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Stephen Crabbe Date

An Analysis of Water Quality in Small Water Treatment Plants and Households in the Yucatán Peninsula, Mexico

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Bachelor of Arts

Lake Forest College

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An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

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Abstract

An Analysis of Water Quality in Small Water Treatment Plants and Households in the Yucatán Peninsula, Mexico

By Stephen Crabbe

Background: With over 884 million people lacking access to safe water, private water vendors offer drinking water to communities where public utilities do not exist or are inadequate. Due to the informal nature of many private water vendors, there is often little regulation of the quality of water they provide or the premium they charge their customers. Living Waters for the World (LWW) is an organization that establishes small water treatment plants in developing countries in order to create a sustainable business that provides communities with safe drinking water at reduced cost.

Objective: This research project examined the quality of the water produced by the LWW treatment plants to determine if it meets World Health Organization (WHO) guidelines for drinking water. This study also analyzed household water samples from LWW customers in order to assess the drinking water quality in the home and determine household risk factors for recontamination of LWW drinking water.

Methods: LWW water treatment plants in four communities in Mexico were selected for this study. Water quality at the plant was tested by membrane filtration to measure the concentration of total coliforms (TC) and E. coli. Households in the study communities were surveyed, and water samples were collected from home water storage containers and also tested for TC and *E. coli*.

Results: LWW water treatment plants produced water that met WHO and national guidelines for *E. coli*, but only 48% of the plant samples met the guidelines for TC suggesting inadequate treatment by some plants. Household samples had higher TC and *E. coli* concentrations (31.3 and 2.3 fold, respectively) than plant samples ($p<0.0001$, $p=0.0130$). No household characteristics were significant predictors of household water contamination with *E. coli*.

Discussion: The LWW water treatment plants produced water that consistently met WHO and national standards for *E. coli* but not TC. Recontamination occurs in stored household water before it is consumed. Further research is needed to analyze the source of LWW drinking water contamination. Improved hygiene education for LWW customers may help reduce in-home contamination and improve the quality of the water at the point of use.

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And lastly I would like to give thanks to my family, who has shown me the real value of community service, and has equipped me with the passion and courage to do great things.

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Objectives

The objective of this research was to determine the quality of water that delivered by Living Waters for the World (LWW), a private sector water vendor, to customers in the Yucatán Peninsula, Mexico. Furthermore, the goal was to determine whether there are specific household factors that may lead to recontamination of LWW water while stored and consumed in the home.

Specific objectives

- Assess levels of *Escherichia coli* and total coliforms in water samples after treatment from the LWW water treatment plant.
- Compare *E. coli* and total coliform levels between samples leaving the treatment plant and samples from in-home water storage containers.
- Determine household demographic information, socioeconomic status, hygiene knowledge and drinking water consumption behaviors of LWW customers.

Hypotheses

- LWW plants produce water that meets WHO guidelines for drinking water (less than 1 *E. coli* or total coliforms per 100 mL sample).
- Higher *E. coli* and total coliform concentrations will be found in household water samples compared to water samples from LWW water treatment plants.
- Water samples from households with children under the age of 5 will have higher total coliform and *E. coli* concentrations.

- Water samples from households with head of households having only a primary school education or less, who are self employed or do not currently work will have higher total coliform and *E. coli* concentrations in their household drinking water.
- Total coliform and *E. coli* concentrations will be higher in water samples from households who do not have access to indoor sanitation facilities

Introduction

Over 884 million people worldwide and 78 million in Latin America lack access to safe drinking water (UNICEF and WHO 2010c). Drinking water contaminated with bacteria, protozoan, and viral pathogens has been shown to cause enteric disease, leading to increased morbidity and mortality from diarrhea. The public sector has not been able to build and maintain sufficient safe water infrastructure to meet the vastly growing demand for safe drinkingwater and as a result, many private water vendors have entered the marketplace to fill the gaps where public utilities don't reach or provide substandard service (Kjellen and McGranahan 2006).

The market for private water vendors is growing rapidly and types of water vending can include standpipe operators, tanker truck distributers, and water distributors who deliver directly to homes. The informal nature of these businesses makes it difficult for governments to regulate the quality of the water they provide or the prices they charge. Further research is necessary to determine why individuals choose to purchase water from private water vendors and to assess the quality of the water they provide.

Living Water for the World (LWW) is a non-profit organization whose goal is to create partnerships between communities in the United States and in developing nations in order to build sustainable small water treatment plants and vending businesses in the developing nations and help meet the need for quality drinking water in these communities. This study is part of an evaluation of LWW small water treatment plants in the Yucatán Peninsula, Mexico to determine the quality of the water being consumed by their customers.

Specifically, we examined whether the LWW water treatment plants produced water that met WHO and national guidelines for drinking water quality and whether the quality of the water stored in households was worse than the water produced in the plants due to recontamination because past studies have shown that water from improved sources, usually with higher quality show greater contamination after collection and storage (Wright et al. 2004). Differences in water quality from plants and in the households may be due to a variety of factors including the household and town demographics, hygiene knowledge and practices (Table 10).

It is important to understand the services provided by these small, private water vendors because of their increasing role as a community-based drinking water intervention world-wide. Private water vendors' ability to reach consumers where public water interventions have been inadequate has led to increasing influence, and it is important to be able to assess the quality of water they produce, and the potential health impacts of their drinking water product.

Background and Significance

Water and Sanitation

The WHO estimates that 884 million people lack access to improved water sources, and over 2.6 billion people do not have access to improved sanitation (UNICEF and WHO 2010c). In 2000, the Water Supply and Sanitation Collaborative Council (WSSCC), a global multi-partner organization aimed at improving access to safe water and sanitation, established three specific targets for water supply and sanitation: 1) reduce the proportion of people without access to hygienic sanitation facilities by one half by 2015, 2) reduce the proportion of people without access to a sustainable source of quality drinking water by one half by 2015 (where quality water is defined as meeting the WHO guidelines for safe drinking water), and 3) provide water, sanitation and hygiene for all by 2025 where hygiene was defined as full coverage of hand washing, safe disposal of feces, as well as safe water handling and storage (UNICEF and WHO 2000). The United Nations set forth eight goals, called the Millennium Development Goals (MDGs) aimed at increasing equality and reducing poverty worldwide, and among these, was the target to reduce the number of people who do not have access to safe water and improved sanitation by half by 2015 (UN 2011). Since 2000, increases in coverage of 7 and 10% globally for improved sanitation and water access respectively have occurred. However, if drastic improvements towards the MDGs are not made, then in 2015 an estimated 2.7 billion people will not have access to improved sanitation and 672 million will be without improved drinking water sources, reaching the MDG for water access and missing the sanitation target by 13% (UN 2010; UNICEF and WHO 2010c).

The burden of lack of access to safe water sources and improved sanitation falls heavily on people in developing nations and is even a more common problem for people living in rural areas compared to those living in urban environments (UNICEF and WHO 2010c). Rural populations account for around 84% of the people lacking access to improved water sources and sanitation services (UNICEF and WHO 2000).

Definitions of improved drinking water and sanitation

The WHO defines improved drinking water sources as those with technology that is most likely to deliver safe water to individuals, such as household connections to piped water, public standpipes, boreholes, protected wells, and rainwater catchments (WHO 2004). It is important to note that unprotected wells, springs, water sold from vendors and tanker trucks fall under the heading of "unimproved water sources." (WHO 2004).

Indicators of water quality

Pathogens are often spread in low concentrations into water supplies making them difficult and expensive to detect. But some micro-organisms can be used to indicate pathogen presence in water, however the relationship is not always a direct correlation (Ashbolt et al. 2001; EPA 2009). Ashbolt et al. (2001) describe three types of microbial indicators: 1) process indicators, 2) fecal indicators, and 3) index organisms.

Coliform bacteria are found in the intestines of warm-blooded mammals and are shed into the environment in excreta (Ashbolt et al. 2001; EPA 2010). Total coliform bacteria may occur in human intestines, but are also found in animal excreta, soil, and from other non-human sources (EPA 2010). Total coliforms are considered process indicators and used for drinking water analysis to suggest the evidence of other contaminants, however they do not directly correlate to pathogen contamination. The presence of total coliforms in treated drinking water indicates incomplete treatment, treatment failure, or post-treatment contamination.

Fecal coliforms and *E. coli* are more closely linked to fecal contamination from warm-blooded mammals than total coliforms, although both can be found in the environment from non-fecal sources (Ashbolt et al. 2001; EPA 2010). Fecal coliforms and *E. coli* are less useful as environmental indicators of water quality due to the possibility of non-fecal origins, but they are generally good indicators of fecal contamination in drinking water (EPA 2010). *E. coli* is not only recommended as an indicator of fecal contamination, but can also be used as an index organism along with Enterococci (a fecal streptococci bacteria), because their presence often occurs with *Vibrio cholerae*, *Salmonella*, *Cryptosporidium parvum*, and other water-borne bacteria shed into the environment along with excreta (EPA 2010; NRC 2004).

Water quality standards

For the purpose of this study, good quality drinking water was defined as water that meets the WHO guidelines for drinking water. According to the WHO International Guidelines for Drinking water Quality (2008), 100 milliliter samples of treated and untreated drinking water should have less than 1 *E. coli* and less than 10 total coliforms, respectively (WHO 2008). Although the WHO develops guidelines for drinking water quality, describes water quality monitoring systems, and how to respond to contamination, national drinking water standards and regulations are left up to national governing bodies. In Mexico, the ministry of health (SDS) allows for 2 total coliforms and 0 fecal coliforms or *E. coli* per 100-milliliter sample of drinking water (SDSM 1994).

Table 1. Drinking water standards recommended by the WHO and SDS Mexico, measured as colony-forming units (CFU) in 100 mL

Parameters	WHO	Mexico
Total Coliforms	**0 CFU**	**2 CFU**
Fecal Coliforms/ *E. coli*	**0 CFU**	**0 CFU**

1. (WHO 2008) 2. (SDSM 1994)

Membrane filtration

Membrane filtration is a method to detect bacterial indicator organisms in water (Ashbolt et al. 2001). The presence of these indicators can suggest fecal contamination is present in the water. However some of the microorganisms used as indicators, such as total coliforms and *E. coli* can occur naturally in water environments and therefore can not be taken as direct evidence of pathogen presence (Ashbolt et al. 2001).

Membrane filtration involves passing a measured volume of water sample, through a nitrocellulose, 0.45 μm pore membrane, which allows the passage of water and some small particles, but does not allow passage of bacteria (Bartram and Pedley 1996). Recommended sample volumes for treated or partially treated drinking water are 100 and 10 mL (Bartram and Pedley 1996). After placing the filter on a surface containing selective media to facilitate growth of the target bacteria, the filters are incubated at a temperature between 36-38 or 44.5° Celsius for total coliforms and fecal coliforms, respectively, for approximately 18-24 hours for bacterial growth (Ashbolt et al. 2001; Bartram and Pedley 1996; OXFAM-DelAgua 2000). At the end of the incubation period, it is then possible to enumerate the total concentration of indicator bacteria in the sample by counting the number of colonies formed; accuracy in estimating the bacterial concentration is enhanced if between 20 and 100 CFUs are present on the filter (Bartram and Pedley 1996; OXFAM-DelAgua 2000).

Sources of Contamination

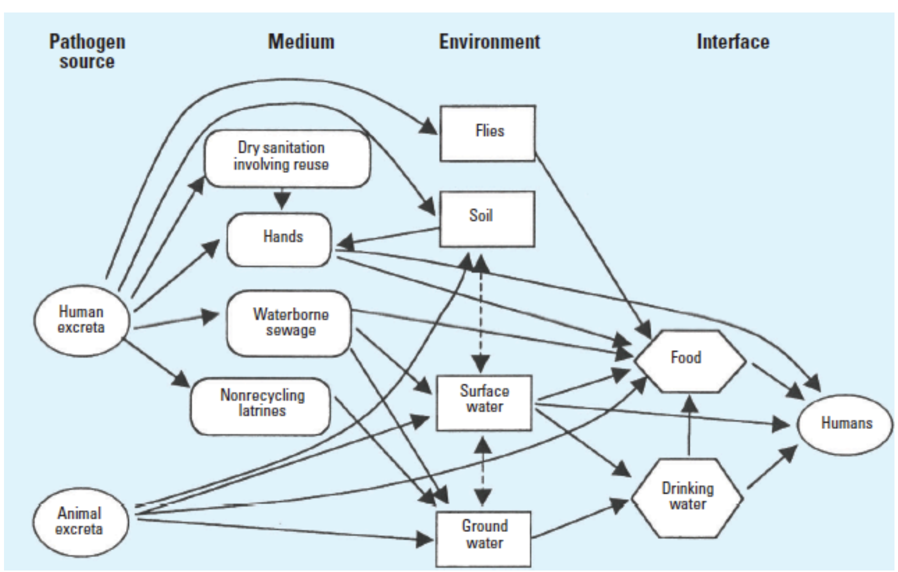


Figure 1. Transmission pathways of Fecal-oral contamination (Prüss et al. 2002)

Enteric pathogens originate from human and animal feces and can be transmitted in a variety of routes, including through contaminated drinking water, food sources, insect vectors, and person-to-person contact (Figure 1) (Ashbolt 2004; Prüss et al. 2002). Pruss et al. (2001) created a table of risk from fecal contamination in the environment based on different scenarios and levels of water, sanitation and hygiene service (Table 2). Fecal contamination of drinking water can occur at the water source, from waterborne sewage, soil runoff, or direct contact with human and animal excreta (Figure 1). Unfortunately, even if improvements are made at the source to protect the quality of drinking water, many routes of transmission may occur in the home from hands or food contaminated with fecal matter (Figure 1).

Table 2. Levels of risk for environmental fecal-oral pathogen contamination, (Prüss et al. 2002)

Scenario	Description	Environmental fecal–oral pathogen load
VI	No improved water supply and no basic sanitation in a country that is not extensively covered by those services, and where water supply is not routinely controlled	Very high
Vb	Improved water supply and no basic sanitation in a country that is not extensively covered by those services, and where water supply is not routinely controlled	Very high
Va	Basic sanitation but no improved water supply in a country that is not extensively covered by those services, and where water supply is not routinely controlled	High
IV	Improved water supply and basic sanitation in a country that is not extensively covered by those services, and where water supply is not routinely controlled	High
IIIc	IV and improved access to drinking water (generally piped to household)	High
IIIb	IV and improved personal hygiene	High
IIIa	IV and drinking water disinfected at point of use	High
II	Regulated water supply and full sanitation coverage, with partial treatment for sewage, corresponding to a situation typically occurring in developed countries	Medium to low
I	Ideal situation, corresponding to the absence of transmission of diarrheal disease through water, sanitation, and hygiene	Low

In-home recontamination

Although contamination of drinking water often occur at the source, there are also a number of opportunities for contamination to occur either during transport from the source to the home, or within the households themselves (Clasen and Bastable 2003; Rufener et al. 2010). Wright et al. (2004) found that nearly half the studies that examined the quality of the water at the source and compared it to the quality in household water samples, showed signs of increased contamination with bacteria (total coliforms, fecal coliforms, or *E. coli*) after water collection. Studies where the quality of water was relatively high at the source, such as from improved sources, recontamination before the point-of-use was proportionally higher than water sampled at the source (Wright et al. 2004). Other studies suggest that when water storage containers were covered, there was less contamination with total coliforms and E. coli than in water storage containers with a wide opening (Wright et al. 2004). The narrow openings in storage containers may reduce the contact of fecally contaminated hands or water removal devices. However, hands and cups that have fecal contamination may contaminate water in the storage containers when removing water from containers with both wide and narrow openings (Wright et al. 2004).

