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B.S.
University of South Florida
2012

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

Quantification of *Escherichia coli* Exposure from Drinking Water and Produce in Low-Income Urban Neighborhoods in Accra, Ghana: Implications for Children's Health

By Melissa Sizemore

Urban environments with overcrowded living situations, inadequate access to sanitation, and dysfunctional water and drainage systems have complex, inter-related webs of fecal-oral transmission routes. Poor, urban residents bear the brunt of these exposures, which manifest as higher incidence rates of diarrhea, especially among children. To compare the risks from different fecal-oral transmission routes, a Quantitative Microbial Risk Assessment (QMRA) was applied to evaluate the risks of exposure to fecal contamination for three drinking water scenarios (consumption of sachet, stored household, or municipal tap water) across three child age groups (0-1, 1-2, and 2-5 year olds), and two food scenarios (children 2-5 or 5-12 years old consuming a street food side salad) in Accra, Ghana. The exposure assessment was based on the concentration of the fecal indicator organism *Escherichia coli* (*E. coli*) detected in the three drinking water sources and four produce items that comprise a typical street food side salad (lettuce, cabbage, tomato, and spring onion). Dose estimates from the QMRA were then used to compare the drinking water and food exposure scenarios and identify which represented the greatest microbial risk to children (aged 2-5 years). Consumption of stored household water resulted in the largest average weekly dose estimate (3.61 $\log_{10}$ Colony Forming Units (CFU) *E. coli*), while consumption of sachet water resulted in the lowest average weekly dose estimate (1.62 $\log_{10}$ CFU). Consumption of a street food side salad produced the second largest average weekly dose estimate (3.28 $\log_{10}$ CFU). Although consumption of a street food side salad did not produce the highest dose estimates, 100 percent of produce items collected from four study communities had quantifiable *E. coli* concentrations, indicating the potential for exposure to fecal contamination from consuming raw produce items. Educating residents on safe water storage practices and the importance of disinfecting drinking water and produce prior to consumption will lower these exposures; however, improving the intermittent municipal water supply, which drives water storage practices, and developing safer, alternative sources of irrigation water will best mitigate the health risks associated with these exposures.

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ABBREVIATIONS

WASH	Water, Sanitation and Hygiene
WHO	World Health Organization
AMA	Accra Metropolitan Area
QMRA	Quantitative Microbial Risk Assessment
WRI	Water Research Institute
LOD	Limit of Detection
CFU	Colony Forming Units
GSB	Ghana Standards Board
UNICEF	United Nation's Children's Fund
DALY	Disability Adjusted Life-Years
DHS	Demographic Health Survey
SFP	School Feeding Program
GWC	Ghana Water Company Ltd.
POU	Point of Use
CDC	United States Centers for Disease Control
E. coli	*Escherichia coli*

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I. BACKGROUND

A. Global Childhood Diarrheal Disease Burden

Across the globe, 8.8 million children die before their fifth birthday every year [1]. These deaths are not equally distributed: over half of all deaths in children under the age of five occur in sub-Saharan Africa [1-3]. Diarrhea represents the second leading cause of childhood mortality and is responsible for 11 percent of all childhood deaths [1, 2]. In sub-Saharan Africa, however, diarrhea is more pervasive and is responsible for nearly 16 percent of all childhood deaths [4]. Nearly 90 percent of all these deaths can be attributed to unsafe water, inadequate sanitation and poor hygiene practices, highlighting the importance of water, sanitation and hygiene (WASH) interventions on improving health outcomes [5].

The Millennium Development Goals have declared reducing the under-five child mortality rate by two-thirds and halving the proportion of the population without sustainable access to safe drinking water and basic sanitation global priorities; however, to date, only the water target has been achieved [3]. Access to sanitation among the world's poor has improved, yet 1.1 billion people still practice open defecation globally [2]. Under-five childhood mortality rates have decreased from 90 to 48 deaths per 1,000 live births, but still falls short of the proposed two-thirds reduction [3]. These goals are inextricably linked – a reduction in childhood mortality requires reductions in the global burden of diarrhea, which is dependent on improving access and use of water and sanitation infrastructures.

Investment in WASH interventions is considered one of the most cost-effective strategies for reducing childhood mortality: it is estimated that full household coverage with water and sanitation infrastructure could reduce childhood mortality by 25 deaths per 1,000 live births, equating to a total reduction of 2.2 million child deaths per year [6]. Although the link between water, sanitation and health are well understood, improvements in WASH infrastructures have been slow to fruition. As of 2013, only 30 percent of the population in sub-Saharan Africa have access to improved sanitation facilities, with an estimated 26 percent still practicing open defecation, and 44 percent using unimproved sanitation facilities [7]. Lack of access to improved sanitation facilities – defined by the World Health Organization (WHO) as a flush toilet, piped sewer system, septic tank, pour to flush pit latrine, ventilated improved pit latrine, or composting toilet – leads inevitably to fecal contamination of the environment, ultimately increasing the transmission, exposure, and risk of illness from enteric pathogens

B. Fecal-Oral Exposure Pathways

Gastrointestinal pathogens are spread primarily through fecal-oral exposure routes. The major fecal pathways can be split into two domains: public and private. Exposure through public domains are represented via contact with surface water, use of wastewater irrigation for food crops, contact with open drainage systems and sewers, practice of open defecation, use of public latrines, and contact with contaminated soil – through flooding of sewage drains and/or open defecation. Private exposure domains encompass household sanitation practices, household water quality treatment and storage techniques, personal hygiene behaviors, food preparation practices, the presence of livestock within the home, and insect vectors [8]. The primary fecal-oral exposure pathways have been described for decades using the F Diagram – food, flies, fingers, field and fluids – and are outlined further below [9].

i. Fecal-oral Exposure Pathways: Food

Food may become contaminated with feces along any point of the economic value chain: during production, harvesting, transportation and distribution, processing, or directly in consumers' homes [10]. The majority of the food exposure pathways reside in the private domain, namely personal hygiene behaviors and food preparation practices, while a few are linked to the public domain, namely wastewater irrigation practices. To address some of these issues, the WHO has developed five main keys to safer food – which include keeping food preparation surfaces and utensils hygienic (using safe water), improving hand washing rates and beneficial hygiene behaviors among food preparers, in addition to establishing water quality recommendations for irrigation water [11].

