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Katharine Robb Date

Influences of Household Storage on *E. coli* Concentration in Drinking Water in Northern, Coastal Ecuador

By

Katharine Robb

Master of Public Health
Global Environmental Health

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Karen Levy, PhD
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By

Katharine Robb

B.A., University of Michigan, 2008

Thesis Committee Chair: Dr. Karen Levy, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2011

ABSTRACT

Influences of Household Storage on *E. coli* Concentration in Drinking Water in Northern, Coastal Ecuador

By Katharine Robb

Background: Recontamination of stored drinking water is a known issue. However, a study by Levy *et al*. (2008) observed significant attenuation of *E. coli* concentrations during storage. The observed attenuation could be due to organisms settling to the bottom of storage containers or die-off. Viable organisms that settle could become re-suspended, consumed and cause disease. Die-off, however, signifies loss of infectivity. **Purpose:** We examine: (1) changes in *E. coli* concentration during storage of untreated water under household and control conditions, (2) the impact of agitation of containers on *E. coli* concentrations, in order to understand whether previously observed reductions were due to settling or die-off, (3) the impact of chlorination on the results observed in aims (1) and (2), and (4) the extent of in-home *E. coli* contamination. **Methods:** We quantify changes in contamination during storage, considering household and source water characteristics and controlling for source contamination, utilizing microbiological/physicochemical data and household surveys. Source water was stored under household and control conditions and containers were re-sampled after 24 hours before and after agitation to determine how *E. coli* concentration changed and if settling occurred. **Results**: Without treatment, significant log reduction in *E. coli* concentration was observed under control, but not household conditions. Differences between pre- and post-agitation samples were observed under household but not control conditions, suggesting that settling of viable bacteria occurs under household conditions whereas die-off occurs under control conditions. This may be attributable to biofilms and in-home contamination of household containers. With chlorine treatment, significant log reduction of contamination was observed under both household and control conditions. Differences between pre- and post-agitation samples were observed under control but not household conditions, suggesting die-off of bacteria under household conditions but settling under control conditions. The opposite trend observed with chlorine treatment may be explained by the interaction of chlorine and turbidity. **Conclusion:** Die-off and settling of *E. coli* during storage was influenced by storage conditions (household or control), turbidity and initial source contamination. Significant differences between pre- and post-agitation samples suggest the need for standardized sampling protocols that call for the agitation of water storage containers.

RESUMEN

Las Influencias de Almacenamiento Domestico en la Concentración de *E. coli* en el Agua Potable en la Costa de Ecuador

Por Katharine Robb

Antecedentes: La re-contaminación del agua potable almacenada es un problema conocido. Sin embargo, un estudio realizado por Levy *et al.* (2008) observó la atenuación significativa de las concentraciones de *E. coli* durante el almacenamiento. La atenuación observada podría deberse a las bacterias depositándose en el fondo de los recipientes de almacenamiento o la mortandad de las bacterias. Bacterias viables que se depositan podrían convertirse nuevamente en suspensión donde podrían ser consumidas y causar enfermedades. Mortandad, sin embargo, significa la pérdida de infectividad. **Objetivo:** Examinamos: (1) cambios en la concentración de *E. coli* durante el almacenamiento de agua bajo condiciones del hogar y del control, (2) el impacto de la agitación de los recipientes en las concentraciones de *E. coli*, con el fin de entender si las reducciones observadas previamente se explican por deposición o mortandad, (3) el impacto de la cloración en los resultados observados en los objetivos (1) y (2), y (4) el alcance de contaminación del agua en la casa. **Métodos:** Cuantificamos los cambios en la contaminación durante el almacenamiento, teniendo en cuenta las características de los hogares y de las fuentes de agua. Utilizamos datos microbiológicos / físico-químicos y las encuestas de hogares. El agua de la fuente se almacena en las condiciones del hogar y del control. De los recipientes se tomaron muestras después de 24 horas, antes y después de la agitación para determinar si la concentración de *E. coli* cambió y si la deposición ocurrió. **Resultados:** Sin tratamiento, la reducción significativa de la concentración de *E. coli* fue observada bajo condiciones de control, pero no bajo las condiciones del hogar. Las diferencias entre las muestras tomadas antes y después de agitación se observaron en los hogares, pero no las condiciones de control, lo que sugiere que la deposición de bacterias viables se produce en condiciones del hogar, mientras que la mortandad se produce bajo condiciones de control. Esto puede atribuirse a las bio-membranas y a la contaminación en el hogar de los recipientes domésticos. Con el tratamiento con cloro, se observó una reducción significativa en ambas condiciones del hogar y del control. Las diferencias entre las muestras tomadas antes y después de la agitación se observaron bajo control, pero no en las condiciones del hogar, lo que sugiere mortandad de bacterias en las condiciones del hogar, pero la deposición bajo condiciones de control. La tendencia opuesta observada con el tratamiento con cloro puede explicarse debido a la interacción del cloro y turbidez. **Conclusión:** La mortandad y deposición de *E. coli* durante el almacenamiento fueron influidas por las condiciones de almacenamiento (hogar o control), la turbidez y la contaminación de la fuente inicial. Las diferencias significativas entre las muestras tomadas antes y después de agitación sugieren la necesidad de estandarizar los protocolos de muestreo que requieren la agitación de los recipientes de almacenamiento de agua.

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Acknowledgments

I would like to extend a heartfelt thanks to Shannon Oliver of Emory University and Larissa Anderson of the University of Michigan for their tireless support and camaraderie throughout the entirety of the thesis process, from data collection in Ecuador to data analysis in Atlanta. I would also like to sincerely thank my thesis advisor, Dr. Karen Levy of Emory University, for her guidance. I am grateful to my parents, sisters, brother and my boyfriend, Rahul, for their love and encouragement. Finally, I am indebted to the kindness and insight of Dr. William Cevallos of the University of San Francisco, Quito and the field staff in Borbón Region, Ecuador: Deni David Tenorio, Erenni, Blanca Vega, Mariuxi, Marixa and Jorge Ayoví and Chelo Ortiz. Muchisimas gracias!

TABLE OF CONTENTS

TABLES AND FIGURES

INTRODUCTION

Safe drinking water is a primary determinant of health worldwide and provides the foundation for the control and prevention of many infectious diseases. Each year, 2.2 million people die of diarrheal disease related to unsafe water, 90 percent of them children under five years of age (Pruss et al. 2002). Currently, 884 million people, 16 percent of the world's population, rely on unimproved water sources, such as rivers and unprotected wells, for their drinking water (WHO/UNICEF 2010). Of those relying on unimproved sources, 743 million, 84 percent, live in rural areas (WHO/UNICEF 2010). In many rural areas, treated piped water is not economically or environmentally feasible in the medium or long term. Given the immense need for improved water quality, assessment of drinking water from unimproved sources is necessary in order to design appropriate interventions. Household water treatment and safe storage interventions can lead to striking improvements in water quality and subsequent reductions in diarrheal disease. Successful water quality interventions must take into account local practices and unique water quality characteristics of an area in order to ensure sustainability and effectiveness. For this reason, research regarding household water treatment and source water characteristics is essential.

In places without reliable and pre-treated piped water connections, drinking water is often stored in the home prior to consumption. Numerous studies demonstrate a deterioration of water quality from source to storage, attributable to recontamination in the home from poor storage and hygiene practices (Wright et al.

2004). Unwashed containers and dipping vessels, uncovered containers, and dirty hands often are the culprits. A systematic review of 57 studies measuring bacteria counts in source water and stored water in the home demonstrated that the bacteriological quality of drinking water declined significantly after collection in approximately half of studies reviewed (Wright et al. 2004). The degree of contamination after water collection varied considerable between settings but was proportionately greater where fecal and total coliform counts in source water were low (Wright et al. 2004).

There were no studies in which the overall geometric mean bacteria count or proportion of contaminated samples was significantly lower at the point-of-use (POU); however, the author points out that "while the typical house experiences poorer water quality at point-of-use, there are likely to be a minority of households that do not conform to this general trend within a population" (Wright et al. 2004). For example, water quality improved between source and POU for 16% of households in the studies by Vanderslice and Briscoe (1993). Other researchers have shown that water samples may become less contaminated after they are collected from highly contaminated sources because of die-off as bacteria compete for limited oxygen and nutrients in the water (Momba and Kaleni 2002). Tompkins *et al* found that overnight storage in earthenware containers considerably reduced bacterial numbers but contamination was still at elevated levels (1987). The study also showed that reductions in contamination were more pronounced when the water was collected from an unprotected source (Tompkins 1987).

Researchers in a study by Levy *et al.* (2008) in rural Ecuador observed significant overall reduction of indicator organisms in control and household containers a unique finding compared those of studies reviewed by Wright *et al.* (2004). The design of the study allowed the researchers to observe contamination changes. When study participants filled their water containers, researchers simultaneously filled a control container and took a sample from the source water for microbiological analysis. Both household and control containers were re-sampled on subsequent days to determine how levels of contamination had changed inside the containers. The design not only allowed researchers to control for initial source contamination but also to quantify the amount of recontamination, the difference in contamination between the control and household containers (Levy et al. 2008).

The observed attenuation of microorganisms during storage in both control and household containers raises the question of whether the reductions were due to die-off or settling out of microorganisms. The distinction is important because organisms that settle out could become re-suspended and consumed which may lead to infection. Die-off, however, would mean loss of pathogen infectivity. Researchers in the study by Levy *et al.* collected samples from containers without first agitating the water. During storage, bacteria may attach to sediment in the water and settle to the bottom of containers. Thus, samples from un-agitated water may not be accurate estimates of the contamination level throughout the container. Conversely, the observed reductions could be due to die-off of indicator organisms caused by predation by other

microorganisms, lack of nutrients, or other factors creating unfavorable conditions inside the container. Determining the cause of attenuation and factors that influence attenuation is an important step in reducing the risk of waterborne disease (Levy et al. 2008).

Between 20 and 35 percent of fecal coliforms, *E. coli,* and *enterococci* are associated with settleable particles during normal flow conditions in lakes and rivers (Rehmann and Soupir 2009). Researchers in the U.S. found that more than 80% of fecal indicators were associated with suspended sediments in Chesapeake Bay (Sayler et al. 1975) and 38% were associated with suspended sediments in the Neuse River Estuary (Fries et al. 2006). Factors influencing the degree of settling of bacteria in a water column are thought to be settling velocity, the fraction of bacteria attached to sediment and the re-suspension rate (Rehmann and Soupir 2009).

Evidence for bacteria settling to the bottom of water storage containers is presented in a study by Roberts *et al.* (2001) which showed that when water stored for six hours in the home was agitated, coliform levels increased by 16% in improved buckets (with a lid, handle and spout) and 327% in control buckets. This demonstrates that changes in contamination levels observed during storage, whether increases or decreases may not be accurate estimates of contamination if the water is not agitated prior to sampling.

While the turbidity of water samples in the study by Levy *et al.* (2008) was not recorded, the majority of water was drawn from unimproved sources which generally

have higher levels of turbidity. Turbidity is a measure of the level of suspended organic and inorganic matter in water. It contains nutrients that allow for microbial growth (Lechevallier et al. 1981) and for the attachment of organisms to settle-able particles (Rehmann and Soupir 2009). Researchers using scanning electron photomicrographs have demonstrated that some bacteria are able to embed themselves within turbidity particles or become coated with amorphous material (Lechevallier et al. 1981). When turbid water is chlorinated, the number of colony forming units of bacteria may increases as much as five times, indicating the physical separation of cells from particles in the water (Lechevallier et al. 1981). Particle-associated bacteria generally settle faster and may have different mortality rates than bacteria that are not attached to particles (Faust et al. 1975; Burkhardt et al. 2000). Laboratory studies have documented reduced mortality due to predation or environmental exposures for *E. coli* and *Enterococcus* bacteria when attached to particles in the water (Jin et al. 2004; Davies and Bavor 2000).

Turbidity also influences the effectiveness of chlorine disinfection. LeChevallier *et al*. demonstrated that disinfection efficacy was negatively correlated with turbidity. Results from the study showed that coliforms in high-turbidity water (13 NTU) were reduced to only 20 percent the original count while coliforms in low-turbid water (1.5 NTU) were undetectable. When organic compounds in water react with sodium hypochlorite (chlorine) they form trihalomethanes. This process uses the available chlorine, leaving less free chlorine to inactivate bacteria (Lechevallier et al. 1981). Another possible mechanism for increased survival of coliforms in high turbid water may

be that coliforms embedded within particles do not come into sufficient contact with chlorine for disinfection to take place (Lechevallier et al. 1981). In further experiments, Lechevallier *et al.* found that increasing the turbidity of water samples from 1 to 10 NTU, while keeping chlorine dose constant, resulted in an eightfold decrease in the efficiency of disinfection (Lechevallier et al. 1981).

This goal of this research was to examine the influences of household storage on *E. coli* concentrations in stored drinking water in order to determine if the observed attenuation in the study by Levy *et al.* (2008) represents a true hazard reduction. The study design is similar to that of Levy *et al.* (2008), with the important distinction that samples from stored water were taken both before and after agitation of the stored water in an effort to detect if the observed reductions in water quality were due to die-off or settling of bacteria. In addition, the impact of chlorination on *E. coli* concentrations in stored water under household and control conditions was examined. The aims of this study are to examine:

1. **Changes in *E. coli* concentration during storage of untreated water under household and control conditions**
2. **The impact of agitation of containers on *E. coli* concentrations, in order to understand whether previously observed reductions were due to settling or die-off**
3. **The impact of chlorination on the results observed in aims (1) and (2), above**
4. **The extent of in-home *E. coli* contamination in treated and untreated water.**

Through the analysis of each aim, we can determine whether observed changes in *E. coli* concentration during storage are the result of die-off, settling out, or in-home contamination and examine the factors that contribute to the fate of *E. coli* during storage. The analysis focuses on factors such as source water turbidity, initial concentration of *E. coli* in the source water, chlorine residual and household characteristics that may explain reductions in *E. coli* concentration in stored water and differences between samples taken before and after agitation of storage containers.

METHODS

Study Site:

Research for this study was conducted in seven villages along the Santiago-Capayas-Onzole river system on the northern coast of Ecuador, the same region where Levy *et al.* (2008) carried out their study. Two main ethnic groups reside in the Santiago-Cayapas-Onzole river system: an Afro-Ecuadorian population, which comprises the majority of residents, and the Chachis, an indigenous population. The main economic activities are timber extraction and African palm oil production. In the city of Esmeraldas, 60% of people have no access to basic services like electricity, water and sanitation (Cooper et al. 2006). Water and sanitation infrastructure is rare in the smaller villages of the Esmeraldas region. In a survey of 1,000 households in 21 villages in the region where our study was conducted, 60% of people reported disposing of human waste in the open, either in a hole or into the river (Levy et al. 2008). The river serves as the main water source for 68% of households and 60% of people reported drinking their water without treating it (Levy et al. 2008). Diarrheal disease is common in the region (Eisenberg et al. 2006; Vieira et al. 2007).

The Santiago-Capayas-Onzole river system is a region undergoing intense environmental and social change, largely due to the construction of a new highway. The highway runs along the coast and connects previously remote villages to the outside world, causing changes in resource extraction, land use, and human migration patterns.

Construction of the road has led to intensified logging and deforestation, as well as increased migration into and out of the region (EcoDess 2008).