For example, in Sierra Leon, Clasen (2003) examined the total thermotolerant coliform concentration (TTC), (which can indicate fecal contamination) in water sources improved by OXFAM, unimproved sources, and in household storage containers. In OXFAM, improved sources all but two were free of thermotolerant coliforms, and in the unimproved sources the mean TTC concentration was 407 CFU per 100 mL (Clasen and Bastable 2003). Mean TTC concentrations of 244 and 882 CFU per 100 mL were observed for homes who received their water from improved and unimproved sources respectively, showing that fecal contamination can occur even after the water is drawn from an improved source (Clasen and Bastable 2003). Recommendations aimed at reducing contamination of drinking water through improved transportation and storage practices include the use of a storage vessel that is large and durable enough to be taken to and filled at the water source, with a narrow opening to limit hand access and a spigot to reduce the need for cups and utensils to be inserted into the water container (Clasen and Bastable 2003).

A study in Northern coastal Ecuador, examined concentration of Enterococci and *E. coli* were examined both at the source where the drinking water was collected, and in household storage containers in the days following collection. Concentration of these indicator organisms was found to be higher in the source samples than in household water samples showing natural attenuation of the indicator organisms (Levy et al. 2008). However, household water containers were sampled daily for a week and compared to control stored water samples, and about half of the household containers were found to have higher concentrations of Enterococci and E. coli over the days of they were followed, suggesting recontamination of the water stored in the households (Levy et al. 2008).

Household utensils and drinking vessels are possible sources of contamination when not cleaned properly (Figure 2). An example of this was seen in a study in Bolivia; Rufener et al. (2010) detected *E. coli* in two thirds of drinking vessels and mean concentration of *E. coli* increased from 0 to 8 CFUs in 100 mL, from the source to the point of consumption with the drinking cup. Nearly half of the households reported that they treated their water with solar disinfection or by boiling, but even in *E. coli*-free samples (after disinfection), 35% became contaminated after contact with the drinking vessel (Rufener et al. 2010). This evidence suggests that interventions that target source water quality or provide point-of-use disinfection may not be adequate, and proper hygiene education needs to accompany water disinfection interventions.

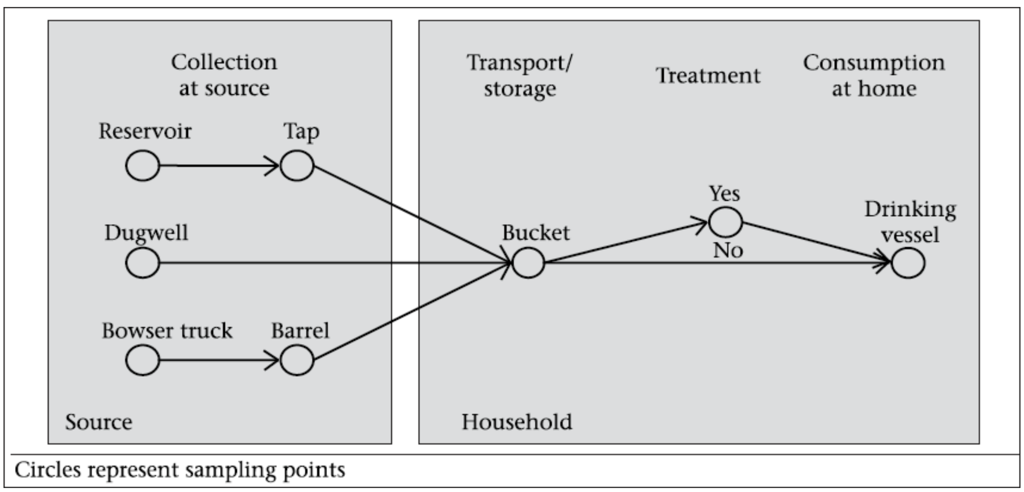


Figure 2. Potential drinking water contamination and sampling points from source to point-of-use (Rufener et al. 2010)

Household water storage and point-of-use treatment

Because of potential poor quality of "improved" sources of drinking water or contamination before the point-of-use, numerous in-home water treatment options have been developed to improve the quality of drinking water before consumption. Among these in-home options are safe storage techniques, in-home filters, solar disinfection, chlorination, and other point-of-use technologies to prevent consumption of contaminated water (Clasen and Bastable 2003). In Bolivia, a case-control study was conducted by providing intervention households with a chlorine disinfectant and storage container with a narrow mouth and spigot. The results showed that the combined in-home intervention was able to reduce total *E. coli* concentration in drinking water and mean diarrhea incidence when compared to the control households (Quick et al. 1999). Other household-level filtration methods, such as the carbon block filter combined with ultraviolet light were found to reduce over 99.9% of bacteria, viruses, and protozoa from contaminated water while being run at 150% of the volume recommended by the manufacturer's standards (Abbaszadegan et al. 1996). A review of water treatment interventions at the source and point-of-use estimated a median reduction in the relative risk of diarrhea incidence of 0.89 (3 studies, 0.42-1.90) and 0.61 (9 studies, 0.46-0.81), respectively (Fewtrell et al. 2005). In a meta-analysis by Arnold et al. (2007), household treatment interventions using chlorine showed a mean reduction in diarrhea prevalence of 29% (RR=0.71, 0.58-0.87). In studies when safe storage and hygiene education was included in the intervention, an 80% reduction (relative risk=0.20, 0.13-0.30) in detectable *E. coli* contamination of household drinking water was observed (Arnold and Colford Jr. 2007).

Health Outcomes Associated with Inadequate Water and Sanitation

Estimates by Pruss et al. (2002) on disease related to water, sanitation, and hygiene indicate that these factors may account for 4% of all mortality and 5.7% of the total disease burden in terms of DALYS (disability adjusted life years). Diarrheal disease alone is the second leading cause of death for children under the age of five years globally, claiming approximately 1.5 million lives yearly (UNICEF and WHO 2009). These deaths are largely due to the increased risk of infection and illness due to poor access to improved water, sanitation, and poor hygiene as well as lack of adequate medical treatment once children are infected in developing countries (Ashbolt 2004; UNICEF and WHO 2009). Kosek et al. (2003), reported mean diarrhea incidence of 3.2 times per year per child in developing areas with 21% of under five mortality due to diarrheal illness (Kosek et al. 2003). In cases of chronic diarrhea, children's nutritional status and cognitive development have also been adversely affected (Guerrant et al. 2002; Guerrant et al. 1999).

Water Treatment and Provision Interventions

Numerous water, sanitation and hygiene interventions have been implemented aiming at reducing diarrheal morbidity, mortality, improving nutritional status, and reducing parasite disease burden. In studies reviewed by Esrey et al. (1991) outcomes were mainly measured in specific pathogens loads measured in water and in excreta, nutritional status and diarrheal morbidity and mortality. Studies that focused on improving water quality at the source alone showed median 15% reduction in diarrheal morbidity (Esrey et al. 1991). Although water quality interventions showed marked improvement in health status, in studies where sanitation and hygiene interventions accompanied improvements in water quality, the disease burdens were reduced by more than 30% (Esrey et al. 1991).

Fewtrell et al. (2005) conducted a meta-analysis of studies that examined hygiene, sanitation, water quantity, and water quality interventions and their effect on diarrhea morbidity. Hygiene interventions that included both hygiene education and hand washing in order to reduce contamination of water and food, were found to be effective at reducing diarrhea morbidity (RR =0.67, CI 95%) (Fewtrell et al. 2005). Interventions aimed at improving water quality at the point-of-use were found to be more effective than previously thought and reduced diarrhea morbidity by 39% (RR=0.61, 0.46-0.81) (Fewtrell et al. 2005). This meta-analysis also suggested that multiple interventions conducted at the same time did not decrease diarrhea morbidity significantly more than single interventions (RR=0.67, 0.59-0.76), possibly due to lack of clarity of intervention goals or improper directing of resources when trying to organize and conduct multiple interventions simultaneously (Fewtrell et al. 2005).

Clasen et al. (2007) reviewed studies that focused on improvement of water quality both at the source and at the point-of-use and their impact on diarrhea morbidity. When pooling the interventions, source-based water quality interventions were found to reduce diarrhea morbidity in all individuals and children under the age of five, but this reduction was not statistically significant (rate ratio=0.87, 0.74-1.02) (Clasen et al. 2007). Household water treatment interventions were found to be effective at reducing diarrhea morbidity for all ages, and household chlorination (RR=0.41, 0.26-0.65), filter use (RR=0.41, 0.21-0.79) and solar disinfection (OR=0.69, 0.63-0.74) were associated with a statistically significant reduction diarrhea morbidity (Clasen et al. 2007).

Hygiene and sanitation Interventions

Improved hygiene behavior in the home, especially surrounding hand washing with soap after defecation and prior to handling of food and water, has shown to be effective in reducing incidence of diarrheal disease (Esrey et al. 1991; Fewtrell et al. 2005). In a study of children under the age of five in Brazil, households that were determined to be unhygienic had a relative risk of 1.9 for diarrhea (CI=95%, 1.7-2.8) (Strina et al. 2003). Increased water quantity can have a positive effect on hygiene behaviors. In 10 studies of interventions to improve hygiene practices, the mean reduction in diarrheal morbidity was 33% (Huttly et al. 1997).

Improvements in access to basic sanitation have also proven effective in reducing diarrhea morbidity. In a study of eight African and Latin countries, using data collected from the standardized Demographic and Health Surveys (DHS) program, diarrhea prevalence was similar in children under the age of five in rural and urban settings and improved sanitation facilities were necessary prior to seeing improved health from improving the quality of the drinking water (Esrey 1996).

Large water storage containers

Large water storage containers have been used as an intervention in homes to decrease the time spent collecting water and increase the quantity of water available for drinking and nondrinking purposes. In communities along the US-Mexico border, the efficacy of large storage containers (2,500 gallons) as an intervention to increase the quantity of water at the home that is free of total coliforms and *E. coli* was examined. The study found that an increased number of the water storage containers had greater than 10 total coliform CFUs in 100 mL at the nine-month follow up than at the baseline (Graham and VanDerslice 2007). The extended period of time that the water was stored in the large containers suggests more opportunities for contamination and also showed a decrease in mean chlorine residual levels over the time of the study (Graham and VanDerslice 2007). However, in contrast to the increased total coliform concentration, a reduction in *E. coli* concentration was observed in the stored tank water was found over the nine-month period (Graham and VanDerslice 2007)(Graham et al., 2007). The tanks did reduce the amount of time spent collecting water and increase the amount of water availablein the household. Increased water quantity in the home has been associated with a positive health impact even without interventions targeted at improving the quality of water (Esrey et al. 1991; Graham and VanDerslice 2007).

Community-level water interventions

Improved water sources connected to households, such as public water systems, although costly in comparison to point-of-use treatment interventions, can often provide protection against disease caused by fecal contamination of water due to the increased quality and quantity of the water supply. A difference in mean monthly rate of diarrhea of 179 to 75 cases per 1000 people in was found when comparing homes without piped water to the home to households with piped water respectively in Uzbekistan (Semanza et al. 1998). However, the study was also able to determine that the drinking water in the distribution system was contaminated because there ws no detectable cholrine residucal in the tap water at the home and implemntation of point-of-use chlorination resulted in a significant reduction of diarrhea (Semanza et al. 1998). This was measured by the lack of chlorine residual found in household water and the reduction in diarrhea rate by households who implemented in home chlorination of the piped water (Semanza et al. 1998). Unfortunately, access to improved water sources is not always sufficient to reduce the burden of diarrheal disease, and further measures are often needed to accompany public utility interventions to increase access to quality drinking water (Ainsworth 2004).

Water Vendors

Although water from water vendors is not considered by the WHO to be an improved source of drinking water due to the lack of ability to regulate the sector, water from vendors is used by households in a variety of locations where public utilities don't exist or do not provide adequate quality of drinking water (Kjellen and McGranahan 2006; WHO 2004). In a study of 10 African cities, 17-76% of homes were found to have in-house connections, leaving many households to fetch water from traditional sources or to purchase water from independent water vendors (Collignon and Venzina 2000). Private water sellers operate most often in urban communities with rapid growth and where the formal government infrastructure is too slow to respond to the population growth and community needs for adequate quantities of good quality water (Collignon and Venzina 2000; Kjellen and McGranahan 2006). Additionally, water vending can provide needed economic stimulus for the population, and in some areas, water vendors make up 2% of the urban workforce (Collignon and Venzina 2000). Private water sellers can be classified into three groups: 1) water vendors that operate a standpipe, well or borehole at a fixed location where individuals come to purchase water and carry it away on their own, 2) water distributors that deliver water to homes using carts, animals, or vehicles to transport storage containers, and 3) water tanker trucks that can carry larger quantities of water and deliver to homes and neighborhoods (Figure 3) (Kjellen and McGranahan 2006).