Fecal contamination introduced during crop production is dependent on the source of irrigation, the fertilization techniques used, and the hand hygiene of farm workers. Irrigating crops with wastewater is a common practice in urban and peri-urban areas in sub-Saharan Africa, as well as other developing regions, due to ease of access and lack of other reliable supplies [12]. Using wastewater for irrigation, however, has numerous health implications because it contains high levels of fecal contaminants that are then deposited directly onto food surfaces. For this reason, the WHO has recommended that water used for irrigation have fecal coliform counts less than 1×10^3 100 ml^{-1} [13]. Additionally, using natural fertilizers – such as cow manure – can introduce fecal contamination onto crops, especially root crops or crops that grow near or in the ground [13].

Contamination that occurs during harvesting, transporting, trading, selling, or preparation of food, is primarily due to poor hygiene practices (i.e. hand washing prior to food contact and preparation) or cross-contaminating food [14]. Failure to practice healthy hygiene behaviors or prevent cross-contamination of foods may introduce or spread fecal contaminants, especially when the food preparation environment is highly contaminated. According to the Centers for Disease Control (CDC), contamination during food processing or cross-contamination in the kitchen is the major contributing factor in one-third of foodborne outbreaks in the U.S. from 1998 to 2002 [14]. In developing regions, humans, animals or flies may track in feces into the food preparation environment, further increasing the risk of contamination. Furthermore, assuming cutlery and food preparation surfaces are washed, the water used to wash them may be re-used, previously contaminated water, leading to the re-contamination of these surfaces [15].

Solid waste management practices too, may play a role in fecal contamination on food and subsequent diarrheal incidence. One study found that storing household waste in the home is associated with the presence of houseflies in the cooking area ($p < 0.0001$), and that the presence of houseflies in the kitchen during cooking was correlated with incidence of childhood diarrhea ($p < 0.0001$) [16].

Consumers may also be exposed to fecal contamination if they eat food directly with their hands, i.e. without the aid of utensils, which is commonly practiced throughout developing regions of the world. One study investigating fecal contamination on hands reported finding an average of 3.5 log colony forming units (CFU) of *Escherichia coli* (*E. coli*) per pair of hands with another study reporting an average of 2.5 log CFU for *E. coli* per pair of hands [17, 18].

The combination of eating without utensils, fecal contamination on hands, and the lack of adherence to hand washing guidelines paves the way for direct ingestion of fecal contaminants.

ii. Fecal-oral Exposure Pathways: Fluids

Fecal contamination of fluids (for this report, fluids refer to drinking water) occurs in a similar method as the food exposure route. Water maybe contaminated anywhere along the production-consumption pathway starting from contamination at the water source, infiltration of fecal contaminants within distribution networks, unsanitary water collection and storage methods, or within the household, at point-of-use (POU).

In developing regions, microbiological contamination of water at its source is typically due to poor community sanitation: open defecation, inadequate access to or utilization of sanitation infrastructure, and lack of or inadequate wastewater treatment leads to deposition of feces into the environment, which is ultimately found in the drinking water supply [19]. Water can also become contaminated during its transportation to supply centers or home taps – intermittent water pressure due to electrical outages in the distribution system can lead to intrusion of wastewater and thus fecal contamination into the piped water supply [8, 20]. Furthermore, water storage practices can introduce fecal contamination – water that is considered safe at the source is subject to frequent exposures to fecal contamination during its collection and storage [21]. Inadequately protected water storage containers (open, uncovered or poorly covered), unsanitary dispensing methods (using fecally contaminated hands or ladles), possible exposure to vectors (flies, rodents, and cockroaches), and lack of cleaning storage containers can all lead to water contamination at its POU. A resent assessment of six countries by United Nations Children's Fund (UNICEF) showed that more than half of households surveyed showed post-source contamination [22]; a meta-analysis of 57 studies also reported similar findings [21].

C. Identifying Fecal Contamination: The Role of Fecal Indicator Organisms

Consuming food or water contaminated with feces increases a person's risk of acute, infectious diarrhea and/or vomiting (gastroenteritis). Gastroenteritis is the second greatest burden of all infectious diseases, responsible for 89.5 million DALYs and 1.5 million deaths worldwide each year [23]. Identifying the causal agents of diarrhea is expensive, both in regards to human and financial capital. In order to cheaply estimate levels and identify potential sources of fecal contamination, fecal indicator organisms – such as total coliforms, thermotolerant fecal coliforms, enterococci, and *Escherichia coli* – have been traditionally used as proxies to testing for enteric pathogens [10, 16, 17, 20, 24, 25]. Fecal indicator organisms are indigenous to the intestines of both humans and warm-blooded animals and can provide evidence for the potential presence of other pathogenic organisms surviving under similar environmental conditions [26].

However, the validity of using fecal indicator bacteria as an indicator for health risks associated with human viruses, protozoa, or even the risk of diarrheal illness, has recently been questioned. Fecal indicator organisms are indigenous to the intestines of both humans and warm-blooded animals and can provide evidence for the potential presence of other pathogenic organisms surviving under similar environmental conditions [26].

Viruses and protozoa cysts have been shown to be more resistant to traditional wastewater treatment processes than traditional fecal indicator bacteria, so the lack of fecal indicator bacteria within a sample may not reflect the safety of drinking water [27]. Furthermore, studies have also shown varied results when using fecal indicator organisms as a predictor for health risks: some report an association between levels of *E. coli* in drinking water with childhood diarrhea while others could not find any relationship between the indicator organism and incidence of diarrhea [17, 26].

D. Health Challenges in Urban Environments

During the 20th century, the world's urban population grew more than tenfold, with the proportion of people living in urban areas tripling (15% to 48%) between the years of 1900 to 2000 [28]. This growth is expected to continue, with projections suggesting that by 2050, nearly 70 percent of the world's population will live in urban environments [29]. With much of the projected urban growth to take place in developing regions, sub-Saharan Africa will be no exception – its population is expected to triple by 2050, with the majority living in urban or peri-urban areas [25]. Coinciding with this rapid urbanization is the rise in urban slum dwellers: as of 2013, it is estimated that 863 million people live in slums, an 8 percent increase since 2000 [30].

