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Water, Worms and Weird Diseases:
Water Quality Variability and Pediatric Health Outcomes in Northern Coastal Ecuador

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B.A., Vassar College, 2010

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health in Global Health
2016

Abstract

Water, Worms and Weird Diseases: Water Quality Variability and Pediatric Health Outcomes in Northern Coastal Ecuador

Background: poor water quality (WQ) is known to contribute to poor health outcomes such as diarrhea and soil transmitted helminth (STH) infections. However, studies attempting to link WQ and waterborne illnesses often find conflicting results while studies of interventions that aim at reducing water contamination successfully show that improved water quality leads to a decrease in waterborne illness. Experts argue that this contrast can be explained by study design and the variability of microbial indicators. **Purpose:** this study intended to: 1) characterize the factors that influence WQ within households (i.e. rural vs urban setting, water source, intermittent water supply, storage, and treatment), 2) determine the association between WQ, diarrhea and STH infections, 3) compare intra- and inter- household WQ variability in order to help guide potential new WQ standards, and inform better study designs. **Methods:** a field study was conducted in the district of Quinindé, Ecuador. Survey data and water samples were collected during household visits. WQ was measured using *E.coli* (EC) and total coliforms (TC) as bacterial indicators. Non-parametric tests, linear and logistic regression were used for data analysis. **Results:** River water had the highest contamination but all other sources of water were also contaminated. Rural households that do not have access to improved municipal water sources had significantly higher contamination (EC: 1.17 $\log_{10}$ MPN/100 mL, TC: 2.87 $\log_{10}$ MPN/100 mL) than urban households (EC: 0.90 $\log_{10}$ MPN/100 mL, TC: 2.49 $\log_{10}$ MPN/100 mL). Boiled water was strongly associated with lower contamination when compared to untreated water (EC: β=-0.73, p<0.0001; TC: β=-0.87, p=0.0002). Storage was a strong predictor for higher contamination (EC: β=0.33, p=0.0014; TC: β=0.62, p<0.0001). For households that used potable water, intermittent water supply was linked with higher rates of storage (OR=2.2, p=0.01). Both diarrhea (OR=1.1, p=0.0475) and STH infections (OR=1.16, p<0.0001) were associated with higher contamination levels. EC was a more accurate microbial indicator than TC. WQ variability was greater within rural households. **Conclusions:** WQ measurements and factors that pose a contamination risk such as storage, water source and treatment should be incorporated into new water safety and risk assessment measures for more accurate monitoring of progress made towards universal access to safe water.

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Acknowledgements

I would first like to express my sincere gratitude to my thesis committee for their guidance throughout this process, especially to Dr. Karen Levy. Thank you for your input and support both in Ecuador and in Atlanta.

Secondly, I want to express my utmost gratitude to the FEPIS staff in Quinindé, especially Dr. Philip Cooper, Dr. Martha Chico, Carlos Sandoval, Adriana Zambrano, Xavier Sanchez and Sofia Loor, for sharing their resources with me, helping me understand their beautiful country, Ecuador, and teaching me the dos and don’ts of field work.

I feel forever indebted to the families enrolled in the ECUAVIDA study for generously sharing their homes, water, food and intimate details of their daily lives with us and ultimately allowing us to tell their stories with our data. Their humility and ability to find joy despite adversity will continue to fuel my passion for global health and universal and equitable access to healthcare for years to come.

To my “Emory crew” and other friends elsewhere, thanks for your moral support and numerous Skype and WhatsApp pep talks. Your fellowship has helped me learn and grow in ways I would have never imagined.

Lastly, I owe everything I have accomplished so far to the supportive and loving family God has blessed me with. Maman et Papa, thank you for all the sacrifices you have made to allow me to pursue my dreams, do and be my best. Comme vous me l’avez toujours dit: seul le travail paye. Amy, thanks for being my biggest cheerleader and reminding me that nothing is impossible.

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Chapter 1: Introduction

Introduction and rationale

Diarrhea is the second leading cause of death in children under five (Liu et al., 2015), and causes considerable morbidity in children(Guerrant et al., 2013). In addition, soil transmitted helminth (STH) infections affect over a billion people worldwide and cause high morbidity in children (Bethony et al., 2006), aggravating the effects of diarrheal disease on child development.

Adequate water quality and quantity is an important factor in the prevention of waterborne illnesses that can cause diarrhea. Universal access to safe drinking water is deemed a global priority as reflected by its inclusion in the Millennium Development Goals (MDGs) in 2000 and in the Sustainable Development Goals (SDGs) in 2015. MDG target 7c states that UN state members will strive to "halve the proportion of the population without sustainable access to safe drinking water and basic sanitation between 1990 and 2015" (Salman, 2005; Bartram et al., 2014).

The World Health Organization (WHO) and United Nations Children's Fund (UNICEF) set standards, goals and monitor progress towards universal access to safe drinking water through the Joint Monitoring Programme (JMP). In 2012, the JMP's report that the MDG target 7c had been accomplished was met with skepticism by public health researchers (e.g., Clasen, 2012; Onda, 2012). Much of this skepticism stems from the measures used by the JMP to monitor progress. The JMP classifies drinking water as water coming from an improved versus unimproved source and relies primarily on national censuses and household surveys to derive its data on access to safe drinking water. However, water quality is widely variable even between two different types of improved sources and affected by factors such as water system maintenance, water delivery and handling at the point of use (Lee & Schwab, 2005). Thus, the current JMP classification system is susceptible to misclassification and overestimation of access to safe drinking water. In order to develop more appropriate water quality standards and goals, more research is needed to help understand the factors that influence water quality variability at the household level.

Problem statement

Although important progress has been made with regards to access to clean water and adequate sanitation and hygiene in developing countries, millions of individuals remain without appropriate drinking water sources (UNICEF/WHO, 2015).

Monitoring progress towards universal access to safe drinking water is challenging for multiple reasons. First, it is costly and difficult to reliably assess microbial water quality at the household level. Microbial water quality is currently measured through enumeration of indicator organisms for fecal contamination, which do not always directly correlate with risk for diarrheal disease (Schmidt, 2014; Levy, 2015). Second, previous research has shown that water quality is highly variable on a temporal and geographical scale (Levy et al., 2009; Luby et al., 2015). This high variability is partly explained by factors such as geographical location (Bain et al., 2014; UNICEF/WHO, 2015), intermittent water supply (Kumpel and Nelson, 2013; Shaheed et al., 2014), water storage (Wright et al., 2004; Levy et al., 2008; Jensen et al., 2012) and water treatment within the household (Gundry et al., 2004).

The JMP's current classification system of drinking water sources as improved and unimproved omits many of these factors, making it prone to underestimation of the real proportion of the population without access to safe drinking water. In fact, many households with access to an improved source of water may still be consuming fecally contaminated water if their improved source of water is not continuously available as is the case in many developing countries. Furthermore, many households in such settings rely on multiple water sources to meet the demand for drinking water.

Although many studies have investigated the link between water quality and risk for diarrheal disease at the household level, there is a need to further characterize water quality variability within and between households that rely on more than one source of drinking water, identify the factors that drive this variability, and investigate associated diarrheal and parasitic disease risks. Such new evidence would provide contextual information for the design and interpretation of studies investigating the relationship between household water quality and diarrheal disease. These new data could also support arguments made for the imminent need for continuous supply of safe drinking water free of fecal contamination, and could help guide new standards and goals for the JMP as it continues to monitor progress towards universal access to safe drinking water worldwide.

Purpose statement

The goal of this research project was to generate data on the factors that influence water quality variability within and between households, as well as the association between water quality and diarrheal and parasitic disease. These data could in turn be used to guide potential policies, standards and goals in the water, sanitation and hygiene global community.

In order to accomplish this objective, six specific aims were established:

1. Identify factors that affect water quality at the household level (i.e. water source, geographical location, water storage, water storage conditions, and water treatment).
2. Identify factors that lead to water storage (i.e. water source, continuous or intermittent potable water supply).
3. Investigate the relationship between water quality and risk of diarrhea within the household.
4. Investigate the relationship between water quality and risk of STH infections within the household.
5. Compare intra- versus inter- household water quality variability.

Significance statement

The Sustainable Development Goals were adopted in 2015 as a reiteration of the MDGs. Goal 6.1 of the SDGs states that member nations will strive to achieve universal and equitable access to safe and affordable drinking water for all by 2030 (UN, 2015). The results of this research project will add to the existing body of knowledge about disparities and barriers to access to safe drinking water for all and provide new information that could inform better policies and monitoring efforts in order to achieve universal access to safe drinking water by 2030.

Definition of terms

Diarrhea: having loose or watery stools at least three times per day, or more frequently than normal for an individual (UNICEF/WHO, 2009).

Soil transmitted helminth infection (STH): an infection caused by intestinal parasites. Most commonly caused by three main types of parasites: roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworm (*Necator americanus* or *Ancylostoma duodenale*) (Bethony et al., 2006). In this study, STH refers to infection with roundworms (*Ascaris lumbricoides*) or whipworms (*Trichuris trichiura*).

Safe drinking water: In terms of microbial safety, safe drinking water is defined as water that contains 0 *E. coli* CFU/ 100 mL (WHO, 2011).

Improved drinking water source: a source that by nature of its construction and design adequately protects the water from outside contamination, in particular by fecal matter. Improved drinking water sources include: household tubed water connections, public standpipes, boreholes, protected dug wells, protected springs and rainwater collections (UNICEF/WHO, 2015)

Unimproved drinking water source: unprotected wells, unprotected springs, vendor provided water, bottled water (when used as the only source of water within the household) and water acquired from tanker trucks are considered unimproved sources of water (UNICEF/WHO, 2015).

Fecal indicator organisms: A group of organisms that indicates the presence of fecal contamination (Ashbolt, 2001). Ideal organisms are specifically found in human feces and easy to grow in the laboratory.

Chapter 2: Literature review

Along with sanitation and hygiene, water quantity and quality is undeniably linked to good health (T. F. Clasen et al., 2015; Wolf et al., 2014). Water-related pathogens mainly cause disease through contact (i.e., bathing in *Schistos*oma-infested water), inhalation and aspiration (i.e., breathing aerosolized *Legionella* particles), and ingestion (i.e., drinking water contaminated with *V. cholera*) (WHO, 2011).

Although major advances have been made with regards to access to clean water and sanitation (UNICEF/WHO, 2015) and reduction of diarrheal disease and soil transmitted helminth infections on the global scale (Liu et al., 2015; Murray et al., 2012) , there remain some barriers with universal access to clean water. In addition, diarrheal disease and soil transmitted helminth infections remains a major cause of mortality and morbidity in the world (Guerrant et al., 2013; Liu et al., 2015; Murray et al., 2012). This literature review examines the burden of disease associated with water related illnesses with a special focus on diarrhea and soil transmitted helminth infections; the advances made on the global scale to guarantee the human right to safe drinking water for everyone; evidence that source water contamination and intermittent water supply leading to water storage at the household level pose limitations to microbiologically safe drinking water; and the effects of safe-drinking water interventions on health.

Burden of disease

Diarrheal disease accounts for about 53% of all water-related illnesses (Bartram & Cairncross, 2010). In 2010, diarrhea was the third leading cause of DALYs worldwide, accounting for approximately 282 982 DALYs or 3.6% of all DALYs (disability-adjusted life years)(Murray et al., 2012). It disproportionately affects children under five, who bear about 90% of the mortality caused by diarrhea (Bartram & Cairncross, 2010). According to the Liu and colleagues (2015), diarrhea is the second leading cause of death in children under five, causing between 448,000 - 750,000 deaths in that age group in 2013. Diarrhea also causes considerable morbidity in children (i.e. chronic malnutrition and stunting) (Guerrant et al., 2013) that surpasses the amount of DALYs associated with the deaths it causes (Bartram & Cairncross, 2010).

The majority of soil-transmitted helminth (STH) infections are caused by three main types of parasites: roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworm (*Necator americanus* or *Ancylostoma duodenale*) (Bethony et al., 2006). Worldwide, it is estimated that about over a billion individuals are infected with at least one type of STH (Bethony et al., 2006). STH infections cause few deaths but are responsible for a great amount of morbidity, especially among children living in impoverished areas (Bethony et al., 2006; Cooper et al., 2011; Strunz et al., 2014). Most of the morbidity they cause is linked to malnutrition, stunting, anemia, and reduced cognitive development (Bethony et al., 2006; Cooper et al., 2011; Strunz et al., 2014), although they are increasingly being linked to other health conditions such as allergic and autoimmune conditions (Cooper et al., 2011).

In 2010, it was estimated that STH infections accounted for between 3 and 8.8 million DALYs (Murray et al., 2012).