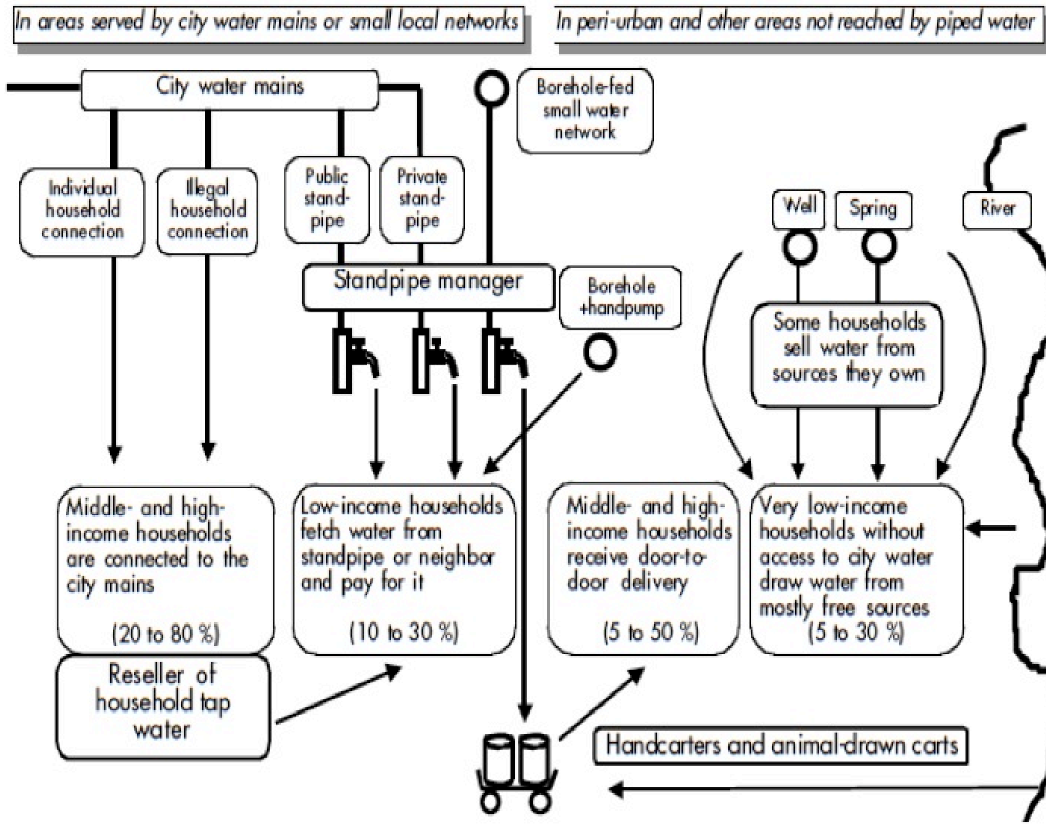


Figure 3. Water vending options for households in African cities, from source to household (Kjellen and McGranahan 2006)

Interestingly, the need for private water vendors is validated by not only their presence, but also by their sustainability in the sector when often charging what could be considered premium prices for their services. In an example from Onitsha, Nigeria, a city of 700,000 people, it was found that the population paid almost $30,000 (adjusted $1= N4.3) for 2.96 million gallons of water per day (MGD) of privately vended water from distributing vendors, standpipe vendors and tanker trucks while only paying $3,000 for nearly the same quantity of water from the public system (Whittington et al. 1991). In some cases, the price was seven times higher for vended water when compared to the public system, which shows both the lack of availability of public water and also the market-driven desire for water from private vendors (Whittington et al. 1991).

Future research on water vendors

Although some water vendors are contracted out by larger public and private water distribution companies to operate public standpipes, many are small-scale entrepreneurs who are highly mobile, serve a small community and are highly unregulated (Collignon and Venzina 2000; Kjellen and McGranahan 2006). The lack of a connection fee and the community knowledge that comes with community vendors also allows for leeway to be given in payments for services. However, this can also lead to situations where households can be forced to pay exorbitant prices for water due to the lack of government regulation (Opryszko et al. 2009). Government involvement in the form of regulation usually only occurs when water vendors begin to encroach on previously established areas served by public utilities or start to take profits away from established water providers (Collignon and Venzina 2000).

Furthermore, the quality of vended water due to contamination at the source or during transportation and storage is almost never regulated (Opryszko et al. 2009). There is often a lack of communication between governments and private sector water vendors that contributes to a system of drinking water vending with no regulations or means to enforce quality (Opryszko et al. 2009). In an analysis of small water enterprises, Opryszko et al. (2009) identifies a number of community-based research questions that have yet to be answered by researchers regarding the sale of water to households by private vendors. Some of these data gaps include the need for analysis of water quality, information on the effects of private vending on hygiene behavior and health, community perception of private water vendors and marketing strategies employed, as well as business model and sustainability analysis (Opryszko et al., 2009).

Water and Sanitation in Mexico

One of the largest Latin American countries in the Americas, Mexico has a vast population of 133,724,226 with over 30% of the population composed of Native American Indians (CIA). The growing country is experiencing a general trend of migration towards urban centers with an urbanization rate at 1.2% yearly and an overall total of 78% of the population residing in urban areas. Unfortunately, spending on social services for water and sanitation has been hampered recently due to a financial crisis, which reduced the country's GDP by 6.5% in 2009 (CONAGUA 2010).

Mexico faces a variety of issues surrounding water including overdraw of its aquifers and groundwater, poor waste management, and stresses on irrigation for agriculture (WB 2007). Currently, Mexico uses 77% of its 409 billion cubic meters of renewable water resources for irrigation, leaving considerably less for domestic consumption (Olson and Saltiel 2007; WB 2007). Overexploitation of water resources is occurring for surface waters but is especially critical for the ground water aquifers in Mexico, where it is a problem in 16% of all aquifers. This situation is leading to increased cost for water retrieval and decreased quality of ground water in coastal regions where salt content is becoming a severe issue (Olson and Saltiel 2007). In addition, poor wastewater management (only 35-15% of wastewater is treated) has led to decreased quality of renewable sources of drinking water in Mexico and spurred new legislation aimed at improving this sector (Olson and Saltiel 2007; Shah et al. 2004; Tortajada 1998). In 1992, the Law of National Waters (Ley de Aguas Nacionales) was enacted, decentralizing much of the duties of wastewater management to regional basin councils in order to include local populations and improve water quality and efficiency (Shah et al. 2004; Tortajada 1998).

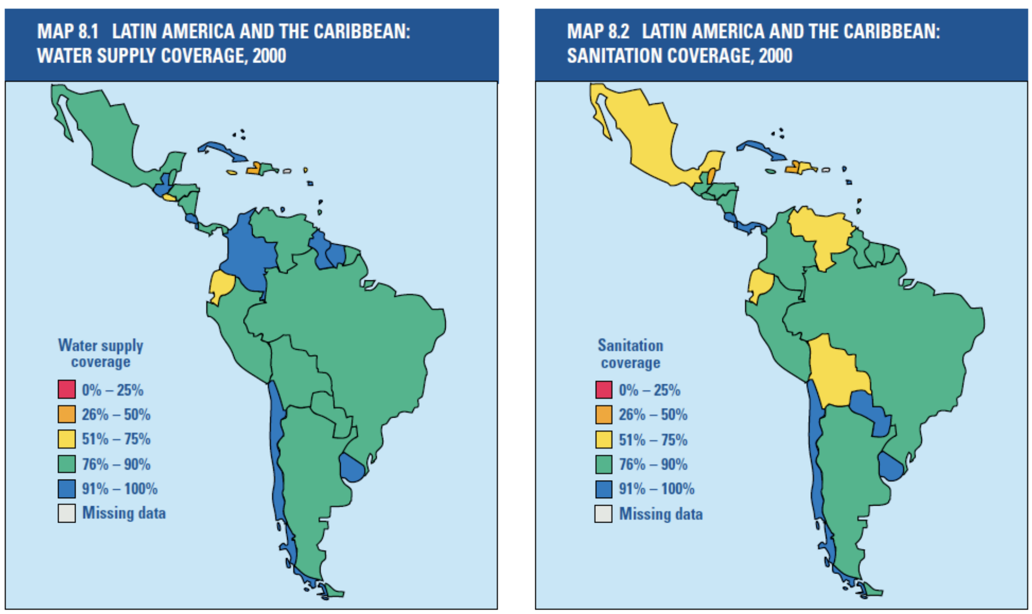


Figure 4. Water and Sanitation Coverage in Latin America and the Caribbean (UNICEF and WHO 2000)

Mexico is above the average in the Americas for mortality in children under five years of age (17 deaths per 100,000 live births) and the percentage of children who are underweight 3.4% (WHO 2010). In a 2008 report by the WHO, 96% of urban households were estimated to have access to improved water sources with 92% having household connections, while 87% of rural households had access to improved water sources and 72% had household connections (UNICEF and WHO 2010a). Furthermore, according to the 2005 census, rural households were more likely to use wells and surface water as sources of drinking water (26.6%) than urban households (2.5%) (UNICEF and WHO 2010a). Similarly, 90% of urban and 68% of rural households had access to improved sanitation (UNICEF and WHO 2010b). In rural settings, 16% of households use shared sanitation facilities and 12% use open defecation as their main disposal of excreta (UNICEF and WHO 2010b). In regard to access to improved sources of water and sanitation, Mexico is situated around the median for countries in Latin America and the Caribbean (Figure 4,5).

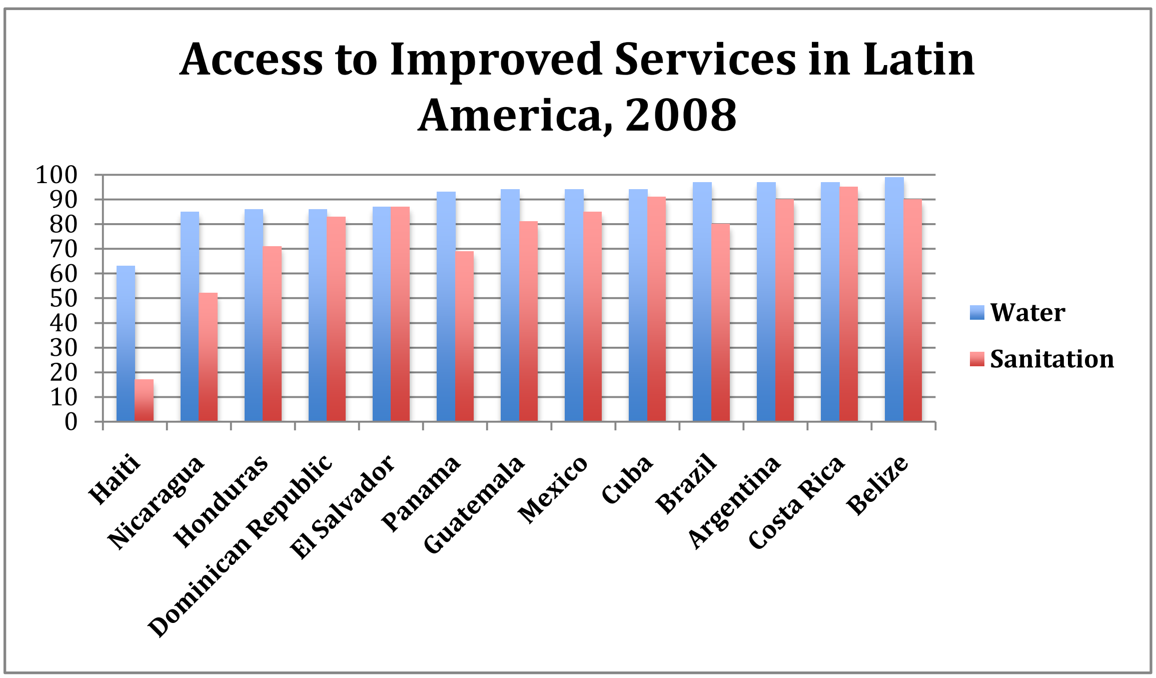


Figure 5. Improved water and sanitation coverage in Latin America and the Caribbean (WHO 2010)

The Yucatán Peninsula

The Yucatán Peninsula (Yucatán) is comprised of the three Mexican states in southeast Mexico: Quintana Roo, Yucatán, and Campeche and had an estimated population of close to 3 million people in 2000 (Gelting 1995). The peninsula is bordered by Belize and Guatemala to the south, Tabasco to the west, as well as the Gulf of Mexico and the Caribbean to the north and east. The peninsula is mostly low elevation, coastal land and is the soil is largely made up of karst, or layers of porous limestone(Gelting 1995). The lack of top soil and high permeability of the land leads to the formation of fewer surface water sources and also allows wastewater to reach the high water table quickly and contaminate possible drinking sources (Gelting 1995). This particular geology also allows salt-water infiltration from the Gulf of Mexico which contributes to additional problems with groundwater quality in the peninsula, even though it has higher than average rainfall than the rest of the country (Gelting 1995; Olson and Saltiel 2007).

The population of the Yucatán is composed of 49.5% indigenous people (3,009,223 estimated total population 2000), which is the highest percentage of the population that identify as Indigenous in comparison to other regions in Mexico (CDI 2006). Indigenous people in the Yucatán often have to face discrimination due to customs and language in school, and 60% adult indigenous are illiterate in Spanish, which causes great difficulty with public schooling and receipt of public services (Benitez and Reyes Gomez 2006; Mijangos-Noh 2009). This poor social standing in the overall Yucatán population is evidenced by 45.4% of the indigenous population being at high or very high marginalization levels, and some indigenous populations have an infant mortality rate of 40 in 1000 live births (CDI 2006). Together, the geographic characteristics along with the presence of a large indigenous population have created an extremely vulnerable population in the Yucatán with regard to drinking water availability, affordability, and quality.

Living Waters for the World

Living Waters for the World (LWW) is a United States-based, non-profit organization that trains and supplies teams to build community-based water treatment plants to sell filtered and disinfected water at a reduced cost in communities identified to need safe water options. Over the last 15 years, LWW teams have installed more than 300 water purification plants in 22 countries worldwide, including over 40 systems in the Yucatán Peninsula of Southeastern Mexico.

Initiating and Operating Partners

LWW works with initiating partners in the United States to identify potential operating partners in communities in the United States and in developing countries that show significant need for a water purification plant due to poor water quality or poor access to good quality, affordable drinking water. Initiating partners are expected to do a preliminary assessment once a potential community has been identified to receive a purification plant. The assessment includes basic demographic information on the potential operators and customers, current water sources in the area, potential local resource suppliers for the construction and maintenance of the plant, barriers to implementation, as well as potential effects on the community after the plant is operational.

Initiating partners are expected to attend Clean Water U, a week-long training school where they learn the plant installation, routine maintenance, and health education modules that accompany plant implementation in the operating community. LWW operates under the mantra of "train the trainer" where the initiating partners are given the tools and knowledge to transfer responsibility for operation, maintenance, and health education to the operating partners on the ground, creating a more sustainable community-level solution to purify water.

Initiating partners bear the majority of the financial burden involved in installation, including travel, parts, and supplies. The operating partners are asked to help supply materials and labor for construction of the water treatment plants. The plants are installed jointly in a central location in the community that is appropriate for production and delivery, usually a church, hospital, school or other central community location. After installation, plant maintenance and health educator courses are conducted with the operating partners in order for the operators to be able to continue the functionality and health outreach in the community after the initiating partners depart. Both sides are expected to sign a three-year covenant where the initiating partners are expected to return to the plant site at least four times, and provide knowledge and support during the first years of plant operation. LWW has established local part suppliers in areas close to installation sites, and operating partners are ultimately responsible for understanding the maintenance of the plant and acquiring replacement parts as necessary to continue proper operation.

Water Purification Process

Although the water treatment plants at each site are subject to local regulations, the basic set-up for each plant installed by LWW and the two partner communities remains the same. Water from an existing water source (regulations in Mexico require a unique water source for each water treatment plant) enters the plant and first passes through a sediment filter, then through a carbon filter to remove any residual chlorine, taste, and odor from the water received from the original source. A water softener removes calcium and magnesium ions, and then the water moves through a 1-micron filter and the reverse osmosis (RO) membrane into a clean water storage tank. From the clean storage tank, the water then passes through either an ultra-violet (UV) or ozone disinfection unit before being bottled and sealed in 20L containers for distribution. All bottled LWW water is distributed in sealed, 20L plastic containers with a narrow spigot.