These unique social and environmental changes mediated by highway construction catalyzed the formation of the ECODESS research group. ECODESS was formed to study how changes in the social and natural environment affect the epidemiology of pathogens that cause diarrheal disease. The group is a partnership between investigators at University of Michigan, Emory University, Trinity College, University of California Berkeley, and the Universidad de San Francisco de Quito. Researchers, physicians, nurses, students, statisticians and field staff make up the organizational team that has been working in the region since 2003. The local project staff imparts a valuable asset as they are familiar with the customs and geography of the region in addition to being skilled in field research methods. All field activities for our research were coordinated with the help of ECODESS.

Local field staff and community health promoters familiar with both ECODESS and the local population served as key informants and research assistants. In addition to a health promoter or local research assistant, the research group was composed of four MPH students: two from the University of Michigan and two from Emory University. We conducted the study in seven villages. Key characteristics of each village are shown in Table 1.

Table 1: Key Characteristics of Study Villages

Community Name	Population	Road Access	Piped Water Available	Households Ever Reporting Chlorine Use (Since 2003)	Households in Community	Percent of Households Using Chlorine
Colon Eloy	943	Yes	Yes	100	328	30.5%
Las Cruces	94	No	No	4	21	19.0%
Punta de Piedra	332	Yes	Yes	10	84	11.9%
San Agusten	316	Yes	No	25	144	17.4%
San Francisco	148	No	No	7	49	14.3%
Santo Domingo	453	No	No	26	138	18.8%
Zancudo	371	No	No	28	91	30.8%

Study Design:

Community selection for the study was based on a combination of logistical and research requirements. A primary consideration was proportion of people reporting past chlorine use. Availability of lodging for our research groups, safety considerations, and electricity were other factors. Study villages needed to either have electricity in order to power laboratory equipment or be close enough to a village with electricity to which we could send samples by boat each day for analysis. Not all study villages had reliable electricity and thus a gas-powered generator was used during periods of no power, ranging from a few hours to three days.

Field activities were completed during two field visits from June 5 through June 20, 2010 (Visit One) and July 3 through July 25, 2010 (Visit Two). Visit One was completed within the communities of Punta de Piedra, Las Cruces, San Francisco, and San Agustín. Visit Two was completed within the communities of Zancudo, Santo Domingo and Colon Eloy.

Community maps, household demographics, and other relevant information about the study communities were collected during a community census carried out by ECODESS in 2009. We placed households in the seven selected communities into one of three treatment groups:

1. **The No-Treatment Group:** This group did not use any water treatment method. **N=67**
2. **The Local Chlorine Group:** This group treated their water with locally available sodium hypochlorite solution without instruction from the research team about dosage. **N=42**
3. **The Commercial Chlorine Group:** We treated this group's water with a commercial available sodium hypochlorite solution according to the dose recommended by the WHO. **N=36**

The "local chlorine" group and the "commercial chlorine" group used chlorine of different concentrations. Households in the "local chlorine" group used their own chlorine to dose their water. The chlorine used by this group was purchased locally by the household. However, some households in this group were temporarily out of chlorine. To these households we lent a bottle of chlorine that we had purchased locally. The "commercial chlorine" group used chlorine that we purchased in a supermarket in Quito, Ecuador. A description of the chlorine concentration and dosing methods can be found in the Laboratory Analysis section.

Using household surveys compiled by ECODESS from 2003 to 2009, we created a list of households in each community that had ever reported treating their water with chlorine (Table 1). Using a random number generator, we randomly assigned these households to one of the two chlorine treatment groups as we considered them more likely to currently use chlorine to treat their water or be willing to let us dose their water with chlorine. The first half of randomly selected households that had reported chlorine use was assigned to the "commercial chlorine" arm of the study. The second half was assigned to the "local chlorine" arm. If there were more houses in a village with reported chlorine use than were needed for the target number of households for either of the aforementioned arms, then the first half of these additional houses were assigned as alternate houses for the "commercial chlorine" arm and the second half were assigned as alternates for the "local chlorine" arm. Households that had not reported chlorine use were selected into the "no-treatment" group using a random number generator. Once the target number was reached for each community, subsequent households on the list were assigned as alternates for the "no-treatment" group. Table 2 shows the study design characteristics of each study village including the selection methodology and the percentage of households in each treatment group.

Table 2. Study Design Characteristics of Study Villages. ‡NT=No-Treatment, LC=Local Chlorine, CC=Commercial Chlorine. † Households selected within each block by stratified random sampling.

Community Name	Water Sources Used	Visit	Household Selection Strategy	Households in Each Group‡ (%)	Households in Study N(Percentage)
Colon Eloy	River (30%) Tap (10%) Well (60%)	2	Block Randomization†	NT=42% LC=42% CC=16%	27 (11%)
Las Cruces	Rain (100%)	1	Stratified Sampling	NT=20% LC=40% CC=40%	5 (24%)
Punta de Piedra	Tap (93%) River (7%)	1	Block Randomization	NT=80% LC=20%	17 (20%)
San Agusten	Rain (47%) River (37%) Well (17%)	1	Block Randomization	NT=53% LC=30% CC=17%	30 (21%)
San Francisco	River (62%) Rain (38%)	1	Stratified Sampling	NT=63% LC=25% CC=13%	10 (20%)
Santo Domingo	River (100%)	2	Census	NT=61% LC=17% CC=22%	20 (15%)
Zancudo	River (100%)	2	Census	NT=29% LC=29% CC=41%	42 (46%)

For inclusion in the study, households needed be in the habit of storing drinking water for a period of at least 24 hours. At study onset, the study population contained one third of households in each treatment group. However, a problem arose in that many households assigned to a certain group opposed their assignment. Because the data on chlorine use used for household assignment was collected as many as seven years earlier, some households had discontinued their use of chlorine. Additionally, some households assigned to the "commercial chlorine" group preferred to be in the "local chlorine" group and vice versa. To deal with this issue we allowed for participants to change treatment groups. Subsequently, instead of having 1/3 of households in each treatment group, 47% of households were in the "no-treatment" group, 29% were in the "local chlorine" group and 24% were in the "commercial chlorine" group.

Household Selection Methodology:

During Visit One, in the villages of Punta de Piedra and San Agustin, block randomization was employed to select households for participation. This was to ensure spatial heterogeneity in the study population. In these larger villages, households were dispersed various distances from the water source and road. Some households

clustered while others were more isolated. The location of the spatial blocks was based on the locations of groups of households in relation to roads and water sources to ensure that certain households were not chosen preferentially. In each spatial section of the community, we assigned a proportional number of households to each treatment group using stratified random sampling. In Las Cruces and San Francisco, households were selected using stratified random sampling. Households in these villages were situated on either side of a river with little clustering of homes.

After Visit One, we made alterations to the inclusion criteria. Eligible households were limited to those using river, well or tap water for drinking or cooking. Households using rainwater were excluded. This reduced the eligible population but allowed us to be able to collect 20L of water for the control containers. Household using rainwater had a limited supply and therefore could not donate sufficient water to fill control containers. More water for control containers provided a better opportunity to study the possible effects of settling during storage. Excluding rainwater users also increased the likelihood that households would be willing to use chlorine as river, well and tap water sources were perceived as dirtier.

During Visit Two, it was logistically feasible to survey the entire community in Zancudo and Santo Domingo. In these villages, all willing households that fit the inclusion criteria were enrolled. Assignment into each arm of the study was done during the initial interview. If households said that they currently treated their water with chlorine they were assigned to the “local chlorine” group. If they did not use chlorine

but were willing to let us dose their water with chlorine they became part of the "commercial chlorine" group. If they did not want chlorine in their water they became part of the "no-treatment" group. In Colon Eloy, the largest community in the study, time and resources did not allow for a census of the community and thus a combination of recruitment at common water collection sources and block randomization, as performed in Visit One, was implemented. See Table 2 for a summary of the study design characteristics of each village.

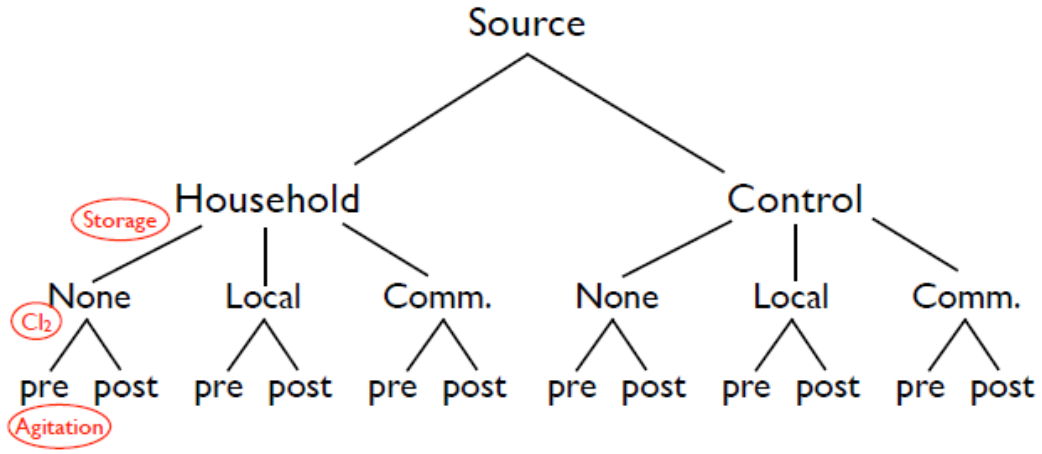
Varied enrollment methodology at each village may have resulted in selection bias. However, given that the study is not of a sensitive nature and there is considerable homogeneity of education level and SES in each village, it is unlikely that selection methodology would have a great impact on the type of households that enrolled in the study with respect to water collection, storage and usage practices.

Sampling Methodology:

From each of the selected households a total of five samples were collected. One sample was collected when the household went to a water source to fill their water container. At this time, we also filled a control container and took it back to the laboratory. Control containers were 20L plastic jerry cans. For reasons previously discussed, during Visit One control containers were filled with approximately five liters of water if the source was rain water and approximately 10L if the source was river, tap or well. During visit Two, all control containers were filled with 20L of water.

After 24 (±3) hours we returned to the study participants' households and the laboratory to collect two more samples from each location. The first sample was always taken prior to agitating the container. The second sample was taken after agitating the container. Through the use of a control container we were able to determine the extent of recontamination that occurred in the home. By collecting a pre- and post-agitation sample from each container we were able to determine if viable bacteria had settled to the bottom of containers during storage. Figure 1 shows a schematic of the study design.

Figure 1: Schematic of Study Design

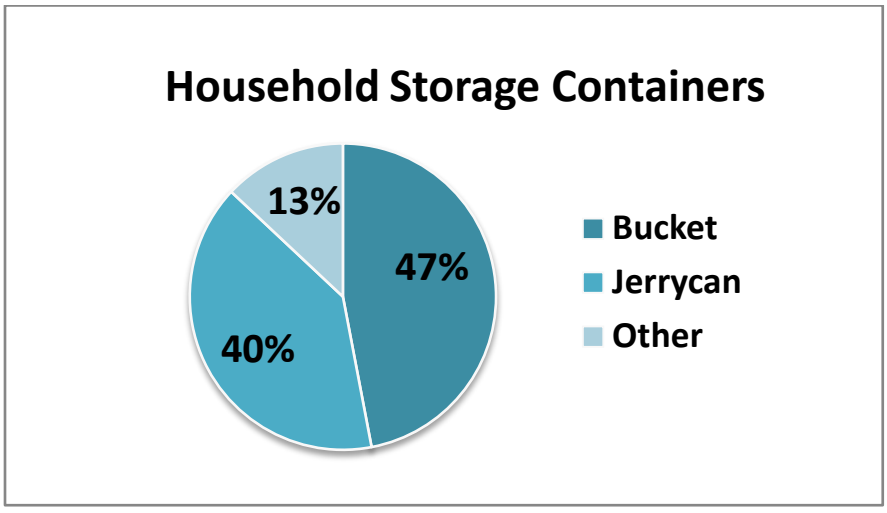


Control vs. Household Study Design:

We collected source water in the control container at the same time that the household collected their water. The control container allowed us to observe changes in microbiological contamination and, when applicable, chlorine residual under control conditions and compare those to changes seen under household conditions. Important

differences and similarities existed between the lab and the household. The lab was situated inside a home or health post and thus conditions such as temperature were the same in both control and household environments. However, households were instructed to use the water the collected, saving half of the water for sampling on the following day. (During Visit One, household were only instructed to save "some.") This meant that water inside the household containers may have been subject to many opportunities for recontamination such as the introduction of unclean dipping utensils or unwashed hands. The water was also being depleted and agitated during the 24 hours of storage in the household. In contrast, the control container was capped and left to sit untouched for 24 hours. Furthermore, the type of container used by each household varied. The majority of households used buckets and jerry cans. Others used plastic gallon jugs, large water barrels or cooking bowls. Figure 2 illustrates the type and percentage of storage containers used by study households.

Figure 2. Household Storage Containers



Data Collection:

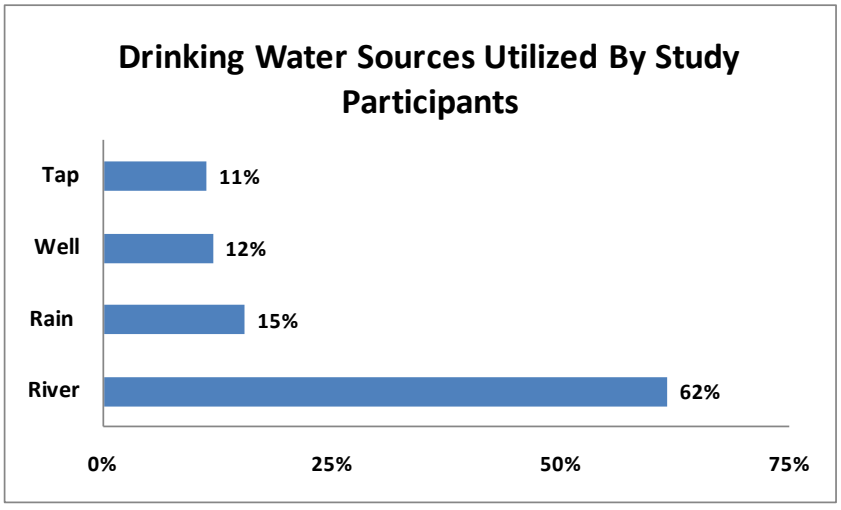
Using a community map, households were located according to their unique household number. If the household fit the inclusion criteria (storing water for at least 24 hours during Visit One and storing water for from a river, tap or well for at least 24 hours during Visit Two) and the resident consented to participate in the study, we administered a survey to assess household water treatment practices and chlorine use. Concurrently, we made an appointment with the resident to collect drinking water with them at their convenience either on the same day or the following day.

At the appointed time, sometimes directly after the initial interview, we met the household member to collect water. Source water characteristics were collected at the time of sample collection. Measurements included turbidity (NTU), temperature (°C), pH and conductivity (mV). Turbidity was measured utilizing a HACH® 2100P Turbidimeter, (Loveland, CO) which was calibrated at the onset of each visit as well as after moving to each new village utilizing STABLCAL® Stabalized Formazin Standards. Temperature, pH and conductivity were measured utilizing a HACH® sensION2 pH ISE Meter, (Loveland, CO) which was calibrated with the same frequency as the turbidimeter utilizing buffered standards of 10.0 pH and 7.0 pH per the calibration instructions included with the instrument. The pH electrode meter was cleaned utilizing the electrode cleaning solution at the onset of each visit and as needed during field visits. The electrode was stored in pH electrode storage solution during times of non-use per the instructions included with the instrument. A Hanna Waterproof Combo

pH/EC/TDS/Temperature Pen (Tampa, FL) was used for six water samples but its use was discontinued due to equipment malfunction.

