130	16 (13)	13 (10)	0.450
Travel in the past year	184 (57.3)	233 (66.6)	0.010**	40 (100)	62 (100)	--	49 (100)	58 (100)	--	28 (25.7)	43 (43)	0.008**	67 (54.5)	70 (53.8)	0.920

Demographics

Within the sample population, the mean age was 12 years old (146.9 months) and the median age was 4 years old (48 months). Males represented a little over 50% of the total population. The most pronounced race in the population was *Mestizo*, or mixed, accounting for 58% of the population. Nearly a third of the population reported Black as their race. This was primarily true for Borbón and Esmeraldas.

Socioeconomic Status

Variables of interest for socioeconomic status included receiving government assistance, employment, housing status, education, and nursery use. 75% of the population in Quito reported that someone in their house had a job, whereas the rural communities and Borbón had fewer people that worked. For the people in Esmeraldas and Quito, there were more cases than controls that received a university degree but this did not hold true for the rural communities and Borbón, which reported that more controls had university degrees than cases.

Medical History

Among the cases and controls, there were 41 participants who tested positive for rotavirus. If a patient was under the age of three years old, his/her guardian was asked information about the patient's rotavirus vaccination history. Of those who reported receiving the full course of the vaccine, 7% tested positive for rotavirus. However, of those that tested positive for rotavirus 100% (7/7) had received the full course of the vaccine. 38 of the 175 patients whose stool samples were tested for parasites were returned positive for one or more parasites. Both cases and controls were found positive for parasites in Borbón and Quito. At the time of analysis, no data on parasitic infections was collected from Quito or rural communities. Patients were also asked about their medication use in the last seven days. There was a significant difference for medication use between cases and controls in Quito and Esmeraldas *(p*=<0.001 and *p*=0.002 respectively), whereas very few participants reported taking medications in Borbón and in the rural communities. As for breastfeeding practices, there were no differences between cases and controls in each site.

Water and Sanitation Practices

Of the 696 respondents to this section, 96% reported using improved water sources at home. As mentioned earlier, improved water sources were classified as a tap inside, a tap outside, a neighbor's tap, rainwater, and bottled water. There were no differences in types of home water sources by site nor between cases and controls for each site. Of the 59 participants who traveled in the last week and answered the questions about water sources, 97% used improved water sources while traveling. Just like with home water sources, there were no differences in types of travel water sources neither by site nor between cases and controls for each site. A tap inside was the most commonly used source of water at home, whereas bottled water was the most commonly used source of water while traveling.

In addition to asking about water sources at home and while traveling, participants were asked about water treatment at home and while traveling. Of those asked about water treatment at home, 44% reported treating their water. Since the p-value was less than 0.05, the null hypothesis was rejected and it was concluded that there was a significant difference in those who treated their water between each site *(p*<0.001) and between cases and controls in each site *(p*=0.004). Of those who were asked about water treatment while traveling, 15% reported treating their water. There were no significant differences in water treatment while traveling between sites and between cases and controls for each site. The primary means of water treatment for both at home and while traveling was boiling water, with 93% of individuals reporting boiling. There were no significant differences between any of the other types of water treatment (Abate, filter, and chlorination).

As for sanitation, 91% of the 687 respondents reporting using improved sanitation sources at home. Improved sanitation sources included pour-over flush, personal latrine, community latrine, septic tanks, and diapers. There were no differences in types of home sanitation sources neither by site nor between cases and controls for each site. Of the 50 participants who traveled in the last week and answered the questions regarding sanitation sources, 94% used improved sanitation sources while traveling. Similar to sanitation at home, there were no difference in types of sanitation sources while traveling neither by site nor between cases and controls for each site. A pour-over flush was the most commonly used source of sanitation at home and while traveling.