The empty resale bottles also go through a cleaning process by which they are washed with soap and water inside and out before being rinsed with a chlorine solution. The bottles are then rinsed with clean water, filled with the purified water and sealed with shrink-wrap. The bottled water is usually stored for less than 24 hours at the plant before being sold to the public.

Water Treatment Plant Production

LWW is trying to create a community-level intervention with the construction and operation of the water treatment plants; each is built to provide water to approximately 300 individuals. The tanks in the LWW plants have an average capacity of 300 gallons and produce approximately 5 gallons per minute of purified water. This means that high capacity plants, which are defined as producing more than 100 bottles per day, will have to treat enough water to fill the storage tanks twice daily. Depending on location, filled and sealed water bottles are either sold at the plant, where customers transport the bottles themselves, or delivered by motor-tricycle or truck to individual customers' homes.

LWW customers

LWW seeks to establish water treatment plants in areas with a specific need for affordable drinking water, which means either the public water supply is not available, does not meet national guidelines for drinking water, or the drinking water options are not affordable for the community members. For this reason, LWW water plant operators set the prices for their vended water at half of the local, private brand name price (when available). The positioning of LWW bottled water as an affordable brand, means that LWW water treatment plants are targeting, but not limited to selling water to community members who are unable to afford other premium brands of drinking water.

MATERIALS AND METHODOLOGY

System Evaluation

In Fall 2009, LWW requested a team of researchers do a review and evaluation of their water purification plant systems in Mexico. The ultimate goal for LWW is to conduct a total health impact evaluation for communities in which their plants are operating. A team of three Emory Rollins School of Public Health students was created to assess the current efforts and evaluate business sustainability of the plants, marketing strategies, consumer characteristics, and the quality of the water provided to and consumed in the homes. Two other researchers have produced reports on the evaluation of LWW plants in Mexico during the Summer 2010. Janelle Hartman collected interview data from plant managers and authored the study, "Evaluation of the Operation and Financial Sustainability of Water Purification Plants in the Yucatán, Mexico." Joanna Galvaz conducted a study on the LWW customers using an original survey and authored the study, "Small Water Enterprises: A cross-sectional study of bottled water consumption in the Yucatán Peninsula, Mexico."

Research Design

This study sought to determine the quality of the water produced by LWW water treatment plants and being consumed by LWW customers. Samples were collected from the final, treated water immediately leaving the system in four local LWW water treatment plants to examine the effectiveness of the treatment systems for removing microbial contamination from the water. Water samples were also collected from the households of LWW water customers for analysis. The goal was to determine if the quality of water actually consumed met WHO guidelines for drinking water and whether recontamination was occurring in homes due to transport of water containers, water storage, or household drinking container conditions.

The quality of the water samples was determined by measuring total coliforms and *E. coli* using the membrane filtration method and 100-milliliter samples. Household surveys were also conducted with a member of each study household to determine socioeconomic status, household water usage practices as well as knowledge and opinions on water quality and hygiene.

Exposure Variables

A number of variables of interest were identified at the plant and household level, and were collected through survey sampling (Appendix A). Number of household members, specifically children under the age of five and children under the age of fifteen, were noted. The structure of the house, including flooring, bathroom or latrine presence in the house, as well as the presence of specific household goods, and monthly spending habits were noted by household survey. Opinions on water usage, hygiene practice, and specific reasons for purchase of LWW water were also recorded. Questions were asked to determine the number of times drinking water bottles were purchased by the household and the number of drinking water containers consumed by the household weekly. Some indicators were noted by observation during the household surveys such as the specific placement within the house of the water storage container, the elevation of its storage, and the method for removal of water from the container for consumption. If the researcher could not make these observations during the survey, questions were formally asked to the head of the household in order to determine this information. The presence of other water sources at the household such as indoor taps, or outdoor pipe-stands were also observed and questions regarding their functionality were included at the conclusion of the survey. A copy of the household survey is included in Appendix A.

Outcome Measures

The presence and concentration of total coliforms and *E. coli* were the main outcome measures of interest. This was measured in samples of both water leaving the treatment plant and samples from household water storage containers.

Study Communities

For this study, LWW water treatment plants and households were identified in four different towns in the Yucatan Peninsula; one in the state of Yucatan and three in the state of Campeche. These locations were selected from a list of 43 LWW operating plants in the peninsula based on two criteria: degree of urbanization and average daily water sales. Ticul, Yucatan and Lerma, Campeche were identified as plants that served urban populations, while Pich, Campeche and Chuina, Campeche delivered to households in rural areas.

Figure 6. Yucatán Peninsula, Mexico and study communities

Furthermore, these study sites were selected based on the quantity of daily water deliveries with Ticul and Pich were identified as *high output* systems (more than 100 20L deliveries daily) and Lerma and Chuina were identified as *low output* systems, delivering less than 100 20L bottles daily. A total of 33, 40, 39, and 24 households were sampled in Ticul, Pich, Lerma, and Chuina, respectively.

LWW water treatment plant characteristics

Table 3. LWW water treatment plant characteristics

Plant	Installation Date	Urban/ Rural	Output (bottles/day)	UV Light	Ozone	Bottle Disinfect.
Ticul	May 2008	Urban	High (120)	Yes	No	Yes
Pich	May 2007	Rural	High (130)	No	Yes	Yes
Lerma	July 2005	Urban	Low (80)	Yes	No	Yes
Chuina	June 2004	Rural	Low (40)	No	Yes	Yes

The four plants studied were initiated at different times by LWW partners, starting with Chuina in June of 2004, followed by Lerma, Pich, and Ticul with installation dates of July 2005, March 2007, and May 2008 respectively. Ticul and Lerma were identified as urban areas, while Pich and Chuina were determined to be operating in rural towns (Table 3) based on smaller population size (under 2000 population in Pich and Chuina). In addition, there was lower percentage of people employed in agriculture (Table 5) in Ticul and Lerma (0.0%) in comparison to Pich and Chuina where 40.3% are agriculturalists. LWW water filtration plants in Ticul and Pich were defined as high output plants (Table 3) because they produced more than 120, bottles (20 L) per day, while Lerma and Chuina produced on average about 80 and 40, bottles per day, respectively.

All plants use the same initial filtration and purification techniques where water is pumped from a private well through a sediment filter, then a carbon filter before reaching a water softener. Water is then filtered by reverse osmosis before disinfection by either ultra-violet light (UV) or ozone (Table 3); Ticul and Lerma used UV light as the final treatment procedure while Pich and Chuina used ozone. All four plants cleaned the bottles used for water storage and delivery using dish soap and water for the outside of the container, and a chlorinated water (concentration 1mg/L) rinse to disinfect the inside of the bottle prior to filling. All the plants have the capacity to fill 300 gallons per hour, and after the bottles are filled, the caps are sealed on using plastic shrink-wrap.

Household Surveys

Households that consumed water delivered from LWW treatment plants were identified by recording customer addresses when water was purchased at the plant site. In addition, locations of potential study households were noted and mapped while participating with the vendor in water deliveries to households in the distribution range. The purpose of the study was explained at the first meeting and permission was requested to return to the participants homes within three days to conduct a household survey and collect a stored water sample. This study was determined not to be human subjects research, so the Emory University IRB did not need to approve the study protocols and surveys (Appendix A). Written informed consent was obtained at the home prior to conducting the survey or collecting the water sample (Appendix B).

Collection of Water Samples

Sampling of water after treatment by the LWW plants started one day prior to the collection of household samples and occurred at the beginning of the day after the first bottles were filled and sealed for sale. A 100 mL Whirl-Pak® bag was filled directly from the distribution nozzle typically inserted into the neck of the bottles for filling. Water samples were also collected from the LWW water treatment plants using the same procedure at the end of each day prior to the filling of the final batch of bottles for sale.

During the visits to each study household, water samples were collected from the storage container. Participants were asked to pour water from the storage container into a drinking cup, and then transfer the water from the drinking cup into a 100 mL Whirl-Pak® bag. Drinking cup was defined as any vessel possibly used for drinking by household members and was chosen by the key informant.

Samples were collected between 9:00 am and 1:00 pm from the households and stored in a portable cooler filled with ice until processing on the same day after the final sample was collected. The placement of the water containers in the household was noted, specifically which room used for water storage and whether the water storage containers were elevated or remained on the floor in the homes. Water extraction methods from the storage containers, such as the use of hand pumps, water storage stands, or direct pouring from the container, was also noted.

Sample Processing

All samples were processed and analyzed on site using sterile techniques in temporary laboratories using membrane filtration method and the Oxfam DelAgua® water testing-kit. Temporary laboratories were established in each town where sampling occurred and consisted of a table cleaned with chlorine solution, the DelAgua kit, individually packaged disposable pipettes, methanol, chlorine solution, bottled, sterile water (not LWW brand, purchased from pharmacy), and a portable incubator. The laboratory station was sterilized prior to processing the water samples daily using a 2% chlorine solution.

Although the WHO and EPA recommend 100 milliliter samples for membrane filtration, for greater sensitivity, the processing samples were reduced to 50 milliliters and 2 milliliters. Samples were processed at two volumes, 2 and then 50 milliliters (in that order) to minimize sample contamination due to laboratory methods and improve validity of results. Water samples were diluted to the proper concentration in 50 mL centrifuge tubes using bottled, sterile water purchased from a local pharmacy. A Millipore filter membrane (47 mm) was placed between the collection and loading chambers of the DelAgua® filter funnel, and the water sample was pulled through the membrane using a hand pump. Next, the membrane along with the bacteria that were unable to pass through was removed using sterilized (using concentrated high heat) forceps and moved onto a Millipore filter pad (47 mm) and placed on a pad in a sterile Petri dish. Prior to placing the filter membrane on the pad, the pads were evenly soaked with 2 milliliters of m-ColiBlue24® broth using a graduated pipette and hand pump. Petri dishes containing membrane filters and pads were incubated at 38±1 °C for 18-24 hours.

The DelAgua® equipment, including forceps were sterilized between each sample using a lighter and methanol, and then allowed to cool prior to processing the following sample. Negative controls of sterile, water purchased from a pharmacy (the same water used for dilutions) were run as the first and last sample every day to ensure adequate sterilization of the DelAgua® equipment. If either the first or second negative controls showed bacterial growth, the daily samples and their dilutions were assigned a null value and not used for analysis. Bacteria colonies were counted after 24 hours, and the results were recorded in a laboratory notebook; red colonies indicated total coliforms and blue colonies indicated *E. coli* presence in the sample.

Each water sample was assigned an identification number that linked it to the household survey from where the sample was taken, and results from water tests were recorded originally in a table that listed sample volume used in the dilution and actual number of colonies counted. If more than 100 coliforms or colonies were counted on a single membrane, the sample was assigned a value of too numerous to count (TNC). It was necessary to convert the colony counts to concentration per 100 mL using the two dilutions for each plant and household sample. The two dilutions for each sample were compared to each other to check for precision (the 50 mL dilution colony count should be approximately 25 times the 2 mL dilution colony count ± 15%) before conversion to 100 mL concentrations, and only matching dilutions were included for statistical analysis. The colonies on the membranes from the 50 mL dilutions were analyzed first, and if there were fewer than 100 colonies, the concentration per 100 mL was determined by multiplying 2 times the colony count. If the 50 mL dilution was identified as TNC, then the 2 mL dilution was used for conversion by multiplying the colony count by 25 to determine concentration of total coliforms or *E. coli* in 100 mL. If the 2 mL dilution had a colony count greater than 100, then the concentration of total coliforms or *E. coli* was given a value of 5000 per 100 mL. These conversions were calculated as survey data and bacteria concentration results were transferred from paper copies to Microsoft Excel 2008 using double entry. The spreadsheet generated by Excel was then imported into and analyzed using SAS version 9.2 (Cary, NC)

Statistical analyses

Data entry and cleaning were performed in Microsoft Excel 2008, and all analyses including multivariate, bivariate, descriptive, and regression statistics were performed using SAS statistical software.

Descriptive statistics (means, medians, ranges, and frequencies) were calculated to analyze differences between the four study communities based on household demographic characteristics, water usage reports, as well as household and water storage information. Descriptive statistics and geometric means were also used to compare outcomes of interest such as the frequency of total coliform and *E. coli* detection and the specific concentration of total coliforms and *E. coli* in plant and household water. Geometric means using log base 10 were used when analyzing bacteria concentrations to correct for non normal distributions in the data. One-way analysis of variance (ANOVA) was used to assess if a statistical difference in concentrations of total coliforms and *E. coli* occurred between water treatment plants, and households in the four study communities.

Linear regression analysis was used to analyze changes in microbiological quality of the water produced in LWW plants and the water stored in the households. The log-transformed concentrations of both *E. coli* and total coliforms were compared using linear regression between plant samples and household samples overall, as well as individually for each of the four towns where the sampling occurred. The outcome of interest were the point estimates of the linear slope, which were used to estimate the order of magnitude of the difference between the geometric mean concentrations of *E. coli* and Total coliforms in the treatment plants and in the household water samples. T-tests were also performed in order to compare the geometric mean concentrations of total coliforms and *E. coli* in water samples from LWW treatment plants and corresponding households

For the logistic regression analyses, the presence of E. coli in a household water sample was the outcome of interest. The probability of a household water sample having an E. coli concentration greater than 1 colony per 100 mL was then examined relative to several possible predictor variables, including household demographics, water storage and usage practices, as well as community characteristics (Table 14). All variables were first analyzed separately with the outcome of interest and then used in a logistic regression in order to control for possible confounding that could occur from other exposure variables.

Logistic model selection was first performed using the stepwise procedure in SAS, and variables were removed based on their p value (<0.05). This procedure removed all but one variable as a predictor of an *E. coli* concentration greater than 1 colony in 100 mL. Backwards procedure was then performed, using SAS software, to create a logistic model, and variables were grouped according to categories such as household demographics and water storage practices. Adjusted odds ratios for the exposure variables were estimated using the model with *E. coli* concentration greater than 1 colony in 100 mL in household water samples as the outcome of interest.

Results

Surveys were completed in 179 households in four communities (Ticul, Pich, Lerma, Chuina) in the Yucatán. Water samples from household storage containers were collected from 135 LWW customers only. A total of 28 water samples were collected from the LWW operating plants in the four towns where households were surveyed.

Household Demographics

The mean number of household members for the four communities was 4.6 with Ticul having the highest mean of 5.7 members per household and Lerma having the lowest with 4.1 members per household (Table 6). Most households (69.3%) had at least one child under the age of 15 and 37.4% had a child under the age of five at the time of the survey (Table 6).

A higher percentage of households in the rural towns had a head of household who only finished primary school or didn't attend school at all with 69.1% compared to 37.7% of urban households (Table 4). Moreover, only 25.5% of urban and 16.9% of rural households were headed by someone that was educated beyond secondary school (Table 4). The majority of urban households were self-employed (46.1%), while the majority of rural households worked in agriculture (40.3%, Table 5).