In addition to the source characteristics mentioned above, data were collected on the type of source (river, well, rain or tap) and the number of people in the source at time of collection, if applicable. Figure 3 shows the drinking water sources utilized by study participants. A 100mL water sample was collected from the source using standard collection procedures with a WhirlPak® bag. The bag was labeled with sample number, time, and date and placed in an iced cooler. All samples were analyzed within five hours of collection and kept on ice in the time between collection and analysis.

Figure 3. Drinking Water Sources Utilized by Study Participants



Before the household member filled their container, we asked if they had washed the container prior to filling and we completed a visual inspection of the container for visible contamination. The control container was filled at the same as the household in a manner consistent with the household's collection method. During Visit

Two, 20L of water were collected inside the jerry can. During Visit One, 5-10L were collected. Control containers were washed daily with detergent and weekly with chlorine.

Chlorine for the "commercial chlorine" arm was purchased at a supermarket in Quito, Ecuador and verification of chlorine concentration was completed using a HACH Digital Titrator (Loveland, CO). "Local chlorine" was purchased from a vendor in the area where the study took place. Prior to deciding on which chlorine would be used for the "local chlorine," chlorine samples were purchased from a variety of venders and the range of their concentrations determined with the HACH Digital Titrator (Loveland, CO). Two gallons of chlorine solution of a representative concentration were chosen for distribution to the local chlorine group if they didn't have their own chlorine available. The concentration of chlorine solution made available to the "local chlorine" group was 2.2% in one gallon and 2.0% in the other. The concentration of "commercial chlorine" was 4.6%.

If the household belonged to either chlorine treatment arm, both household and control containers were dosed with chlorine immediately following collection. We dosed the control container with a volume of chlorine proportional to the amount the household used and corresponding to the volume of water in the container. We recorded the amount of chlorine used and the volume of the water dosed.

If the household was randomly assigned to "local chlorine" arm and they used their own chlorine to dose the water, a 2mL sample of their chlorine was collected in a

WhirlPak® bag for chlorine concentration analysis. Households in the "local chlorine" arm that did not have chlorine available at the time of visit were given chlorine that was purchased in the region in order to dose their water. Residents were instructed to dose their drinking water in the manner in which they usually dose. If the resident was unsure of how to dose (N=8), the health promoter or field assistant helped them dose. As the health promoters and filed assistants are members of the community who often do health education, we presumed that their knowledge of dosing would be a good estimate of the dose that had been recommended to community members.

It was sometimes difficult to ascertain how much chlorine a household in the "local chlorine" group used to dose their water. For instance, some households poured chlorine into their container and we approximated this by pouring the same chlorine into our container for the same amount of time. If a household used something to measure out the dose, we also used the same measuring tool. Nonetheless, the amount of chlorine used to dose control containers was not exactly proportional to the amount used by the household, but rather our best approximation.

If the household was part of the "commercial chlorine arm", we dosed their water and the control water with commercially available chlorine using a pipette in accordance with the dosage recommendations by the CDC Safe Water System initiative of 1.875 mg/L of sodium hypochlorite (NaOCl) (Lantagne 2008). To determine the correct dose volume, we used a measuring tape to calculate the volume of each container.

After collection, residents were instructed to use the water as they normally would. During Visit One, residents were instructed to reserve "some" of the water for re-sampling on the following day. During Visit Two, they were instructed to reserve half of the water collected. The control container was transported to the lab and labeled with the household's number and the date. An appointment was made with the household at the same time on the following day to collect water samples from the stored water.

Twenty-four (± 3) hours after collecting water from the source, we returned to the household to collect two 100mL samples from the stored water and administer a survey regarding storage and usage practices. The first sample was collected before agitating the container. The second was collected after agitating the container. In most cases, agitation of the water was achieved by capping and shaking the container. If the container was too large to be shaken by hand, a plastic pole sterilized with rubbing alcohol was used to stir the water. If the household was part of either chlorine treatment arm, Sodium Thiosulfate was added to the WhirlPak® bag to prevent further action of the chlorine. Two additional 50mL samples were collected from the containers of households in the chlorine treatment groups both before and after agitation in order to determine the chlorine residual levels (free and total) of the pre- and post-agitation samples. All water samples were placed on ice inside a cooler to await laboratory analysis within five hours of collection. Simultaneously in the lab, pre- and post-agitation samples were collected from the control containers 24 (± 3) hours after collection from

the source. Pre- and post-agitation chlorine residual samples were also taken from containers that had been dosed with chlorine.

On several occasions, samples were not collected or were lost by the researchers. Four samples that were collected were not processed because they did not make it to the laboratory within eight hours for processing. On two occasions, households left insufficient water for collection of both a pre- and post-agitation samples. On two occasions we forgot to collect a pre-agitation sample before agitating the container. On one occasion a household was not home to collect samples from the stored water.

Laboratory Methods:

A field laboratory was set up inside a health dispensary or a resident's home. Microbial analysis was performed using the IDEXX QuantiTray 2000 method. One Colilert reagent pillow was added to the Whirlpak® bag containing each 100mL water sample and mixed until the powder dissolved. The contents of the sample were then poured into a QuantiTray and the tray was processed through the IDEXX sealer according to standard methods. Process date and time were recorded the trays were incubated at 37.5°C (+/- 3°C), for a period of at least 24 hours. Only samples incubated between 24 and 28 hours were included in the analysis as per product recommendations of reliability. Residual chlorine analysis was performed with a LaMotte 1200 Colorimeter (Loveland, CO) and recoded in mg/L for free chlorine and total chlorine.

Results for total coliforms were recorded as the number of large and small cells turning from clear to yellow after a 24-hour incubation period, and these numbers were used to calculate the most probable number (MPN) of colony forming units in each sample, using the MPN chart made available by IDEXX. Results for *E. coli* were recorded as the number of large and small cells that fluoresced under UV light after a 24-hour incubation period, and these numbers were used to calculate the MPN of colony forming units for each sample, again, using the MPN chart made available by IDEXX.

The lower and upper detection limits were <1 and 1011.2 MPN/100mL, respectively. The IDEXX Quanti-Tray/2000 has an upper detection limit of 2419.6. However, this MPN is only reached if the overflow well is filled. The overflow well only fills if the sample volume is over 100mL or if a well within the tray does not fill. We did not include the overflow well in our MPN calculations in an effort to maintain consistency. Measurement of 100mL was not consistently exact using the Whirlpak® bag. Therefore, if any well did not fill, we did not include the MPN in our analyses, regardless of whether or not the overflow well was filled. Concentrations above 1011.2 MPN/100mL were recorded as a 1011.2 *E. coli* or total coliform MPN/100mL. As serial dilutions were not possible, an upper threshold of 1011.2 *E. coli* and total coliform MPN/100mL exists.

The ultraviolet lamp brought to the field broke during transport and a new bulb and fixture were purchased in Quito. The lamp sold by the IDEXX Corporation is a 6 watt fluorescent 365-nm long wave UV lamp. The lamp purchased in Quito is a 25 watt 120V

60 hz 320 mA UV lamp. In a laboratory in Atlanta both the lamp purchased in Quito and the lamp recommended by IDEXX were validated using 70 samples processed using the IDEXX method. There was perfect agreement on *E. coli* counts between the two lamps.

As a quality control measures, negative control and duplicate samples were run throughout the study period. A total of 21 negative control samples were run with autoclaved water brought from a laboratory in Quito and processed as if it were a water sample in the study. This amounts to running a negative control on 66% of study days. There were five instances in which the blanks were positive for total coliforms and one that was positive for *E. coli*. It is possible that the autoclaved water used for the "negative controls" was contaminated during transportation or storage. It is also possible that samples were contaminated by the researchers during processing. On each of the days in which the negative control was positive there was at least one other sample that was negative for total coliforms and *E. coli*. Therefore, we believe that the source of the contamination in the "negative controls" occurred during transport and storage and not during sample processing. If contamination had happened during sample processing then it is unlikely that other samples processed on that day would be negative for total coliforms and *E. coli.*

A total of 68 samples were run in duplicate (7% of all samples). The spearman correlation coefficient for *E. coli* concentration was 0.91 (p<0.0001) signifying that there is no evidence to suggest a difference between samples used in the analysis and the corresponding duplicate samples. In presence and absence analysis, there was an 87%

(p<0.0001) correlation. We can therefore conclude that the samples used in the analysis are good estimates of the true *E. coli* concentration.

A gas-powered generator was used to maintain temperatures in the incubator and run the IDEXX sealer when electricity was lost. Power outages occurred with some frequency during Visit Two. There were stretches of time—all less than three hours—in which the generator was turned off in an effort to conserve limited fuel or when the power outages occurred at night and we were temporarily unaware of the loss of power. During various power outages, three samples were caught inside the IDEXX sealer and wells were melted together and thus could not fill. These samples were not used in the analysis.

Analytical Methods:

Water was sampled from a total of 159 households. Surveys of chlorine use practices were completed in 151 households. Chlorine dosing data and container cleanliness data were collected from 144 households. Data on water usage and storage practices were collected from 142 households. These discrepancies are due to laboratory equipment malfunction, households being lost to follow up and lost samples.

Results from the laboratory notebook were entered into a Microsoft Excel (Redmond, WA) database and 10% double entry showed an error of 0.18%, considered acceptable so no data were re-entered. Data from the household surveys were entered into EpiInfo Version 3.5.1 (Atlanta, GA). Double entry of 10% of the data showed an error of 0.56%, also considered an acceptable rate.

Using SAS 9.2 (Cary, NC) data from EpiInfo were merged into a database that contained the laboratory data we collected and census data from ECODESS. Each household number was linked with its corresponding survey, census and water quality data. The Excel file was converted into a tab delimited file and imported into SAS 9.2 for analysis.

The variables for the concentration of both *E. coli* and total coliforms were not normally distributed. A log transformation was performed since a log-normal distribution often best describes positively skewed water quality data (Helsel DR 2002). Total coliforms were not used in the analysis as *E. coli* is a better indicator of recent fecal contamination and the risk of waterborne illness (EPA 1998). The total coliform and *E. coli* MPN counts of zero were changed to 0.5, halfway between zero and the detection limit of one. This was done because the logarithm of zero produces an undefined number.

Using this dataset, the outcome variables analyzed were the paired log difference in *E. coli* counts: (1) between the source and the stored water, to evaluate log reductions during storage; (2) between the pre- and post-agitation samples, to evaluate whether log reductions could be attributable to settling or to die-off of indicator organisms; and (3) between the household and control samples, to evaluate the extent of in-home contamination. Various covariates were analyzed with the above outcome variables including: container-level variables such as container type (small vs. large mouth: classified as less than eight centimeters such as jerry cans and plastic soda

bottles and greater than 8 centimeters such as buckets and rain barrels), whether the container was covered during storage, and whether or not there was visible contamination in the container at the time of water collection, and water characteristic variables such as turbidity, chlorine residual and pH.

Each of the paired log differences (1-3 above) were examined by treatment group and single sample *t*-tests were performed to test the null hypothesis of no difference in *E. coli* concentration between the sample groups.

The difference between household and control samples was made into two binary variables for whether or not the stored water in the home experienced in-home contamination, defined as the difference between the two samples being greater than zero.

All three outcome variables (log reduction from source, log difference between pre- and post-agitation samples, and log difference between household and control samples) were stratified by initial source contamination and turbidity level. *A priori*, it was decided based on the literature that these variables may be important in log reductions and log difference between sample types. Turbidity was thought to be an important factor as bacteria may adhere to particles of sediment that may influence settling behavior and the action of chlorine. Initial source contamination was chosen because studies have shown the initial source contamination level influences the amount of in-home contamination, the effectiveness of chlorine and the die-off of organisms during storage.

In the analysis, both chlorine treatment groups were combined because no significant differences in outcome variables were observed between the two groups. Separate analyses were performed on samples from the no-treatment and chlorine treatment group.

Univariate and multivariate regressions were performed with all plausible covariates. In multivariate regression, variables that were qualitatively similar or were directly related to one another were removed. See A-1 for a list of all covariates used in analysis.

RESULTS

Analyses One and Two: How does storage affect E. coli concentration in untreated water? What is the impact of agitation of containers on E. coli concentration in untreated water?

Aims One and Two are analyzed together as both are necessary to understand the influence of storage on *E. coli* concentrations in drinking water. The analysis of Aim One establishes on how *E. coli* concentrations change during storage (i.e. increase, decrease, stay the same) and Aim Two determines if settling of viable *E. coli* occurs. Both Aims are necessary to answer the question posed in the study by Levy *et al.* (2008): Are the reductions in *E. coli* concentration during storage that Levy *et al.* observed the result of die-off or settling of viable bacteria?

Table 3 and Figures 4 and 5 summarize the central results in the no-treatment group. Figure 4 presents the geometric mean *E. coli* concentration in source and stored water under household and control conditions. Table 3 provides more detailed information on the geometric means and the log differences between sample types. Figure5 shows the mean log reductions from source to stored water and the differences between pre- and post-agitation samples. The household post-agitation sample in Figure 5 is negative because under household conditions the *E. coli* concentration in the stored, agitated water is greater than in the source.

Figure 4: E. coli Concentration by Sample Type: No-Treatment Group. The geometric mean *E. coli* MPN/100mL and coefficient of variation (error bars) are shown.