International efforts around water, hygiene and sanitation

The international community has long recognized the dangers that poor sanitation, hygiene and water cause to the global population and has undertaken various efforts in an attempt to mitigate them (Salman, 2005; Bartram et al., 2014). In 1977, the United Nations Organization (UN) held its first conference on water and sanitation in Mar del Plata, Argentina. During this conference, it urged all its members to "make a commitment to programs that provide water for urban and rural areas by 1990" (Salman, 2005). In 1980, the UN declared the years 1981-1990 to be the International Water Supply and Sanitation Decade and all member states committed to "bring about a substantial improvement in the standards and level of services in drinking water and sanitation" (Salman, 2005; Bartram et al., 2014). Due to slow progress and difficulty monitoring advances, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) created the Joint Monitoring Programme for Water Supply and Sanitation (JMP) in 1990 (Cairncross et al., 2014). In that same year, the World Summit for Children also called for universal access to safe drinking water and sanitary means of excreta disposal by 2000 (Bartram et al., 2014). In 2000, the UN assembly general adopted the Millennium Declaration and the Millennium Development Goals (MDGs) (Salman, 2005; Bartram et al., 2014). Target 7c of MDG 7 (ensuring environmental sustainability) states that member countries will strive to "halve the proportion of the population without sustainable access to safe drinking water and basic sanitation between 1990 and 2015" (Salman, 2005; Bartram et al., 2014). It was not until 2002 that the UN recognized "water as a human right", stating that every one is entitled to "sufficient, safe, acceptable, physically accessible and affordable water for personal and domestic uses" (Salman, 2005; Bartram et al., 2014). In 2003, the years 2005-2015 were declared the "International Decade for Action, Water for Life" with the goal to have "a greater focus on water-related programs and projects to achieve the internationally agreed, water related goals" (Salman, 2005).

MDG target 7c and JMP monitoring

Although it was not specifically created for that purpose, the WHO/UNICEF JMP is primarily responsible for monitoring progress towards MDG target 7c since its adoption in 2000 (Bartram et al., 2014; Bain et al., 2014; Clasen, 2012). Use of an improved source is used an indicator to monitor progress towards access to safe drinking water around the world and measured through national censuses and household surveys (Bain et al., 2014; Bartram et al., 2014; UNICEF/WHO, 2015). The JMP has classified sources of water as improved or unimproved based on whether or not they are "protected from outside contamination, in particular from contamination with fecal matter" (Bain et al., 2014). According to those criteria, household tubed water connections, public standpipes, boreholes, protected dug wells, protected springs and rainwater collections are considered improved sources (UNICEF/WHO, 2015). On the other hand, unprotected wells, unprotected springs, vendor provided water, bottled water (when used as the only source of water within the household) and water acquired from tanker trucks are considered unimproved sources of water (UNICEF/WHO, 2015). However, this classification system for water safety is just a surrogate measure of water quality since it assumes that improved water sources are less susceptible to fecal contamination by design but does not take into account variability in water system maintenance, water delivery and handling at the point of use. In its Guidelines for Drinking Water Quality, the WHO emphasizes that a continuous effort must be made to maintain drinking water quality at the highest possible level. In terms of microbial contamination, the WHO identifies *E. coli* as the most reliable fecal contamination microbial indicator and defines safe drinking water as water that contains 0 *E. coli* CFU/ 100 mL (WHO, 2011).

In 2012, the UN declared that the goal to reduce the population of people using unimproved water source by half had been achieved in 2010 (Clasen, 2012; UNICEF/WHO, 2015). In 2015, it is estimated that 91% of the global population now uses an improved drinking water source (UNICEF/WHO, 2015). This leaves 663 million people who still lack improved water sources (UNICEF/WHO, 2015). Eight out of ten of those individuals live in rural areas (UNICEF/WHO, 2015).

Is the mission really accomplished? MDG criticism and skepticism

The report that MDG target 7c was achieved early and that the international community was on track to deliver universal access to safe drinking water to all was met with skepticism by some public health experts. Onda et al. (2012) and Clasen (2012) argue that MDG target 7c has not been fully met because the binary indicator of use of improved vs unimproved water sources used by the JMP to measure progress does not directly address water quality, quantity and access. Similarly, Shaheed et al. (2014a) argue that the microbiological safety of drinking water in households with an improved source of water is often jeopardized by three interrelated factors: water storage, contaminated and intermittent piped water supplies and household water management practices. The JMP itself is aware of its own limitations regarding drinking water safety and sustainability and specifically addressed them in its 2011 report entitled "Drinking Water: Equity, Safety and Sustainability" (Clasen, 2012; Onda et al., 2012; UNICEF/WHO, 2015). The report discusses the results of a rapid assessment of drinking water quality (RADWQ) conducted in five countries that revealed that 13-32% of improved water sources were contaminated at levels exceeding WHO guidelines in four out of five countries (UNICEF/WHO, 2015). A literature review commissioned by the JMP in 2012 established that at least 1.8 billion people globally used a source of fecally contaminated drinking water (UNICEF/WHO, 2015).

Multiple studies external to the JMP have also reported on the issue of improved water source contamination. In a study using data from the JMP RADWQ, Onda et al. (2012) estimated that in 2010, 1 billion people had access to microbiologically unsafe improved sources of water. Although their study used thermotolerant coliforms instead of *E.coli* as an indicator of fecal contamination, their model highlights the fact that if microbial contamination and sanitary risk are taken into account, improved water sources are not as safe as they are thought to be. In a meta-analysis of 319 studies, Bain et al. (2014) also determined that although improved sources of water were less likely to be contaminated than unimproved sources of water, over 25% of samples from improved sources contained fecal contamination in about 73 studies. Contamination of water sources was more likely in low-income countries and rural areas (Bain et al., 2014.). In a cross sectional study of 914 peri-urban households in Cambodia, Shaheed et al. (2014b) also found that improved sources of drinking water did not meet WHO microbial water quality standards. In their study, piped water from taps contained an average of 520 *E. coli* CFU /100 mL and rainwater contained an average of 1500 *E. coli* CFU/100 mL with no statistically significant difference between those two improved sources of water (Shaheed et al., 2014b). Similarly, in a study of 207 households in rural Peru, Heitzinger et al. (2015) established that although over 90% of study participants used an improved source of water, about 47% of source water samples collected were positive for *E. coli*. Additionally, in a meta-analysis of 45 studies with a total number of 10 934 source water samples, Shields et al. (2015) found that 46% of all source water samples were fecally contaminated. Piped water at the source was the least contaminated type of water, with 25% of samples positive for fecal contamination (Shields et al., 2015). They report that piped water was significantly less contaminated than other improved and unimproved sources (Shields et al., 2015).

Less than optimal access

The current JMP indicators also did not measure access adequately. Only 64% of those with access to improved sources of water have access to piped water in their household (UNICEF/WHO, 2015), meaning that the other 36% still have to travel outside of their home to gain access to water. In addition, of those who have access to piped water on plot in the developing world, supply is often intermittent (Lee & Schwab, 2005). This not only puts a strain on those individuals in terms of time and effort spent collecting water, but it also restricts the quantity of water available for immediate use in the household and jeopardizes optimal water quality since water has to be handled, transported and stored prior to being used, putting it at higher risk for contamination.

In a review of the literature, Lee and Schwab (2005) identify loss of adequate disinfectant residual, low water pressure, intermittent service and ageing of water distribution infrastructure as factors that can affect piped water supply quality. They state that intermittent water supply has become the norm rather than the exception in many developing countries, with up to 60% of the population with household connections having intermittent supply in Latin America and the Caribbean (Lee& Schwab, 2005).

Intermittent water supply encourages water stagnancy and growth of microorganisms (Lee and Schwab, 2005). The literature these authors reviewed showed that certain diarrheal illness outbreaks have been linked to interrupted piped water service in the developing world (Lee & Schwab, 2005).

In a quantitative microbiological risk assessment (QMRA), Hunter et al. (2009) established that piped water interruptions in Uganda lead people to consume water from unimproved sources. Their model predicted that even with a few days of interrupted improved water service, individuals are at significantly higher risk of rotavirus, cryptosporidium and enterotoxigenic *E. coli* infection; negating the cumulative health benefits of drinking from an improved source (Hunter et al., 2009).

In a study comparing water quality in a community with continuous water supply and another one with intermittent water supply in India, Kumpel and Nelson (2013) reported higher levels of contamination with *E. coli* in source water obtained in households with intermittent supply. Only 0.7% of tap water samples were contaminated in households with continuous water supply as opposed to 31.7% of tap water samples in households with intermittent water supply (Kumpel & Nelson, 2013). They hypothesize that this higher level of contamination could be due to backflow, resuspension of bacteria or release from biofilms in the pipes when the supply is reestablished (Kumpel & Nelson, 2013).

In a literature review, Shaheed and colleagues (2014a) report that daily piped water supply duration ranges from 2 to 16 hours on average in various developing countries, and this intermittent water supply leads to poor water quality since low pressures in the pipes can lead to water contamination. These authors argue that intermittent service leads individuals to use unimproved water sources and household water storage, which pose significant health risks (Shaheed et al., 2014a).

Even when populations that previously had intermittent water supply obtain uninterrupted services, they often continue to engage in household water storage (Kumpel & Nelson, 2013; Shields et al., 2015).

In order to better assess access to safe and adequate drinking water, the WHO developed service level ladders that link the distance traveled to collect water to the quantity available at the household level and the associated public health risks. These levels stress the importance of access to water in adequate quantities but were not used in formal monitoring and reporting by the JMP prior to 2015. A similar service ladder has been proposed for monitoring the post-2015 sustainable development goals related to universal access to hygiene, sanitation and water (Shaheed, 2014a; UNICEF/WHO, 2015).

Household water storage and its effects on water quality

Suboptimal access to a continuous source of safe drinking water leads to water storage within the household. Storage and handling of water prior to consumption increase the risk of fecal contamination. In a study conducted in 50 rural Pakistani households, Jensen and colleagues (2002) found that water from sources with low levels of fecal contamination (<100 *E. coli* CFU/ 100 mL) was more likely to be heavily contaminated once it was stored in the household. Interestingly, water from heavily contaminated sources (>1000 *E. coli* CFU/100 ml) contained less fecal contamination once it was stored in the household. The authors attributed their results to potential bacterial die off in heavily contaminated source water. They also undertook a randomized trial to determine whether storage vessel characteristics affected water quality. They selected households located in villages that used unimproved sources of water (i.e. unprotected wells and surface water) and gave 34 households narrow-necked water pitchers and 33 households wide-necked pitchers that were traditionally used for water storage. Although there were still instances of high contamination in both types of vessels, they found that *E. coli* counts for water stored in narrow-necked pitchers were significantly lower than those for water stored in wide-necked pitchers (Jensen et al., 2002).

In a systematic meta-analysis of 57 studies comparing microbiological contamination of source water and stored water, Wright et al. (2004) report varying results depending on study setting. In about half the studies reviewed, significant contamination after collection was reported. Although no statistically significant reduction in contamination was reported in any of the studies they reviewed, they note that many of them indicated decline in indicator organisms counts after collection (Wright et al., 2004). Their results indicate that household water contamination is proportionately greater where source water is relatively uncontaminated and most likely from an improved source (Wright et al., 2004). They also found that water stored in covered recipients was less likely to be contaminated (Wright et al., 2004).

In a controlled study of water storage in rural Ecuador, Levy et al. (2008) followed contamination trends of stored water from collection to day 5 of storage. They found source water to be significantly more contaminated than water in the household from time of collection up to day 3 of storage and attributed their results to bacterial settling and die off (Levy et al., 2008). Their study design included a control stored water sample, which allowed them to deduce household recontamination levels (Levy et al., 2008). Only 46% of their household samples showed recontamination with *E. coli* after one day of storage, indicating that there are differences in factors that lead to contamination at the household level (Levy et al., 2008). They established that water stored in uncovered and wide-mouthed containers (opening >8 cm wide) was more likely to be recontaminated in the household (Levy et al., 2008).

In their cross-sectional study of 914 peri-urban households in Cambodia, Shaheed and colleagues (2014b) observed that *E. coli* colony counts more than doubled during storage of piped water and other improved sources such as rainwater. Heavy bacterial contamination was associated with certain handling practices such as uncovered storage containers and hand or receptacle dipping into the storage container (Shaheed et al., 2014b).

Shields et al. (2015) had similar results in their meta-analysis of 45 studies. Their results indicated that water stored within the household was more likely to be contaminated than water at the source regardless of source types although samples from piped sources were less likely to be contaminated than samples from non-piped sources (Shields et al., 2015). The JMP also replicated those findings in a pilot study aiming at incorporating water quality testing in national surveys, in which water at the household level was often more contaminated than water at the source (UNICEF/WHO, 2015).