Travel

Two-thirds of the entire study population reported traveling at least once in the last year. Across each study site, cases reported traveling more in the last year than cases (p=0.010). However, only 59 respondents reported travel in the last week across the four sites. With the exception of Quito, every site had more cases than controls that traveled. The distribution of travel between study sites and their destinations is depicted below in Figure 6. Residents of the rural communities surrounding Borbón traveled often to Borbón and Esmeraldas but not as much to Quito. Residents of Borbón experienced a high amount of travel between Borbón and Esmeraldas, as well as between Borbón and Quito.

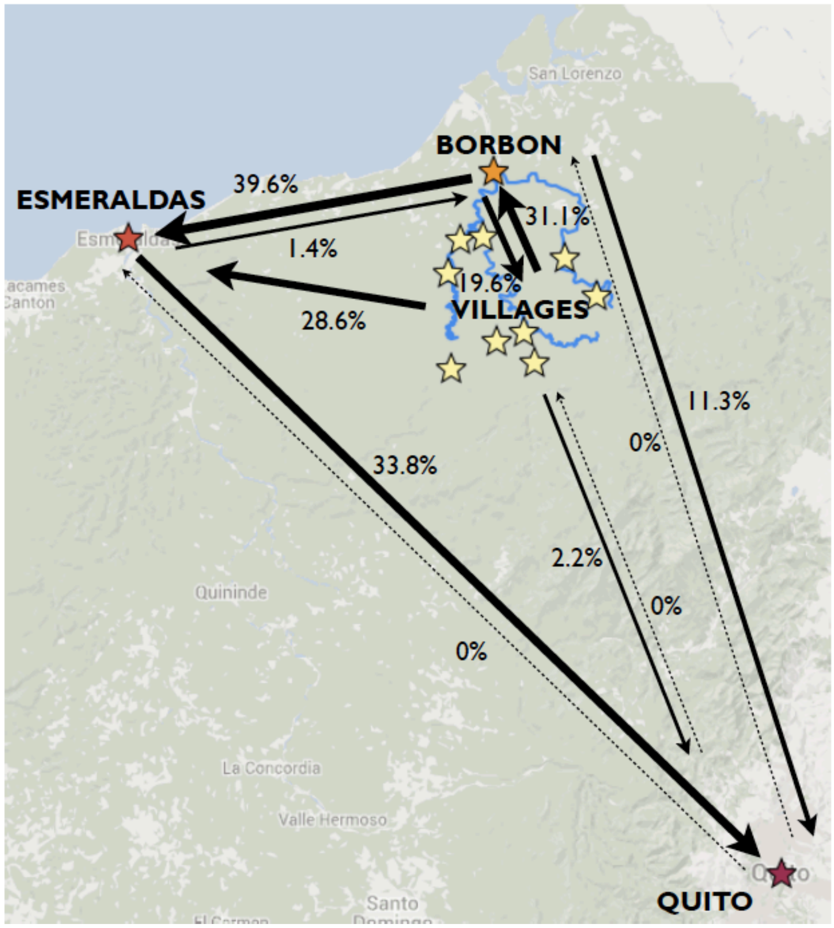


Figure 6: Distribution of travel between study sites.

Residents of Quito did not report travel between Quito and any of the other study sites, though the participants reported travel to areas near the study sites, including the Province of Esmeraldas.

Comparison of Water and Sanitation Practices in Rural versus Urban Sites

When comparing water systems between rural versus urban sites, there were significant differences in each of the types of water systems. Nearly 50% of the rural communities used rainwater as their primary source of water, whereas 45% of Borbon used bottled water, 51% of Esmeraldas and 85% of Quito used a tap inside.

As for sanitation, there were significant differences in nearly each of the types of sanitation practices. In the rural communities, almost 30% of participants use a hole in the ground. Conversely, 44% of Borbon use septic tanks, 45% of Esmeraldas and 74% of Quito use pour-over flush latrines.