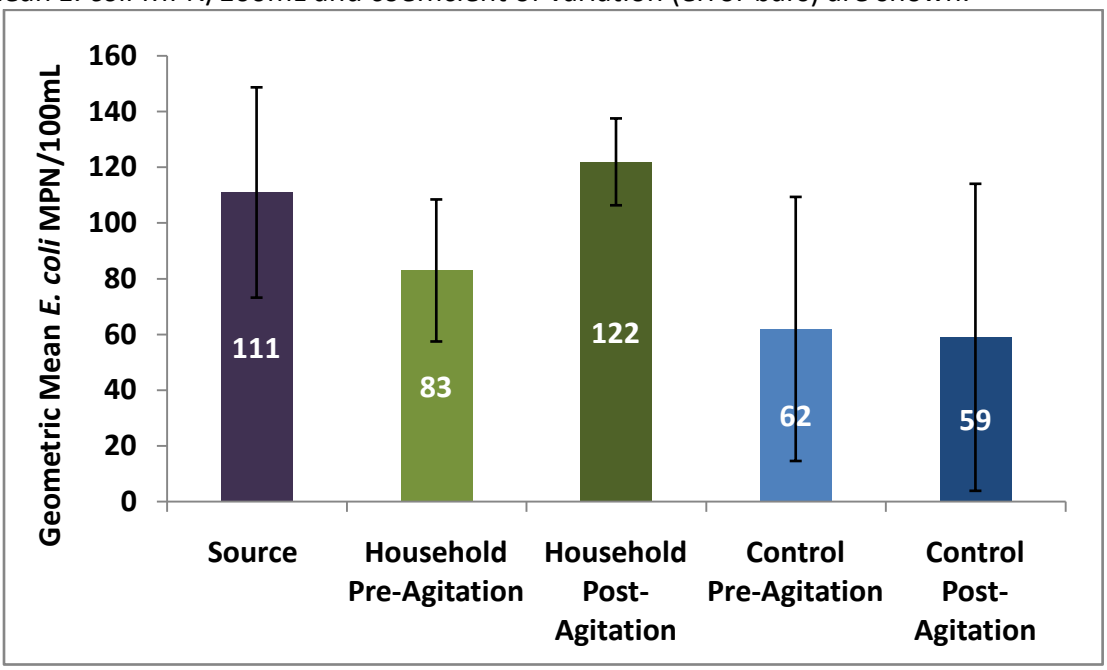
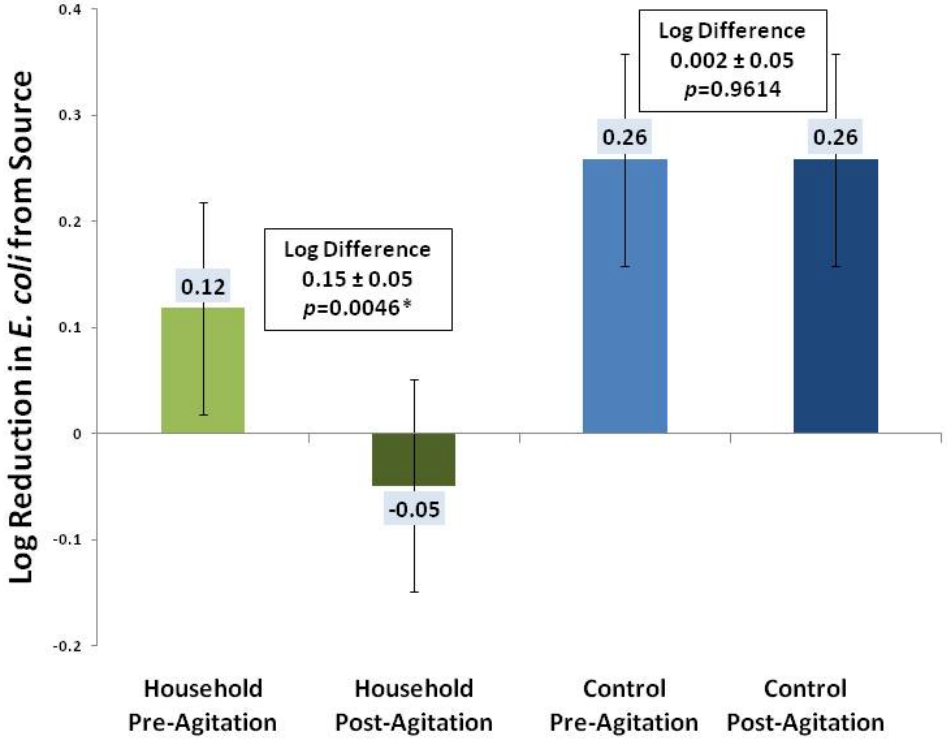


Table 3: Comparison of *E. coli* Concentration in Source, Household and Control Samples. Geometric mean *E. coli* concentrations and the log differences between samples in the no-treatment group are shown. Reported *p*-values test the null hypothesis that the log difference between samples is zero using single sample *t*-tests.

				Mean, Standard Error and T-test for Log Difference between Samples			
Sample Type	N	Geometric Mean E. coli MPN /100mL	95% CI	Source vs. Stored Water	Pre-Agitation vs. Post-Agitation	Household vs. Control Pre-Agitation	Household vs. Contol Post-Agitation
Source	66	111.4	57.4-216.1	--	--	--	--
Household Pre-Agitation	65	83.3	44.3-156.6	0.12 ± 0.09 0.2086	0.15 ± 0.05 0.0046*	0.16 ± 0.11 0.1498	--
Household Post-Agitation	65	121.5	67.9-217.2	-0.05 ± 0.10 0.6131			0.31 ± 0.10 0.0048*
Control Pre-Agitation	64	62.0	31.0- 124.0	0.26 ± 0.09 0.0034*	0.002 ± 0.05 0.9614		
Control Post-Agitation	65	59.4	24.5-119.9	0.26 ± 0.07 0.0007*		--	

Figure 5: Log Reduction in *E. coli* concentration by Sample Type and the Difference between Pre- and Post-Agitation Samples: No-Treatment Group. Reported *p*-values test the null hypothesis that the log difference between samples is zero using single sample *t*-tests. The bars show the mean log reduction in *E. coli* concentration (MPN/100mL) from the source to stored water. Text boxes show the mean log difference in *E. coli* concentration (MPN/100mL) between pre- and post-agitation samples in the household and control samples, respectively.



Under control conditions, significant reduction in contamination from the source is observed in both pre- and post-agitation samples (pre-agitation 0.26 ± 0.09, p=0.0034) (post-agitation 0.26 ± 0.07, p=0.0007). The mean log difference between pre- and post-agitation samples in water from control containers is very close to zero (0.002 ± 0.05, p=0.9614).

Under household conditions (green bars), the pre-agitation samples show a mean log reduction of 0.12 ± 0.09 (p=0.2086) from source to stored water. The post-agitation samples show a mean log increase of 0.05 ± 0.10 (p=0.6131). Neither change in contamination from the source is significant. However, the mean log difference between the pre- and post-agitation samples in the household is significant (0.15 ± 0.5; p=0.0046).

In-home contamination, defined as the log difference between household and control sample, is significant in the post-agitation household samples (0.31 ± 0.10; p=0.0048). The pre-agitation samples do not show significant in-home contamination (0.16 ± 0.11, p=0.1498).

In summary, storage conditions influence *E. coli* concentrations in the no-treatment group. There is evidence to suggest that settling of *E. coli* occurs during storage under household conditions but die-off occurs under control conditions.

The Impact of Turbidity and Source Contamination

As stated in the methods section, all outcome variables are stratified by both turbidity level and initial source contamination level as the literature suggests that these are important influences on changes in *E. coli* concentrations during storage.

Initial *E. coli* concentration and turbidity level vary by source type (Figure 6). River and stream sources are the most contaminated while source water from household taps and rain barrels is the least contaminated. Turbidity is highest in highly contaminated sources and lowest in sources with little contamination.

Figure 6: Mean *E. coli* Concentration and Turbidity by Source Type. The geometric mean *E. coli* concentration (*E. coli* MPN/100mL) and turbidity level (NTU) are shown by source type. Parentheses following source type show the number of households in the study collecting water from each source.

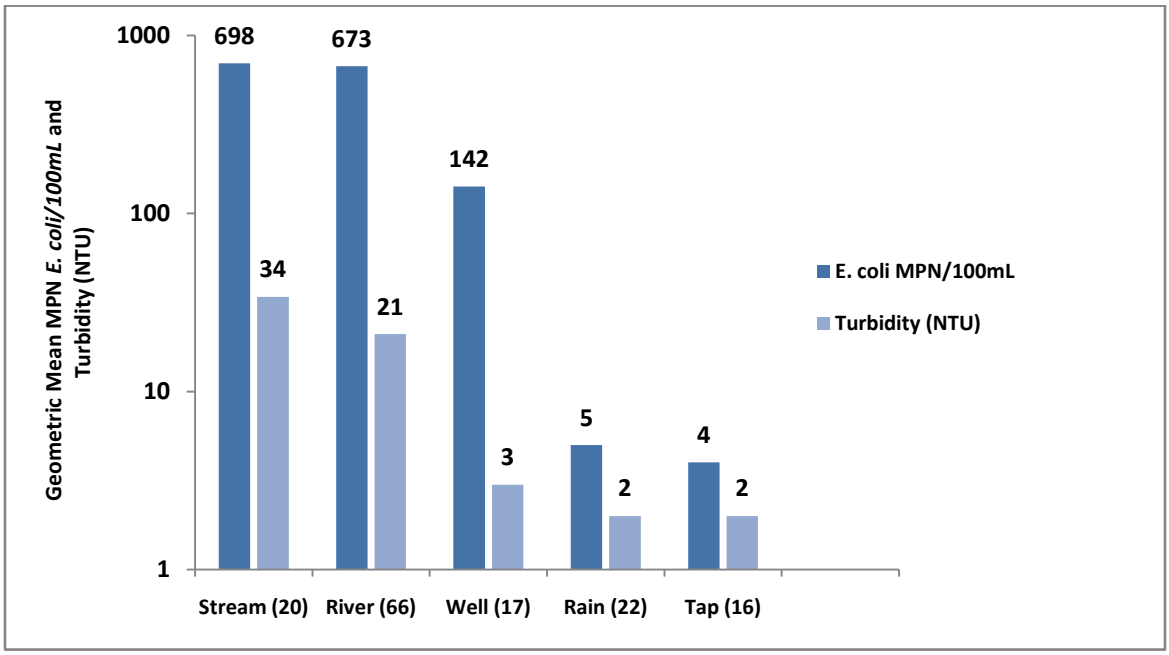
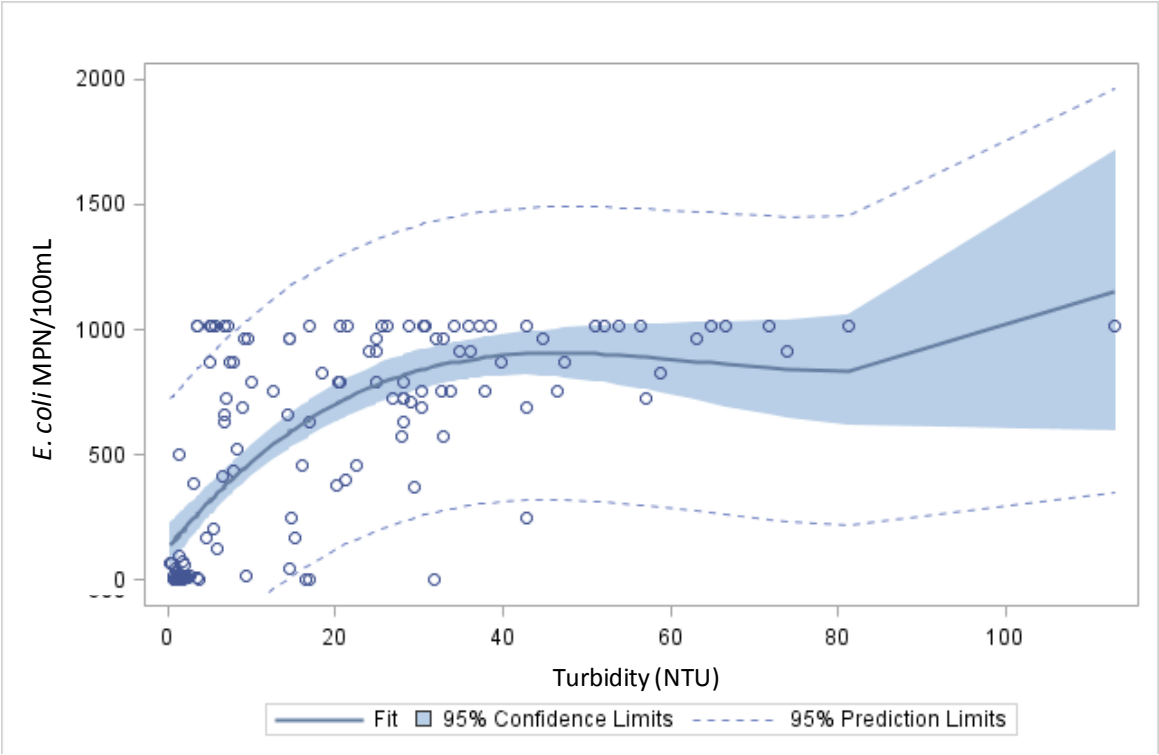


Figure 7 shows the relationship between turbidity and *E. coli* concentration (MPN/100mL) in source water. The relationship between turbidity and *E. coli* concentration follows a log linear curve (r-squared=0.61, p<0.0001). Source contamination increases exponentially with increased turbidity until approximately 40 NTU. At this point, source contamination flattens out and does not increase with increasing turbidity.

Figure 7: Regression Fit for Source Turbidity and *E. coli* Concentration. Source *E. coli* concentration (*E. coli* MPN/100ml) and source turbidity level (NTU) are plotted along with a log linear regression fit showing confidence and prediction limits. The upper detection limit was 1011.2 *E. coli* MPN/100mL.



Turbidity

From Source to Stored Water

Table 4 shows log reductions in *E. coli* concentration from source to stored water stratified by turbidity level (≥ 10 NTU and < 10 NTU), demonstrating that *E. coli* concentrations during storage respond differently at high and low turbidity levels.

Water from high turbidity sources (≥ 10 NTU) is associated with a significant reduction in contamination from source to stored water, with the exception of the household post-agitation sample. Under control conditions, a mean log reduction of 0.32 ± 0.12 (p=0.0153) in the pre-agitation sample and 0.27 ± 0.11 (p=0.0251) in post-

agitation sample is observed. Under household conditions, in the pre-agitation sample a mean log reduction of 0.29 ± 0.13 (p=0.0307) is observed. However, post-agitation, the mean log reduction is smaller, 0.19 ± 0.11 (p=0.0837), and does not represent a significant log reduction.

Table 4: Log Reductions in *E. coli*/100mL from Source to Stored Water Stratified by Turbidity Level. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

	High Turbidity Source (≥10 NTU)				Low Turbidity Source (<10 NTU)			
	Household		Control		Household		Control	
Sample	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value
Pre-	0.29 ± 0.13	0.0307*	0.32 ± 0.12	0.0153*	-0.07 ± 0.13	0.6114	0.19 ± 0.13	0.1372
Post-	0.19 ± 0.11	0.0837	0.27 ± 0.11	0.0251*	-0.30 ± 0.15	0.0576	0.25 ± 0.10	0.0185*

Water from low turbidity sources is associated with no significant change in *E. coli* concentration from source to stored water in the pre-agitation samples. However, in the post-agitation samples under control conditions a significant reduction in contamination from the source (0.25 ± 0.10; p=0.0185) is observed. Under household conditions, a marginally significant increase in contamination from the source is observed (0.30 ± 0.15; p=0.0576). The effect of increased contamination from the source is not observed in the samples from high turbidity sources. While the pre-agitation samples from water stored under both household and control conditions do not show a significant difference in *E. coli* concentration from the source, upon

agitation, the change from the source is significant; albeit in opposite directions depending on the storage conditions.

Difference between Pre- and Post- Agitation Samples

Table 5 show the log difference between pre- and post-agitation samples stratified by turbidity level. While stratification by turbidity demonstrates differing results when looking at log reductions in *E. coli* concentration from the source, turbidity level does not change the results of the analysis when looking at the log difference between pre- and post-agitation samples. Household pre-and post-agitation samples, when stratified by turbidity level, remain significantly different from each other while control samples do not show any difference between pre- and post-agitation samples.

Table 5: Log Difference between Pre- and Post-Agitation Samples in the No-Treatment Group Stratified by Turbidity Level. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

High Turbidity Source (≥10 NTU) N=31				**Low Turbidity Source (<10 NTU) N=33**			
HH		**CN**		**HH**		**CN**	
Mean ± SE	**p-value**	**Mean ± SE**	**p-value**	**Mean ± SE**	**p-value**	**Mean ± SE**	**p-value**
0.09 ± 0.04	**0.0418***	**0.05 ± 0.04**	**0.2068**	**0.23 ± 0.09**	**0.0166***	**-0.5 ± 0.09**	**0.5498**

Source Contamination

From Source to Stored Water

Table 6 shows the relationship between initial source concentration (*E. coli* MPN/100mL) and the log difference between source and stored water. When stratified by high and low contamination of the source water, the importance of initial source

contamination is demonstrated. The high contamination samples all show significant reduction in *E. coli* concentration from source to stored water while the low contamination sources do not show a significant change in contamination from the source, with the exception of the post-agitation samples under household conditions, which demonstrate a mean log increase in contamination from the source.