Two randomized control trials conducted in Malawi (Roberts et al., 2001) and Benin (Gunther and Schipper, 2013) tested the effectiveness of improved water storage containers designed to prevent hand dipping and keep stored water covered at all times. Roberts and colleagues (2001) reported a 69% reduction in water contamination with fecal coliforms and an insignificant 31% reduction in diarrhea in the households using their improved container. Gunther and Schipper (2013) reported a 70% reduction risk of contamination and up to a 25% reduction in diarrhea incidence in households that used their improved water storage container compared to their control group. Those two studies reinforce the importance of hygiene and good water storage practices at the point of use when water storage cannot be avoided.

Water quality variability and reliability of indicator organisms

There is a multitude of waterborne organisms associated with poor health outcomes. However, testing for specific pathogens in water is costly, time intensive and inefficient (Levy, 2015). Therefore, we mainly rely on bacterial indicators of water quality to assess for the likelihood that drinking water may be contaminated with pathogenic organisms. Good indicator organisms are those that are specifically found in human feces and are easy to grow in the laboratory. The WHO currently recommends using *E. coli* and fecal coliforms to test for potential fecal contamination (Levy et al., 2009; Levy et al., 2012; Gruber et al., 2014), although other indicators such as total coliforms and enterococci are also commonly used. These assays have been developed and primarily used in temperate environments that greatly differ from the tropical climates encountered in areas that are most affected by waterborne diseases. The use of fecal coliforms in such settings has specifically been questioned by many experts since they are known to occur naturally in tropical environments, regardless of fecal contamination (Moe et al., 1991; Levy et al., 2012; Gruber et al., 2014). In an epidemiological study conducted in the Philippines, Moe and colleagues (1991) concluded that *E. coli* was more suitable than fecal coliforms, enterococci and fecal streptococci as a fecal contamination indicator and predictor of diarrheal disease in tropical settings. They also established that fecal contamination with *E. coli* only posed a great risk of diarrhea when the bacteria counts were above 1000

CFU/100 mL (Moe et al., 1991). In a comparative study of five assays using E. coli, enterococci and somatic coliphage in rural Ecuador, Levy et al. (2012) determined that *E. coli* culture using mI agar was the only sensitive method to detect an association between water contamination and diarrheal disease outcome. Gruber et al. (2014) found similar results in a meta-analysis of 14 studies in which a significant association between water contamination and diarrhea was only detected when *E. coli* was used as an indicator as opposed to fecal coliforms.

Even when using organisms with higher sensitivity such as *E. coli*, there are still temporo-spatial issues with water quality measurements. In a ten-months long study of water quality in a community that mainly relied on unimproved water sources in rural Ecuador, Levy and colleagues (2009) observed significant variability in *E. coli* counts of source water on an hourly, daily and weekly basis. Water quality was also affected by season (higher *E. coli* counts during the wet season) (Levy et al., 2009). Additionally, in a two-year long prospective longitudinal cohort study in Bangladesh, Luby et al. (2015) assessed household drinking water quality and diarrheal disease incidence. They found that water quality varied greatly over the course of their study. Most households had intermittently contaminated water samples, and 69% of them had at least one instance of contamination with more than 100 *E. coli* CFU /100 mL (Luby et al., 2015). They established that higher contamination levels of water samples preceding report of diarrhea were associated with higher incidence of child diarrhea within households (Luby et al., 2015).

Those studies emphasize the fact that current study designs that only sample water at one point in time and from only one source or household water storage container may not truly capture the quality of the water consumed by household members and the associated risk of developing diarrhea. Many studies are therefore at risk of over- and under-estimating the true effects of water quality on health.

Does clean water really matter? Evidence supporting efforts for clean water

It is both secular and scientific knowledge that poor water quality and poor health outcomes, especially diarrheal disease, are linked. In the late 1800s, John Snow, "the father of epidemiology" was the first to provide scientific evidence to suggest that water and sanitation play an important role in the transmission of pathogens that are usually contracted through the fecal-oral route (Schmidt 2014; Levy, 2015). Many twentieth century public health initiatives in high-income nations were undertaken under such assumptions, focusing on universal access to high quality and reliable water and sanitation.

Policies and funding for public health interventions tend to be heavily data driven. Funders tend to focus on diseases and conditions with the highest burden of disease and invest more in cost-effective interventions. In the arena of water, hygiene and sanitation, data is often conflicting and hard to interpret. In an editorial article, Schmidt (2014) discussed the fact that most studies at the beginning of the public health shift towards improving safe water access were based on a "before and after" model. The scientific community then adopted a case-control approach to studies of interventions aimed at reducing diarrhea and reported that they reduced disease cases by 20-30% (Schmidt, 2014). After concerns of selection bias and confounding were raised, there was a shift to randomized controlled trials, which reported diarrhea case reduction rates of 30-50% (Schmidt, 2014). Many consider those studies to be highly flawed because they mainly rely on self-report and tend to be unblinded. In the hopes of producing more accurate data, recent studies have moved towards village cluster randomized control trials (Schmidt, 2014) and objective outcomes such as anthropometry.

The most recent global burden of disease study in 2010 reported that inadequate access to water and sanitation only accounted for 0.9% of the total global burden of disease (Clasen et al. 2014; Schmidt, 2014) as opposed to 6.8% in 1990. Although many experts agree that major advances have been made towards better health in the world; water, hygiene and sanitation experts who work in the field are very skeptical of those latest estimates (Clasen et al., 2014; Schmidt, 2014). Clasen et al. (2014) attribute those numbers to inappropriate statistical methods. Schmidt (2014) and Wolf (2014) underscore the importance of using high quality continuous water supply as the gold standard for studies of water and health instead of the JMP classification of improved and unimproved sources. Clasen et al. (2014) and Schmidt (2014) also argue that diarrheal disease is not the only health outcome affected by water, sanitation and hygiene.

Indeed, water availability and quality affect many other health conditions such as vector-borne illnesses, soil transmitted helminth infections and respiratory diseases but those outcomes are not as thoroughly studied as diarrhea (Guerrant et al., 2013; Strunz et al., 2014). In a meta-analysis of 94 studies on the association between water, sanitation, hygiene and STH infections, Strunz et al. (2014) reported that access to piped water and use of treated water were both associated with lower of STH infections.

In a meta-analysis of 84 studies on the effect of water and sanitation interventions on self-reported cases of diarrhea, Engell And Lim (2013) reported that the effects of sanitation and water on health were much smaller than previously thought. They found the use of unimproved water sources as opposed to improved water sources as defined by the JMP was associated with a risk ratio 1.33 of developing diarrhea (Engell and Lim, 2013). They also reported no statistical difference found between piped water and other types of improved water (Engell and Lim, 2013). On the other hand, in a systematic review of 28 studies investigating the effects of water quality at the point of use and household water treatment and safe storage on diarrhea and cholera, Gundry et al. (2004) found a clear association between improved water quality, household water treatment and safe storage and reduced rates of cholera disease. Wolf and colleagues (2014) found similar results in a systematic review of 61 studies. In addition, they constructed a model that indicated that high quality continuous water supply is associated with statistically significant greater reductions in diarrhea than basic piped water and other improved water sources (Wolf et al., 2014). In a meta-analysis of 46 studies, Fewtrell et al. (2005) found that point of use treatment interventions reduced risk of diarrhea by about 31%. Although studies of interventions have been able to show that improving water quality at the point of use reduces risk of diarrheal disease, it has been more difficult to find a strong association between poor water quality and diarrheal disease. In an editorial article, Levy (2015) argues that this discrepancy is most likely due to the variability and lack of specificity of water quality indicators. Furthermore, in another editorial, Schmidt (2014) concludes that “the literature on the impact of water, sanitation and hygiene is unreliable in its entirety and only represents the results from those trials and studies that are feasible”.

Summary

Although it is commonly believed that poor quality water leads to poor health outcomes, including diarrhea and many other diseases, it has been difficult to show a clear association using currently available scientific methods and study designs. Despite this lack of definitive evidence and strong data, the international public health community continues to make universal access to safe water a priority, as illustrated by the recent declaration of access to clean water as a human right (United Nations, 2010) and the inclusion of universal access to water, hygiene and sanitation in the sustainable development goals (United Nations, 2015). The research presented here aims at better characterizing the factors that remain a barrier to access to safe water for all; specifically source water contamination, intermittent water supply and household water storage and how they affect water quality variability at the household level.

Chapter 3: Materials and methods

Introduction

This research project aimed at identifying factors that affect drinking water quality within and between households in Northern Coastal Ecuador. In particular, we were interested in geographical setting (rural vs urban), type and reliability of the water supply (potable water, well, bottled water, other), reported water treatment, and water storage within the household. We were also interested in correlating water quality to potential infectious disease outcomes such as diarrhea and STH infections. We used a cross-sectional survey approach to answer our questions.

Population and sample

The district (cantón) of Quinindé is located in the province of Esmeraldas in Northern Coastal Ecuador (see figure 1), with a population of 26,844 inhabitants in 2010 (Gobierno Autonomico Descentralizado Municipal del Cantón Quinindé, 2015). It is estimated that 22% of its population is urban (primarily living in the town of Quinindé) and 78% is rural. In 2010, according to the Ecuadorian national office for planning and development, only 31% of the population in the district (cantón) of Quinindé had access to potable water distributed by a public system (SENPLADES, 2014). The municipal government of Quinindé estimates that about 55% of the urban population has access to potable water while 77.5% of the total population in the district obtains their drinking water from other sources such as wells and rivers (Gobierno Autonomico Descentralizado Municipal del Cantón Quinindé, 2015).

All participants in the present study were parents (mostly mothers) or caretakers of children enrolled in the ECUAVIDA cohort study led by the Fundación Ecuatoriana Para Investigaciones en Salud (FEPIS) (for full description of study design, see Cooper et al. (2011)). This project was hosted by FEPIS as a complement to the ECUAVIDA cohort study. In total, 163 households were visited out of the 2244 households that are currently enrolled in the cohort (Cooper et al., 2015). The households visited in this study were not chosen at random from the full study population, but rather based on household visits occurring through the ECUAVIDA study that overlapped with the timing of this study.

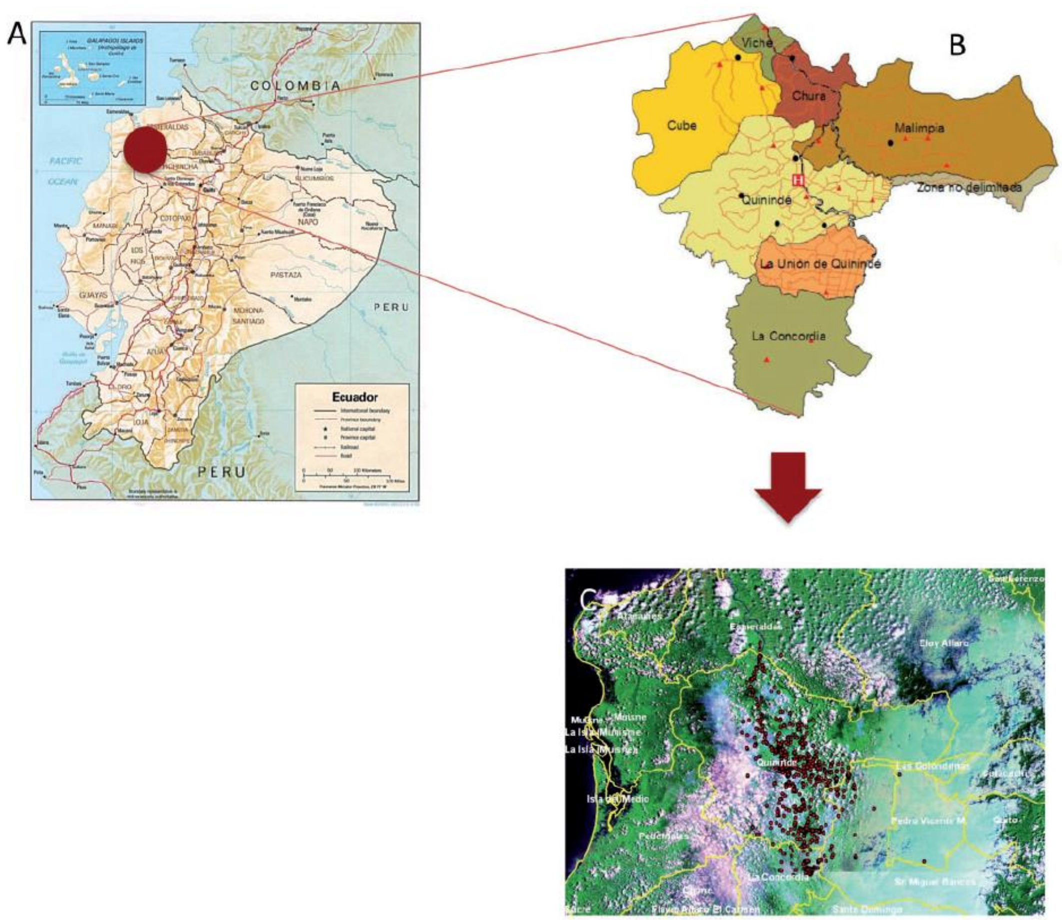


Figure 1: ECUAVIDA study site. (A) Map of Ecuador showing location of district of Quinindé (shaded circle). (B) Map of parishes within the district of Quinindé. (C)

Geographical location of households of all cohort participants. Source: Cooper et al. (2015).