Table 4. Head of household education level in the Yucatán, Mexico

Community	n	Education Level (%)				
		None	Primary	Secondary	Prep.	Tech./Univ.
Urban	*106*	*8.5*	*29.2*	*36.8*	*13.2*	*12.3*
Ticul	31	9.7	6.5	54.8	16.1	12.9
Lerma	75	8.0	38.7	29.3	12.0	12.0
Rural	*71*	*9.9*	*59.2*	*14.1*	*11.3*	*5.6*
Pich	47	8.5	57.5	17.0	12.8	4.3
Chuina	24	12.5	62.5	8.3	8.3	8.3
Total	**177**	**9.0**	**41.2**	**27.7**	**12.4**	**9.6**

Table 5. Head of household occupation in the Yucatán, Mexico

Community	n	Occupation (%)				
		None	Agric.	Self	Employee	Tech./Adv.
Urban	*106*	*26.5*	*0.0*	*46.1*	*17.6*	*9.8*
Ticul	31	3.2	0.0	67.7	16.1	16.1
Lerma	75	36.6	0.0	36.6	18.3	8.5
Rural	*71*	*31.9*	*40.3*	*9.7*	*12.5*	*5.6*
Pich	47	47.9	35.4	6.3	4.2	6.3
Chuina	24	0.0	50.0	16.7	29.2	4.2
Total	**177**	**28.7**	**16.7**	**31.0**	**15.5**	**8.0**

Note: Employee was defined as working for someone else. Technical and advanced occupations required technical school or university training.

Table 6. Household characteristics of study communities in the Yucatán, Mexico

Community	n	Mean # per HH (min-max)	HH with child <5 yr. %	# people who sleep per room (%)				
				1	2	3	>4	Average
Urban	*106*	*4.6 (1-16)*	*37.8*	*6.6*	*35.8*	*29.2*	*28.3*	*3.0*
Ticul	31	5.7 (2-16)	35.5	3.2	19.4	41.9	35.5	3.2
Lerma	75	4.1 (1-8)	38.7	8.0	42.7	24.0	25.3	2.8
Rural	*73*	*4.7 (1-11)*	*37.0*	*6.1*	*42.5*	*21.9*	*30.1*	*3.0*
Pich	49	4.5 (1-9)	42.9	6.1	44.9	20.4	28.6	3.0
Chuina	24	5.0 (2-11)	25.0	4.2	37.5	25.0	33.3	3.0
Total	**179**	**4.6 (1-16)**	**37.4**	**6.1**	**38.5**	**26.3**	**29.1**	**3.0**

Sanitation Facilities and Hygiene knowledge

Flush toilets were found in over 98% of households in this study, the remaining households utilized pit latrines. Interestingly, while 63% of households reported that they received training in hygiene or water purification techniques, such as boiling or adding chlorine, only 7% of these households identified LWW as the source of this information. Schools and local clinics were much more common places where people learned hygiene behaviors (93% of households).

All households reported that they washed their hands prior to preparing and eating food. While only 40.7% reported using bottled water to wash their hands, 96.6% of households said they believed there were microbes in the tap water that made piped water unsafe to drink. This was supported by the fact that over 70% of households claimed that tap water was unsafe to drink even occasionally, and 95% reported that they noticed better health when they drank bottled water. Furthermore, 81.5% of households surveyed responded that it was necessary for both adults and children to drink bottled water, and over 50% believed that the water they drink could impact their health.

Water use practices

The information from the household surveys indicated that households in the different towns purchased between 5.0 and 5.7, 20L bottles per week in the four sites which when compared to household occupancy, meant that about 1.3 bottles were consumed per individual during the week (Table 7). All households (100%) reported that they used bottled water exclusively for drinking, and around 90%

reported using bottled water for food preparation (Table 7). Rural homes were more likely to report using bottled water for cooking (97.3%) than households in urban locations (Table 7). When surveyed, 53.7 and 41.2% of households reported using bottled water for brushing their teeth and taking care of an infant respectively (Table 7). Again, rural households were more likely to report using bottled water for these purposes than urban houses.

Table 7. Household consumption and bottled water use in the Yucatán, Mexico

Community	n	Mean # bottles cons./ week (min-max)	Mean # bottles cons./HH member	% Using purified water for HH activities			
				Drink	Cook	Baby	Teeth
Urban	*106*	*5.1 (1-21)*	*1.3*	*100*	*83.0*	*28.3*	*29.3*
Ticul	31	NA	NA	NA	64.5	NA	NA
Lerma	75	5.1 (1-8)	1.3	100	90.7	28.3	29.3
Rural	*73*	*5.3 (1-11)*	*1.2*	*100*	*97.3*	*66.7*	*78.1*
Pich	49	5.0 (1-10)	1.2	100	95.9	64.3	77.5
Chuina	24	5.7 (2-12)	1.2	100	100	80.0	79.2
Total	**179**	**5.2 (1-21)**	**1.3**	**100**	**88.8**	**41.2**	**53.7**

Note: “NA” values in Ticul were not answered by respondents. When respondents did not use vended bottled water then potable water/household taps were used.

Because the household surveys included both LWW customers and households that purchased other brands of water, we sought to determine the reason for purchasing the particular brand of water consumed by each household. Over 60% of houses surveyed said their primary reason for their brand choice was either price or convenience. Households in Lerma, an urban town, were the most likely to report that their choice was based on price or convenience, with 41.9 and 35.6% of households reporting that they purchase bottled water for these reasons respectively. About 70% of households in Chuina chose a specific brand of water in order to reduce sickness, and over 65% of the households we interviewed in rural towns said their reason for bottled water purchase was health related. Nearly 25% of households reported purchasing bottled water because they preferred the taste.

The most frequent response for how individuals knew water (either bottled or household tap) was safe to drink was the absence of chlorine smell and taste; almost 35% of households reported that this was the key indicator for safe water. The next most common answer was the clarity of the water with 22.6% of households reporting this to be reason they knew water was safe. Trust in the seller, lack of microbes due to filtration, and bottles that are sealed were each mentioned by 12 to 15% of households as reasons they knew the water they purchased was safe to drink.

Table 8. Water storage in households in the Yucatán, Mexico

		Room (%)		**Method of Removal (%)**				**Elev.**
Comm.	**n**	Bedroom Livingroom	Kitchen	Pour	Pitcher	Hand-pump	Stand	Yes (%)
Urban	*106*	32.3	67.7	41.2	8.5	13.6	39.0	59.3
Ticul	31	55.6	44.4	60.0	10.0	30.0	0.0	30.0
Lerma	75	22.7	77.8	34.7	8.2	10.2	46.9	65.3
Rural	*73*	28.9	71.1	34.3	22.4	10.4	32.8	43.9
Pich	49	38.1	61.9	34.9	25.6	9.3	30.2	23.6
Chuina	24	20.8	79.2	33.3	16.7	12.5	37.5	65.2
Total	**179**	30.3	69.7	35.9	15.9	11.7	35.7	51.2

Note: Pour was defined as being poured straight from the storage container into a drinking cup. Pitcher was defined as any other hand-held container to store water.

The location where water is stored in the house, as well as the method used to remove water from the storage container, are potentially important determinants of whether or not drinking water remains free of microbial contamination in the household prior to consumption. Water was most commonly stored in the kitchen, while 30.3% of households reported storing their water in the bedroom or living room (Table 8). The most common methods for removing water from the household storage containers were to pour water straight from the container (35.9%) or use an elevated tap stand with spigot (35.7%, Table 8). The least common method for water removal from the storage container was a hand-pump that screws onto the top of the storage container. Only 11.7% of households were observed using this method, but more urban households and 30% of households in Ticul used hand pumps to remove water from storage containers (Table 8). More rural households kept household water containers on the floor, and nearly 70% of households in Ticul and Pich (Table 8). About 65% of households in Lerma and Chuina stored water containers in an elevated location, such as on a table, chair, or water stand (Table 8).

Microbial water quality

Microbiological water quality was measured by calculating the concentration of *E. coli* and total coliforms in 100 mL samples. Samples with a concentration of greater than 1 *E. coli* CFU per 100 mL were defined as contaminated because they did not meet WHO guidelines for drinking water.

Table 9. LWW water treatment plant and household water contamination

Comm.	**n (pl.)**	**n (HH)**	**Detectable TC (%)**		**Detectable *E. coli* (%)**		**Mean TC**		**Mean *E. coli***	
			Pl.	HH	Pl.	HH	Pl.	HH	Pl.	HH
Urban	*15*	*106*	*66.7*	*92.8*	*0.0*	*20.3*	*7.3*	*175.7*	*1.0*	*2.2*
Ticul	8	31	37.5	100.0	0.0	16.1	2.3	523.2	1.0	3.1
Lerma	7	75	100.0	86.8	0.0	23.7	19.4	70.4	1.0	2.2
Rural	*13*	*73*	*30.0*	*97.0*	*0.0*	*31.8*	*1.4*	*99.7*	*1.0*	*2.6*
Pich	7	49	40.0	100	0.0	50.0	2.6	328.1	1.0	4.4
Chuina	6	24	20.0	91.7	0.0	0.0	1.4	12.4	1.0	1.0
Total	**28**	**179**	**52.0**	**94.8**	**0.0**	**25.9**	**4.2**	**312.9**	**1.0**	**2.4**

Note: Pl. Detectable total coliforms (TC) and E. coli are measured as >1 CFU per 100 mL. All means are geometric means in CFU per 100 mL. Total Coliforms were abbreviated as TC, and households were abbreviated as HH.

Table 10. Comparison of the log transformed concentration of total coliforms and *E. coli* between LWW water treatment plant and household water samples using t-test

Community	**n (plants)**	**n (HH)**	**Total coliform concentration**		**E. coli concentration**	
			t-statistic	p-value	t-statistic	p-value
Urban	*15*	*68*	*5.15*	*<0.0001*	*1.70*	*0.0926*
Ticul	8	31	6.81	*<0.0001*	1.10	0.2806
Lerma	7	37	1.51	0.1650	1.32	0.1938
Rural	*10*	*66*	*8.45*	*<0.0001*	*1.80*	*0.0762*
Pich	7	42	6.40	0.0013	1.76	0.0858
Chuina	6	24	5.27	0.0003	-	-
Total	**28**	**134**	**7.99**	*<0.0001*	**2.51**	**0.0130**

Note: *E. coli* was not detected in any LWW water treatment plant or household water samples in Chuina,

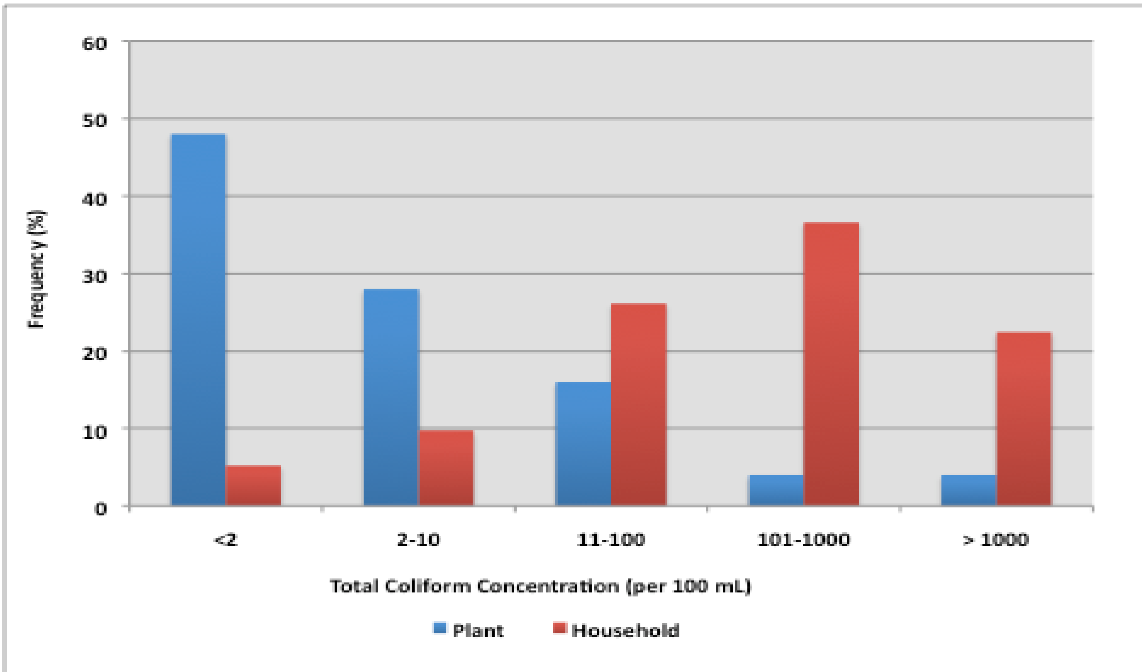


Figure 7. Distribution of total coliform concentrations in water samples from treatment plants and households in Ticul, Lerma, Pich, and Chuina

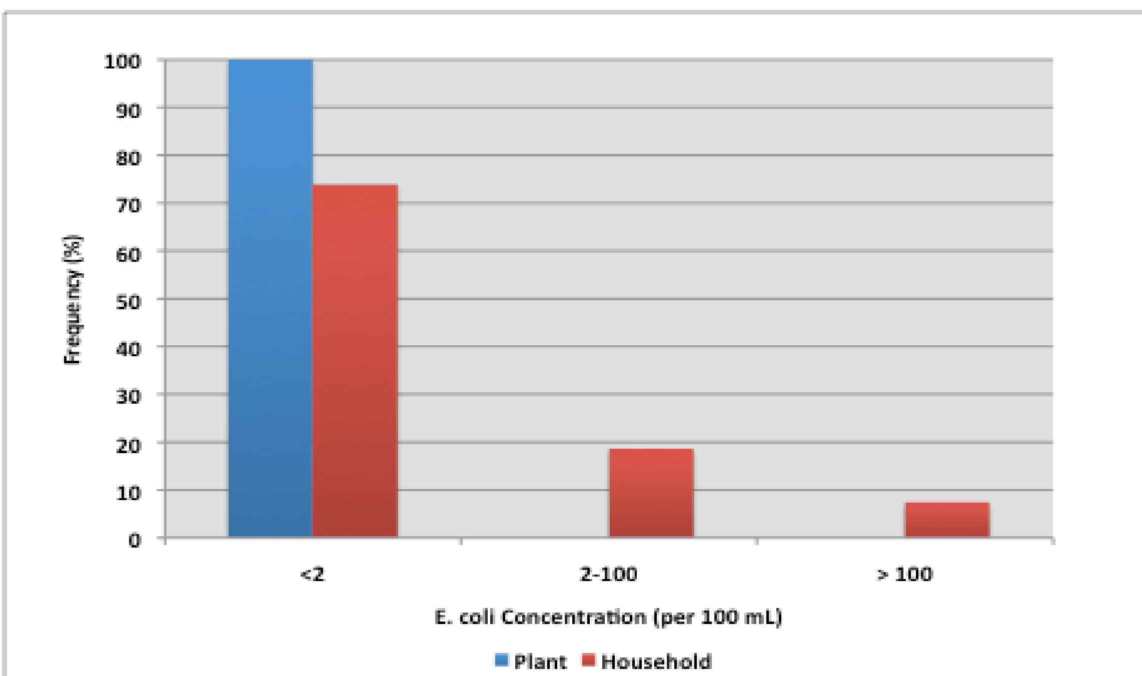


Figure 8. Distribution of total coliform concentrations in water samples from treatment plants and households in Ticul, Lerma, Pich, and Chuina