Water and sanitation infrastructures responsible for limiting human exposures to fecal contaminants are being greatly outpaced by this rapid growth. Public latrines attempting to address the sanitation issue still leave much to be desired: long queues, large pit openings, poorly maintained facilities, and the large distance required to access them limit the frequency of their use, specifically among children [28, 31]. As a result, children in poor urban areas commonly practice open defecation, causing rampant fecal contamination of the environment [31]. Since children infected with enteric pathogens shed more pathogens in their feces and are more likely to engage in activities that increase their exposure to feces (crawling on floors, mouthing objects, etc.), they are more likely to become infected with enteric pathogens [32, 33]. This exposure is cyclical in nature, and child feces are they key – UNICEF has identified safe disposal of child feces as the critical link to reducing fecal-oral transmission of enteric pathogens, and ultimately child mortality [2].

Urban environments with overcrowded living situations, inadequate access to sanitation, and dysfunctional water and drainage systems have complex, inter-related webs of fecal exposure pathways. Because many poor, urban residents reside in slums, which rarely have formal sanitation systems, they bear the greatest risk to fecal exposure. A citizen of a low-income urban area can be exposed to fecal contaminants across an array of settings. These can be through direct contact with feces in the environment – which is a common due to open defecation practices and the use of flying toilets as a means for waste removal – or indirectly through contact with contaminated soil, drinking water, public latrines, open drains, wastewater-irrigated crops, floodwaters, flies, and recreational waters. These higher exposure risks manifest into higher incidence rates of diarrhea for slum dwellers, especially among immunocompromised groups such as children, the elderly and pregnant women [34]. As the WHO has recognized, when sanitation infrastructure and basic social services are lacking, urban settings are among the world's most life-threatening environments to children. For cities with piped water, sanitation, drainage systems, and waste removal services, childhood mortality rates are generally 10 per 1000 live births; for cities without these services, rates are 10 to 20 times higher [28].

E. The Urban Environment: Accra, Ghana

The Accra Metropolitan Area (AMA), like other African cities, is urbanizing rapidly. Urban growth over the past fifty years has ranged from 4.2 to 5.6 percent, with a current growth rate of 4.4 percent per year, according to the 2000 census [8, 31, 35]. It is also the most densely populated area in the country, with nearly 900 persons per square kilometer and a population totaling to 3 million [25]. This rapid urbanization has outstripped adequate water, sanitation, and housing infrastructures and as a result, 60-80 percent of Accra's population resides in slums [25, 31, 36] and roughly one-third of households do not have sanitation facilities [31, 35, 36]. Residents of these areas rely primarily on vendors for their food and water supplies and on public latrines for their sanitation needs [31]. However, Accra's public latrines do not adequately protect users from contact with feces, and the food and water supplied by vendors may be adulterated with fecal pathogens.

i. Sanitation in Accra

Residents across all sub-metro areas of Accra have ranked sanitation as their top priority [out of seven public services] for broadening coverage and improving service quality, and for good reason: currently, 30 percent of households in Accra do not have access to sanitation facilities within their homes and thus rely on public latrines for their sanitation needs [31, 35]. Another 30 percent of households rely on the pan/bucket system for their sanitation needs, and four percent report using plastic bags or open defecation in drains as their primary sanitation strategy [35].

Accra's urban drainage system, which was originally intended to collect storm water, has become the primary receptacle for the city's sewage. Of the homes that have sanitation facilities, only a third are connected to the central sewage system, leaving an estimated 50-90 percent of households in the AMA to discharge their sewage directly into the urban drainage system [25, 31, 36]. Although Accra has sewage treatment plants, less than a quarter are operational, leaving the majority of human sewage in the AMA untreated [25].

Inadequate access to sanitation and the absence of sewage treatment is contaminating the urban environment with sewage, which may explain the prevalence of diarrhea in Ghana. With a disability Adjusted Life Year (DALY) or 2.06×10^{-2} per person per year, just beneath that of HIV/AIDS (2.59×10^{-2}), diarrheal disease is one of the top contributors to Ghana's overall morbidity [12]. It is also the second leading reason cited by those seeking care at a primary healthcare center [36], with over 3.5 million cases of gastroenteritis diagnosed in children under five between June 2003 and December 2004 [4]. Moreover, it is estimated that 12 percent of children under five years of age die from diarrhea in Ghana [20]. Estimates for treating these cases ranges from 5.5 to 8.9 million USD annually [4].

ii. Food Production and Dietary Habits in Accra

Food Production

Food production in urban Accra is similar to those found in other urban centers of developing countries – nearly all (90%) of the vegetables consumed in the city are produced in urban or peri-urban farms [12, 25]. Farmers in the AMA produce a mix of exotic crops (lettuce, cabbage, spring onions, and cauliflower) and traditional crops (tomatoes, okro, and hot peppers) [25]. Due to the lack of reliable access to water for crop production, farmers rely on wastewater from Accra's urban drainage system for crop irrigation [10, 12, 25, 37].

However, multiple studies have shown that wastewater used for urban farming in Accra, Ghana has fecal coliform levels up to 1×10^{7} 100 ml^{-1}, which are well above the WHO recommended levels (1×10^{3} 100 ml^{-1}) [10, 24, 36]. Irrigating crops with this water has implications for food safety: crops had counts of fecal coliforms similar to the wastewater itself (1×10^{6} g^{-1} to 1.1×10^{7} g^{-1}) [24, 36]. Because many enteric pathogens are waterborne, consuming crops irrigated with wastewater presents a health risk, especially if food is eaten raw.

Dietary Habits

The typical Ghanaian diet is comprised of: green leafy vegetables (including lettuce), beans, garden eggs (type of small eggplant), plantains, fish, banku (maize), fruits (such as apples, mangoes and oranges), okro, agushie (pumpkin seeds), lettuce, carrots bread, cassava, and cucumbers [38]. One study looking at food frequency consumption patterns among Ghanaians found that nearly every respondent (96-98%) eats maize, fish, tomatoes, onions and peppers on a daily basis [39].

Although the majority of Ghanaian residents consume three meals each day, very few cook all three meals at home [38, 39]. Street vendors are filling this gap, providing cheap, ready-to-eat foods to the masses. The most common street food customers are the poor, who rely exclusively on street food for their daily needs, and workers/students, who rely on street food while they are away from home [12, 40]. Although food vendors increase food access and security to these populations, they are also viewed as a major vector for spreading foodborne diseases [15, 40].

Most of the foods served by vendors are traditional meals accompanied with a raw side salad, comprised of locally grown lettuce, cabbage and spring onions [41]. While consumption of salads is not part of the typical Ghanaian diet, it is estimated that 200,000 people, roughly 7 percent of the AMA population, consume such supplemental salads each day [10]. Of the 850-1250 tons of lettuces sold annually in Accra's markets, 60 percent are sold to street food vendors, 38 percent are sold to restaurants, and only 2 percent are purchased by households [10, 37].