Research design

Following a brief introduction to the study and its goals, participants were asked to participate in the study by answering a survey and offering samples of household drinking water for microbial contamination analysis in the laboratory. All participants gave verbal and written consent before data was collected. STH infection was determined previously by the ECUAVIDA study by stool sample examination; for a description of procedures see Cooper at al. (2011, 2015).

Procedures

The data was collected during household visits held for the ECUAVIDA cohort study. Most household visits were carried out in conjunction with the 5 and 8 year-old follow-up cohort visits of the study team, although some additional households were also visited if they were located in the same vicinity as the households being visited for the routine follow-up. During the visit, the caretaker answered a series of questions regarding access to drinking water, use of potable water, water purification, water storage and occurrence of diarrhea (defined as three or more loose stools per day) within the household in the past week. If s(he) reported that water was stored in the house, an inventory of all storage containers containing water during the visit was recorded.

Once the survey interview was completed, the participant was asked to provide drinking water samples of all the sources household members drink from. Every storage container was sampled as well as all direct sources of water such as taps and wells. If s(he) reported that water was consumed both from a direct source (i.e., tap or well) and from containers after storage, both were sampled. Similarly, if water was reported to be consumed from a direct source (i.e., tap or well) and after treatment and storage in a container, both untreated and treated water sources were sampled. All drinking-water sources in the house were sampled, except for the rare instances when potable water service was interrupted during the visit. Although it was a rare instance (n<5), containers that contained water from more than one source (for instance rain and well water) were not sampled to avoid classification bias. The informant was asked to collect the water as (s)he would normally do for consumption, and this water was then poured from the drinking vessel into Whirl-Pak bags containing sodium thiosulfate (NASCO Corp., Fort Atkinson, WI) to neutralize residual chlrorine, then placed on ice for transport to the laboratory. Duplicate samples were collected for every third sample.

All samples were processed within 8 hours of collection, and tested for total coliform and *E. coli* using Colilert Quantitray 2000 (IDEXX Laboratories Inc, Westbrook, ME). The samples were incubated at 35ºC for 24-28 hours before the results for total coliform and *E. coli* were read. One negative control of sterile distilled water was processed at the end of each day and incubated with the daily samples. If the negative control was positive (n=1), the results for all the samples from that day were discarded.

Instruments

All surveys were administered in Spanish language. The survey questions were read to the participant and answers were recorded electronically with an Android phone (Samsung) using the data collection application Open Data Kit (www.opendatakit.org). Survey questions are presented in Appendix A.

Data Analysis

All data analysis was performed using SAS 9.2 (SAS Inc., Cary, NC). Bacterial counts were $\log_{10}$ transformed prior to analysis. Counts below the lower limit of detection (<1 MPN/100 mL) were substituted with 0.5 MPN/100 mL and counts above the upper limit of detection (2419.6 MPN/100 mL) were substituted with 2420 MPN/100 mL prior to log transformation.

The data collected was not normally distributed, therefore non-parametric tests (Wilcoxon Rank Sum and Kruskal-Wallis) were conducted on continuous outcomes (*E. coli* and total coliform log MPN/100 mL). Chi-square tests were performed for binomial outcomes (diarrhea, storage).

In order to control for data clustering at the household level, linear and logistic generalized estimating equations (GEE) models were created. First, simple linear regression models were created, followed by multiple linear models (excluding collinear covariates) to identify confounding factors.

Ethical Considerations

The study protocol was reviewed the Institutional Review Board (IRB) at Emory University (Atlanta, GA) and the Universidad Central del Ecuador (Quito, Ecuador). IRB approval for this project was waived by the IRB at Emory University, since it was being performed within a cohort study population at the request of the host organization (FEPIS).

The protocol for the ECUAVIDA cohort study had previously been approved by the ethics committees of the Hospital PedroVincente Maldonado and the Universidad San Francisco de Quito.

Limitations and delimitations

Since the study was conducted within a pre-selected population that was already part of a cohort study, the sample used is not completely random and therefore decreases the generalizability of our findings. This study is also limited by the self-reported nature of cross-sectional surveys that can introduce information bias. Certain questions in the survey asked about past events, which can lead to recall bias. In addition, questions about hygiene and sanitary conditions are subject to bias since participants may not always feel comfortable sharing personal information. Furthermore, due to logistical and financial constraints, water quality data was only collected at one point in time and not longitudinally, which could have provided more insight into water quality variability, especially if data had been collected in more than one season.

Chapter 4: Results

Descriptive statistics

A total of 163 households were visited and a total of 438 drinking-water samples were collected between the months of July and September 2015. One-hundred and fifty (92%) of the houses that were visited during this study had at least one child who had completed his/her 8 years old follow up visit for the ECUAVIDA study within the past year.

Two-thirds of the houses were located in an urban area while the other third were located in a rural area. At least one child under the age of 5 lived in about half of the households visited. Approximately one quarter of the households reported using more than one source of drinking-water. The most reported drinking-water source was municipal potable water (52%), followed by well (31%) and bottled water (31%). The vast majority of households had drinking-water stored in a container on the day of our visit. Less than half of participants reported treating water prior to consumption, with the most reported method being boiling. Table 1 summarizes descriptive statistics about the households.

Households with access to municipal potable water (*n*=106) were predominantly located in an urban area. The average number of years since potable water installation in the house was 4.89 with a range of 0 to 35 years. Most households reported experiencing potable water supply interruptions, and one-third experienced daily disruptions. Table 2 summarizes descriptive statistics on potable water access and supply.

We collected between 1-21 samples in each household, (average: 2.7 samples/household). More than half those samples came from a tap in or outside of the household, with well, bottled, river and rain water comprising the remainder. Twenty-nine percent of samples were reported to be treated, with boiling being the most common method. Sixty-six percent of samples came from a storage container after being stored for an average of 1.57 days (range:1-8 days). Eighty-five percent of storage containers were reported by the survey participant as washed either with chlorine, soap or both prior to storage. Table 3 summarizes descriptive statistics on water samples.

About a quarter of participants (24%) reported that at least one family member living in the household had been ill with diarrhea in the week prior to the visit. (see Table 1) Seventeen-percent of children who had an 8-year follow-up visit for the ECUAVIDA study tested positive for either an *Ascaris lumbricoides* or *Trichuris trichiura* infection (see Table 1).

Characteristics	Number (%)
Geographical location	
Urban	109 (67)
Rural	54 (33)
Presence of children under 5 in household	86 (53)
Drinking Water Sources (not mutually exclusive)	
Potable water	85 (52)
Rain	4 (2)
River	13 (8)
Well	50 (31)
Bottled water	50 (31)
Households with more than one drinking water source	39 (24)
Stored water present in the house on day of survey	134 (82)
Reported drinking water treatment prior to consumption	73 (45)
Drinking water treatment method (self-reported)	
chlorine	25 (34)
filter	2 (3)
boiling	53 (73)
Treated drinking water available at time of survey	51 (70)
Diarrhea case in the household within the last week	39 (24)
Households with at least one child who participated in 8 year-old follow up visit within the past year	150 (92)
Child with helminth (*Ascaris lumbricoides* or *Trichuris trichiura*) infection (at time of 8 year follow up visit)	25 (17)

Table 1: Household characteristics (n=163)

Characteristics	Number (percentage)
Geographic location	
Urban	95 (90)
Rural	11 (10)

Length of time since installation of potable water on plot(range)	4.89 years (0-35 years)
Frequency of supply interruptions for more than one hour a day	
never	26 (25)
daily	29 (28)
more than once a week	9 (9)
at least every week	11 (11)
once a month to every other week	25 (24)
monthly or less often	3 (3)

Table 2: Households with potable water on plot (n=106)

Characteristics	Number (%)
Source	
Potable water	238 (54)
Rain	9 (2)
River	26 (6)
Well	109 (24)
Bottled water	56 (13)
Mean time bottle opened prior to consumption (range)	4.14 days (1-97 days)
Treatment prior to consumption	128 (29)
chlorine	59 (46)
filter	4 (3)
boiling	65 (51)
Stored water	290 (66)
Recipient covered	176(61)
Mean time water stored before sampling (range)	1.57 days (1-8 days)
Recipient washed with chlorine or soap prior to storage	246 (85)

Table 3: Drinking-water samples (n=438)

Factors affecting water quality

Geographical location

In order to assess whether geographical location affected water quality, the *E. coli* (EC) and total coliforms (TC) counts expressed in $\log_{10}$ MPN/100 mL for water collected in urban and rural areas were compared. As shown in figure 2a, the mean and median *E. coli* counts for samples collected in rural zones were 1.17 and 1.20 log_{10} MPN/100 mL respectively; while the mean and median *E. coli* count for samples collected in urban zones were 0.90 and 0.72 log_{10} MPN/100 mL. Figure 2b shows that the mean and median TC counts in rural zones were 2.87 and 3.38 log_{10} MPN/100 mL, while the mean and median TC counts in urban zones were 2.49 and 3.38 log_{10} MPN/100 mL. There was an average 0.27 and 0.38 log_{10} difference between rural and urban concentrations of EC and TC, respectively. Water samples collected in rural settings were significantly more contaminated than samples collected in urban settings by Wilcoxon Rank-Sum test (EC: p=0.01; TC: p=0.01) (Figures 2a and 2b).

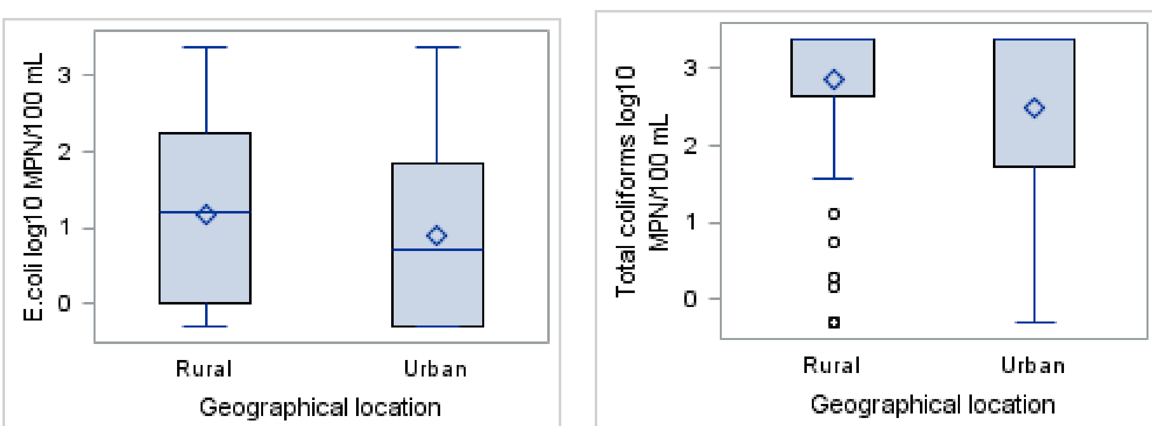


Figure 2a Figure 2b

Figure 2: Boxplots of E. coli (TC) and total coliforms (TC) concentrations by geographical location. Blue line represents median, diamond represents mean.

Intermittent water supply

In order to establish whether intermittent supply of potable water affected water quality, the EC and TC counts expressed in log_{10} MPN/100 mL of potable water samples taken in households that reported intermittent water supply were compared to the counts of samples taken in households that reported continuous water supply. The mean and median EC counts for households with intermittent water supply were 0.85 and 0.72 $\log_{10}$ MPN/100 mL while the mean and median EC counts for households with continuous water supply were 0.45 and -0.30 $\log_{10}$ MPN/100 mL (Figure 3a). The mean and median TC counts for households with intermittent water supply were 2.49 and 3.17 $\log_{10}$ MPN/100 mL while the mean and median TC counts for households with continuous water supply were 2.08 and 3.2 $\log_{10}$ MPN/100 mL (Figure 3b). There was an average 0.40 and 0.41 $\log_{10}$ difference between continuous and intermittent concentrations of EC and TC, respectively. A Wilcoxon Rank sum test indicated that samples collected from houses with an intermittent tap water supply had statistically higher mean scores of EC log MPN/100 mL than households with a continuous supply (p=0.0055).