Comparison of Water and Sanitation Practices while Traveling

When investigating changes in water practices with short-term travel within the study region, the null hypothesis was rejected because there were significant differences in those who used improved versus unimproved water sources when traveling (p=0.03). 98% of those who used improved water sources at home continued to use improved water sources while traveling, while 2% of those who used improved water sources changed to unimproved water sources while traveling. 100% of those who used unimproved water sources at home continued to use unimproved water sources while traveling. The numbers and percentages are shown in Table 3.

	Improved Water While Traveling	**Unimproved Water While Traveling**
Improved Water at Home	58 (98.31)	1 (1.69)
Unimproved Water at Home	0 (0)	1 (100)

Table 3: Changes in water practices between home and travel.

When investigating changes in sanitation practices with short-term travel within the study region, there were no significant differences in those who used improved versus unimproved sanitation sources when traveling. 94% of those who used improved sanitation sources at home continued to use improved sanitation sources while traveling, while 6% of those who used improved sanitation sources changed to unimproved sanitation sources while traveling. 100% of those who used improved sanitation sources at home continued to use improved sanitation sources while traveling. The numbers and percentages are shown in Table 4.

	Improved Sanitation While Traveling	**Unimproved Sanitation While Traveling**
Improved Sanitation at Home	46 (93.88)	3 (6.12)
Unimproved Sanitation at Home	1 (100)	0 (0)

Table 4: Changes in sanitation practices between home and travel.

Risk Factors for Diarrheal Disease, Parasites, and Rotavirus

To determine if water and sanitation practices were risk factors for diarrheal disease, parasites, and rotavirus, logistic regression models were created. While controlling for age, race, sex, improved sanitation at home, improved water at home, and treating water at home, there were no associations for these risk factors and their impact on parasites and rotavirus. However, in the all-cause diarrheal disease model, one variable of interest offered significant protection against diarrhea. Of those who treated water at home, their odds for having diarrhea were 0.6 times the odds of those who didn't treat water, while controlling for race, age, sex, improved sanitation at home, and improved water at home (0.436, 0.824; p=0.0016). No other results were of interest from these models.

Chapter 5 – Discussion, Conclusion, and Recommendations

Discussion

Characteristics of the study sample were consistent with what was expected for the population for each of the study sites. The median age of the study participants is of particular interest because current diarrheal research emphasizes the global burden of diarrheal disease among children under the age of five years old. There were significant differences with some demographic and socioeconomic variables between sites but this disappeared when divided into cases and controls by site. The lack of differences in cases and controls suggests that it is appropriate to compare them. While there were not a lot of differences between cases and controls, a few risk factors emerged from the data. For almost all of the sites, reported medication use was higher in cases than in controls. This may be because people with diarrhea were taking medications for treatment or a result of drug-induced diarrhea. While we attempted to exclude patients who reported taking antibiotics in the last seven days, there are several medications besides antibiotics that are known to cause diarrhea. This may have been the instance for some of the cases.

As for the picture of water and sanitation practices in rural and urban sites, there were significant differences between the sites. Rural communities primarily utilized unimproved water and sanitation systems. Borbón, a larger community, used a mix of improved and sanitation practices. Esmeraldas appeared more similar to Quito, with larger percentages of the population using improved sources of water and sanitation. These results align with the idea that rural communities lack access to more improved water and sanitation sources than in more urban areas. The number of cases from the urban areas though suggests that these improved sources of water and sanitation might not be as effective in reducing the transmission of pathogens in these sites. This may be in part because of compromised systems, compliance, or mismanagement of the water and sanitation systems.

Surprisingly, there were not a lot of significant differences between cases and controls in regards to their water practices. Although the majority of cases reported using improved water sources, they still presented with diarrhea. Traditionally, it is thought that improved water sources could alleviate many of the waterborne pathogens that result in diarrhea or other diseases. Reviews have concluded that a piped water system could have a significant impact on childhood mortality due to diarrheal diseases (Fewtrell and Colford 2005; Clasen 2007). However, current research is divided on the subject of 'improved' water sources and their impact on diarrhea reduction. Literature reviews show that despite having improved water sources, such as taps inside, that the microbial content in the water varied inconsistently due to low pressure in the piped system and household contamination from handling practices or improper storage (Clasen et al 2007; Wolf et al. 2014). These results regarding improved water sources suggest that it may not the type of water source that you use but how you use it that can have the greatest impact on health.