Except for those still breastfeeding (under the age of two), Ghanaian children do not have a special diet and are weaned directly into the adult diet (most likely due to convenience for caregivers) [42-45]. The 2008 Ghana Demographic Health Survey (DHS) found that overall, 81 percent of children aged 6 – 35 months consumed foods such as dark leafy green vegetables, fish, meat, poultry, eggs, and pumpkins, mirroring dietary patterns found in adults [45]. Since children split their time between home and school, their primary dietary inputs are via School Feeding Programs (SFPs) and home-cooked meals. SFPs, sometimes referred to as food-for-education programs, have been instituted primarily as an incentive for increased educational participation and improved nutritional status in school-aged children. Since 2005, the Ghanaian government has implemented the Ghana SFP, through which school children are fed one nutritious meal per day; current estimates place student participation in program at 34 percent [46]. The only guideline for food procurement under the program is that the food must be grown and produced by local farmers. As such, SFP meals vary greatly by region but are generally planned around three core food items: rice, cowpeas, and fortified corn soya blend; protein foods (eggs, meat and fish) are served at least once a week with fruit (typically oranges) [42].

For Ghanaian children under the age of two, breastfeeding is still an important source of food. Because it is one of the most cost-effective strategies for preventing diarrhea and diarrheal deaths, the WHO recommends exclusively breastfeeding children for the first six months of life; however, only 33 percent of infants in sub-Saharan Africa are exclusively breastfed during this time [2, 47]. Estimates for Ghana are slightly better, with 46-66 percent of mothers in Ghana practicing exclusively breastfeed during this time [44, 47]. While exclusive breastfeeding practices decline dramatically after 6 months, breastfeeding practices in general last much longer: breastfeeding in Ghana typically lasts for a median of twenty months [45, 47]. Among mothers of 9-23 month old children, 89 percent report that they are still currently breastfeeding (n=298), highlighting the importance of breast milk as a food source for young children [47].

Once a child stops exclusive breastfeeding, and thus are introduced to complimentary foods and drinking water, their risk of diarrheal illness and death increases. Infants who are not exclusively breastfed for the first six months experience a seven-fold increased risk of death from diarrhea when compared to infants who were exclusively breastfed [5]. This is because the food and water consumed by the child may be contaminated with enteric pathogens. Although Ghanaian mothers are educated on the importance of exclusive breastfeeding for the first six months, roughly one-third (n=199) of mothers still report giving water to their child before they are six months old and one-fifth also introduce complementary foods at this time [44].

iii. Drinking Water Sources in Accra

The Ghana Water Company Ltd. (GWC) supplies water service to the AMA, but city growth has outstripped its capacity to provide reliable service to residents. Though the GWC's service coverage is technically 80 percent of the AMA, less than half of residents have access to a house or yard connection, and less than 10 percent have a reliable in-house connection [48]. Access to the GWC service is also greatly dependent on household wealth: while roughly 70 percent of medium wealth and over 90 percent of high wealth households have private piped water indoors, less than 30 percent of poor households report having a connection [31]. Municipal pipes in the AMA have been observed throughout the urban drainage system [20], which, combined with low pressure from frequent power outages, could cause contaminated wastewater to intrude into the distribution system [8, 20].

Households without access to the GWC system typically acquire water through private vendors (who sell water by the bucket, bottle or sachet), public taps/boreholes, water kiosks, and private water truck services [35]. Of the vended water sold, sachets (which are 500mL polyethylene plastic bags of water) are the most popular choice among AMA residents, especially the poorest of the poor [49]. A recent DHS in Ghana revealed that more than one-third of residents in the AMA rely primarily on sachet water for their drinking water needs and roughly half of households report sachet use [48, 49]. Sachets have gained popularity among AMA residents because of their convenience, low price, and perception that sachet water is of higher quality than GWC tap water [48].

Because of the widespread use of sachet water, the Ghana Standards Board (GSB) has implemented regulations that require vendors to certify the safety of their products. In theory, sachet water goes through some sort of filtration – generally a combination of carbon and sand filters – and in some instances, ultraviolet disinfection before entering the sachet bag. Vendors who prove the safety of their products are given a "GSB certification seal" to use in their product packaging. However, some sachet vendors are home-based businesses that have little to no business assets and as such, the water they fill their bags with may not be certified. Inspection of sachet products throughout the AMA reveal that many sachet bags' GSB seals were absent or fake [48].

While the safety of vended water throughout the AMA is generally unknown, sachet water is considered safe and its use has been found to be protective against childhood diarrhea. One study found that children from households that do not use sachet water as their primary drinking water source were 77 percent more likely to experience diarrhea in the previous two weeks then their counterparts [49]. Another study, however, found a positive association between a household's source of drinking water and the incidence of diarrhea (p=0.002), with one-third of children drinking vended water reporting diarrhea within the previous two weeks [31]. However, the latter study was conducted in 2005, before sachets were commonplace – and under GSB's regulations – and the vended water in Accra was most likely water sold from a household with a piped connection to the GWC [49].

F. Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment (QMRA) is one tool that has been used to quantify public health risks. QMRAs are an adaptation to traditional risk assessments, utilizing statistical mathematical models to estimate likelihoods of adverse health outcomes in a population, given microbial concentrations, frequency and duration of contact [50]. This strategy is particularly advantageous because it can calculate risk estimates for rare disease outcomes, can easily assess differences in risk for vulnerable populations, and it allows for risk-comparisons between different exposure routes [13, 51]. Because QMRAs allow policy-makers to discern which exposure routes are of the greatest concern for their citizens, they are the WHO-recommended strategy for educating decision-makers on how to create effective sanitation interventions [13].

The QMRA framework has four primary steps: hazard identification, exposure assessment, hazard characterization, and risk characterization. During hazard identification, information regarding the pathogen is collected, such as its presence in food and associated health effects if consumed. Exposure assessment identifies the scope, duration and frequency of contact with the microbe, while hazard characterization integrates knowledge on dose-response relationships and probability of infection given its consumption. In the final stage, risk characterization, the information from the prior three steps are integrated into computational models that develop the risk estimate [50].