Figure 9. Distribution of total coliform concentrations in water samples from treatment plants and households in Ticul, Lerma, Pich, and Chuina

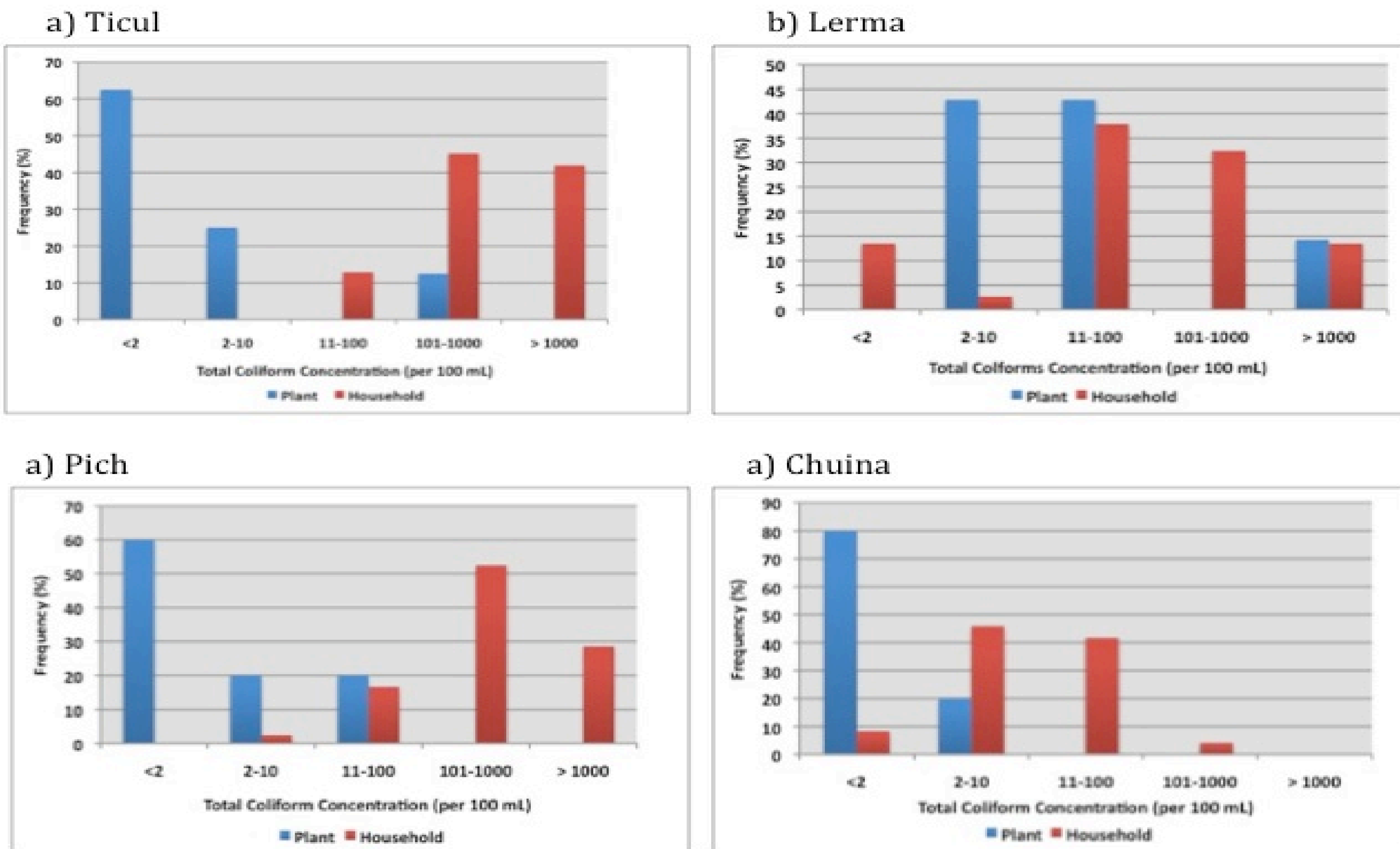
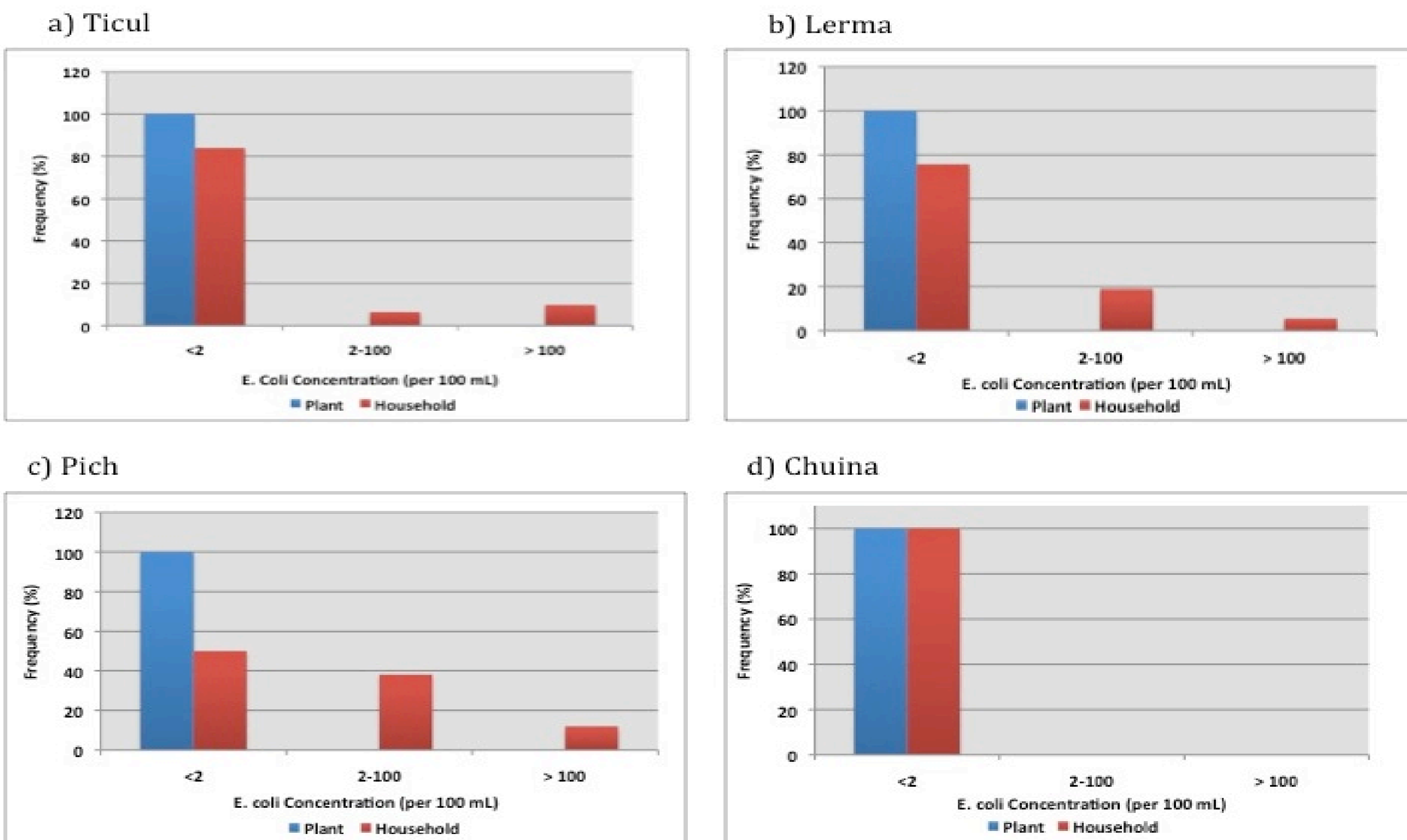


Figure 10. Distribution of *E. coli* concentrations in water samples from treatment plants and households in Ticul, Lerma, Pich, and Chuina



Water quality in LWW water treatment plants

Analysis of the 28 water samples from the water treatment plants showed relatively good quality, only 52% of samples from the water treatment plants had detectable levels of total coliforms, and the geometric mean was 4.2 CFU per 100 mL (Table 9). Few of the water treatment plant samples had high levels of contamination: 8% had a total coliform concentration of 100 CFU or greater in 100 mL while 4% had a concentration of greater than, or equal to 1000 CFU per 100 mL (Figure 7). Analysis of the difference between total coliform concentrations in the samples from the different water treatment plants was conducted using ANOVA and showed that there was no statistically significant difference between concentrations of total coliforms in the water samples from the four LWW water treatment plants ($p=0.0778$). The water samples from the water treatment plant in Lerma had the most frequent total coliform detections (100%), while Chuina had the smallest proportion of water samples with detectable levels of total coliforms (20%, Table 9). Similarly, the water treatment plant in Lerma had the highest geometric mean concentration of total coliforms with 19.4 CFU per 100 mL, and Chuina had the lowest with 1.4 CFU per 100 mL water sample. Almost 15% of water samples from the water treatment plant in Lerma had total coliform concentrations over 1000 CFU per 100 mL (Figure 9b). None of the water samples from the water treatment plants had detectable levels of *E. coli* (Table 9, Figure 8).

A difference approaching significance ($p=0.0595$) was detected between urban and rural LWW plants when comparing concentration of total coliforms per 100 mL; urban plant samples had a higher geometric mean concentration (7.3

CFU/100 mL) than did rural plant samples (1.4 CFU/100 mL) (Table 9). When comparing the differences in concentration of total coliforms between low and high output plants, no statistical difference was found ($p=0.3127$). The mean concentration of total coliforms for the water treatment plants in Lerma and Ticul was found to be higher than in the water treatment plants producing more bottles per day.

Quality of household water

Household water quality was consistently worse than the water quality of samples collected directly from the LWW water treatment plants. When assessing total coliform concentrations in the household water samples, almost 95% of the household samples had detectable coliform concentrations in 100 mL (Table 9). Almost 60% of household samples had a concentration of total coliforms greater than 100 CFU per 100 mL and the geometric mean concentration of total coliforms in household water samples was 132.9 CFU per 100 mL (Figure 7, Table 9). Household water samples with the highest geometric mean of total coliform concentrations were Ticul and Pich, with 523.2 and 328.1 CFU per 100 mL, respectively (Table 9). In Ticul, over 80% of household water samples had a total coliform concentration of greater than 100 CFU per 100 mL (Figure 9a). The lowest total coliform concentrations in household water samples were in Chuina, where the geometric mean concentration was 12.4 CFU per 100 mL (Table 9). The ANOVA statistical test showed that the total coliform concentrations were significantly different between the households in the LWW study sites ($p<0.0001$).

E. coli concentrations in household water were much lower than total coliform concentrations, and mean household *E. coli* concentrations ranged from 1.0 to 4.4 per 100 mL in Chuina and Pich, respectively (Table 9). *E. Coli* was detected in 25.9% of household water samples (Table 9), but less than 10% of household water samples had a concentration of 100 CFU per 100 mL or greater (Figure 8). The highest mean *E. coli* concentration in household water samples was in Pich (4.4 CFU per 100 mL, Table 9). In Pich, nearly 12% of household water samples had an *E. coli* concentration of greater than 100 CFU per 100 mL (Figure 10c).

Even though more than half of the treatment plant samples had total coliforms, the geometric mean total coliform concentration in the plant samples was only 4.2 CFU per 100 mL compared to 132.9 CFU per 100 mL in the household water samples (Table 9).

The water treatment plant in the town of Lerma had the highest amount of total coliform contamination, with 100% of plant samples having a concentration greater than 1 colony per 100 mL and the geometric mean total coliform contamination was 19.4 coliforms per 100 mL (Table 9). The next highest total coliform concentration in plant samples was found to be Pich with a geometric mean of 2.6 colonies per 100 mL (Table 9).

Although more rural households showed evidence of total coliform concentration, geometric mean concentrations for urban households were 175.7 colonies per 100 mL, higher than 99.7 colonies per 100 mL in rural households (Table 9). Chuina was the only town where 100% of the household and treatment plant samples had no detectable *E. coli* concentrations (Figure 10d, Table 9). A statistically significant difference was found between the mean concentrations of *E. coli* in household water samples (p=0.0080).

Differences between LWW water treatment plant and household water quality

Water samples from the LWW water treatment plant in Lerma had the poorest quality of water based on geometric mean total coliform concentration, however household water samples in Lerma had the lowest frequency of detectable total coliforms and one of the lowest geometric means of total coliform concentration (70.4 CFU per 100 mL, Table 9). Ticul and Pich had the worst household water quality in terms of geometric mean total coliform and *E. coli* concentration, however the LWW water treatment plants in Ticul and Pich produced relatively good quality in regards to the number of water samples with detectable total coliforms (37.5, 40.0% respectively), and geometric means of total coliforms (2.3, 2.6 CFU per 100 mL respectively, Table 9).

Linear Regression Analysis of Recontamination

Linear regression analysis was used to examine the relationship between the microbiological quality of the water produced in the LWW plants and the water stored in the households. The log-transformed concentrations of both *E. coli* and total coliforms were compared using linear regression between the plant samples and household samples overall, as well as individually for each of the four towns where the sampling occurred. Point estimates of the linear slope were used to estimate the order of magnitude of the difference between the concentrations of *E.*

coli and total coliforms in the water samples collected at the treatment plants and in the household water samples.

When comparing all the plant and household samples, the household samples had a concentration of total coliforms 31 times greater than the plant samples ($p<0.0001$, Table 11). When examining *E. coli* concentrations, household samples had a 2.3 times higher concentration than plant samples ($p= 0.0130$, Table 11).

Ticul had the greatest difference in total coliform concentrations between plant and household samples. Household samples had an overall mean concentration 170 times greater than that of the plant samples ($p<0.0001$, Table 11). Only Lerma did not have a statistically significant difference in total coliform concentrations between the samples from the plant and the samples from the households.

The greatest difference between *E. coli* concentrations in samples from the water treatment plants and from households was in Pich where household samples had 4.4 times the *E. coli* concentration of the plant samples ($p=0.0858$, Table 11). When examining the *E. coli* results from all four towns, although the concentration of *E. coli* increased between the water plants and the households, the linear relationship between the treatment plant and household water samples were not statistically significant ($p=0.2806$, Table 11).