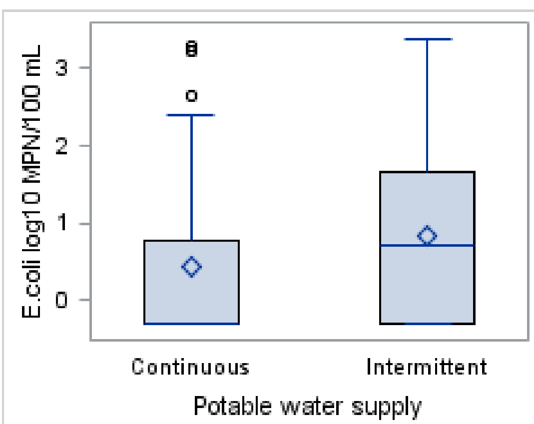


Figure 3a

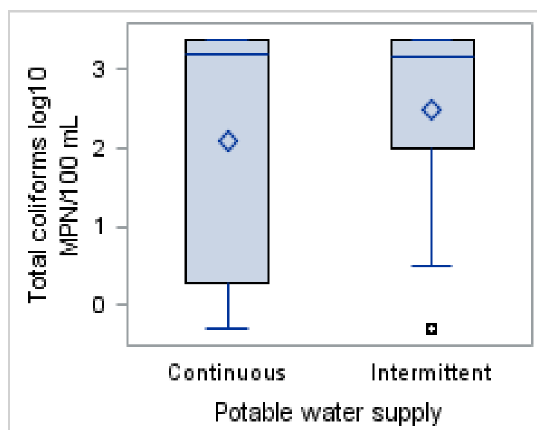


Figure 3b

Figure 3: Boxplots of *E.coli* (EC) and total coliforms (TC) $\log_{10}$ MPN/100 mL in households with intermittent vs continuous water supply. Blue line represents median, diamond represents mean, and circles represent outliers.

Storage

In order to ascertain whether storage prior to consumption affected water quality, EC and TC counts expressed in $\log_{10}$ MPN/100 mL of stored and non-stored water samples were compared. The mean and median EC counts for stored samples were 1.06 and 1.09 $\log_{10}$ MPN/100 mL while the mean and median EC counts for non-stored samples were 0.85 and 0.49 $\log_{10}$ MPN/100 mL (Figure 4a). The mean and median TC counts for stored samples were 2.76 and 3.38 $\log_{10}$ MPN/100 mL while the mean and median TC counts for non-stored samples were 2.37 and 3.03 $\log_{10}$ MPN/100 mL (Figure 4b). There was an average 0.21 and 0.39 $\log_{10}$ difference between stored and non-stored concentrations of EC and TC, respectively. Wilcoxon Rank Sum tests indicated that mean rank scores for EC and TC $\log_{10}$ MPN/100 mL for stored samples were significantly higher than samples that were never stored (EC: p= 0.0462 ; TC: p<0.0001).

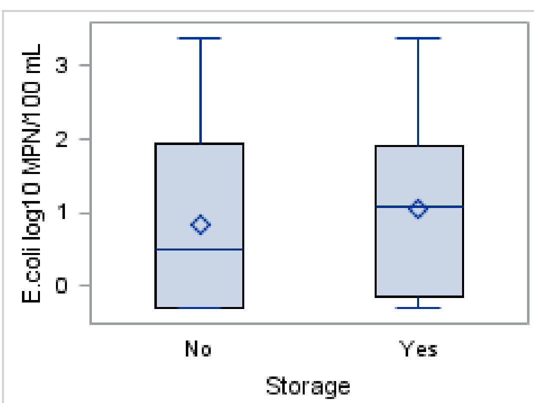


Figure 4a

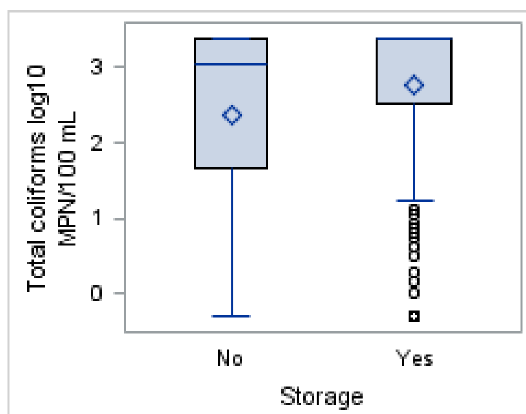


Figure 4b

Figure 4: Boxplots of *E.coli* (EC) and total coliforms (TC) $\log_{10}$ MPN/100 mL for stored and non-stored water samples. Blue line represents median, diamond represents mean, and circles represent outliers.

Source

To assess differences in level of contamination between various water sources, EC and TC counts expressed in $\log_{10}$ MPN/100 mL were compared for samples taken from tap, well, river, rain or bottled water. As seen in Figure 5a, the highest EC mean and median counts were observed in river water, at 1.95 and 2.27 $\log_{10}$ MPN/100 mL. The lowest EC mean and median counts were observed in rain water at 0.28 and 0 $\log_{10}$ MPN/100 mL. Similarly, the highest mean and median TC counts were observed in river water at 2.93 and 3.38 $\log_{10}$ MPN/100 mL (Figure 5b). The lowest TC counts were observed in bottled water with a mean of 2.55 $\log_{10}$ MPN/100 mL and a median of 3.38 $\log_{10}$ MPN/100 mL (Figure 5b). A Kruskal-Wallis test revealed that there were significant differences in mean rank scores of EC log MPN/100 mL between the five sources of water (tap, bottled, well, rain and river) ($\chi 2$ (4)= 51.81, p<0.0001). The same results were obtained when a Kruskal-Wallis test was performed with TC log MPN/100 m L ($\chi 2(4)$=27.77, p<0.0001).

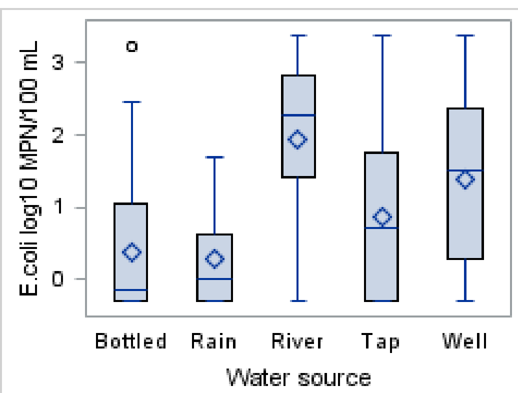


Figure 5a

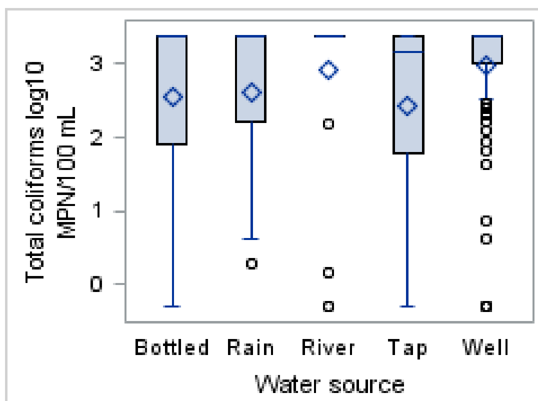


Figure 5b

Figure 5: Boxplots of *E.coli* (EC) and total coliforms (TC) $\log_{10}$ MPN/100 mL by water source (bottled, rain, river, tap and well). Blue line represents median, diamond represents mean, and circles represent outliers.

Treatment

In order to measure water treatment compliance and determine whether reported water treatment had an effect on water quality, EC and TC counts expressed in log10 MPN/100 mL were compared based on treatment method (none vs boiling vs chlorine vs filter). As seen in Figures 6a and 6b, the least contaminated samples were those that had been reported to as boiled. Boiled samples had an EC mean and median count of 0.49 and -0.30 $\log_{10}$ MPN/100 mL; and a TC mean and median of 2.11 and 3.09 $\log_{10}$ MPN/100 mL. The means and median EC and TC counts for filtering and chlorine are similar to the mean and median counts for samples that were not treated. Figures 6a and 6b show that filtered samples have the highest mean and median EC and TC counts, even relative to no treatment. Kruskal-Wallis tests revealed a significant difference in mean rank scores for EC and TC based on treatment group (EC: $\chi2$ (3)= 21.09, p=0.0001; TC: $\chi2$(3)=8.07, p=0.04). The mean rank scores for boiled water were significantly lower than those for non-treated, chlorinated and filtered water.

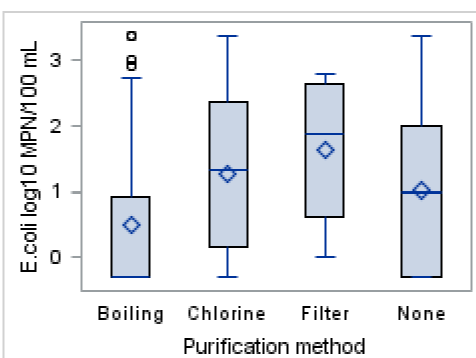


Figure 6a

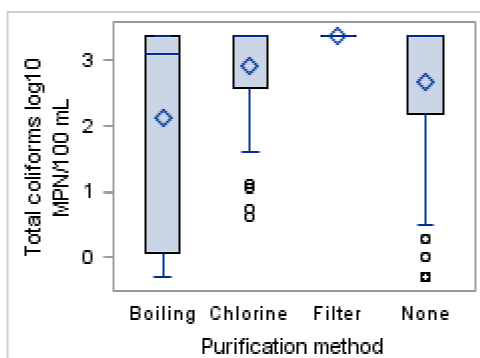


Figure 6b

Figure 6: Boxplots of *E.coli* (EC) and total coliforms (TC) $\log_{10}$ MPN/100 mL by water purification method (none, boiling, chlorine or filter). Blue line represents median, diamond represents mean, and circles represent outliers.

Simple linear regression

In order to examine the association between water quality and sample characteristics while controlling for clustering by household, a series of simple linear regression models were analyzed to calculate estimated EC and TC log_{10} MPN/100 mL based on each water sample characteristic (geographical location, intermittent water supply, storage, source, treatment). All results are displayed in Table 4.

When EC log_{10} MPN/100 mL was used as predictor, there was a statistically significant association between source and higher contamination levels in water samples, with river water having the highest association with elevated levels of microbial contamination (β=1.62). Location in a rural area (β=0.44), storage (β=0.43) and water purification with chlorine (β=0.34) and filters (β=0.76) were all significantly associated with higher levels of contamination when TC log_{10} MPN/100 mL was used as predictor. Boiling had a negative association with contamination levels when both EC log_{10} MPN/100 mL (β=-0.53) and TC log_{10} MPN/100 mL (β=-0.51) were used as separate predictors.

	Outcomes			
Predictors	**EC log_{10} MPN/100 mL**		**TC log_{10} MPN/100 mL**	
	β (SE)	p-value	β (SE)	p-value
Geographical location (reference= urban)				
Rural	0.25 (0.16)	0.1148	0.44 (0.13)**	0.0012
Water supply (reference= continuous)				
Intermittent	0.35 (0.23)	0.1284	0.38 (0.29)	0.1998
Storage (reference=no)				
Yes	0.17 (0.10)	0.0965	0.43 (0.11)**	0.0002

Source (reference=rain)				
Tap	0.65 (0.23)**	0.0041	-0.16 (0.29)	0.5805
Bottled	0.23 (0.24)	0.3426	-0.03 (0.32)	0.9285
Well	1.20 (0.24)**	<.0001	0.41 (0.29)	0.1582
River	1.62 (0.30)**	<.0001	0.34 (0.32)	0.2775
Treatment (reference=none)				
Boiling	-0.53 (0.18)**	0.0027	-0.51 (0.23)**	0.0280
Chlorine	0.15 (0.18)	0.3893	0.34 (0.14)**	0.0133
Filter	0.47 (0.41)	0.2543	0.76 (0.15)**	<.0001

Table 4: Simple linear regression GEE models for factors predicting water quality (geographical location, water supply, storage, source and treatment). ** indicates *p*<0.05.

Multiple linear regression

To further examine the association between water quality and water sample characteristics, control for possible confounding variables and for clustering by household, two separate multivariate linear regression models were analyzed. Water source and storage are collinear variables since rainwater can only be stored. The variable characterizing water supply only applies to potable water. For this reason, Model 1 was analyzed with the following variables as predictors: geographical location, storage, and treatment. Model 2 was analyzed including the following variables as predictors: geographical location, source, and treatment. Tables 5 and 6 summarize the results of Models 1 and 2 for EC $\log_{10}$ MPN/100 mL and TC $\log_{10}$ MPN/100 mL respectively.