Table 6: Log Reductions in *E. coli*/100mL from Source to Stored Water Stratified by Source Contamination Level. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

	High Contamination Source (≥100 MPN *E.coli*/100mL)				Low Contamination Source (<100 MPN *E. coli*/ 100mL)			
Sample	HH		CN		HH		CN	
	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value
Pre	0.33 ± 0.09	0.0011*	0.32 ± 0.09	0.0010*	-0.29 ± 0.18	0.1236	0.13 ± 0.18	0.4808
Post	0.21 ± 0.08	0.0091*	0.27 ± 0.08	0.0025*	-0.55 ± 0.21	0.0152*	0.23 ± 0.14	0.1101

Water from highly contaminated sources stored under control conditions shows a mean log reduction of 0.32 ± 0.09 (p=0.0010) in the pre-agitation sample and a mean log reduction of 0.27 ± 0.08 (p=0.0025) in the post-agitation sample. Under household conditions, the mean log reduction is 0.33 ± 0.09 (p=0.0011) in the pre-agitation sample and 0.21 ± 0.08 (p=0.0091) in the post-agitation sample. These results suggest that water from highly contaminated sources undergoes significant reduction in *E. coli* concentrations during storage, regardless of whether or not the container is agitated.

Samples from low contamination sources demonstrate strikingly different results than samples from highly contaminated sources. Under control conditions, the mean

log reductions in *E. coli* concentration are not significant (pre-agitation 0.13 ± 0.18, *p*=0.4808) (post-agitation 0.23 ± 0.14, *p*=0.1101). In the household, the post-agitation household samples demonstrate a significant mean log increase in *E. coli* concentration during storage of 0.55 ± 0.21 (*p*=0.0152). The pre-agitation samples, though not significant, also show a mean log increase in contamination from the source of 0.29 ± 0.18 (*p*=0.1236).

Difference between Pre- and Post- Agitation Samples

The log difference between pre- and post-agitation samples, when stratified by source contamination level, follows the same pattern as when stratified by turbidity. Table 7 shows the log difference between pre- and post-agitation samples stratified by source contamination level. While stratification by source contamination demonstrates differing results when looking at log reductions in *E. coli* concentration from the source, source contamination level does not change the results of the analysis of the log difference between pre- and post-agitation samples.

Table 7: Log Difference between Pre- and Post-Agitation Samples in the No-Treatment Group Stratified by Source Contamination Level. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

High Contamination Source (≥100 *E.coli* /100mL) n=41				**Low Contamination Source (<100 MPN *E. coli* / 100mL) N=24**			
HH		**CN**		**HH**		**CN**	
Mean ± SE	**p-value**	**Mean ± SE**	**p-value**	**Mean ± SE**	**p-value**	**Mean ± SE**	**p-value**
0.09 ± 0.04	**0.0213***	**0.05 ± 0.03**	**0.1072**	**0.25 ± 0.12**	**0.0490***	**-0.08 ± 0.11**	**0.4758**

Turbidity and Source Contamination

From Source to Stored Water

Table 8 shows how *E. coli* concentration changes during storage in water from high contamination and high turbidity sources, high contamination and low turbidity sources, and low contamination and low turbidity sources.

Table 8: Log Reductions of *E. coli*/100mL between Source and Stored Water Stratified by Turbidity and Source Contamination Level. Reported *p*-values test the null hypothesis that the log difference between samples is zero using single sample *t*-tests.

				Turbidity							
				High (≥ 10 NTU) N=31					Low (< 10 NTU) N=33			
				HH		CN			HH		CN	
		Sample	N	Mean ± SE	p-value	Mean ± SE	p-value	N	Mean ± SE	p-value	Mean ± SE	p-value
Source *E. coli* Level/ 100 mL	High (≥ 100 CFU) N=42	Pre	30	0.37 ± 0.11	0.0024*	0.33 ± 0.13	0.0152*	11	0.35 ± 0.18	0.0766	0.23 ± 0.06	0.0031*
		Post		0.26 ± 0.08	0.0037*	0.29 ± 0.12	0.0193*		0.24 ± 0.15	0.1443	0.17 ± 0.07	0.0279
	Low (< 100 CFU) n=24	Pre	1	--	--	--	--	22	-0.28 ± 0.17	0.1077	0.17 ± 0.18	0.3576
		Post		--	--	--	--		-0.57 ± 0.19	0.0070*	0.28 ± 0.14	0.061

Stratified analysis reveals significant log reduction in household and control containers in both the pre-and post-agitation samples when water is taken from highly contaminated and high turbidity sources. The mean log reductions amongst sample types range from 0.26 to 0.37.

Samples from low turbidity, highly contaminated sources show that even in low turbidity waters, high *E. coli* concentration is an important predictor of reductions in *E. coli* concentration during storage under control conditions. A mean log reduction of 0.23 ± 0.06 (p=0.0031) in the pre-agitation sample and 0.17 ± 0.07 (p=0.0279) in the post-agitation sample are observed under control conditions. However, under

household conditions, the mean log reduction in contamination is not significant (pre-agitation: p=0.0766; post-agitation: p=0.1443).

Greater variability in the direction of changes in *E. coli* concentration is observed in samples of low turbidity and low source contamination, as water from low turbidity, low contamination sources is more likely to experience in-home contamination events under household conditions. This is demonstrated by the fact that under household conditions, the post-agitation samples undergo a 0.57 ± 0.19 mean log increase in contamination (p=0.0070). Under control conditions, the post-agitation samples undergo a mean log reduction of 0.28 ± 0.14 (p=0.0610).

Difference between Pre- and Post- Agitation Samples

Stratification by both turbidity level and source contamination level has little effect on the mean log difference between pre- and post-agitation samples (Table 9). However, unlike in the previous stratified analyses, there is not a significant difference between pre- and post-agitation samples in household, low turbidity and high contamination samples (0.11 ± 0.09, p=0.2604).

Table 9: Log Difference between Pre- and Post-Agitation Samples Stratified by Turbidity and Contamination Level. Reported p-values test the null hypothesis that the log difference between samples is equal to zero using single sample t-tests.

			Turbidity							
			High (≥ 10 NTU) N=31				Low (< 10 NTU) N=33			
			Household		Control		Household		Control	
		N	Mean ± SE	p-value	Mean ± SE	p-value	N	Mean ± SE	p-value	Mean ± SE	p-value
Source *E. coli* Level/ 100 mL	High (≥ 100 CFU) N=42	29	0.09 ± 0.04	0.0474*	0.04 ± 0.04	0.2986	11	0.11 ± 0.09	0.2604	0.06 ± 0.05	0.2522
	Low (< 100 CFU) N=24	22	--	--	--	--	1	0.29 ± 0.13	0.0337*	-0.10 ± 0.12	0.4152

Effects of Covariates on the Difference between Pre- and Post-Agitation Samples: No Treatment Group

Multivariate and univariate regressions were performed to further explore the relationship between pre- and post-agitation samples in the no-treatment group. Univariate analysis demonstrates differing results for pre- and post-agitation samples and differing results for control versus household conditions and therefore each is analyzed separately. In univariate analysis, only temperature of the source water is associated with the difference between pre- and post- agitation samples in the household (0.06 $\pm$ 0.03; p=0.0417). Table A-2 shows the full model of the effects of covariates (adjusted and unadjusted) on the log difference between pre- and post-agitation samples in the household. In the full model, conductivity (-0.06 $\pm$ 0.03; p=0.0350) and pH (-3.00 $\pm$ 1.41; p=0.0402) are associated with the difference between the pre- and post-agitation samples, however the full model is not significant (p=0.2564). After using backwards selection, source water *E. coli* MPN/100mL (-.0004 $\pm$ 0.0002; p=0.0392), conductivity (-0.03 $\pm$ 0.02; p=0.0462), and pH (-1.71 $\pm$ 0.88; p=0.0508) and a household having washed their collection container prior to water collection (-0.25 $\pm$ 0.12; p=0.0409) are all associated with a log decrease in the difference between pre- and post-agitation samples in the household (Table 10). Temperature (0.09 $\pm$ 0.04; p=0.0182) is associated with a log increase in the difference between pre- and post-agitation samples in the household.

Under control conditions, multivariate and univariate analyses of the log difference between pre- and post-agitation samples do not provide any variables that are significantly associated with the outcome (Table A-3).

Table 10: Effects of Covariates on the Log Difference between Pre- and Post- Agitation Samples under Household Conditions: No Treatment Group. Multivariate Analysis was performed using backwards elimination. The reported p-values test the null hypothesis that the parameter estimate is zero.

Parameter	Parameter Estimate	Standard Error	*p*-value
Intercept	**10.47**	**6.14**	**0.0938**
***E. coli* source contamination**	**-0.0004**	**0.0002**	**0.0392***
Conductivity of source	**-0.03**	**0.02**	**0.0462***
Temperature of source	**0.09**	**0.04**	**0.0182***
pH of source	**-1.71**	**0.88**	**0.0508***
Washed container (Y/N)	**-0.25**	**0.12**	**0.0409***

Analysis Three: How does chlorination influence changes in E. coli concentration during storage and what is the impact of agitation of containers on E. coli concentration in chlorinated water?

The local and commercial chlorine groups are combined for analysis because, despite differences in chlorine dosage (See A-4a) there is not a significant difference in the mean log reduction between the two groups in either the household or control containers (See Table A-4b). Geometric mean log reductions from source to stored water in the chlorine group are presented in Figure 8. Table 11 provides more information about the log differences between sample types in the chlorine group.

Figure 8: Mean Reduction in Contamination from Source to Stored Water: Chlorine Group. The bars with boxes in the middle show the geometric mean log reduction in *E. coli* MPN/100mL from the source to stored water. Error bars show the coefficient of variation of the geometric mean. The text box on the left shows the mean log difference in *E. coli* MPN/100mL between pre- and post-agitation samples under household conditions. The text box on the right shows the difference between pre- and post-agitation samples under control conditions. Reported *p*-values test the null hypothesis that the log difference between samples is zero using single sample *t*-tests.

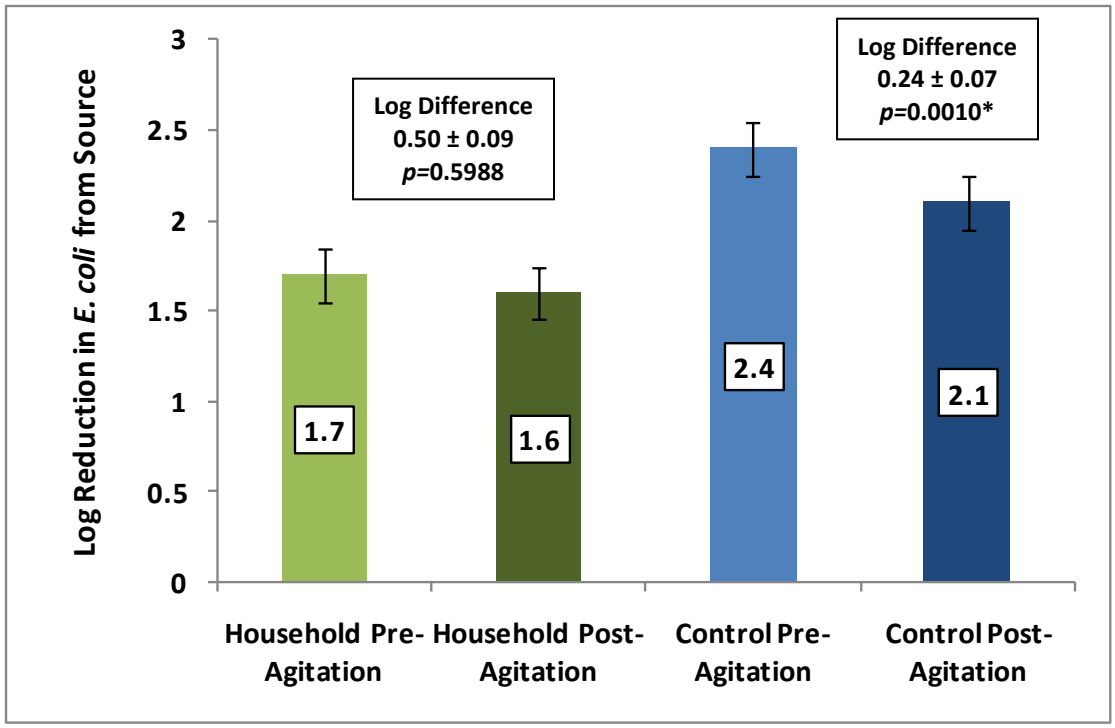


Table 11: Mean contamination by Sample Type and the Log difference between Samples: Chlorine Group. The geometric mean of *E. coli* concentration (MPN/100mL) and the 95% confidence interval by sample type are shown in the left-hand portion of the table. On the right, the log differences in *E. coli* concentration (MPN/100mL) between samples are shown. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

				Mean, Standard Error and T-test for Difference between Samples			
Sample Type	**N**	**E. coli MPN**	**95% CI**	**Source vs. Stored Water**	**Pre-Agitation vs. Post-Agitation**	**Household vs. Control Pre-Agitation**	**Household vs. Contol Post-Agitation**
Source	**70**	**198.5**	**111.3-354.2**	--	--	--	--
Household Pre-Agitation	**72**	**4.0**	**2.3-6.8**	**1.72 ± 0.15 <.0001***	**0.50 ± 0.09 0.5988**	**0.68 ± 0.13 <.0001***	--
Household Post-Agitation	**73**	**4.3**	**2.5-7.2**	**1.65 ± 0.14 <.0001***			**0.84 ± 0.10 <.0001***
Control Pre-Agitation	**72**	**0.8**	**0.6-1.1**	**2.13 ± 0.15 <.0001***	**0.24 ± 0.07 0.0010***		
Control Post-Agitation	**72**	**1.5**	**1.0-2.1**	**2.40 ± 0.15 <.0001***		--	

Significant log reductions are seen during storage for both control (2.4 $\pm$ 0.15 pre-agitation, 2.1$\pm$ 0.15 post-agitation) and household containers (1.7 $\pm$ 0.15 pre-agitation; 1.6 $\pm$ 0.14 post-agitation) (all *p*-values <0.0001), demonstrating that chlorine use significantly reduces *E. coli* concentrations. However, concentrations of *E. coli* under household conditions are significantly greater than *E. coli* concentrations under control conditions (0.68 ± 0.13 pre-agitation; 0.84 ± 0.10 post-agitation) demonstrating reduced chlorine effectiveness and significant in-home contamination (p<0.0001) in both the pre- and post-agitation samples (Table 12). There is a significant difference between pre- and post-agitation samples under control conditions (0.24 $\pm$ 0.07;

p=0.0010) but not under household conditions (0.5 ± 0.09; *p*=0.5988). This effect is the opposite of what was observed in the no-treatment group.

Turbidity and Source Contamination

Tables 12 and 13 show the log difference between pre- and post-agitation samples in the chlorine treatment group stratified by turbidity level and initial source contamination level, respectively. The only significant difference between pre- and post-agitation samples is observed under control conditions when stratified by high turbidity level (Table 12) and by high initial source contamination level (Table 13). In high turbidity samples, the effect is the opposite of that seen in samples that are not chlorinated. In the no-treatment group there is a significant difference between pre- and post- agitation samples in the household but not under control conditions. In the chlorine group, there is a significant difference between pre- and post-agitation samples under control but not household conditions.