Logistic regression analyses of E. coli detection in household drinking water

Logistic regression analysis was used to examine potential predictor variables from the household surveys and their relationship to detectable *E. coli* concentration (>1 CFU/100 mL) in household water samples. Only one of the exposure variables included in the logistic regression was significantly associated with presence of *E. coli* in household water samples; having dirt or cement floor (in comparison to tile) was associated with a higher likelihood of E. coli contamination of household water (OR=2.434, CI=1.019, 5.813, Table 12).

Table 11. Linear regression analysis of log transformed concentration of total coliforms in water samples, between water treatment plants and households

Community	n (plant)	n (HH)	Parameter estimate	10^PE	p-value
Ticul	8	39	2.23	169.8	<0.0001
Lerma	7	72	0.56	3.6	0.1701
Pich	7	44	2.10	126.6	<0.0001
Chuina	6	24	0.95	9.0	<0.0001
Total	**28**	**159**	**1.50**	**31.3**	**<0.0001**

Note: Bacterial concentrations were log transformed in order to normalize the data. 10^PE is taken in order to reverse the log transformation and gives the linear coefficient, or multiplier for concentration between water treatment plants and household water samples.

Table 12. Linear regression analysis of log transformed concentration of *E. coli* in water samples, between water treatment plants and households

Community	n (plant)	n (HH)	Parameter estimate	10^PE	p-value
Ticul	8	39	0.36	2.3	0.2806
Lerma	7	72	0.34	2.2	0.1938
Pich	7	44	0.65	4.4	0.0858
Chuina	6	24	0.00	1.0	-
Total	**28**	**159**	**0.36**	**2.3**	**0.2806**

Note: Bacterial concentrations were log transformed in order to normalize the data. 10^PE is taken in order to reverse the log transformation and gives the linear coefficient, or multiplier for concentration between water treatment plants and household water samples. No p-value is given for Chuina because no difference was observed between water treatment plants and household water samples.

Table 12. Logistic regression analysis of household characteristics and their relationship to *E. coli* detection in household water samples.

Variable	Unadjusted Odds Ratio	95% CI	Adjusted Odds Ratio	95% CI	p-value	Model
Rural (yes)	0.524	0.244, 1.123	0.647	0.222, 1.889	0.4261	
Child under 15 (yes)	0.509	0.226, 1.149	1.052	0.313, 3.532	0.9343	
Child under 5 (yes)	0.560	0.256, 1.226	0.468	0.121, 1.820	0.2734	
Primary education only (yes)	0.745	0.343, 1.616	1.154	0.449, 2.968	0.7657	Demographics
Agriculture/Self Employed (yes)	0.397	0.140, 1.123	0.394	0.123, 1.263	0.1170*	
Dirt or cement floor (yes)	2.172	0.986, 4.783	2.434	1.019, 5.813	0.0452**	
More than 4 people per room (yes)	1.246	0.576, 2.696	0.790	0.276, 2.261	0.6609	
Elevated bottle storage (yes)	0.448	0.189, 1.058	0.378	0.104, 1.377	0.1368*	
Use of a stand for distribution (yes)	1.245	0.505, 3.073	0.760	0.175, 2.814	0.6170	
Open jar for storage (yes)	0.808	0.277, 2.359	0.880	0.254, 3.049	0.8402	Storage and Consumption
Price is reason for purchase (yes)	1.232	0.475, 3.196	1.318	0.410, 4.237	0.6433	
Convenience is reason for purchase (yes)	0.970	0.391, 2.410	1.079	0.395, 2.948	0.8821	
Consume 1 bottle per person/week (less)	1.427	0.592, 3.439	1.598	0.630, 4.057	0.3239	
Primary transportation is walking (yes)	1.344	0.571, 3.162	0.517	0.102, 2.614	0.4247	
Health clinic identified in town (no)	>999.999	<.001, >999.999	1.690	<.001, >999.999	0.9987	Town and Health Clinics
Medium/far distance travel to health clinic (yes)	>999.999	<.001, >999.999	>999.99	<.001, >999.999	0.9399	
Learned hygiene from LWW (yes)	0.419	0.124, 1.419	0.745	0.186, 2.983	0.6773	

Note: The exposure variables were divided into groups based on common relationships in order to enhance power of the logistic regression.
**Statistically significant (95% CI)
*Approaches statistical significance (95% CI)

Discussion

The purpose of this evaluation was to determine if LWW water treatment plants in Yucatán, Mexico were producing water that met WHO and national standards for total coliform and *E. coli* concentration and to determine if those standards were being maintained throughout water transport and storage in the homes. This study also tried to identify any differences in the LWW plants, towns where they were located, or household characteristics that might lead to differences in the proportion of samples that were contaminated or the concentration of total coliforms and *E. coli* in the water from the study communities.

Household Characteristics

Demographic and socioeconomic information on the LWW patrons were collected by household surveys administered to the head of households identified as having purchased LWW within the last two days. About 52% of households in this study who purchased LWW were identified as having the head of the household reach an education level of primary school, while only 22% of respondents reported having received an education higher than secondary school. Recent data from UNESCO (2008) on education in Mexico, shows that 94% of students complete at least five years of education, and secondary school enrollment is around 72%, which indicates that the LWW customers in the study areas were under educated compared to the national median. Furthermore, 76.4% of respondents claimed that the head of the household was self employed, worked in agriculture, or had no formal occupation. Taken together, these results indicate that the study population had lower education and socioeconomic status than the national average and meets the goal of LWW to provide more vulnerable populations with treated drinking water at lower prices compared to the premium bottled brands available in the area. Low educational attainment and low socioeconomic status have been shown to be important factors that are associated with decreased frequency of safe hygiene practices and water treatment techniques (Fewtrell et al. 2005; Jalan and Ravallion 2003; Schmidt and Cairncross 2008).

Price and convenience were the two main reasons identified by LWW customers for purchasing water from LWW water treatment plants. Interventions such as LWW water treatment plants that aim to increase the quantity of water available to customers because of their home delivery service, or affordable price have been shown to reduce the risk of incidence of diarrhea by 25% (relative risk=0.75, 0.62-0.91) (Fewtrell et al. 2005).

Water Consumption Patterns

The households that purchase LWW water also have a mean number of members between 4.1 and 5.0, with 37.4% of households having a family member below the age of five. Larger households and the proportion of LWW customer households with children could lead to an increase in hand contact with household water, which has been show to increase contamination of household water (Jensen et al. 2002; Schmidt and Cairncross 2008). Our study households consumed on average 5.2 bottles per week, which is slightly more than one 20 L bottle per member per week. Consuming multiple bottles per week could reduce exposure to household contamination because the storage time per bottle is shorter. However recontamination of household water has been observed to occur within 1 day and therefore it was not surprising to see higher *E. coli* concentrations in our household water samples compared to samples from the water treatment plants. In a study by Levy et al. (2008), 45.5% of household water containers had an increase in *E. coli* concentration in the first day.

All study households reported using only bottled water for drinking, 88.8% of households reported using bottled water for cooking, 41.2% for taking care of the baby, and 53.7% for brushing their teeth. Exposure to enteric pathogens is not limited to contaminated drinking water. The use of unsafe sources of water for food preparation could lead to adverse health outcomes such as diarrhea (Schmidt and Cairncross 2008).

Previous studies have reported that household water samples were consistently of poorer quality than source water samples, especially when the source was considered, "improved" or had low concentrations of *E. coli.* Point-of-use water treatment can reduce microbial contamination of household drinking water and possibly reduce enteric illness (Clasen et al. 2007; Wright et al. 2004). Household chlorination has been reported to reduce diarrhea morbidity in a number of studies (pooled RR=0.61, 0.46-0.81) (Clasen et al. 2007). Interestingly, 34.9% of households surveyed in this study reported that the way they knew water was safe to consume was if it lacked a chlorine flavor or odor. This may be due to a lack of trust in the public water system as delivering a safe product, and an association of chlorine taste with piped municipal water. Introduction of a point-of-use water treatment intervention involving chlorine addition to household water, in addition to the current community-based intervention may be hindered by a lack of acceptance by households in these study communities.

Improved sanitation interventions and increased access to basic sanitation has been shown to reduce diarrhea incidence by 32% (Fewtrell et al. 2005). All but four respondents reported having a bathroom with a flush toilet in their homes (pit latrines were used by the other four households), indicating almost universal access to improved sanitation. The lack of variability in sanitation practices between households in our study communities prevented sanitation facilities from being included as an exposure variable in our logistic analysis to predict detectable *E. coli* contamination in household water samples.

Hygiene education can be an important intervention for improving water quality (Fewtrell et al. 2005). Among LWW customers we studied, 63% of households reported learning water disinfection techniques or receiving hygiene education and of these, 93% of households said they had learned the skills from a clinic or through school. This suggests that the LWW health education program has not had significant penetration in the community they serve.

LWW Water Treatment Plants

Our results indicate that LWW water treatment plants produced relatively good water quality. Nearly 50% of water samples from LWW plants had less than 2 total coliforms per 100 mL and none of the water treatment plant samples had detectable concentration of *E. coli*. Mexico's national standards for drinking water are less than or equal to 2 total coliform CFU per 100 mL sample (SDSM 1994). Half of the LWW water treatment plant samples in the study communities produced water at or above the quality demanded by the Ministry of Health. Total coliform concentration in water samples can vary on a daily and hourly basis due to seasonality, natural attenuation, or settling and multiple samples at different time points during the day give a more accurate estimate of the actual total coliform and *E. coli* concentrations (Levy 2009). Total coliforms are considered a process indicator, meaning that they do not directly correlate to the presence of pathogens in the water, but rather their presence suggests the possibility of contamination because of inadequate treatment or treatment failure (Ashbolt et al. 2001; EPA 2009). The presence of total coliforms in water samples from LWW water treatment plants should be reason for concern, indicating that the disinfection process might not be removing all of the bacteria present in the water.

All of the water samples from the LWW water treatment plants did meet WHO and national standards for *E. coli* in drinking water (<1 CFU/100 mL). The lack of fecal bacteria detected in the drinking water after LWW treatment suggests that the LWW water treatment plants were effective in removing pathogens. This may be due to their multi-step design, which includes reverse osmosis, and either UV light or ozone disinfection.

Although a difference in concentration of total coliforms approaching statistical significance (p=0.0595) was detected between urban and rural LWW treatment plants, these results may have been confounded by the use of UV irradiation in urban LWW water treatment plants and ozone in rural water treatment plants. If the concentration of total coliforms in crude water entering the plants from the private wells is the same for all four locations, the disinfection process may contribute to the difference in total coliform concentration. Further research is needed to analyze the efficacy of the LWW water treatment plants for eliminating total coliforms and *E. coli* from source water.

There was no significant difference in total coliform concentration in samples from the low and high output plants (p=0.3127), although the mean total coliform concentration in samples from the water treatment plants in Lerma and Ticul was higher in the samples from the water treatment plants that produced more bottles per day. Although not statistically significant, these findings may suggest that running the water treatment system more frequently may provide higher water quality of that the higher output plants are better operated and maintained.

Household Water Quality

Water quality in the households was determined by quantifying the concentration of total coliforms and *E. coli* in households sample that were removed from the stored water container and transferred into a drinking cup used in the home. Therefore, the concentration of bacteria in these water samples was representative of what was actually being consumed by patrons.

Household water quality was expected to be worse than water treatment plant samples, and 94.8% of household water samples had a total coliform concentration of greater than 1 CFU per 100 mL. This suggests that bacterial contamination occurs before consumption of water in the study households.

When analyzing the fecal contamination indicator, *E. coli* concentration greater than 1 CFU per 100 mL was found in 25.9% of household samples, and the mean concentration in household samples was 2.4 colonies per 100 mL which is greater than the WHO and national standards for drinking water quality. Chuina was the only town where no *E. coli* was found in any household samples, which may be because of the fact that most households served by the LWW plant in the Chuina only received and consumed one bottle of drinking water daily, not allowing long periods of water storage in homes. Pich showed the highest concentration of *E. coli* in household samples; 50% of household samples were positive for *E. coli* and the mean concentration was 4.0 colonies per 100 mL.

Recontamination

The difference between the concentrations of total coliforms and *E. coli* in plant and household samples for all four study communities were found to be statistically significant (Point estimate=31.3, $p<0.0001$; Point estimate=2.3, $p=0.0130$). We observed an average log increased level of *E. coli* contamination of 0.9 between water samples from LWW water treatment plants and household water samples, which is similar to the increase seen in a study by Levy et al. (2008) where a 0.6-1.2 log increase was seen in *E. coli* concentration in contaminated household water supplies. A study comparing water quality at the water source and in household water samples in South Africa had over 95% source water samples negative for *E. coli*, while *E. coli* was detected in 8-25% of household water samples (Genthe et al. 1997). The study results from Genthe et al. (1997) were similar to the results seen in this study where LWW water treatment plant samples had no detectable *E. coli*, but detectable *E. coli* was found in over 25% of household water samples.

This difference in water quality between plants and homes suggests that water is being contaminated between the LWW water treatment plants, but prior to consumption in the home. According to Rufener *et al.* (2010) contamination of the drinking water can be occurring at a number of points along the pathway to consumption by individuals in places such as the storage of water in unsafe containers, the handling of water with contaminated hands, or at the point right before consumption due to use of a contaminated drinking container. This evaluation of LWW water treatment plants was not able to analyze the exact route of contamination, but it does document that there was significant deterioration in water quality after it left the treatment plant.

There was no indication from this study that the LWW customers were aware of any possible in-home contamination and whether household water contamination could harm perception of the LWW brand. This study is unable to determine if the increase in *E. coli* concentration in household water supplies resulted in adverse health outcomes, and if that had an effect on the perception of the LWW brand by its customers.

Several factors, such as location of drinking water storage, the type of storage container used for drinking water, and the method of water removal from the storage container, were examined to determine if there were any significant predictive associations with detectable *E. coli* contamination in household water samples. Past studies have implicated household water storage techniques and the use of dipping cups or hands in storage containers as sources of bacterial contamination at the point-of-use (Jensen et al. 2002; Levy et al. 2008). However, when adjusting for potential confounding variables, only having a dirt or cement floor as opposed to a tile floor in the house was found to be significant (p=0.0452), where the odds of contamination with *E. coli* for people with a dirt or cement floor was 2.4 times that of people who had a tiled floor in their home. Having a dirt or cement floor may indicate socioeconomic status in these four study communities, which would suggest that lower socioeconomic status was associated with higher concentrations of bacteria in household water samples.

Strength and Limitations of this Study

During the microbiological analyses, sterile bottled water was to dilute plant and household water samples as well as to run negative controls as the first and last sample processed each day during water testing. No positive results, defined as 1 total coliform or colony of *E. coli* per 100 mL, were observed in any of the negative controls suggesting that the purified water used did not affect the water microbiology results.

This study was limited due to the cross-sectional nature of its design and small sample size. The small sample size did not allow us to determine specific household and town characteristics that might explain the differences we observed between the levels of total coliform and *E. coli* contamination at the plant and household level. Furthermore, small sample size, recall bias, and possible association of the researchers with LWW may have lead to a decreased and inadequate reporting of diarrhea in children under the age of five which caused us to exclude this outcome from the statistical analysis.