The validity of the QMRA is dependent on the validity of assumptions and model inputs. Exposure assessment – where the breadth of these assumptions is made – requires outlining patterns of consumption, identifying pathways of exposures (from production to consumption), and creating exposure scenarios to identify variations in risk. All of these inputs require substantial assumptions on human behavior, pathogen survival, and even the amount of the organism consumed. Small variations in model inputs can lead to enormous variations in the risk estimates; therefore it is imperative to select valid model inputs and limit the amount of assumptions made within the model – ideally using parameter estimates based off of confirmed data. Two ways to address these issues are to propagate parameter uncertainty estimates through the model – using Monte Carlo simulations – and conduct sensitivity analyses that help identify primary drivers of fecal contamination.

Two recent studies (Seidu et al., 2008; Barker et al., 2014) have applied QMRA methodologies to assess risks associated with vegetable production and consumption of street food salads, and two other studies (Machdar et al., 2013; Labite et al., 2010) applied their assessments to Accra's municipal water supply. Each of these studies focused their assessment on adult exposures, specifically as they relate to adult consumers and farmers, rather than children, who bear the brunt of diarrheal diseases.

G. Study Objectives

While these studies provided a solid foundation for assessing annual risk of infection and DALYs associated with adult exposures to fecal microbes, there is still a large information gap on child exposures. In order to address these gaps, this study aims to identify which exposure pathway poses the greatest microbial risk to children in low-income urban settings; therefore, the goal of the study was three-fold:

1. Describe the microbiological quality of the AMA's drinking water sources and raw produce by estimating concentrations of the fecal indicator organism *E. coli*
2. Create exposure scenarios based on water and produce consumption patterns reported by the study population, specifically those that relate to children in low-income urban settings
3. Quantify the risks of exposure to *E. coli* via drinking water and food ingestion utilizing measured microbial data

Once the QMRA has been completed, this study will help identify which fecal-oral exposure pathways present the greatest risk to children in low-income urban settings. The results of this study will be used by policy-makers and other key actors to implement evidenced-based interventions that target pathways that pose the greatest risk, with the goal of ultimately reducing children's diarrheal disease burden in low-income urban settings.

II. MANUSCRIPT

A. Introduction

The world's population is urbanizing rapidly. It is projected that, by 2050, nearly 70 percent of the world's population will live in urban environments [29]. With much of the projected urban growth predicted to occur place in developing regions, sub-Saharan Africa will be no exception – its population is expected to triple by 2050, with the majority living in urban or peri-urban areas [25]. Coinciding with this growth is the rise of urban slum dwellers: as of 2013, it is estimated that 863 million people live in slums, an 8 percent increase since 2000 [30]. Urban environments with overcrowded living situations, inadequate access to sanitation, and dysfunctional water and drainage systems have complex, inter-related webs of fecal exposure pathways. Poor, urban residents residing in slums bear the brunt of these exposures, which manifest as higher incidence rates of diarrhea, especially among immunocompromised groups such as children, the elderly and pregnant women [34]. When sanitation infrastructure and basic social services are lacking, urban settings are among the world's most life-threatening environments for children. For cities with piped water, sanitation, drainage systems, and waste removal services, childhood mortality rates are generally 10 per 1000 live births; for cities without these services, rates are 10 to 20 times higher [28].

Ghana's ability to provide water, sanitation, and housing infrastructures to its populace is greatly outpaced by rapid urban population growth. As a result, 60-80 percent of Accra's population reside in slums, and roughly one-third of households do not have sanitation facilities [31, 35, 36]. Accra's urban drainage system, which was originally intended to collect storm water, has become the primary receptacle for the city's sewage. It is estimated that 50-90 percent of households in the Accra Metropolitan Area (AMA) discharge their sewage directly into the urban drainage system [25, 31, 36].

Gastrointestinal pathogens are spread predominantly through fecal-oral exposure routes. The primary fecal-oral exposure pathways have been described for decades using the F Diagram – food, flies, fingers, field and fluids [9]. Preventing contact between excreta and the environment will reduce the risk of exposure to gastrointestinal pathogens [34]. In Accra, improper use of the drainage network and the absence of sewage treatment [25, 52] has contaminated the urban environment with sewage, and compromised the safety of the water and food supplies [8, 12, 20].

Previous studies assessing the microbiological quality of drain water in the AMA have reported concentrations of fecal microbes consistent with those found in raw sewage [8, 20, 37, 53]. Some municipal water pipes in the AMA have been placed in the urban drainage system [20], which, combined with low pressure from frequent power outages, could lead to intrusion of contaminated wastewater into the water distribution system [8, 20]. Additionally, the intermittent municipal water supply across the city causes residents to store water in the household, which increases the risk of fecal contamination [21, 22, 54], and consume water from alternative sources, such as sachets or local wells [20], the safety of which are unknown [48].

The state of Accra's urban drainage system also has implications for the safety of the city food supplies. While drain water is highly contaminated, it is also free and readily accessible, making these waterways the primary source of irrigation water for urban farmers [10, 12, 25, 37]. The World Health Organization (WHO) has recommended that water used for irrigation have a fecal coliform concentration less than 1×10^3 100 ml^{-1} [13]. However, multiple studies have shown that wastewater used for urban farming in Accra has fecal coliform levels up to 1×10^7 100 ml^{-1}, which are well above the WHO recommended levels [10, 24, 36]. Because many enteric pathogens are waterborne, consuming crops irrigated with wastewater presents a health risk, especially if food is eaten raw.

While a majority of Ghanaian residents consume three meals a day, very few cook all three at home [38, 39]. Ghana's rapid urbanization and post-independence industrial development has increased the number of people working away from their homes [55, 56], which has increased the demand for inexpensive, ready-to-eat food. Street food vendors are filling this gap and street food consumption is increasing across many of Ghana's cities [12, 57]. The most common street food customers are the poor, who rely almost exclusively on street food for their daily needs, and workers/students, who rely on street food while they are away from home [12, 40]. Although most of the foods served by vendors are traditional meals, they are often accompanied by a raw side salad. These salads are typically comprised of locally grown lettuce, cabbage and spring onions [12]. While consumption of salads is not part of the typical Ghanaian diet, it is estimated that 200,000 people, roughly 7 percent of the AMA population, consume such supplemental salads each day [10].