Table 12: Log Difference between Pre- and Post-Agitation Samples in the Chlorine Treatment Group Stratified by Turbidity Level. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

	High Turbidity Source (≥10 NTU) N=45				Low Turbidity Source (<10 NTU) N=26			
Difference between Pre- and Post-Agitaiton	HH		CN		HH		CN	
	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value
	0.05± 0.12	0.6787	0.41 ± 0.10	0.0004*	0.06 ± 0.12	0.6416	0.02 ± 0.02	0.1474

Table 13: Log Difference between Pre- and Post-Agitation Samples in the Chlorine Treatment Group Stratified by Source Contamination Level. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

	High Contamination Source (≥100 *E.coli* /100mL) N=53				Low Contamination Source (<100 MPN *E. coli* / 100mL) N=15			
	HH		CN		HH		CN	
Difference between Pre- and Post-Agitaiton	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value
	0.09 ± 0.11	0.4321	0.28 ± 0.08	0.0012*	0.07 ± 0.12	0.5972	0.09 ± 0.15	0.5485

When the log difference between pre- and post-agitation samples is stratified by both turbidity and source contamination level (Table 14), once again, the only significant difference is observed in under control conditions in samples from highly contaminated, high turbidity sources (0.39 ± 0.11, p=0.0010). Turbidity and initial source contamination proved to be better predictors of log reductions form the source rather than of the log difference between pre- and post-agitation samples in both the chlorine and no-treatment groups.

Table 14: Log Difference between Pre- and Post-Agitation Samples in the Chlorine Treatment Group Stratified by Source Contamination Level and Turbidity Level. Reported *p*-values test the null hypothesis that the log difference between samples is equal to zero using single sample *t*-tests.

			Turbidity							
			High (≥ 10 NTU) N=31				Low (< 10 NTU) N=33			
			Household		Control		Household		Control	
		N	Mean ± SE	p-value	Mean ± SE	p-value	N	Mean ± SE	p-value	Mean ± SE	p-value
Source *E. coli* Level/ 100 mL	High (≥ 100 CFU) N=42	40	0.12 ± 0.13	0.3353	0.39 ± 0.11	0.0010*	14	-0.03 ± 0.21	0.8800	0.001 ± 0.001	0.3356
	Low (< 100 CFU) N=24	3	-0.06 ± 0.12	0.6595	0.64 ± 0.63	0.4167	11	0.15 ± 0.15	0.3614	0.05 ± 0.04	0.1669

Effects of Covariates on the Difference between Pre- and Post-Agitation Samples: Chlorine Group

Univariate and multivariate analyses were performed to further explore the relationship between the difference in pre-and post-agitation samples in the chlorine treatment group.

Under control conditions in univariate analysis, the turbidity of the source is significantly associated with the difference between pre- and post-agitation samples but the association is not meaningful as the parameter estimate is only 0.01 $\pm$ 0.003 (p=0.0030). Samples collected during Visit Two compared to Visit One (0.43 $\pm$ 0.14; p=0.0034) and samples collected from a river or stream source compared to a well, tap or rain water source (0.34 $\pm$ 0.14; p=0.0179) are associated with a log increase in the difference between pre- and post-agitation samples. Table A-5 shows the unadjusted and adjusted effect of all covariates on the log difference between pre- and post-agitation samples under control conditions in the chlorine group.

Under household conditions in univariate analysis, the presence of visible contamination in the container at the time of collection compared to no visible contamination (-0.38 $\pm$ 0.18; p=0.0343) and the use of a safe container, defined as a container with an opening less than 8 cm wide (-0.44 $\pm$ 0.17; p=0.0113) are associated with a log decrease in the difference between the pre- and post-agitation samples. Table A-6 shows the unadjusted and adjusted effect of all covariates on the log difference between pre- and post-agitation samples stored under household condition in the chlorine group.

In multivariate analysis of the full model under control conditions, turbidity (0.01 $\pm$ 0.01; p=0.0286) and *E. coli* concentration of the source (0.0006 $\pm$ 0.0003; p=0.0503) are associated with log increase in the difference between pre- and post-agitation samples (Table A-5). However, the relationship between source *E. coli* concentration and the difference between pre- and post-agitation samples is not meaningful as the parameter estimate is only 0.0006.

In multivariate analysis of the full model under household conditions, no covariates were significantly associated with the difference between pre- and post-agitation samples (Table A-6).

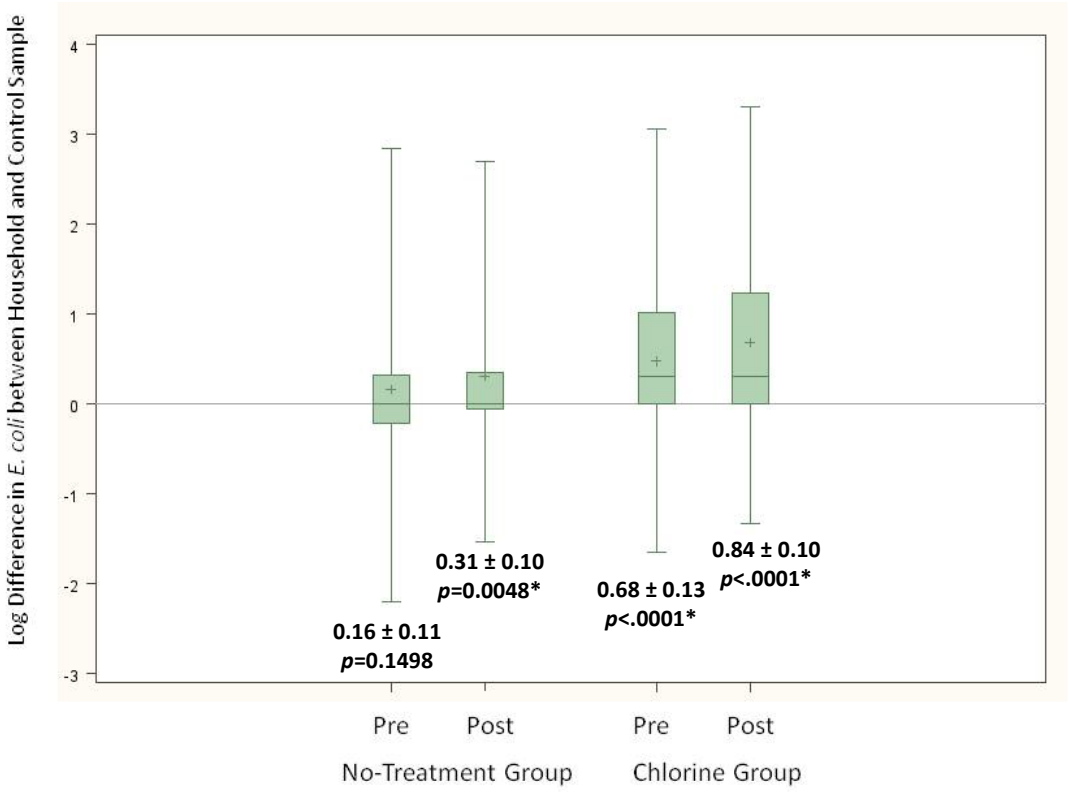
Analysis Four: What is the extent of in-home E. coli contamination in treated and untreated water?

Analysis Four sheds insight on in-home contamination, the difference in *E. coli* concentration between water stored under household and control conditions. This analysis helps to explain the changes in *E. coli* concentrations that occur during storage and the difference between effectiveness and efficacy of chlorine.

Figure 9 shows the difference between household and control samples, which represents in-home contamination, by treatment group and sample type. There is no evidence to suggest that in-home contamination occurs in the pre-agitation sample in the no-treatment group as the difference in *E. coli* concentration between water stored under household and control conditions is 0.16 ± 0.11 (p=0.1498). However, once agitated, the difference between household and control samples increases to 0.31 ±

0.10 (p=0.0048) indicating significant in-home contamination in the post-agitation sample of the no-treatment group. In the chlorine group, significant in-home contamination is apparent in both pre- and post-agitation samples. The mean log in-home contamination in the pre-agitation sample is 0.68 ± 0.13 (p<0.0001) and 0.84 ± 0.10 in the post-agitation sample (p<0.0001).

Figure 9: In-Home Contamination by Treatment Group and Sample Type. Reported p-value tests the null hypothesis that there is no difference between control and household samples.



Untreated water shows increased odds of in-home contamination when it is from a low turbidity or low contamination source. However, treated water shows the opposite effect. In the chlorine group, the odds of in-home contamination of highly contaminated water is 5.7 (95% CI: 1.6 to 20.4) times the odds of in-home

contamination of low-turbidity water in the pre-agitation sample (two-sided p=0.0053). In the post-agitation sample, the odds of in-home contamination of highly contaminated water is 2.7 (95% CI: 0.8 to 9.1) times the odds of in-home contamination of water from low contamination sources (two sided p=0.0958). This relationship; however, is not significant.

Similar results are observed when examining the effect of turbidity on chlorinated samples. The odds of in-home contamination of high turbidity water is 6.8 (95% CI: 2.3 to 19.8) times the odds of in-home contamination of low turbidity water in the pre-agitation sample (two sided p=0.0003). In the post-agitation sample, the odds of in-home contamination of high turbidity water is 2.7 (95% CI: 1.0 to 7.2) times the odds of in-home contamination of low turbidity water (two sided p=0.0498).

In both the pre- and post-agitation samples, treatment group (no-treatment or chlorine) is not associated with whether or not the water experienced in-home contamination (p=0.1770 in pre-agitation samples; p=0.3319 in post-agitation samples).

Levels of free chlorine in containers are not statistically different between household and control containers (p=0.7093) (Table 15), nor are they different when stratified by turbidity level (p=0.9632) and source contamination level (p=0.5265).

Table 15: Comparison of Free Chlorine Level in Control and Household Containers. Reported *p*-value tests the null hypothesis that there is no difference between control and household samples.

Sample Type	N	Mean	Std Err	Min	Max
Control	**63**	**0.92**	**0.22**	**0**	**10.6**
Household	**76**	**0.79**	**0.25**	**0**	**16.2**
p=0.7093					

There is, however, a statistically significant difference in the amount of contamination in containers after storage under household and control conditions in the chlorine group (p=0.0197) (Table 16). The arithmetic mean *E. coli* concentration (MPN/100mL) in the control is 10.7 ± 6.4 while in the household is 52.5 ± 16.4.

Table 16: Comparison of *E. coli* Contamination in Control and Household Containers in the Chlorine Group. Reported *p*-value tests the null hypothesis that there is no difference between control and household samples.

Sample Type	N	Mean	Std Err	Min	Max
Control	**72**	**10.7**	**6.4**	**0**	**456.9**
Household	**73**	**52.5**	**16.4**	**0**	**829.7**
p=0.0197*					

This difference in contamination of water stored under household and control conditions demonstrates the difference between efficacy—how well chlorine works under controlled conditions—and effectiveness—how well chlorine works under household conditions.

DISCUSSION:

This goal of this study was to examine the influence of household storage on *E. coli* concentrations in stored drinking water in northern, coastal Ecuador. We visited seven villages and gained the participation of 145 households. Forty-six percent of households participating in the study did not use any form of treatment for their drinking water. The remaining fifty-four percent treated their water with chlorine.

This study provides a follow-up to a previous study by Levy *et al.* (2008) in which significant log reductions in *E. coli* concentration were observed in water stored under household and control conditions. A primary motivation for the study presented in this paper is to determine if the reduction in contamination observed by Levy *et al.* (2008) represented a true hazard reduction or if the observed reduction is the result of settling of viable microorganisms to the bottom of storage containers. The aims of this study were to examine:

(1) Changes in *E. coli* concentration during storage of untreated water under household and control conditions

(2) The impact of agitation of containers on *E. coli* concentrations, in order to understand whether previously observed reductions were due to settling or die-off

(3) The impact of chlorination on the results observed in aims (1) and (2), above

(4) The extent of in-home *E. coli* contamination in treated and untreated water.

Through analysis Aims One and Two we can determine whether observed reductions are the result of die-off or settling out of *E. coli* and examine the factors that

contribute to the fate of *E. coli* during storage of untreated water. Aim Three examines the effect of chlorine on changes in *E. coli* concentration during storage and before and after agitation of storage containers. The results of analyses of chlorinated water are compared to results from untreated water. Lastly, given the differences in *E. coli* concentration between household and control conditions after storage, Aim Four examines the effects of source water characteristics on in-home contamination during storage. Table 17 summarizes the results of each aim.

Table 17: Summary of the Results. The main results of each aim are shown along with stratified analyses of turbidity and source contamination. Conclusions for each aim are in the rightmost column. When applicable, tables are referenced where the quantitative results can be viewed.

			Main Result	Turbidity	Source Contamination	Conclusions
No-Treatment Group	Control	Aim One	Significant reduction between source and stored water (Table 3)	Significant reduction in high turbidity (Table 4)	Significant reduction in high source contamination (Table 6)	Evidence suggest that die-off occurs under control conditions, even with no treatment.
		Aim Two	No significant difference between pre- and post-agitation (Table 3)	No difference in effect between high and low (Table 5)	No difference in effect between high and low (Table 7)	There is no evidence to suggest that settling of bacteria occurs under control conditions.
	Household	Aim One	No significant changes between source and stored water (Table 3)	Significant reduction in high turbidity (Table 4)	Significant reduction in high source contamination (Table 6)	Significant reduction in contamination during storage is observed in water from high turbidity and high contamination sources. In aggregate, no significant change occurs.
		Aim Two	Post-agitation significantly greater than pre-agitation (Table 3)	No difference in effect between high and low (Table 5)	No difference in effect between high and low (Table 7)	There is evidence to suggest that significant settling of viable bacteria occurs under household conditions.
	Household vs. Control	Aim Four	Significant in-home contamination only in post-agitation (Table 3)	In-home contamination less likely in high turbidity	In-home contamination less likely in high source contamination	This finding supports the literature: Highly contaminated and turbid sources are less likely to experience in-home contamination.
Chlorine Group	Control	Aim Three	Significant reduction between source and stored water (Table 11)	--	--	A significant log reduction during storage is expected with the use of chlorine. The reduction is greater under control conditions.
		Aim Three	Post-agitation significantly greater than pre-agitation (Table 11)	Significant difference only in high turbidity (Table 12)	Significant difference in only high source contamination (Table 13)	The difference between pre- and post- occurs only in high turbidity and high contamination waters. Turbidity particles may block the action of chlorine.
	Household	Aim Three	Significant reduction between source and stored water (Table 11)	--	--	A significant log reduction during storage is expected with the use of chlorine.
		Aim Three	No significant difference between pre- and post-agitation (Table 11)	No significant difference in effect between high and low (Table 12)	No significant difference in effect between high and low (Table 13)	There is no evidence to suggest that settling occurs in the household. This may be because bacteria on the bottom of containers come into greater contact with chlorine during water usage.
	Household vs. Control	Aim Four	Significant in-home contamination in pre and post (Table 11)	In-home contamination more likely in high turbidity	In-home contamination more likely in high source contamination	This finding is not consistent with the literature. However, households that choose low turbidity sources and effectively dose with chlorine may also take other measures to prevent in-home contamination
Overall Effect			Larger reductions are observed under control conditions.	Turbidity increases log reductions (no-treatment group) but has little impact on differences between pre- and post- agitation	Source contamination increases (no treatment group) log reductions but has little impact on differences between pre- and post-agitation	There is a large difference in effectiveness (under household conditions) and efficacy (under control conditions) of reductions in *E. coli* concentration during storage.

Aim 1: Examine changes in E. coli concentration during storage of untreated water under control and household conditions.