Of more importance than the type of water source may be water treatment and its impact on diarrhea risk. For this population, treating water at home was protective against diarrhea. Boiling was the main method of water treatment but there were little data to determine if one method was more effective than another method. Boiling has been a common form of water treatment since Hippocrates recommended it in 350 B.C. (Montgomery 2007). Although there are concerns for recontamination of the boiled water, especially with handling and storage, boiling properly can reduce pathogens in the water (Mintz 1995).

As for information regarding human movement, water and sanitation practices, and their risk for diarrheal diseases, the data are mostly inconclusive. This is largely in part because of such a small sample size for those who traveled. The confidence intervals are too wide in each of the logistic regression models to determine with certainty if water and sanitation practices affected the transmission of pathogens. Of those who did travel in the last week, very few changed their water and sanitation practices. There was, however, a significant difference in those who used improved versus unimproved water sources when traveling, meaning that there is evidence that types of water sources are not independent of traveling. These results posit that there may be a different mediating factor in the pathway between exposure and diarrhea other than changes in water and sanitation practices when traveling.

Limitations and Delimitations

The number of limitations inherent with this research may have compromised the results. The case-control study design has strengths and weaknesses. On the one hand, a case-control design is good for studying the outcome variable – diarrheal disease – and the controls matched on age; thus, eliminating some selection bias. However, diarrheal disease can have numerous causes, so the relationship between water and sanitation practices and diarrhea incidence may have been confounded by etiologic agents. Additionally, the small sample size impacted the quantity and quality of data. Even though sample size calculations were initially done to determine the number of cases and controls needed at each site, the calculations did not take into consideration the number of cases and controls reporting travel in the last week. Although more participants responded with travel in the last year, no further data on travel in the last year was collected because of a chance of recall bias. Therefore, the data does not adequately address the question about travel in the last week and water and sanitation practices.

Conclusion

In conclusion, this case-control study used a quantitative and laboratory-based approach to determine how human movement may affect the transmission of pathogens between urban and rural areas and if water and sanitation practices while traveling contributed to the risk of diarrhea. Despite very little data about human movement, the results still provided a possible risk factor for diarrheal disease in this population. The findings from this project contribute to existing public health literature about the importance of water treatment as opposed to the type of water system and how this plays a role in diarrheal disease. Furthermore, these findings shift the focus of water and sanitation interventions while simultaneously providing a picture of how these practices during travel may not directly contribute to the transmission of pathogens in this population, as previously hypothesized.

Recommendations

The question of how travel between urban and rural areas can impact the transmission of diarrheal disease cannot fully be answered with the current data. Once whole genome sequencing of the stool samples is complete, there will more conclusive data to answer how travel is affecting strain circulation in this study region. Additionally, this same research can be replicated with a larger sample size to better assess how water and sanitation practices change from home to travel and how ultimately this could be a risk factor for diarrheal disease.

The significant results from this study though do suggest that water and sanitation systems can only truly be effective when used properly. Instead of proposing more countrywide strategies, more immediate efforts can be directed towards education about water and sanitation systems, specifically about water treatment methods. This inexpensive strategy has implications for travel too. Regardless of whether an individual uses improved or unimproved water sources, treating the water can reduce the odds of diarrhea for that individual.

Point-of-use treatment is already a huge initiative for travelers in developed countries who are visiting developing areas. This initiative could easily transition to developing countries, where residents experience a lot of travel between urban and rural areas. Until vaccines against diarrheagenic *E. coli* and other pathogens become more effective and readily adopted by developing countries, water treatment strategies offer the most protection from diarrheal diseases.