In Model 1, when controlling for geographical location and treatment, storage had a significant association with higher EC (β=0.33, *p*=0.0014) and TC (β=0.62, *p*=<0.0001) counts. When controlling for storage and treatment, location in a rural zone (β=0.44) had a significant association with higher TC counts (*p*=0.0006) but only a marginally significant association with higher EC counts (*p*=0.0813). Similarly, when controlling for geographical location and storage, filtering water (β=0.49) had a significant association with higher TC counts (*p*=0.0187), and boiling maintained a significant and strong negative association with both EC (β=-0.72, *p*<0.0001) and TC (β=-0.87, *p*<0.0001) counts.

In Model 2, when controlling for geographical location and treatment, water source had a significant association with EC log_{10} MPN/100 mL. River (β=1.60) and well (β=1.15) water have the highest significant association (*p*<0.0001). Filtering water was also associated with higher EC and TC counts in Model 2 (β= 0.57, *p*=0.0342; β=0.91, *p*<0.0001). As previously observed, boiling water had a significant negative association with EC and TC counts in Model 2.

	Model 1: geographical location, storage and treatment β (SE)	**p-value**	**Model 2: geographical location, source and treatment β (SE)**	**p-value**
Constant	1.12		0.50	
Geographical location (ref= urban)				
Rural	0.29 (0.16)	0.0813	-0.10 (0.18)	0.5653
Storage (ref= not stored)			N/A	
Stored	0.33 (0.10)**	0.0014		

Treatment (ref=none)				
Chlorine	0.01 (0.19)	0.9658	-0.22 (0.19)	0.2326
Filter	0.34 (0.43)	0.4303	0.57 (0.27) **	0.0342
Boiling	-0.73 (0.18) **	<0.0001	-0.72 (0.18)**	<0.0001
Source (ref=rain)	N/A			
Tap			0.48 (0.24)**	0.0482
Bottled			-0.04 (0.27)	0.8691
Well			1.15 (0.24)**	<.0001
River			1.60 (0.31) **	<.0001

Table 5: Multiple linear regression GEE models. Model 1 uses geographical location, storage and treatment as predictor variables and EC $\log_{10}$ MPN/100 mL as outcome measure. Model 2 uses geographical location, source and treatment as predictor variables and EC $\log_{10}$ MPN/100 mL as outcome measure. indicates *p*<0.05.**

	Model 1: geographical location, storage and treatment β (SE)	**p-value**	**Model 2: geographical location, source and treatment β (SE)**	**p-value**
Constant	2.84		2.64	
Geographical location (ref= urban)				
Rural	0.44 (0.13) **	0.0006	0.23 (0.15)	0.1283
Storage (ref= not stored)			N/A	
Stored	0.62 (0.11)**	<.0001		
Treatment (ref=none)				
Chlorine	0.13 (0.17)	0.4369	0.07 (0.12)	0.5789
Filter	0.49 (0.21)**	0.0187	0.91 (0.13)**	<.0001
Boiling	-0.87 (0.23)**	0.0002	-0.62 (0.24)**	0.0101

Source (ref=rain)	N/A		
Tap		-0.16 (0.26)	0.5448
Bottled		-0.16 (0.30)	0.5828
Well		0.29 (0.26)	0.2612
River		0.31 (0.28)	0.2531

Table 6: Multiple linear regression models. Model 1 uses geographical location, storage and treatment as predictor variables and TC $\log_{10}$ MPN/100 mL as outcome measure. Model 2 uses geographical location, source and treatment as predictor variables and TC $\log_{10}$ MPN/100 mL as outcome measure. ** indicates *p*<0.05.

Factors leading to water storage

As observed in the results reported above, water storage is associated with higher contamination levels. In order to identify drinking-water characteristics that favor water storage, water sources and potable water supply were compared for households that reported having stored water in the house on the day of the visit as opposed to those that did not, using chi-square tests. Water storage frequency was significantly different based on drinking water source type ($\chi 2$ (4)= 44.97, $p<0.0001$) and intermittent water supply in households with tap water ($\chi 2$ (3)= 8.51, $p=0.0035$).

In order to identify an association between water storage and water supply in households with potable water, a logistic regression model was analyzed, controlling for clustering by household. The model predicted an odds ratio of 2.2 [1.21,3.99] of being stored for samples taken in households experiencing intermittent water supply as opposed to those with continuous water supply ($p=0.01$).

Factors predicting diarrhea

Water quality has previously been linked with diarrheal disease. In order to determine whether water quality and factors that determine water quality such as water source and water storage were linked to diarrheal disease outcomes in this study, logistic regression models and chi-square tests were performed.

Two logistic regression models were analyzed to determine an association between diarrheal disease as an outcome and EC $\log_{10}$ MPN/100 mL and TC $\log_{10}$ MPN/100 mL as separate predictors, controlling for clustering by household. Table 7 summarizes those results.

The likelihood of having at least one case of diarrhea in the household within the last week was significantly associated with increases in both EC (OR= 1.10 [1.00-1.21], p=0.0475) and TC concentrations ($\log_{10}$ MPN/100mL) (OR= 1.09 [1.02-1.16], p=0.02). The above results report that water source is significantly linked to water quality. Therefore, a chi-square test was performed to test whether there is an association between water source and diarrheal disease outcomes. There was a significant difference between different water sources and diarrhea incidence (χ^2 (4)=13.89, p=0.0077).

Although the results above indicate that water storage is associated with worse water quality, it was not significantly associated with diarrheal disease outcomes ($\chi^2(1)$= 0.38, p=0.54).

Water quality and STH infection

In order to determine whether there is an association between helminth infection and water quality, two logistic regression models using helminth infection within the last year as an outcome and EC log_{10} MPN/100 mL and TC log_{10} MPN/100 mL as separate predictors, while controlling for clustering by household, were analyzed. Table 7 summarizes those results.

There was a significant association between EC log_{10} MPN/100 mL and helminth infection within the last year (OR=1.16 [1.04, 1.30], *p*<0.0001).

	Predictor			
Outcome	*E. coli* log_{10} MPN/100 mL		Total coliforms log_{10} MPN/100 mL	
	OR [95% CI]	p-value	OR [95% CI]	p-value
Diarrhea	1.10 [1.00, 1.21]**	0.0475	1.09 [1.02, 1.16]**	0.02
Helminth (*Ascaris lumbricoides* or *Trichuris trichiura*) infection	1.16 [1.04-1.30]**	<0.0001	1.09 [0.98-1.21]	0.0893

Table 7: Logistic regression models for diarrhea within the last week and helminth infection within the last year, using EC log_{10} MPN/100 mL and TC log_{10} MPN/100 mL as predictors. ** indicates p<0.05.

Intra- versus inter- household water quality variability

Many households in this study reported storing water and using different sources of water. As observed above, water quality is significantly associated with storage and water source, therefore it is intuitive to think there is bound to be variability in drinking-water quality within and between households. Figures 7a-d display the distribution of median EC and TC counts per household and how they varied based on geographical location. In order to examine the difference between water quality variability within the same household and water quality variability among distinct households, a Kruskall-Wallis test was performed. When *E. coli* was used as a microbial indicator, water quality was found to significantly vary more between two different households than within each household ($p<0.0001$). When the data was stratified by geographical location, this finding held true for households located in urban settings ($p<0.0001$). There was no significant difference in variability within and between households in rural settings.

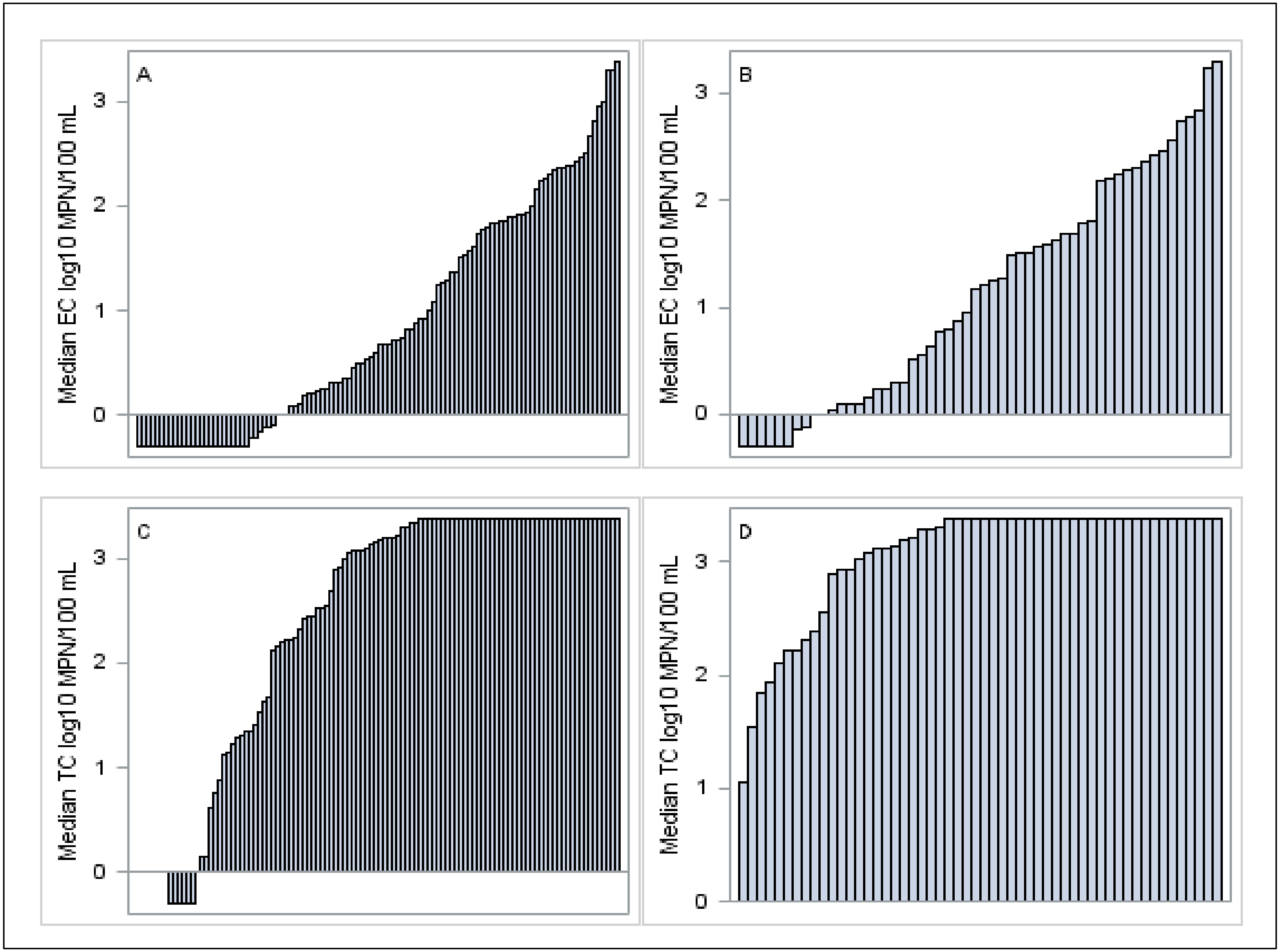
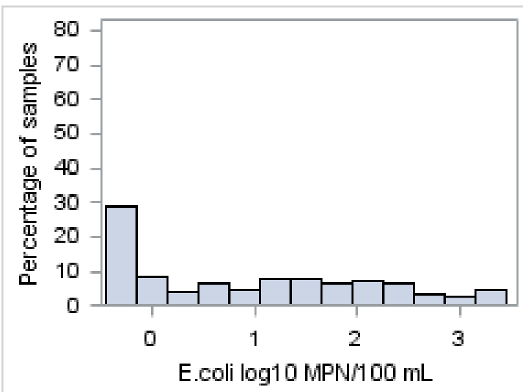


Figure 7: Median EC log_{10} MPN/100 mL and TC log_{10} MPN/100 mL counts for all samples by geographical location. Each bar represents as distinct household. (A) Median EC log_{10} MPN/100 mL for urban households (n=109). (B) Median EC log_{10} MPN/100 mL for rural households (n=54). (C) Median TC log_{10} MPN/100 mL for urban households (n=109). (D) Median TC log_{10} MPN/100 mL for rural households (n=54).

Water quality measurements variability based on microbial indicator

As seen in Figures 8a-b, EC and TC counts had different distributions in this study. As seen in Figure 8a, a greater proportion of samples had lower counts of EC than TC, with about 30% not surpassing the lower limit of detection (<1 MPN/100 mL). Conversely, about 60% of samples reached the upper limit of detection (>2419.6 MPN/100 mL) when TC was used a microbial indicator (Figure 8b).