To determine if changes in *E. coli* concentration occurred during storage, the *E. coli* concentration in the stored water samples was subtracted from the corresponding source water samples. If the log difference between stored and source water is equal to zero then we conclude that no change in the concentration of *E. coli* occurred during storage. If the log difference between stored and source water is greater than zero we conclude that the concentration of *E. coli* has decreased during storage. If the log difference between stored and source water is less than zero, then we conclude that *E. coli* concentration has increased during storage.

Untreated water stored under control conditions demonstrates significant log reductions in *E. coli* concentration over 24 hours (0.26 ± 0.09; p=0.0034). However, untreated water stored under household conditions does not demonstrate significant log reduction in *E. coli* concentration over the same period (0.12 ± 0.09; p=0.2086). In the study by Levy *et al.*, the mean log reduction over 24 hours is 0.18 ± 0.84 (p=0.05) in water stored under household conditions and 0.24 ± 0.80 (p=0.013) in water stored under control conditions. The results of the two studies are similar under control conditions but differ under household conditions.

Control Conditions:

Under control conditions, both studies observed significant log reductions from source to stored water and the reduction was of a similar magnitude. In this study, after

storage for 24 hours, untreated water is reduced from a geometric mean of 111 ± 38 *E. coli* MPN/100mL to 62 ± 47 *E. coli* MPN/100mL in the pre-agitation sample and 59 ± 55 *E. coli* MPN/100mL in the post-agitation sample. This represents a 44% (CI 27% to 80%) decrease in contamination from the source in the pre-agitation sample and a 47% (CI 23% to 95%) decrease in contamination from the source in the post-agitation sample. For reference, chlorine treatment under control conditions results in a 100% (CI 98.6% to 100.0%) decrease in *E. coli* concentration in the pre agitation sample and a 99.2% (CI 97.9% to 100.0%) decrease in the post-agitation sample over 24 hours. Given that under control conditions the post-agitation sample is not significantly different than the pre-agitation sample (0.002 ± 0.05 p=0.9614), there is strong evidence to suggest that, overall, die-off of *E. coli* occurs during storage under control conditions.

The die-off of *E. coli* during storage under control conditions is influenced by turbidity and initial source contamination level. Under control conditions in the no-treatment group, significant log reduction in contamination *is not* observed in samples from low turbidity and low contamination sources. However, significant reduction in contamination *is* observed from high turbidity and high contamination sources. This is expected, as cleaner water, in the absence of recontamination events, has little opportunity to experience significant changes in contamination. In the high contamination group, the geometric mean contamination at the source (*E. coli* MPN /100mL) is 647.5 ± 0.76 while the geometric mean in the low contamination group is 1.74 ± 3.12.

Other studies have observed decreases in microbial concentrations during storage. For example, water quality improved between source and POU for 16% of households in the studies by Vanderslice and Briscoe (1993). Other researchers have shown that water samples may become less contaminated after they are collected from highly contaminated sources because of die-off as bacteria compete for limited oxygen and nutrients in the water (Momba and Kaleni 2002). Tompkins *et al* found that overnight storage in earthenware containers considerably reduced bacterial numbers but contamination was still at elevated levels (1987).

Nonetheless, while the reduction in *E. coli* concentration of water stored under control conditions is significant, the *E. coli* concentration is still not within an acceptable range for drinking water standards, commonly believed to be less than 10 *E. coli* CFU/100mL in low resource settings. However, the observed reduction in *E. coli* concentrations reaffirms the results observed by Levy *et al.* and demonstrates that storing water for at least 24 hours under safe conditions, in this context, may reduce the hazard posed by fecal contamination of drinking water, even when no treatment method is used. This is true under control conditions; however, water stored under household conditions undergoes a markedly different effect.

Household Conditions:

Under household conditions, untreated, stored water does not experience a significant change in *E. coli* concentration from the source in the pre- or post-agitation samples. A 25% (CI 21% to 27%) *decrease* in the geometric mean from the source to stored water is observed in the pre-agitation samples and a 9% (-7% to 45%) *increase* in

the geometric mean is observed from the source to the post-agitation samples. In the study by *Levy et al.*, researchers observed significant log reductions in *E. coli* concentration in the home, as well as under control conditions. This may be attributable to the fact that in the study by Levy *et al.*, 22% of samples were taken from households that reported treating their water with chlorine or boiling. This variation in the treatment of water may be one reason why a significant log reduction in contamination under household conditions was observed and may also explain why the standard error calculations are much larger in the in study by Levy *et al*. However, since water stored under control conditions was not treated in the study by Levy *et al.* this does not explain the larger standard errors observed under control conditions. After removing treated water and corresponding control containers

Aim Two: To examine the impact of agitation of containers on E. coli concentrations, in order to understand whether the previously observed reductions were due to settling or die-off.

Without treatment, significant log differences between pre- and post-agitation samples were observed under household conditions but not under control conditions, suggesting that settling-out of viable bacteria occurs in the household whereas die-off occurs under control conditions. The opposite trend is observed for containers treated with chlorine.

Evidence for bacteria settling to the bottom of water storage containers is presented in previous studies. Roberts *et al.* demonstrated that when water stored for

six hours in the home was agitated coliform levels increased by 16% in improved buckets—with a lid, handle and spout—and 327% in unimproved buckets (2001).

Settling of *E. coli* in household storage containers is influenced by turbidity and source *E. coli* concentration. Water from highly contaminated and highly turbid sources is associated with a mean log reduction of 0.37 ± 0.11 (p=0.0024) in the household pre-agitation sample and 0.26 ± 0.08 (p=0.0037) in the household post-agitation sample. While contamination does increase in the post-agitation sample, it still represents a significant log reduction from the source. The difference between household pre-and post-agitation samples from highly contaminated and highly turbid sources is 0.09 ± 0.04 (p=0.0474). A similar trend is observed in highly turbid and contaminated water stored under control conditions. (Pre-agitation: 0.33 ± 0.13; p=0.0152)(Post-agitation: 0.29 ± 0.12; p=0.0193)(Difference between pre-and post-: 0.04 ± 0.04; p=0.2986) These results provide evidence to suggest that in highly contaminated and highly turbid water, significant die-off of *E. coil* occurs. Under household conditions, significant settling occurs in addition to significant die-off.

Untreated water from both low contamination and low turbidity sources demonstrates increased *E. coli* concentration during storage and a significant difference between pre-and post-agitation samples. Under household conditions, a significant mean log increase of .57 ± 0.19 (p=0.0070) in the post-agitation sample is observed. The pre-agitation sample also demonstrates an increase *E. coli* concentration but this increase is not significant. This finding is consistent with the literature: water from less

contaminated sources is more likely to experience significant in-home contamination (Wright et al. 2004). Interestingly, there is a difference between the pre- and the post-agitation samples in the household even in water from low turbidity and low contamination sources (0.29 ± 0.13, p=0.0337) which is likely due to in-home contamination events, as water stored under control conditions did not show this effect. This finding demonstrates the importance of sampling from containers after agitation, even when the water is of low turbidity and low *E. coli* concentration as the pre- and post-agitation results differ significantly. The finding also shows the importance of storing water under control conditions in order to determine if in-home contamination occurs.

In multivariate analysis of untreated water stored under household conditions, household members having reported washing their container prior to filling compared to households that did not report washing their containers is associated with a 0.27 ± 0.12 (p=0.0249) log decrease in the difference between pre- and post-agitation samples. This signifies that in recently-cleaned containers, pre- and post-agitation *E. coli* concentrations are more similar, possibly because cleaning reduces the presence of biofilm.

Biofilm is implicated as a possible cause of increased microbiological contamination during storage. Bacterial build-up on the inner walls of water-storage containers contains microorganisms that may break loose from the sides during filling and agitation. The bacteria can form particulate suspensions that are home to

significant numbers of viable bacteria (Jagals et al. 2003). A study by Jagals *et al*. in 2003 observed that total coliforms and *E. coli,* however, were not directly associated with levels of biofilm in containers and were most likely introduced intermittently from the domestic environment**.** The authors also note that because *E. coli* only occurred intermittently and in low numbers compared to total coliforms and *C. perfringens*, *E. coli* was not effectively supported by the biofilm and probably died off after a while in the container water (Jagals et al. 2003).

Under household conditions in univariate analysis, the presence of visible contamination (an indication of biofilm) in the container at the time of collection compared to no visible contamination is associated with a 0.38 ± 0.18 log decrease in the difference between pre- and post-agitation samples (p=0.0343). This is the opposite effect of what is observed in water that is not treated with chlorine. A possible explanation is that biofilm on the sides of the containers contributes to increased contamination of the water column so that there is not a significant difference between the contamination on the bottom of the container and the contamination at the top of the water column. This may also be because the biofilm blocks the action of the chlorine in the same way it blocks the chlorine at the bottom of the storage containers.

In summary, without treatment, significant log differences between pre- and post-agitation samples are observed under household conditions, but not under control conditions. This suggests that viable bacteria enter into household containers (possibly through unwashed or uncovered containers) and settle to the bottom during storage.

Under control conditions, where re-contamination events do not occur, die-off of bacteria takes place during storage. The opposite trend, however, is observed for containers treated with chlorine.

Aim Three: To determine how E. coli concentration changes during storage of chlorinated water and the impact of agitation on the difference between pre- and post-agitation samples from chlorinated water.

Chlorinated water experienced significant log reduction in *E. coli* concentration under household (pre-agitation: 1.72 ± 0.15; post-agitation: 1.65 ± 0.14) and control (pre-agitation: 2.13 ± 0.15; post-agitation: 2.40 ± 0.15) conditions (all *p*-values < 0.0001) Unlike samples from untreated water, pre- and post-agitation samples of chlorinated water are similar to one another under household conditions, suggesting that die-off of *E. coli* occurs. Chlorination appears to effectively address the biofilm and container cleanliness issues observed with untreated water. Under control conditions, the significant difference between pre- and post-agitation samples provides evidence to suggest that bacteria that settle to the bottom of storage container are not effectively disinfected by chlorine.

The effect of die-off of *E. coli* under household conditions and settling of *E. coli* under control conditions does not change when the differences between pre- and post-agitation samples are stratified by turbidity level and source contamination level.

While there is evidence for die-off of *E. coli* under household conditions, this does not mean that the *E. coli* concentration in the household lower than the

concentration under control conditions. *E. coli* concentrations are significantly greater in containers stored under household conditions (p=0.0197). The arithmetic mean *E. coli* MPN/100mL is 10.7 ± 6.4 when stored under control conditions and 52.5 ± 16.4 under household conditions (Table 17). The difference between effectiveness and efficacy of chlorine may be due to the fact that household containers are subject to in-home contamination.

Mixing of the water is another important difference between household and control containers. The household containers are used by the household over the course of 24 hours and are thus subject to more agitation, whereas the control containers were left untouched in the lab. This in-household mixing may allow particles embedded in sediment to come into contact with free chlorine and become inactivated. Yet, it cannot be the act of mixing alone that results in there not being a difference between pre- and post-agitation samples under household conditions because under household conditions in the no-treatment group there is a significant difference between pre- and post-agitation samples.

Through agitation during daily use, bacteria inside household containers may have a better chance of coming into contact with chlorine. Therefore, household containers should have less free chlorine as it should be depleted through the disinfection on in-home contamination and newly suspended particles. Despite this, WHO recommended chlorine residual levels are met in both household and control containers and the levels of free chlorine in containers are not statistically different

between household and control containers (p=0.7093) (Table 16) nor are they different when stratified by turbidity level (p=0.9632) and source contamination level (p=0.5265).

One possible explanation of increased contamination in the post-agitation sample under control conditions is that sediment may block the action of chlorine, preventing it from coming in contact with bacteria. Scanning electron micrographs show that bacteria in turbid waters can be embedded within turbidity particles and/or coated with amorphous materials (Lechevallier et al. 1981). If particles containing bacteria settle to the bottom of containers they may be less likely to come into contact with chlorine. A bacterium within the water column that is not attached to sediment is thus more likely to be disinfected than a bacterium attached to sediment. In experimentation, Lechevallier *et al*. (1981) also found that chlorinating turbid water increased the number of standard plate count bacteria by as much as five times. The researchers offer the explanation that the increase in plate count is the result of the physical separation of cells from particles. In other words, chlorine unmasks bacteria within the sediment. It is possible that agitating the container allows chlorine to come into greater contact sediment particles that before were buried on the bottom of containers, unmasking the bacteria in higher numbers in the post-agitation sample. In further experiments, Lechevallier *et al.* found that increasing the turbidity of water samples from 1 to 10 NTU, while keeping chlorine dose constant, resulted in an eightfold decrease in the efficiency of disinfection (Lechevallier et al. 1981). Particle-associated bacteria generally settle faster and may have different mortality rates than

bacteria that are not attached to particles (Faust et al. 1975; Burkhardt et al. 2000). Laboratory studies have documented reduced mortality due to predation or environmental exposures for *E. coli* and *Enterococcus* bacteria when attached to particles in the water (Jin et al. 2004; Davies and Bavor 2000).

The interaction of turbidity and chlorination may explain the difference between pre-and post agitation samples under control conditions. *E. coli* attached to sediment settles to the bottom of containers where it is shielded from the action of chlorine. Under control conditions, there is no agitation during storage that would cause *E. coli* to become dislodged from turbidity particles or come into increased contact with chlorine. The same effect may be taking place under household conditions but recontamination events populate the upper water column with *E. coli*, making the pre- and post-agitation samples more similar. The plausibility of this hypothesis is increased by the fact that household samples are significantly more contaminated than control samples even with the use of chlorine.

The use of a safe container, defined as a container with an opening less than 8 cm wide, is associated with a 0.44 $\pm$ 0.17 log decrease in the difference between the pre- and post-agitation samples (p=0.0113). This is expected as safer containers may allow fewer bacteria embedded in settle-able particles to enter during storage, resulting in more similar pre- and post-agitation samples. However, people in the chlorine group who had visible contamination on their containers were 3.2 times as likely to use safe

containers (two-sided p=0.0225). This may be due to the fact that it is more difficult to wash inside a container with a smaller opening.

In the control group, samples collected during Visit Two compared to Visit One are associated with a 0.43 ± 0.14 log increase in the difference between pre- and post-agitation samples (p=0.0034). This is likely attributable to the fact that in Visit Two, 20L of water was used in the control containers—at least twice as much water as was used in Visit One. The greater volume may allow settled particles to be less disturbed during the collection of the pre-agitation sample. Furthermore, water sources in Visit Two were more likely to be from more highly contaminated and turbid sources. Accordingly, samples collected from a river or stream source compared to a well, tap or rain water source are associated with a 0.34 ± 0.14 log increase in the difference between pre- and post-agitation samples (p=0.0179). Higher water volume, higher turbidity and higher contamination level may be the driving forces behind the significant difference between pre- and post-agitation samples under control conditions. However, the volume of household containers is not associated with the difference between pre- and post-agitation samples (p=0.6692) (household water volume data are only available in the chlorine group).

Aim Four: To examine the extent of in-home E. coli contamination in treated and untreated water?

In the fourth research question, we examine the influence of in-home contamination, defined as the difference between corresponding household and control *E. coli* concentrations, during storage.