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Appendix A

Below is an adapted paper version of the electronically administered survey in English. The survey was catered to each site. This particular survey was for Quito.

Individual Interview

Form I

Date:
Start Time:
End Time:
Administer consent: ☐ Yes ☐ No
Sample ID Number:
Location: ☐ Emergency Room ☐ Internal Medicine ☐ Dermatology ☐ Pediatrics
☐ Other: _______________

A. Registration Information

1. *Ask to see cedula card.*
   Birth Date: _____ / ______/ ___________
   day month year

2. Sex: ☐ Male ☐ Female

3. ☐ Case ☐ Control

4. Who is answering the questions? ☐ Same subject ☐ Father, Mother or Guardian

5. Where do you live within the Metropolitan District of Quito? ________________
   If outside of Quito, exclude participant.

6. *Only ask if seven years old or less.*
   Did you bring your vaccine card today? ☐ Yes ☐ No

7. If yes, did the child receive the 1st dose of rotavirus vaccine? ☐ Yes ☐ No Date:

8. If yes, did the child receive the 2nd dose of rotavirus vaccine? ☐ Yes ☐ No Date:

B. Recruitment Information

9. Have you lived in Quito for more than six months? ☐ Yes ☐ No
   If no, exclude participant.

10. In the last seven days, have you had diarrhea? ☐ Yes ☐ No
Hint: Diarrhea is defined as three or more loose or watery stools in 24 hours. If a control says yes, exclude participant.

11. In the last seven days, have you taken a pill, syrup, or injection? ☐ Yes ☐ No

12. If yes, what medicine did you take? _____________

13. Did you (the enumerator) observe the bottle, label, or prescription? ☐ Yes ☐ No

C. Demographic Information

14. Race:
☐ Afro-Ecuadorian/Black ☐ Mixed Race
☐ Chachi/Indigenous ☐ Other: __________ ☐ N/R

15. How were you delivered?
☐ Vaginally ☐ C-section ☐ Don't know / can't remember ☐ N/R

16. *For children under three months:* Have you attended a nursery in the last month?
☐ Yes ☐ No

17. Is the child breastfeeding?
☐ Never breastfed ☐ No longer breastfeeds ☐ Exclusively breastfeeds
☐ Breastfeeds and bottle or solid food ☐ N/R

D. Symptoms

Control Symptoms

18. Why did you visit the clinic today?
☐ Diaper Rash ☐ Scabies ☐ Rash
☐ Dermatitis ☐ Abscess ☐ Skin spots
☐ Fungus ☐ Leishmaniasis ☐ Acne
☐ Shingles ☐ Other: ___________

Diarrhea Symptoms

19. In the last seven days, how many times did you have diarrhea? ______

20. If you said you did have 3 or more stools in one day:
Was it watery?
☐ Yes ☐ No ☐ N/R
Was it with mucous and slimy?

☐ Yes ☐ No ☐ N/R
Was it with blood?
☐ Yes ☐ No ☐ N/R

21. What was the cause of the symptoms?
☐ Water ☐ Food ☐ Germs ☐ Hemorrhoids
☐ Bad air ☐ Terror ☐ Evil eye ☐ Night dew ☐ Don't know
☐ Teething ☐ Other: ___________ ☐ N/R

E. Travel

Now I am going to ask you some questions about your travel in the last twelve months (the last year). Later I will ask you some questions about your travel in the last seven days (the last week).

22. During the last 12 months, have you left Quito?
☐ Yes ☐ No ☐ N/R

23. During the last 12 months (the last year), which of the following destinations did you visit?

Where did you go?	**How many trips? (Circle one)**	**How many days? (Circle one)**
☐ Guayaquil	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ San Lorenzo	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Esmeraldas	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Other community in Esmeraldas Province	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Guayaquil	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Santo Domingo	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Other: ______________	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ N/R	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7

24. In the past year, why did you travel? (select all that apply)

☐ Work ☐ School ☐ Shopping
☐ Medical reasons ☐ Visit Family ☐ Religious reasons
☐ Vacation ☐ Other: _______________ ☐ N/R

Now I am going to ask you some questions about your travels in the last week.