With a Disability Adjusted Life Year (DALY) of 2.06×10^{-2} per person per year, just beneath that of HIV/AIDS (2.59×10^{-2}), diarrheal disease is one of the top contributors to Ghana's overall morbidity [12]. It is also the second leading reason cited by those seeking care at a primary healthcare center [36], with over 3.5 million cases of gastroenteritis diagnosed in children under five between June 2003 and December 2004 [4]. Moreover, it is estimated that 12 percent of children under five years of age die from diarrhea in Ghana [20]. Identifying the fecal-oral transmission routes that pose the greatest risk and the number and types of people exposed to these pathways can help decision-makers implement the most effective strategies for reducing childhood mortality and improving the health of the urban populace.

Quantitative Microbial Risk Assessment (QMRA) is one tool that has been used to quantify public health risks. This WHO-recommended strategy is particularly advantageous because it can calculate risk estimates for rare disease outcomes, can assess differences in risk for vulnerable populations, and it allows for risk-comparisons between different exposure routes or different health outcomes [13, 51]. Several studies have recently applied QMRA methodologies to assess risk from various fecal-oral exposures within urban environments. Seidu et al. (2008) and Barker et al. (2014) examined risks associated with wastewater-irrigated vegetable production and consumption of street food salads in Accra, while Labite et al. (2013) and Machdar et al. (2013) focused their assessments on Accra's water supplies.

Another QMRA by Katukiza et al. (2014) examined risks associated with water and soil exposures in Kampala, an urban slum in Uganda. Except for the study by Katukiza et al., who integrated a child exposure model for accidental ingestion of environmental surface and grey water, each of these studies focused their assessment on adult exposures, rather than children, who bear the brunt of diarrheal diseases.

While these studies provided a solid foundation for assessing risks associated with adult exposures to enteric pathogens, there is still a large information gap on child exposures. In order to address these gaps, this study aims to identify which pathways pose the greatest risk of exposure to fecal contamination for children in low-income urban settings; therefore, the goal of the study was three-fold:

1. Describe the microbiological quality of the AMA's drinking water sources and raw produce in four low-income neighborhoods in urban Accra by estimating concentrations of the fecal indicator organism *E. coli*
2. Create exposure scenarios based on water and produce consumption patterns reported by the study population, specifically for children in these low-income urban settings
3. Quantify the risks of exposure to *E. coli* via drinking water and food ingestion utilizing measured microbial data

B. Methods

The SaniPath study was conducted from July 2011 through November 2012 in four low-income neighborhoods in Accra, Ghana. This study aimed to identify and describe the sources and movement of human fecal contamination in the study neighborhoods, as well as the behaviors of children and their caregivers that put them at risk of exposure to fecal microbes. The data sources pertaining to this analysis are: 1) household demographic surveys assessing Water Sanitation and Hygiene (WASH) practices; 2) nursery description questionnaire; 3) school description questionnaire and student self-report surveys; and 4) environmental sampling and testing of drinking water and produce items. The Emory University Institutional Review Board approved all research methods and data collection techniques (Appendix A).

i. Study Site

Neighborhood Selection

Four resource-poor neighborhoods in the AMA were selected for this study: Alajo, Bukom, Old Fadama and Shiabu (Figure 2.1). Neighborhoods were selected to capture a range of environmental and social conditions (Table 2.1). Additional selection criteria were based on feasibility, field logistics, receptivity of community members, and the safety of the field team.

Neighborhood Characteristics

Alajo is a neighborhood within the Accra North neighborhood, one of Accra's wealthiest areas. It is situated furthest inland and is bordered by the Odaw River. Although it is the wealthiest neighborhood in this study, Alajo is still an informal neighborhood, which is typically characterized by poor road and drainage networks, in addition to inadequate water and electricity access [34]. Bukom is located on the coast, in the heart of downtown Accra. Neighborhood infrastructure is based on the remains of a late nineteenth century settlement; few households have a latrine and thus rely on public facilities for sanitation needs. Old

Fadama is an illegal settlement built on top of a large electronic-waste dumping site [58]. It covers approximately four acres of what was a former wetland of the Korle Lagoon just northwest of Accra's Central Business District. It is best known for the Agbogbloshie market, where the majority of residents work sorting through the garbage searching for metals to sell. Nearly every household relies on public bathhouses and latrines for their sanitation needs. Shiabu is another coastal neighborhood, just west of the city center. It is a very diverse neighborhood; illegal settlements surround the beach and gated houses, with access to municipal sewage services, are in the northern region [53].

ii. Data Collection

Trained staff at the TREND group conducted all survey data. Trained staff at the Water Research Institute (WRI) collected environmental samples and performed microbiological analyses. Questionnaires were administered verbally, and questions were asked in a non-leading fashion. All data collection and sampling sites were selected by neighborhood liaisons that had intimate knowledge of the four neighborhoods.

Household Survey

A household survey was conducted in 200 hundred houses in each neighborhood from March to September 2012. Those surveys collected information on: household demographics; water sources used for drinking, hygiene and cooking; dietary habits and consumption patterns; sanitation and hygiene practices; and access to WASH facilities (Appendix B). Surveys were conducted during morning and afternoon hours to household members present and willing to participate.

Nursery Description Questionnaire

Nursery description questionnaires were given to the classroom teacher after the conclusion of the observation period. The questionnaire captured children's food and drinking water sources as well as WASH information for the classroom under observation (Appendix C).

School Description Questionnaire & Student Self-Report Surveys

Primary school data collection was conducted in each neighborhood with 27 schools participating; both private and public schools were included in the study. Data collection occurred over a period of five hours, between 07:00-12:00. Data was collected on WASH infrastructure via administering a questionnaire to the school headmaster, health coordinator or a teacher. The questionnaire covered drinking water access and available food options for students (Appendix D) while information on children's eating habits were collected via a student self report form (Appendix E). The self-report form asked children about which water sources they used, the foods they had eaten, and their latrine use for that day. To minimize reporting bias, children were asked to close their eyes and rest their head on their desk. A raised hand indicated a positive response. A maximum of three classes at each school participated in the exercise.