Chlorine Group

In the chlorine group, there is significant in-home contamination in the pre- and post-agitation samples (Figure 9). The mean log in-home contamination in the pre-agitation sample is 0.68 ± 0.13 (p<0.0001) and 0.84 ± 0.10 in the post-agitation sample (p<0.0001). As hypothesized in the analysis of Aim Three, significant in-home contamination events in the chlorine group may make the pre-agitation sample in the household more similar to the post-agitation sample. (In the no-treatment group there is not significant in-home contamination apparent in the pre-agitation samples and accordingly, there is a significant difference between the pre- and post-agitation samples in the household.) Significant in-home contamination in the chlorine group should increase chlorine demand in household samples; however, there is no evidence to suggest that chlorine residual level differs between household and control conditions (Table 16). This could be due a lack of sensitivity of the colorimeter.

The analysis of the log difference between household and control samples in the chlorine group demonstrates the difference between efficacy and effectiveness of chlorine. The wide confidence intervals in the log reductions observed under

households and control conditions are indicative of the range of chlorine dosages and concentrations and the variety of source water *E. coli* concentration and turbidity levels.

In the Chlorine Group, the odds of in-home contamination of highly contaminated water is 5.7 (1.6 to 20.4) times the odds of in-home contamination of low-turbidity water in the pre-agitation sample (two-sided p=0.0053). While not significant, the odds of in-home contamination in the post-agitation samples from highly contaminated water is 2.7 (0.8 to 9.1) times the odds of in-home contamination of water from low contamination sources (two sided p=0.0958).

Similar results are observed when examining the effect of turbidity on chlorinated samples. The odds of in-home contamination of high turbidity water is 6.8 (2.3 to 19.8) times the odds of in-home contamination of low turbidity water in the pre-agitation sample (two sided p=0.0003). In the post-agitation sample, the odds of in-home contamination of high turbidity water is 2.7 (1.0 to 7.2) times the odds of in-home contamination of low turbidity water (two sided p=0.0498). These results are the opposite of what is observed in water that is not treated and the opposite of what the literature suggests.

Evidence from previous studies shows that water from highly turbid and contaminated sources is less prone to in-home contamination events. However, in this setting, in-home contamination of chlorinated water is much more likely in water with higher concentrations of *E. coli* and higher turbidity levels. This may be due to household level factors. People who choose low turbidity sources and effectively dose

their water with chlorine (resulting in lower *E. coli* concentrations) may also take other measures to prevent in-home contamination.

No-Treatment Group

There is no evidence of in-home contamination in the pre-agitation samples in the no-treatment group. However, in the post-agitation samples, the odds of in-home contamination of water from highly contaminated sources is 0.32 (0.11 to 0.94) times the odds of in-home contamination of water from less contaminated sources (two sided p=0.0406). The odds of in home-contamination of water from high turbidity sources is 0.32 (0.14 to 1.05) times the odds of in-home contamination of water from low turbidity sources in the post-agitation samples (two sided p=0.0663). These results are consistent with the literature that demonstrates that highly contaminated water experiences less in-home contamination than cleaner water.

Attention to the differential effects of in-home contamination on *E. coli* concentration in stored water is important when considering factors influencing changes in *E. coli* concentration during storage and the difference between samples taken before and after agitation of storage containers.

CONCLUSION:

Currently, 16% of the world's population, 884 million people, rely on unimproved drinking water sources (WHO/UNICEF 2010). Given the great need for improved water quality, assessment of drinking water from unimproved sources is necessary in order to design appropriate interventions. Successful interventions must take into account local practices, source water quality and storage characteristics in order to ensure sustainability and effectiveness. The results of this study increase our understanding of how *E. coli* concentrations respond given a set of source water characteristics, treatment and storage practices and can be used to inform improved water treatment, storage and sampling techniques.

Die-off and settling of *E. coli* during storage is influenced by storage conditions (household or control), turbidity and initial source contamination. Without treatment, significant reduction in *E. coli* concentration is observed during storage under control, but not household conditions. Differences between pre- and post-agitation samples are observed under household conditions but not under control conditions, suggesting that settling of viable bacteria occurs in the household whereas die-off occurs under control conditions. This may be due to biofilms and in-home contamination of the household container. With chlorine treatment, significant log reduction of *E. coli* concentration is observed under household and control conditions. Log differences between pre- and post-agitation samples are observed under control conditions, but not household conditions, suggesting die-off of bacteria under household conditions but settling of

viable bacteria under control conditions. The opposite trend observed for water treated with chlorine may be explained by the interaction of chlorine and turbidity.

The distinction between die-off and settling of microorganisms during storage is important. Organisms that settle out could become re-suspended, consumed, and lead to infection. Die-off, however, signifies loss of pathogen infectivity. Storage of water under control conditions allows for the quantification of in-home contamination. The results of this study suggest that in-home contamination is a significant contributor to the changes in *E. coli* concentration that occur during storage. My research provides increased understanding of the fate of *E. coli* under a variety of source, storage, and treatment conditions and demonstrates that storing water for 24 hours under safe conditions (i.e. control), in certain contexts, may reduce hazards posed by fecal contamination of drinking water. However, in-home contamination still jeopardizes human health.

Determining factors influencing changes in microbial concentrations during storage is an important step in reducing waterborne disease. Future implications may include recommendations on reducing microbiological contamination of water through storage prior to consumption when more effective treatment options are not available or acceptable. Results may also be used to inform research about the efficacy and effectiveness of chlorine in turbid waters, given the differences in *E. coli* concentration observed under household and control conditions and between pre- and post-agitation samples.

The results of this study provide opportunities for future studies. The effect of settling of viable bacteria to the bottom of storage containers in both treated and untreated water merits further investigation. Different types of pathogens may be associated with settle-able particles. A study regarding the types of enteric pathogens in both chlorinated and untreated water would shed further insight on the risk posed by re-suspension of microorganisms. Furthermore, our study only examines the effect of water storage over 24 hours. Understanding how *E. coli* concentrations change over shorter and longer time periods is of interests as different cultures and families consume water at different rates. Also of relevance, would be an examination of how container size influences the difference between pre- and post-agitation samples.

Our research findings have implications for water sampling protocols as well. We demonstrate that sampling from water storage containers without first agitating the containers can yield erroneous estimates of the concentration of *E. coli*. Even samples from chlorinated water with low turbidity and low initial source contamination have differing *E. coli* concentrations before and after agitation of storage containers. The differences between samples are more dramatic when sampling from highly turbid and contaminated sources. Currently, there exists no methodological standard for sampling from water storage containers. This study demonstrates the necessity of standardized water quality sampling protocols, calling for agitation of storage containers prior to sampling.

In conclusion, *E. coli* concentrations in stored water in northern, coastal Ecuador are influenced by a variety of factors including storage conditions (household vs. control), chlorine residual, and turbidity and *E. coli* concentration in source waters. When planning research projects, evaluating water treatment technologies and interpreting results, future water quality studies should consider the influence of in-home contamination, settling and die-off on the concentration of microorganisms in stored water.

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APPENDIX:

Table A-1: List of All Possible Covariates Used in Univariate Analysis

Chlorine concentration at time of dosing	Source Contamination: *E. coli* MPN/100mL	Visit (1,2)	Washed container before filling(Y/N)
Free Cl (pre-agitation)	Container volume	pH	Container stored on ground (Y/N)
Free Cl (post-agitation)	Safe container (Y/N)	Conductivity (mV)	Covered container(Y/N)
Total Cl (pre-agitation)	Safe extraction method(Y/N)	Temperature (°C)	Water transferred between containers during storage (Y/N)
Total Cl (post-agitation)	Visible contamination in container (Y/N)	River source (Y/N)	
Turbidity (NTU)		People in river Y/N	
		Has water been used (Y/N)	

Table A-2: The Effect of Covariates on the Log Difference between Pre- and Post-Agitation Household Samples: Full Model. The reported p-values test the null hypothesis that the parameter estimate is zero.

	Unadjusted			Adjusted		
Parameter	**Parameter Estimate**	**Standard Error**	***p*-value**	**Parameter Estimate**	**Standard Error**	***p*-value**
Intercept	-	-	-	**20.50**	**10.28**	**0.0533**
MPN E. coli in Source	**-0.0002**	**.0001**	**0.1087**	**-0.0002**	**0.0003**	**0.4582**
Turbidity of Source	**-0.004**	**0.002**	**0.1301**	**-0.0005**	**0.004**	**0.8946**
Safe Container	**-0.05**	**0.11**	**0.6248**	**-0.18**	**0.19**	**0.3686**
Safe Extraction	**-0.11**	**0.11**	**0.3012**	**0.008**	**0.19**	**0.9680**
Visible Contamination	**-0.04**	**0.11**	**0.7091**	**-0.009**	**0.14**	**0.9490**
Covered Container	**0.19**	**0.10**	**0.0717**	**0.19**	**0.12**	**0.1120**
Container on Floor	**-0.05**	**0.13**	**0.7120**	**-0.14**	**0.19**	**0.4861**
People in River	**0.14**	**0.16**	**0.3635**	**-0.08**	**0.24**	**0.7528**
Conductivity of Source	**-0.0002**	**0.0009**	**0.8351**	**-0.06**	**0.03**	**0.0350***
Temperature of Source	**0.06**	**0.03**	**0.0417***	**0.08**	**0.05**	**0.1047**
pH of Source	**0.03**	**0.05**	**0.5913**	**-3.00**	**1.41**	**0.0402***
River Source	**-0.17**	**0.10**	**0.1089**	**-0.22**	**0.29**	**0.4477**
Visit Number	**-0.13**	**0.10**	**0.2075**	**-0.21**	**0.23**	**0.3580**
Washed Container	**0.13**	**0.11**	**0.1524**	**-0.25**	**0.14**	**0.0776**
Water Transferred	**-0.14**	**0.17**	**0.3854**	**-0.02**	**0.31**	**0.9467**
Water used	**-0.18**	**0.19**	**0.3663**	**-0.12**	**0.27**	**0.6622**

Table A-3: The Effect of Covariates on the Log Difference between Pre- and Post-Agitation Control Samples in the No-Treatment Group. The reported p-values test the null hypothesis that the parameter estimate is zero.

	Unadjusted			Adjusted		
Parameter	**Parameter Estimate**	**Standard Error**	***p*-value**	**Parameter Estimate**	**Standard Error**	***p*-value**
Intercept	-	-	-	**4.73**	**9.89**	**0.6340**
E. coli count in source	**0.0002**	**0.0001**	**0.1201**	**0.0001**	**0.0002**	**0.4901**
Turbidity	**0.001**	**0.002**	**0.5462**	**0.0002**	**0.003**	**0.9295**
People in river (Y/N)	**-0.11**	**0.36**	**0.2632**	**-0.16**	**0.20**	**0.4326**
Conductivity	**-0.0006**	**0.0008**	**0.4949**	**-0.01**	**0.02**	**0.6775**
Temperature	**-0.04**	**0.03**	**0.1388**	**-0.02**	**0.04**	**0.5658**
pH	**0.0008**	**0.05**	**0.9856**	**-0.54**	**1.31**	**0.6828**
River source (Y/N)	**0.11**	**0.09**	**0.2286**	**-0.15**	**0.24**	**0.5192**
Visit (1, 2)	**0.04**	**0.09**	**0.7001**	**-0.06**	**0.21**	**0.7883**

Table A-4: Comparison of Chlorine Arms. Reported *p*-values test the null hypothesis that the difference between the two groups is zero.

A:

	Original Cl concentration (%)	Volume of Water Dosed (L)	Volume of Cl used to dose (mL)	Concentration of Cl in water (mg/L)	Free Cl Residual HH1 (mg/L)	Free Cl Residual CN1 (mg/L)
Local	2.2 ± 0.1	32.5 ± 9.0	4.4 ± 1.2	0.4 ± 0.07	1.3 ± 0.5	1.6 ± 0.4
Commerical	4.5 ± 0.0	16.3 ± 2.8	0.79 ± 0.1	0.2 ± 0.02	0.2 ± 0.03	0.3 ± 0.09
p-value	<.0001*	0.094	0.0051*	0.0125*	0.0251*	0.0038*

B:

	Mean Log reduction HH0	Mean Log reduction HH1	Mean Log reduction CN0	Mean Log reduction CN1	Dif. Btw pre and post HH	Dif. Btw pre and post CN
Local	1.6 ± 0.2	1.7 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	-0.06 ± 0.1	0.1 ± 0.05
Commerical	1.8 ± 0.2	1.5 ± 0.2	2.5 ± 0.2	2.1 ± 0.2	0.2 ± 0.1	0.4 ± 0.1
p-value	0.5605	0.6544	0.4573	0.8333	0.1972	0.0795

Table A-5: The Effect of Covariates on the Log Difference between Pre- and Post-Agitation Control Samples: Chlorine Group. The reported p-values test the null hypothesis that the parameter estimate is zero.

Parameter	Parameter Estimate	Standard Error	*p*-value	Parameter Estimate	Standard Error	*p*-value
Intercept	-	-	-	-3.12	5.01	0.5363
Turbidity	0.01	0.003	0.0030*	0.01	0.01	0.0286*
Conductivity	0.0002	0.001	0.9023	-0.003	0.008	0.6846
pH	-0.003	0.07	0.9648	0.15	0.49	0.7551
Temperature	-0.10	0.05	0.0787	0.04	0.10	0.7046
Free Cl in post sample	-0.05	0.03	0.1305	-0.04	0.04	0.2996
E. coli in source sample	0.0003	0.0002	0.1599	-0.0006	0.0003	0.0503*
People in River (Y/N)	0.37	0.23	0.1155	0.53	0.27	0.0565
River Source (Y/N)	0.34	0.14	0.0179*	0.38	0.30	0.2156
Visit (1 or 2)	0.43	0.14	0.0034*	0.39	0.22	0.0903

Table A-6: The Effect of Covariates on Log Difference between Pre- and Post- Agitation Household Samples: Chlorine Group. The reported p-values test the null hypothesis that the parameter estimate is zero.

	Unadjusted			Adjusted		
Parameter	**Parameter Estimate**	**Standard Error**	***p*-value**	**Parameter Estimate**	**Standard Error**	***p*-value**
Intercept	-	-	-	-6.79	6.90	0.3317
E. coli count in source	0.0001	0.0002	0.5078	-0.00007	0.0005	0.8996
Free Cl in post sample	-0.03	0.04	0.5118	-0.02	0.05	0.6409
People in River (Y/N)	0.36	0.29	0.2249	0.63	0.43	0.1544
Volume of HH container	-0.001	0.002	0.6692	-0.0008	0.004	0.8570
Total Colifroms in Source	0.0003	0.0003	0.3716	0.0005	0.0010	0.6098
Visible Contamination	-0.38	0.18	0.0343*	-0.07	0.27	0.8006
River Source (Y/N)	-0.11	0.19	0.5558	-0.22	0.83	0.7922
Turbidity	0.005	0.005	0.2771	0.01	0.01	0.1062
Temperature	-0.03	0.07	0.6945	0.16	0.20	0.4220
Safe Extraction	-0.27	0.17	0.1236	0.05	0.52	0.9262
Safe container	-0.44	0.17	0.0113*	-0.38	0.56	0.4950
Washed Container	-0.23	0.21	0.2709	-0.11	0.31	0.7227
Container stored on floor	0.04	0.20	0.8257	-0.05	0.35	0.8894
Container Covered	-0.05	0.19	0.8015	-0.09	0.29	0.7717
Container Transferred (n=8)	1.27	0.41	0.0030*	1.21	0.10	0.2236
pH	-0.0006	0.09	0.9948	0.10	0.21	0.6427
Visit	0.07	0.18	0.7051	0.03	0.34	0.9202
Conductivity	0.0005	0.002	0.7826	-0.0004	0.001	0.7999