25. During the last week, have you left Quito?
☐ Yes ☐ No ☐ N/R

26. During the last week which of the following destinations did you visit?

Where did you go?	How many trips? (Circle one)	How many days? (Circle one)
☐ Guayaquil	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ San Lorenzo	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Esmeraldas	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Other community in Esmeraldas Province	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Guayaquil	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Santo Domingo	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ Other: ______________	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7
☐ N/R	0 1 2 3 4 5 6 7 8 9+	0 1 2 3 4 5 6 7

27. In the past week, why did you travel? (select all that apply)

☐ Work ☐ School ☐ Shopping
☐ Medical reasons ☐ Visit Family ☐ Religious reasons
☐ Vacation ☐ Other: _______________ ☐ N/R

Now I am going to ask you about your water and sanitation practices while outside of Quito in the last week.

28. What type of sanitation system did you use when traveling?
☐ Personal Latrine ☐ Neighbor or family member's latrine
☐ Community latrine ☐ Flush toilet ☐ River
☐ Hole in the ground ☐ Open defecation ☐ N/R

29. In the past week, what was your source of drinking water while traveling?
☐ River ☐ Well ☐ Rainwater
☐ Tap water inside your home ☐ Tap water inside another home
☐ Tap water outside of the home ☐ Purchased water ☐ N/R

30. In the past week, did you treat your drinking water while traveling?
☐ Yes ☐ No ☐ N/R
↓
5a. If yes, how did you treat it?
☐ Boiling ☐ Chlorination ☐ Filter ☐ Sunlight
☐ Let settle ☐ Avate (larvacide) ☐ Other: ___________ ☐ N/R

F. Household Characteristics

Now I am going to ask you about your water and sanitation practices while in your house in the last week.

31. What type of sanitation system did you use at home?
☐ Personal Latrine ☐ Neighbor or family member's latrine

☐ Community latrine ☐ Flush toilet ☐ River
☐ Hole in the ground ☐ Open defecation ☐ N/R

32. In the past week, what was your source of drinking water at home?
☐ River ☐ Well ☐ Rainwater
☐ Tap water inside your home ☐ Tap water inside another home
☐ Tap water outside of the home ☐ Purchased water ☐ N/R

33. In the past week, did you treat your drinking water at home?
☐ Yes ☐ No ☐ N/R
🡻
5a. If yes, how did you treat it?
☐ Boiling ☐ Chlorination ☐ Filter ☐ Sunlight
☐ Let settle ☐ Avate (larvacide) ☐ Other: ___________ ☐ N/R

G. Animal Contact

34. Do you have regular contact with animals?
☐ Yes ☐ No ☐ N/R
🡻
If yes:
34a. What kind of contact do you have with animals?

****Select all that apply:*
☐ Raise animals ☐ Have animals in home ☐ N/R
☐ Contact with animals around the house ☐ Other: ____________

34b. With what animals have you come in contact?
☐ Cow ☐ Broiler chickens ☐ Native chicken
☐ Pig ☐ Cat ☐ Dog ☐ Wild animal
☐ Other: ____________ ☐ N/R

H. Socioeconomic Status

35. The house that your family lives in is…
☐ Owned ☐ Rented ☐ Loaned
☐ Other: ____________ ☐ N/R

36. Currently, do you (or someone in your house) have a steady job?
☐ Yes ☐ No ☐ N/R

37. Does your family receive the “bono” (government aid)?
☐ Yes ☐ No ☐ N/R

38. What is your highest level of education?

☐ None (0 yrs) ☐ Primary (6 yrs) ☐ Secondary (12 yrs)

☐ High school (12 yrs) ☐ University or Technical school (13+ yrs)

☐ Other: ___________ ☐ N/R