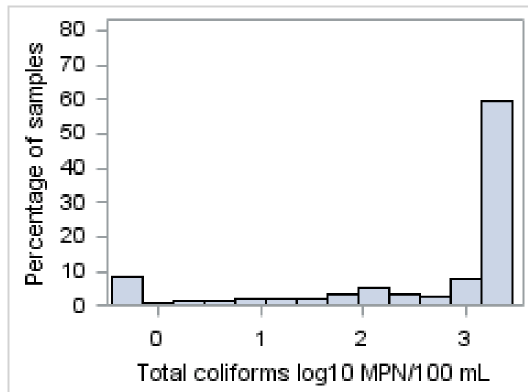


Figure 8a

Figure 8b

Figure 8: Distribution of EC log_{10} MPN/100 mL (n=438) and TC log_{10} MPN/100 mL (n=422) counts for all samples.

Chapter 5: Discussion

The study presented here was conducted to identify the household characteristics (geographical location, water source, intermittent potable water supply, water treatment and storage) that affect water quality, characterize factors that lead to water storage within the household, investigate the relationship between water quality, diarrheal disease and soil-transmitted helminth infections, and compare water quality variability within and between households. Survey data and water samples were collected and analyzed to ascertain pertinent associations.

Factors affecting water quality

Geographical location, intermittent potable water supply, storage, source and treatment were the five characteristics evaluated in this study.

Households in rural settings tended to have higher contamination levels for both EC and TC when compared to urban households. However, as seen in Figure 2, the mean difference between urban and rural settings was small (0.27 EC $\log_{10}$ MPN/100 mL 1.30 and 0.38 TC $\log_{10}$ MPN/100 mL). Non-parametric tests that did not control for other factors and clustering by household revealed a statistically significant difference for both EC and TC counts based on geographical location. However, simple linear regression controlling for clustering for household only found a significant association between geographical location and TC counts (β=0.44, p=0.0012). Similarly, when multiple linear regression was used, only model 1 (controlling for storage, treatment and clustering by household) found a significant association between geographical location and water quality for TC (β= 0.44, p=0.0006) and not for EC. Those inconsistent results indicate that geographical location on its own is not strongly associated with water quality in this study.

For households that used potable water, intermittent water supply was only significantly associated with higher water contamination when EC was used a predictor in a non-parametric test that did not control for other factors and clustering by household (p=0.0055). However, in simple and multiple linear regression models controlling for other factors and household clustering, no significant association was found. Those results indicate that intermittent water supply did not affect water quality in this study.

As reported in previous studies (Levy et al., 2008; Shaheed, Orgill, Ratana, et al., 2014; Shields et al., 2015; Wright et al., 2004), water storage within the households in this study was found to lead to higher levels of drinking-water contamination within the household. Storage was significantly associated with both higher EC and TC counts in non-parametric tests (0.21 $\log_{10}$ and 0.39 $\log_{10}$ difference in concentrations for EC and TC respectively) . Although simple linear regression controlling for household clustering only showed a positive association between water storage and contamination for TC counts, multiple linear regression Model 1, controlling for geographical location, water treatment and household clustering, found significant associations between storage and EC and TC counts.

In this study, water sources were individually compared instead of classifying them into improved and unimproved categories as recommended by the JMP (UNICEF/WHO, 2015). Our intent was to demonstrate that all sources are subject to contamination regardless of their JMP classification. In fact, when stratified by source type, none of the groups were completely free of EC or TC (Figure 5). Rainwater, considered an improved source, was the least contaminated but had a very small sample size (*n*=9). Rainwater was then followed by bottled water, tap water and well water and river water. It is not surprising that river water would be most contaminated since it is classified as unimproved. Statistically significant differences in water quality among all five water types were detected by non-parametric tests for both EC and TC. Simple and multiple linear regression models only found a significant association between water source and water quality for EC counts. In both the simple linear regression model controlling for household clustering effects, and the multiple linear regression model controlling for geographical location and treatment as well as clustering by household; tap, well (improved sources by JMP criteria) and river water (unimproved source by JMP criteria) were significantly associated with higher level of contamination by EC when compared to rain water (Tables 4 and 5). These results echo the findings of previous studies (Bain et al., 2014; Heitzinger et al., 2015; Onda et al., 2012; Shaheed, Orgill, Montgomery, et al., 2014; Shaheed, Orgill, Ratana, et al., 2014; Shields et al., 2015) and support the argument that classifying water sources into improved and unimproved categories is not a sufficient and adequate surrogate for quantitatively assessing microbiological water safety.

Water treatment prior to consumption was perhaps the strongest predictor of water quality in this study, with consistent results across all three analytical methods used. Non-parametric tests, simple linear regression and multiple linear regression models found significant associations between water treatment and both EC and TC counts. When compared to untreated water, boiling proved to be most effective treatment modality (see Tables 4, 5 and 6). The effect of boiling was most likely made evident by the fact that a majority of our boiled samples were collected right after they had been boiled, since we conducted most of our visits in the morning hours when women would be cooking and cleaning their homes. Chlorination was assessed through self-report only, due to logistical challenges. Anecdotally, individuals reported using chlorine for their wells on an infrequent basis. Filters varied by household but most were part of commercially sold plastic water dispensers. Those were also observed to be old and sometimes have moldy residual in them, which would perhaps explain why samples were significantly more contaminated than samples that were not treated (see Tables 4, 5 and 6).

Factors predicting water storage

As previously discussed, results in this study show that water storage leads to higher levels of microbiological contamination. As previously reported by Shaheed et al. (2014a), we found that intermittent water supply was a significant predictor for water storage. Water source was also a significantly associated with water storage when analysis was conducted using a chi-square test that did not control for other factors and clustering by household ($\chi 2$ (4)= 44.97, $p<0.0001$). Those results should be interpreted with caution as some water sources such river and rainwater are always stored.

In the present era of emerging new diseases, storage has more public health implications than just risk of gastrointestinal illness from contaminated water. With the spread of mosquito-born viruses such as zika and chikungunya in Latin America, and long-term issues of dengue and malaria, storage of water, if uncovered, also constitutes risk for larval development of mosquito disease vectors.

Factors predicting diarrhea

Our results finding a significant association between higher water contamination levels and the likelihood of having at least one case of diarrhea in the household were consistent with those of previous studies (Gundry et al., 2004; Levy et al., 2012; Moe et al., 1991; Wolf et al., 2014). Our predicted odds ratios of 1.10 [1.00, 1.21] ($p=0.0475$) and 1.09 [1.02, 1.16] ($p=0.02$) of having at least one case of diarrhea in the household in the last week for every one unit increase in $\log_{10}$ EC MPN/100 mL and $\log_{10}$ TC MPN/100 mL, respectively, were relatively small compared to the odds ratio of 1.29 [1.02-1.65] of having at least one case of diarrhea in a household for every one unit increase in $\log_{10}$ EC MPN/100 mL reported by Levy et al. (2012) in a rural region of Ecuador similar to our study site.

Factors predicting STH infection

In their meta-analysis, Strunz et al. (2014) reported that access to piped water and use of treated water was associated with lower odds of STH infection, suggesting that microbiological water quality played a role in the transmission of STH. In this study, results indicated that household water quality is associated with STH infection. Our logistic regression model predicted an odds ratio of 1.16 [1.04, 1.30] of having at least one positive case of STH infection in the last year for every one unit increase in $\log_{10}$ EC MPN/100 mL ($p<0.0001$). Although previous studies have investigated the relationship between STH infection, household water access and quality (Strunz et al., 2014) through survey and questionnaires; to the best of our knowledge, this study is the first study to report a significant association between an objective measure of water quality (EC counts) and STH infection. These results add to the small body of literature investigating the link between water, sanitation, hygiene and STH infections and support public health experts who advocate for more comprehensive long term approaches to the eradication of STH than simple mass drug administration (Strunz et al., 2014).

Intra- versus inter-household water quality variability

As indicated by Figure 7, water quality variability was significantly greater between households than within households in urban settings but not in rural settings, justifying our use of models that controlled for clustering by household. Our results indicate that rural households rely more heavily on various sources with broadly varying water quality, making exposure risk assessment more difficult to perform in such settings. These findings also indicate that in urban areas, where water tends to be less contaminated, water quality measurements can actually be used to assess differences between households. Whereas in rural areas, where drinking water is more contaminated, and with overall higher variability within each household, water quality measurements are not as useful to discern differences between households. This highlights the importance of taking initial source water conditions into account when thinking about using microbial water quality indicators in health studies.

Water quality measurements variability based on microbial indicators

When *E. coli* was used as an indicator, association measures tended to be more significant and have narrower confidence intervals. In addition, as shown in Figure 8, a greater proportion of samples had counts within the limits of detection (1-2419.6

MPN/100 mL) when *E.coli* was used as an indicator as opposed to total coliforms. Overall, as indicated by the wider range of detection noted in this study, *E. coli* was a more accurate microbial indicator of water quality than total coliforms in this study, similar to findings reported in other studies (Gruber et al., 2014; Levy et al., 2012; Moe et al., 1991). These findings support the notion that when possible, *E. coli* should be used as a microbial indicator for water quality, especially in tropical settings.

Limitations and future directions

As expected of every study, this study was subject to a number of limitations. Our results should be interpreted with caution as the statistical methodology used was simplistic and might not account for confounding variables and some biases inherent to the study design. Although non-parametric tests were used and reported, they are not fit to accurately interpret the data presented since there is a need to control for clustering by households. The chi-square and Kruskal-Wallis tests used to determine associations between water quality, water storage and water sources could only indicate statistical differences between source types but did not allow for further characterization of these differences. It would be of interest to perform more powerful data analysis to further characterize the differences between water source types. Similarly, it would be of interest to further characterize storage factors that lead to water contamination such as length of time stored, type of storage recipient used. Although our study did find an association between household water quality and STH infection, it is important to note that most of the cases were in school aged children who spend the majority of their days outside of the household. Further analysis controlling for other risk factors for STH infection (i.e., sanitation, water quality at school, use of shoes) would provide more definitive results.

A number of the variables used in this study such as diarrhea within the last week, intermittent water supply and water treatment were subject to information and recall bias as they were self-reported. The high number of diarrhea cases in this study could be due to recall bias or reporting bias, although this would likely have been a bias observed across all subjects. Although unlikely, there could have also been a seasonal enteric illness circulating in the community. With respect to intermittent water supply reports, it would be informative to compare official logs of potable water service interruptions from the municipal potable water agency with interruption frequencies reported by study participants.

Although rural settings had higher levels of water contamination than urban settings, we did not detect a statistically significant difference. This is most likely due to misclassification of geographical location in some cases. At the time of the study, there were a lot of urbanization and modernization efforts in the district of Quinindé, including the installation of potable water and sewer systems in the periphery of the city of Quinindé, which at times made it difficult to classify a household as urban or rural. Future studies in Quinindé and in similar quickly developing settings should establish set definitions for the terms “urban” and “rural” to avoid such difficulties.

Finally, it would have been ideal to collect serial water quality measurements in the same households over a longer period of time to investigate other factors such as seasonality and better assess water quality variability in households over time.

Conclusion

In summary, this study sought to identify factors that influence drinking water quality at the household level, characterize the association between water quality and diarrheal and parasitic disease, and assess water quality variability within and between households. In this study, *E. coli* was determined to be a more suitable water quality indicator. At the household level, water treatment was found to strongly impact water quality. Boiling water prior to consumption lead to significantly lower drinking water contamination levels when compared to untreated, chlorinated and filtered water. Water source and storage also were significantly associated with differences in household water quality. Storage was more likely to occur in households that experienced intermittent potable water supply. Although modest, there was a positive association between higher water contamination levels and risk of diarrheal disease and STH infection within our study population. Finally, water variability was found to be greater between and within households in urban settings as opposed to rural settings in which intra- household variability was greater.

Recommendations

The results of this study have several implications. As the global health community embarks on a new journey to accomplish the sustainable development goals by the year 2030, close attention should be paid to the ways in which progress and accomplishment of those goals are executed and measured.

As it relates to SDG 6.1 (“member nations will strive to achieve universal and equitable access to safe and affordable drinking water for all by 2030” (UN, 2015)), public health experts recommendations that the current improved vs. unimproved water source classification does not adequately assess water safety (T. F. Clasen, 2012; Onda et al., 2012) were supported by the findings in this study. The JMP should therefore develop more accurate risk assessment methods that include microbiological water quality measurements and take into account factors that influence variability in such measurements efforts including type of microbial indicator used and baseline water contamination levels.

Until universal and equitable access to safe and affordable drinking water is reached, public health authorities in developing nations should continue to educate populations on the health risks associated with poor water quality. Such education endeavors should include recommending the avoidance or minimization of water storage, the use of low risk water sources and effective point of use water treatment such as boiling. Local and national governments should also make efforts to minimize potable water service interruptions.

Better study designs are needed to help guide our understanding of the relationship between water quality and waterborne diseases. When designing health studies that use water quality indicators in the developing world, special consideration should be given to baseline water contamination levels and intra- vs. inter- household water quality variability.