Environmental Samples

Produce and drinking water samples were collected after the administration of the household questionnaire. Due to logistical constraints however, samples were only collected from households surveyed from 07:00-12:00; households interviewed in the afternoons and weekends were excluded. Produce items were sampled from market vendors on a per unit basis (for instance, a whole head of lettuce) and were placed in either a 1L or 3L Whirl-Pak® (Nasco, Fort Atkinson, WI, USA) bag by the participant, who was asked to place the produce item using whatever means of handling they normally practice. Food Environmental Sample Forms (Appendix F) were completed at the time of sampling to record the food-handling practices of the participant. Stored household drinking water was collected in the same fashion, with participants asked to place 500mL of water into a Whirl-Pak® bag using whatever means they normally dispense their water. Sachets, sealed plastic bags that hold 500mL of water, were either donated or purchased from study participants. Municipal piped water was collected in sterile 20L containers by study staff at the Water Research Institute (WRI). Environmental Sample Collection Forms for stored household and sachet water (Appendix G) and municipal piped water (Appendix H) were completed in conjunction with sample collection to record storage container characteristics, water collection processes, and surrounding environmental conditions. All environmental samples, except municipal piped water, were stored on ice and returned to the WRI within six hours for processing. Due to the large size (20L) of the municipal piped water collection containers, samples were not stored on ice but were instead collected at the conclusion of each neighborhood visit and were transported immediately to the WRI for processing.

iii. Data Management

All paper documents completed in the field were entered into a central Access database (Microsoft, Redmond, WA, USA) managed by the study team. Double entry of 25 percent of all forms was completed to ensure data quality.

iv. Laboratory Methods

All environmental samples were processed for *E. coli* by technicians at the WRI within six hours of collection using U.S. EPA method 1604 [59]. *E. coli* assays were performed directly for drinking water samples using three volumes: 1mL, 10mL, and 100mL. Prior to testing, produce samples were first bathed in 500mL of PBST (PBS with 0.04% Tween-80), incubated at room temperature for 10 minutes, vigorously shaken for 30 seconds, gently massaged, and then shaken again for 30 seconds. *E. coli* assays were then performed using three volumes: 0.1mL, 1mL, and 10mL of the PBST rinse.

v. Statistical Methods

Statistical analyses were conducted in SAS 9.4 (SAS, Cary, NC, USA). All statistical tests were evaluated at an alpha level of 0.05. Drinking water consumption rates for children were calculated from responses to the household survey. Drinking water consumption data was limited to children under 5 years; therefore, consumption rates were stratified into the following age groups: 0-1 year olds, 1-2 year olds, and 2-5 year olds. In the cases where multiple produce items were collected and processed in a single Whirl-Pak® bag, the number of produce items washed were reported and an average concentration value per item was calculated. In order to assess the *E. coli* concentration per gram of produce item, concentration values were standardized by weight per produce item (Table 2.2).

The total volume of the three environmental sample dilutions was used to estimate final *E. coli* microbial concentrations for each sample. *E. coli* results that were too numerous too count or too dirty to count were excluded from analysis; concentrations below the limit of detection (LOD) were deemed non-quantifiable and were assigned a value of half the LOD.

Final concentration estimates for all organisms were log-transformed and then analyzed for arithmetic mean, standard deviations, minimums and maximums. *E. coli* concentration estimates were analyzed using ANOVA methods to examine differences between environmental sources and neighborhoods. Where significant differences were detected, outlying values were imputed separately into the models to establish more accurate exposure estimates for that specific neighborhood or source. In order to address the variability of parameter distributions, 1,000 Monte Carlo simulations were generated for each model input.

vi. Exposure Assessment

Two different exposure models were used to estimate the dose of *E. coli* ingested via drinking water and consumption of a raw street food side salad. The first model examined drinking water ingestion and estimated risk for three scenarios: exposure scenario A was based on the consumption of sachet water; exposure scenario B was based on the consumption of stored household water; and exposure scenario C was based on the consumption of municipal piped water. For each scenario, exposure doses were calculated based on the reported volume of drinking water ingested daily for three age groups (0-1 years, 1-2 years, 2-5 years), as identified in the household questionnaire. The second model examined consumption of a raw street food salad and estimated exposure dose for two scenarios: scenario A was based on a child aged 5-12 years consuming a full serving of salad, three times per week; and scenario B was based on a child aged 2-5 years consuming half a serving of salad, one time per week.

Model Equations

In order to estimate the dose of *E. coli* consumed in each scenario, two equations were used: one for drinking water exposures and the other for food exposures. For the drinking water model, the dose of ingested organisms was defined as:

(1) $$E_{DW} = C_W L$$

where the exposure dose (E_{DW}) is directly proportional to C_W, the *E. coli* concentration estimated per 100mL of each drinking water source (sachet water, stored household water, and municipal piped water), and L, the volume of water consumed by each age group (0-1 year olds, 1-2 year olds, or 2-5 year olds) per day (L per day). Results for *E. coli* dose estimates are reported as Colony Forming Units (CFU) consumed per day.

For the food exposure model, the dose of ingested organisms was defined as:

(2) $$E_F = C_F I F$$

where the exposure dose (E_F) is directly proportional to C_F, the *E. coli* concentration estimated per gram of each raw salad item (lettuce, cabbage, tomato, or spring onion), I, the total mass of each produce item consumed per salad (g per salad), and F, the frequency of salad consumption per week. Results for *E. coli* dose estimates are reported as Colony Forming Units (CFU) consumed per week.

Model Parameters

Model parameters and key assumptions used for food and drinking water exposures are presented in Table 2.2 and Table 2.3, respectively. There is currently no information available on the consumption of individual produce items in Ghana, but estimates of salad serving size have been previously described by Fung et. al (2011) [41]. Salad size was measured at 20g per meal and reported as being primarily comprised of lettuce and cabbage (>75%), with varying accompaniments; therefore for the purpose of this analysis, salad was assumed to be 37.5% lettuce, 37.5% cabbage, 20% tomato and 5% spring onion, and the estimates of *E. coli* concentrations on these produce items were weighted accordingly in the final exposure assessment.

Although no data exists on children's consumption of street food salads, it is likely that children do consume them. Ghanaian children are typically weaned directly to the adult diet (most likely due to convenience for caregivers) [42-45]. Since Ghanaians have reported consuming street-vended food on average three times each week [41], we assumed that children accompanying the caretaker would also consume portions of the vended food. In order to address the possibility of a young child's (aged 2-5 years) exposure to contaminated raw produce, scenario B represents the likelihood of a child being fed a portion of the caregiver's salad, in this case a dose equating to half that of a full portion (10g). Given that some of the most common street food customers are students, who rely on street food while they are away from home [12, 40], it is likely that school-aged children also consume street food side salads. To address this possibility, scenario A represents the likelihood of a child (aged 5-12 years) eating a raw street food side salad three times per week while at school.