A truly random sample was not achieved by this study both in the selection of the sites of the LWW water treatment plants nor in the households selected in each town, which may not allow the study results to be generalizable. The sites were selected from an abbreviated list given to the researchers by LWW managers, where resources were identified and available in order to best complete the study. Furthermore, four sites needed to be selected in order to fulfill the research goals of comparing high and low production of the plants as well as the operation of these treatment systems in urban and rural settings, limiting the possibility of random selection. When choosing households to survey in the study areas, it was necessary to identify who were LWW patrons, these households were identified by participating in delivery of bottled drinking water along with the LWW plant operators. The study households may have believed that the researchers were representatives of LWW, and this may have influenced their answers to some of the survey questions. The microbiological analyses of water quality required processing the samples within 24 hours, and therefore limited the time available to conduct household surveys to the hours between 8 A.M. and 4 P.M. Any households without members available during these times were not represented in the study.

This study sought to determine if there was a difference in the concentration of total coliforms and *E. coli* in water samples taken from the LWW plants and in homes of LWW customers. This was in order to determine if LWW was meeting their goal of not only providing water that met national and WHO standards for drinking water but also if the water actually being consumed in homes met this standard as well. Due to limited resources, this study focused on testing bacterial concentration in LWW drinking water at only two points along the path to consumption; immediately after passing through the treatment system and from a drinking vessel right before consumption. This study did not test the microbiological quality of the source water and therefore did not examine the change in water quality due to the LWW treatment system. The study also it does not analyze or seek to determine the specific point of contamination after leaving the water treatment system, so no inferences can be made as to the cause of microbial growth in the stored household drinking water.

Conclusions

1. All water samples tested directly from the LWW treatment systems had no detectable *E. coli,* or (<2 CFU/100 mL). Approximately half of the samples taken from LWW water treatment plants did not meet WHO and national standards of having less than 2 colonies of total coliforms in 100 mL.

2. A larger proportion of households had total coliform and *E. coli* concentrations of greater than 1 colony per 100 mL compared to samples taken from LWW plants. The higher frequency of bacterial contamination in LWW water stored in households suggests that water becomes contaminated sometime after water is treated by LWW water treatment plants but prior to consumption by individuals in the household.

3. The geometric mean concentration of total coliforms and *E. coli* was higher in samples collected from households compared to samples collected directly from the LWW treatment plants. This result also suggests contamination of household drinking water occurs after treatment by LWW plants.

4. Water samples from urban LWW plants generally had higher concentrations of total coliforms than samples from rural LWW plants; however this difference was not statistically significant at the 95% confidence level. Rural LWW plants differed from urban plants not only in the populations that they serve, but also the two rural plants used ozone disinfection, while the two

urban plants we studied used UV light as the final disinfecting process. The difference seen might be due to the different in treated water quality may be due to the disinfecting methods employed at the different LWW plants or may be due to differences in source water quality.

5. There was no significant difference between the concentration of total coliforms and *E. coli* in water samples collected in rural vs. urban households.

6. Most LWW customers reported having learned about hygiene, importance of maintaining water quality, and methods for household disinfection of water, but very few households claimed to have learned these practices from LWW educators. The health education aspect of LWW water interventions does not seem to be implemented well in the communities served by LWW water treatment plants.

7. The logistic regression analysis of *E. coli* contamination in household water suggested that having dirt or cement floor in comparison to a tiled floor in the household was a significant risk factor for water contamination when controlling for other possible confounding household characteristics. Urban vs. rural household locations was not a statistically significant predictor of *E. coli* contamination in the household water samples.

Recommendations

1. Examine why over 50% of water produced at the LWW plants still has total coliforms. The presence of total coliforms in treated water suggests inadequate treatment or treatment failure. Water samples could be collected at the source water and different points in the treatment process and tested for total coliforms to determine where the problem is in the system.

2. Increase the hygiene and drinking water safety education component of LWW intervention in communities currently served by LWW water treatment plants. This could be done by reaching out more effectively to current customers and then expanding to all community members as a whole. Possible partnerships with local schools or health facilities should be explored in order to reach more community members.

3. Explore point-of-use treatment options to disinfect water stored in households. LWW plants produce water with no detectable *E. coli* and significantly lower total coliform concentrations than are detected in the household water samples. The water consumed in the households is of lower quality than that produced by LWW plants. This could adversely affect the health of consumers as well as damage the reputation LWW has in the community for providing water that meets the WHO guidelines for drinking water.

Future Research

Although private water vendors are not a new phenomenon, relatively little research has been done to quantify their activities, the market they serve, or determine the specific quality of water they provide. Further research is necessary to calculate the impact that LWW has as a community-based drinking water intervention. A similar study with larger sample size may better characterize important household practices that lead to contamination of LWW water after treatment, which would help direct additional interventions to improve the quality of water consumed in homes.

Analysis of the source water used by LWW plants should be conducted to determine the concentration of total coliforms and *E. coli* in the water prior to treatment. These concentrations should be compared to the concentrations of total coliforms and *E. coli* after treatment to determine the efficacy of the LWW water treatment system.

Efforts should be made to determine the points where contamination occurs after the treated water leaves the LWW system. Possible sources of contamination could be the bottles used to transport and store LWW water, as well as the behaviors and handling practices in the home, as well as any cup or bowl used to serve drinking water. Research could be conducted to test the concentration of total coliforms and *E. coli* after each of these points of possible contamination. Treated LWW water could be bottled in LWW containers and then kept in a controlled environment and the concentration of total coliforms and *E. coli* could be tested every 12 hours to determine if any bacteria growth occurs due contamination of the bottle alone. Furthermore, collecting water samples from in-home water storage containers directly and after contact with a drinking vessel could provide information on where point-of-use interventions should be directed.

During this research, multiple other private vendors were identified and found to be operating in the same geographic location as LWW. An analysis of their pricing, target customers, marketing strategies as well as the quality of their vended water could provide a market analysis for LWW, and would add to our understanding of private water vendors as a community drinking water intervention.

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Appendix A: Household Survey

Survey

Survey Number ____________ Name of interviewer ______________________

Read consent to interviewee.
Was Consent Given? Yes No

Name of town _________________

	Section 1.00: Demographics	
1.01	Sex	1. Male 2. Female
1.05	What year were you born?	Year ______
1.10	Marital status	1. Single 2. Married 3. Divorced 4. Widow 5. Cohabitating
1.15	Religion affiliation	1. Catholic 2. Presbyterian 3. Evangelical 4. Other Christian_______________
1.20	How long have you practiced (name religion stated above)?	Years________________
1.25	How often do you attend religious services	1. Once a week 2. More than once a week 3. 1-3 times a month 4. Less than once a month 5. Never
1.30	What is the name of your church	
1.35	Education level? How many years of education?	Years /grade______________ Never attended school _____
1.40	What do you do?	

1.45	Specify type of work	1. Housewife 2. Agricultural/farm labor 3. Informal business: craft, fruit sales 4. Works for others (non farm) 5. Jobs requiring higher education
1.50	How many people live in your house?	Total_______
1.55	What is the age and gender of each household member?	1. Sex____ Age____ 2. Sex____ Age____ 3. Sex____ Age____ 4. Sex____ Age____ 5. Sex____ Age____ 6. Sex____ Age____ 7. Sex____ Age____ 8. Sex____ Age____ 9. Sex____ Age____ 10. Sex____Age_____

	Section 2.00: Wealth, access and assets	
2.01	If there are children under five, has the child been sick with diarrhea in the past month?	1. Yes 2. No
2.05	How long has child been sick	Time_________
2.10	Do you use the local clinic	1.Yes 2. No
2.15	How far is the clinic/hospital/doctor	Distance
2. 20	How much does it take to travel to your medical facility?	Time________
2.25	What mode of transportation do you use when you have to go to the clinic/hospital/doctor?	
2.30	What is your primary mode of transportation? How do you get around	1. Walking 2. Bicycle 3. Motorcycle 4. Car 5. Taxi 6. Public transportation
2.35	Transportation expenses (approximately)	___________________(Month) Would rather not answer _____
2.40	Medical expenses (approximately)	___________________(Month) Would rather not answer _____
2.45	Bills (electric, water) expenses (approximately)	___________________(Month)

		Would rather not answer _____
2.50	Food expenses (approximately)	___________________(Month) Would rather not answer _____
2.55	Other expenses (approximately)	___________________(Month) Would rather not answer _____
2.60	Number of bedrooms in house	
2.65	How many people sleep in each room?	
2.70	(Observe: Type of flooring in the house)	1. Dirt 2. Cement 3. Tile 4. Other___
2.75	(Observe: Type of walls in the house)	1. Cement 2. Tin 3. Straw 4. Other
2.80	(Observe: Type of roof in the house)	1. Cement 2. Tin 3. Straw/thatched
2.85	Which ones of the following items do you have in your house, how many?	Beds _______ Hammocks _____ Bicycles_________ Motorcycles_______ Car/truck_______ Radio_________ TV_________ Refrigerator_________ Telephone/cell__________ Washing machine___________ Other _______________
2.90	How many bathrooms do you have?	0 _____ (g to q 2.50) 1 o more_________ (write total and skip q 2.50)
2.95	Type of sanitation facility	1. Flush toilette 2. Latrine 3. Other____________

	<u>Section 3.00: Water consumption</u>	
3.01	What is your primary source of bottled water?	1. Bottled 2. Tap 3. Well 4. Lake, river water 5. Rain water 6. Other__________

3.05	Do you purchase bottled water?	1. Yes 2. No 3. Bottled water is free
3.10	How much does a bottle of water cost?	Price____________
3.15	What brand of bottled water do you consume?	Name of brand/location ----------------------------------
3.20	Is water delivered or fetched by someone?	1. Delivered 2. Someone fetches water If someone fetches, who? _________
3.25	How often is water delivered?	
3.30	How many bottles of water do you consume a week?	
3.35	How many times a week does someone fetch water?	
3.40	Have you learned about health and hygiene practices? (Hand washing, risk of disease, etc)	1. Si 2. No
3.45	If yes to 3.40, where did you learn?	
3.50	How long ago did you learn?	
3.55	Why do you prefer the brand of water you consume?	1. Price 2. Convenience 3. Taste 4. Avoid getting sick 5. Health and wellbeing 6. Other ____________
3.60	Do you believe bottled water you consume is safe tor drink?	1. Si 2. No
3.65	How do you know water is good enough to drink?	1. Others say 2. No bugs 3. The way water looks 4. No microbes in water 5. Water comes in a sealed bottle 6. Taste 7. Has chlorine 8. Trust in brand/seller
3.70	How do you know if water is not good enough to drink	
3.75	What type of water do you use for the following activities?	1. Drinking ___________________ 2. Brushing teeth______________ 3. Washing dishes______________ 4. Washing clothes_____________ 5. Caring for the baby___________ 6. Bathing/shower______________ 7. Cook/prepare food ___________

	Section 4: Attitudes and beliefs about bottled water					
	I am now going to read you some statements related to water consumption. For each statement please tell me whether you agree, somewhat agree, disagree or somewhat disagree. If you do not know please let me know.	Do not know	Disagree	Somewhat disagree	Somewhat agree	Agree
4.01	Washing your hands before eating is very important	9	1	2	3	4
4.05	It is necessary to use bottled water to wash my hands	9	1	2	3	4
4.10	The brand of bottle water I drink tastes better than other brands.	9	1	2	3	4
4.15	The brand of bottle water I drink is the only one I consider to be safe to drink	9	1	2	3	4
4.20	I don't believe drinking bottle water is important	9	1	2	3	4
4.25	Only children and the elderly need to drink bottled water, not everyone needs to drink it	9	1	2	3	4
4.30	I don't get sick as often when I only drink bottled water	9	1	2	3	4
4.35	There are microbes in tap water that can cause disease	9	1	2	3	4
4.40	Drinking tap water every once in a while is ok	9	1	2	3	4
4.45	The type of water I consume has no impact on my overall health	9	1	2	3	4

This is the last question, thank you for participating in this survey.

*LWW health education module, and the center for Global Safe Water Survey and templates were used to create this survey.

Appendix B

Carta de consentimiento a participar en un Estudio

Titulo: Evaluación del agua de LWW en hogar

Investigador principal: Stephen Crabbe, Candidata al MPH, Rollins School of Public Health, Emory University

Favor de leer esta carta de consentimiento con cuidado antes de dedique a participar en este estudio.

Introduction and Purpose of the research study: Esta siendo invitado a participar en un estudio de investigación para evaluar a LWW (Aguas Vivas para el Mundo) y examinar si recontaminación esta pasando en hogar. Me interesa que usted participe en este estudio ya que usted ha comprado el agua de LWW. Van a haber aproximadamente 300 familias que serán encuestados y muestra de agua examinaran. Estamos conduciendo este estudio para Aguas Vivas Para el Mundo y para la realización de mi tesis de maestría en la Universidad Emory bajo la dirección de la doctora Christine Moe.

Procedimiento: Si usted estad de acuerdo en participar en este estudio le voy a hacer preguntas acerca de las usas del agua en su casa y información demográfico, debe pasar menos de 20 minutos. Recogeré una muestra de 100 mL del agua de su garrafón en casa para examinar.

Riesgos y beneficios: En este momento no parece haber ningún riesgo ni beneficio, político o social asociados con participar con esta estudia. Pero, la información que usted nos de añadirá al conocimiento acerca de que causa el éxito y sustentamiento de sistemas de tratamiento de agua.

Confidencialidad: No voy a incluir su nombre en los resultados del estudio, recibirá un numero de identificación al azar. Todo los documentos estarán protegidas bajo llave en un lugar seguro. Puede haber gente fuera del estudio que vea los documentos. Agencias y departamentos y comités de Emory que están acargo de las reglas y politicas de estudios de investigación tienen derecho a ver estos documentos. Nosotros mantendremos privados todos los documentos producidos como la ley lo requiere.

INFORMACION DE CONTACTO: Si usted tiene alguna pregunta, le invito a preguntarlas ahora. Si usted tiene alguna pregunta en otro momento, puede ponerse en contacto conmigo por email a sjcrabb@emory.edu o +001 520-429-2555. También puede contactar a la doctora Christine Moe a clmoe@emory.edu.

PARTICIPACION VOLUNTARIA Y RETIRO DEL ESTUDIO: Su participación en este estudio es voluntaria. Puede negarse a participar o negarse a responder cualquier pregunta que no quiera responder. Si usted decide participar en este estudio y cambia de opinión en cualquier momento puede retirarse del estudio. No habrá ninguna consecuencia negativa si usted decide participar o no participar en este estudio. No va a ser compensado por su participación en este estudio.

Acuerdo: Le daré una copia de esta carta de consentimiento para sus records. Si usted esta de acuerdo a participar en este estudio por favor firme en la siguiente línea.

Participante: ______________________________ Date: ______________