Finally, as demonstrated by the association between diarrhea, STH infection and water quality found in this study, water quality plays an important role in health and is linked to many conditions that are associated with great morbidity in children and other vulnerable populations. Investing in water, sanitation and hygiene interventions is not only a way to uphold the human right to safe drinking water but also a sustainable and comprehensive approach to reducing the burden of disease in the world.

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Appendix A: Questionnaire form for surveys

*Note: actual surveys were administered using a smart phone and answers recorded electronically.

Número del hogar (*House ID*):
Número del niño (*Patient ID*):

Fecha (*Date*):
Hora (*Time*):

Observador(a) (*Enumerator name*):

<u>A-Datos de la vivienda (*Description of household*)</u>

1-Cantón (*District*):

- ☐ Quinindé
- ☐ La Concordia
- ☐ Otro
- ☐ Especifique otro canton (district- other):

2- Parroquia (*Parish*):

- ☐ Rosa Zárate
- ☐ Cube
- ☐ Chura
- ☐ Malimpia
- ☐ Viche
- ☐ La Union
- ☐ La Concordia
- ☐ Las Golondrinas
- ☐ Otra
- ☐ Especifique otra parroquia (parish-other):

3- Nombre del barrio o recinto (*Neighborhood name*):

4- La vivienda se encuentra (*House location*):

- ☐ En un barrio urbano
- ☐ En un barrio peri-urbano
- ☐ En un recinto
- ☐ En las afueras del recinto
- ☐ Completamente alejada del recinto

<u>B- Fuentes de agua de la vivienda (*Water sources*)</u>

1- Agua Potable (*Potable water access*)

a) Hace cuanto tiempo que vive en esta casa? (meses) (*How long have you lived in this house in months?)*

b) Usted tiene agua potable en su casa? (*Do you have potable water in your house?*)

c) Siempre ha tenido agua potable en esta casa? (*Have you always had potable water in this house?*)

d) Siempre ha utilizado agua potable en esta casa? (*Have you always used potable water in this house?*)
e) Desde cuando tiene agua potable en su casa? (ano/mes) (*Since when do you have potable water in this house?* (year/month))

f) Si no se recuerda la fecha exacta, approximativamente cuanto tiempo en anos? (*if you do not recall the exact date, please give an estimated number of years*)

2- Uso del agua potable (*Potable water utilization and supply*)

a) Desde cuando utiliza agua potable en su casa? (ano/mes) (*Since when do you use potable water in your house (year/month)*)

b) Si no se recuerda la fecha exacta, approximativamente cuanto tiempo en anos? (*if you do not recall the exact date, please give an estimated number of years*)

c) Cortan con frecuencia el agua potable? (*Do you often experience potable water service interruption?*)

☐ Si (*Yes*)
☐ No (*No*)

d) Con que frecuencia? (*If yes, how often?*)

☐ Nunca (*never*)
☐ Cada día (*daily*)
☐ 1 a 3 veces por semana (*1-3 times a week*)
☐ Más de 3 veces por semana (*more than 3 times per week*)
☐ 1 a 3 veces por mes (*1-3 times a month*)
☐ Más de 3 veces por mes (*more than 3 times a month*)
☐ Una vez cada 2 meses (*once every 2 months*)
☐ 1 a 2 veces por año (*1 to 2 times a year*)
☐ Otro (*other*)
☐ Especifique otra frecuencia (*specify other*):

3- Que tipo de agua usan para tomar en esta casa? (*What type of water do you use for drinking in this house?*)

☐ Red publica de agua potable (*municipal potable water*)
☐ Botellon/botellas (*bottled water*)
☐ Pozo (*well*)
☐ Pozo con instalacion a tanque elevado (*well connected to a tank*)
☐ Pozo con instalacion (bomba de agua) (*well connected to a pump*)
☐ Rio, vertiente, acequia (*river*)
☐ Rio, vertiente, acequia con instalacion a tanque elevado (*river connected to a tank*)
☐ Rio, vertiente, acequia con coneccion (*river connected to a pump*)
☐ Tanquero (*tank*)
☐ Lluvia (*rain*)
☐ Otro (*other*)

4- Si usan agua potable del municipio, a donde está la fuente? (*If you use municipal potable water, where is the source?*)

- ☐ Llave adentro (*tap inside the house*)
- ☐ Manguera afuera (*hose outside the house*)
- ☐ Llave en la casa del vecino (*tap in the neighbor's house*)
- ☐ Manguera afuera de la case del vecino (*hose outside the neighbor's house*)
- ☐ Otro (*other*)
- ☐ Especifique otro lugar de la fuente (*specify other location*):

5- Si usan agua de pozo, a donde está la fuente? (*if you use well water, where is the source?*)

- ☐ En el hogar (*on the household plot*)
- ☐ En la casa del vecino (*on the neighbor's plot*)
- ☐ Comprado (*commercial well*)
- ☐ Otro (*other*)
- ☐ Especifique otro lugar del pozo (*specify other location*):

6- Tiene niños que tienen menos de cinco años en esta casa? (Are there children under the age of 5 in this house?)

- ☐ Si (*Yes*)
- ☐ No (*No*)

7- Que tipo de agua utiliza para dar de beber a su niño/a de menor edad que ha empezado a ingerir alimentos que no sea leche materna? (What type of water do you give your youngest weaned child to drink?)

- ☐ Red publica de agua potable (*municipal potable water*)
- ☐ Botellon/botellas (*bottled water*)
- ☐ Pozo (*well*)
- ☐ Pozo con instalacion a tanque elevado (*well connected to a tank*)
- ☐ Pozo con instalacion (bomba de agua) (*well connected to a pump*)
- ☐ Rio, vertiente, acequia (*river*)
- ☐ Rio, vertiente, acequia con instalacion a tanque elevado (*river connected to a tank*)
- ☐ Rio, vertiente, acequia con coneccion (*river connected to a pump*)
- ☐ Tanquero (*tank*)
- ☐ Lluvia (*rain*)
- ☐ Otro (*other*)

8- Si usan agua potable del municipio, a donde está la fuente? (if you use well water, where is the source?)

- ☐ Llave adentro (*tap inside the house*)
- ☐ Manguera afuera (*hose outside the house*)
- ☐ Llave en la casa del vecino (*tap in the neighbor's house*)

- ☐ Manguera afuera de la case del vecino (*hose outside the neighbor's house*)
- ☐ Otro (*other*)
- ☐ Especifique otro lugar de la fuente (*specify other location*):

9- Si usan agua de pozo, a donde está la fuente? (if you use well water, where is the source?)

- ☐ En el hogar (*on the household plot*)
- ☐ En la casa del vecino (*in the neighbor's plot*)
- ☐ Comprado (*commercial well*)
- ☐ Otro (*other*)
- ☐ Especifique otro lugar del pozo (*specify other location*):

<u>D- Metodos de purificacion del agua (*Water purification methods*)</u>

1- Tratan el agua que toman los miembros de la vivienda? (*Do you treat the water that your family members drink?*)

- ☐ Si (*Yes*)
- ☐ No (*No*)

2- Como se tratan el agua que toman? (*How do you treat the water that you drink?)*

- ☐ Ningun (*no treatment*)
- ☐ Hervida (*boiling*)
- ☐ Con cloro (*with chlorine*)
- ☐ Avate (*vermicide*)
- ☐ A sentar (*settling*)
- ☐ No sabe (*I don't know*)
- ☐ Con filtro (*filter*)
- ☐ Otro (*other*)

3- Como se tratan el agua que toman los ninos? (*How do you treat drinking water for your children?*)

- ☐ Ningun (*no treatment*)
- ☐ Hervida (*boiling*)
- ☐ Con cloro (*with chlorine*)
- ☐ Avate (*vermicide*)
- ☐ A sentar (*settling*)
- ☐ No sabe (*I don't know*)
- ☐ Con filtro (*filter*)
- ☐ Otro (*other*)

4- Tiene agua tratada hoy? (do you have treated water in your house today?)

- ☐ Si (*Yes*)
- ☐ No (*No*)

5- Tiene agua tratada hoy para los ninos? (do you have treated water for your children in your house today?)

- ☐ Si (*Yes*)
- ☐ No (*No*)

<u>E- Almacenamiento del agua (*Water storage*)</u>

1- Tiene agua almacenada en su casa hoy? (*Do you have stored water in your house today?*)

☐ Si (*Yes*) ☐ No (*No*)

2- Inventario (*inventory*)

a) Tipo de recipient (*type of recipient*):

- ☐ Balde (*bucket*)
- ☐ Olla (*large cooking pot*)
- ☐ Jarra (*pitcher*)
- ☐ Botella pequena (*small bottle*)
- ☐ Galon (*gallon*)
- ☐ Caneca
- ☐ Pomita
- ☐ Poma
- ☐ Tanque de plastico (*plastic tank*)
- ☐ Tanque metalico (*metal tank*)
- ☐ Tanque de cement (*cement tank*)
- ☐ Otro (*other*)
- ☐ Especifique otro tipo de recipient (*specify other type of recipient*):

b) Fuente (*Source of water*):

i-Especifique la fuente de agua potable (*location of potable water source*):

ii-Especifique otro lugar de la fuente (*other location*):

iii-Si usan agua de pozo, a donde esta la fuente? (*if you use well water, where is the source?*)
iv- Especifique otro lugar del pozo (*other location*):
v- El recipiente esta tapado? (*is recipient covered?*)
vi-El agua esta tratada? (*is the water treated?*)
vii-El agua esta en la nevera? (*is the water in the fridge?*)
viii- Cuantos recipientes de esto tipo estan en la casa? (*number of similar recipients in the household*)

F- Riesgos de infermedades infecciosas (*Infectious disease risks*)

1- Las personas en esta vivienda se banan en (*household members bathe)*:
- ☐ Un cuarto de bano o una ducha (in *a bathroom*)
- ☐ El patio con tanque de recoleccion de agua (*outdoors with a bucket*)
- ☐ El rio o estero (*in the river*)

2- En la ultima semana, algun miembro de la familia tuvo diarrea? (*In the last week, did anyone in the family have diarrhea?*)

☐ Si (*Yes*) ☐ No (*No*)

3- Diarrea (*Diarrhea cases*)

a) Cual es el sexo de la persona que tuvo diarrea? (*Sex*)
b) Cuantos años tiene? (*Age*)

G- Coleccion de pruebas (*Sample collection*)

1-Numero de la prueba (*Sample number*):
2- El agua esta almacenada? (*Is water stored?)*
3- Fuente da la prueba (*source*):
4- Especifique la fuente de agua potable (*specify location of potable water source*):
5- Especifique otro lugar de la fuente(*other location*):
6- Si usan agua de pozo, a donde esta la fuente? (*if they use well water, where is the source?*)
7- Especifique otro lugar del pozo (*specify other well location):*
8- Hace cuanto tiempo, en dias, que el botellon esta abierto? (*if bottled water, how long has the bottle been opened in days?)*
9- Esta el agua tratada? (*Is the water treated?*)
10- En que tipo de recipiente esta almacenada el agua? (*In what type of container is the water stored?*)
11- Especifique otro tipo de recipient (*other type of recipient*):
12- Esta el recipiente tapado? (*is recipient covered?*)
13- Hace cuanto tiempo, en dias, que el agua esta almacenada? (*How long has the water been stored, in days?*)
14- Ha lavado el recipiente antes de almacenar agua? (*Has the recipient been washed before storing water?*)

☐ Con agua (*with water*)
☐ Con jabon (*with soap*)
☐ Con cloro (*with chlorine*)
☐ Con cloro y jabon (*with chlorine and soap*)
☐ No sabe (*I don't know*)

H- Observaciones de las practicas higienicas en el hogar (*Structured Observations*)

1- A donde están los botellones u otros recipientes que contienen agua para beber? (*Where are the recipients containing drinking-water placed?)*

☐ En la mesa (*on a table*)
☐ En el piso (*on the floor*)
☐ En el meson (*on a counter*)
☐ Otro (*other*)
☐ Especifique otro lugar (*other location*):

2-Cuando recoge las muestras, contamina el agua con las manos? (*when the participant collects water samples, does s(he) contaminate the water with her/his hands?*)

3- Usan recipientes sucios para sacar o tomar agua? (*Do household members use recipients that appear dirty/soiled to collect or drink water?*)

4- Hay un recipiente dedicado para sacar agua? (*Is there a dedicated recipient/tool to collect water?*)

5- Como recoge el agua? (*How does the participant collect the water sample?*)

☐ Se saca el agua (*participant collects the water with a recipient*)
☐ Vacia el agua (*participant pours water*)
☐ No se aplica (*not applicable*)

6- Tienen dispensador (que funciona) para botellones? (*Is there a functioning water dispenser in the house for bottled-water?*)