C. Results

i. Neighborhood Demographics, Drinking Water Sources, and Dietary Habits

Two hundred household surveys were conducted in each of the four study neighborhoods (Figure 3.1). Reported household demographics for each neighborhood are presented in Table 3.1. Bukom had the largest percentage of participants reporting that they owned their own home (80%), corresponding with the historical nature of the neighborhood. While the predominant religion in Alajo, Bukom and Shiabu is Christianity, Old Fadama residents are predominantly Muslim (60.5%). Old Fadama also reported the lowest percentage of households with formal education (43.5%), in conjunction with the lowest reported percentage of households with their own sanitation (1%) or bathing facility (3%) within the compound. Across all neighborhoods, household size ranged from four to five people and nearly half of all households reported having at least one child under the age of five living with them. All study neighborhoods reported at least 10 percent of children having diarrhea within the previous two weeks, with Old Fadama reporting the highest prevalence at 25 percent.

Respondents identified three sources for their drinking water: sachets, tap from the municipal piped network, and tap from a polytank (Table 3.2). Across all study neighborhoods, respondents identified sachet water as their primary source of drinking water. Roughly 75 percent of study households in Alajo, Bukom and Shiabu reported sachet water as their primary drinking water source while the remaining respondents identified tap water from the municipal piped distribution system; less than 1 percent of households reported relying on water from a water storage polytank. Residents of Old Fadama, however, were most likely to rely almost exclusively on sachet water, with 93 percent of respondents identifying sachet water as their primary drinking water source (p-value<0.001). Across all neighborhoods, schools and nurseries reported using municipal piped water as their primary drinking water source.

Dietary habits varied across each study neighborhood (Table 3.3). Frequency of child-purchased vendor food at school varied by neighborhood, with 20 to 33 percent of Bukom, Old Fadama and Shiabu respondents reporting that their child purchased vended food at school every time they went. Conversely, only 2 percent of Alajo respondents reported that their child purchased vended food at school every time. Alajo residents were also more likely to report that their child never purchased vended food at school (p-value=0.009). For adults, household frequency of purchasing vended food varied across neighborhoods. Across all neighborhoods, 35 to 50 percent of respondents reported purchasing vended food a few times a week. In Old Fadama, however, residents rely heavily on vended food for their daily needs; over half of the residents surveyed reported purchasing vended food everyday. Residents of Alajo and Shiabu, however, were more likely to report never purchasing vended food compared to the other neighborhoods (p-value<0.001). Consumption of raw produce was evenly reported across all neighborhoods, with 70 to 80 percent of respondents reporting that they eat raw produce at least a few times per week.

ii. Microbial Concentrations for Environmental Samples

Drinking Water

Drinking water sources in Accra had a range of *E. coli* concentrations (Table 3.4). Sachet water showed the lowest frequency of contamination, with *E. coli* only detected in 1 out of 60 samples. The concentration of *E. coli* detected in the single quantifiable sample was 0.44

log_{10} CFU per 100mL. Stored household water showed both the highest frequency and the highest concentration of *E. coli*. Out of 62 samples, 58 (93.5%) were quantifiable for *E. coli*. The average concentration of *E. coli* detected in stored household water was 1.78 log_{10} CFU per 100mL, with a maximum observed concentration of 4.30 log_{10} CFU per 100mL. The distributions of the log-transformed *E. coli* concentration for sachet and stored household drinking water samples are presented in Figure 3.2.

E. coli concentrations in municipal piped water did not vary by neighborhood except for Old Fadama, where the municipal piped water had significantly higher mean levels of *E. coli* (1.08 log_{10} CFU per 100mL) compared to the other study neighborhoods (0.30 log_{10} CFU per 100mL) (p-value=0.0003). The lowest detected *E. coli* concentration was equivalent across all neighborhoods (0 log_{10} CFU per 100mL). Maximum observed *E. coli* concentration, however, was higher for Old Fadama (3.03 log_{10} CFU per 100mL) compared to the other study neighborhoods (1.51 log_{10} CFU per 100mL). The distributions of the log-transformed *E. coli* concentration for municipal piped drinking water samples, by neighborhood, are presented in Figure 3.2.

Produce Items

Every produce item tested had quantifiable *E. coli* concentrations (Table 3.4). The highest observed concentration for *E. coli* was on lettuce, with an average value of 2.41 log_{10} CFU per g. The *E. coli* concentration detected on lettuce ranged from -0.57 log_{10} CFU per g to 4.08 log_{10} CFU per g. Spring onion had the lowest observed concentration of *E. coli*, with an average value of 0.47 log_{10} CFU per g. The lowest *E. coli* concentration detected on spring onion was -1.11 log_{10} CFU per g and the highest value was 2.29 log_{10} CFU per g. The distributions of the log-transformed *E. coli* concentration for produce items are presented in Figure 3.3.

iii. Exposure Assessment

Drinking Water Exposure Scenarios

The distribution of the reported daily volume of water consumed for each age group is reported in Table 2.3 and Figure 3.4. Average volume of water consumed increased with age: children aged 0-1 years old consumed on average 0.56 L of water per day; children aged 1-2 years old consumed on average 1.07 L of water per day; and children aged 2-5 years old consumed on average 1.49 L of water per day. Because the average volume of water consumed increased with child age, microbial doses in drinking water were lowest for children aged 0-1 years old and highest for children aged 2-5 years old in all three scenarios.

The arithmetic means, medians, and 95 percent confidence intervals for *E. coli* doses in drinking water are presented in Table 3.5. Scenario A (consumption of sachet water) resulted in the lowest *E. coli* exposure with an estimated average dose of 0.33 $\log_{10}$ CFU consumed per day for children aged 0-1 years and 0.77 $\log_{10}$ CFU consumed per day for children aged 2-5 years old. Scenario B (consumption of stored household water) resulted in the highest *E. coli* exposure with an estimated average dose of 2.32 $\log_{10}$ CFU consumed per day for children aged 0-1 years and 2.76 $\log_{10}$ CFU consumed per day for children aged 2-5 years old. The distributions of log-transformed *E. coli* exposure for drinking water scenario A and scenario B are presented in Figure 3.5.

Because *E. coli* concentration levels in municipal piped water were significantly higher in Old Fadama compared to the other study neighborhoods (p-value=0.0003), a separate exposure estimate was calculated for scenario C (consumption of municipal piped water). For children aged 0-1 years old residing in Old Fadama, the estimated average dose of *E. coli* consumed each day was 0.97 $\log_{10}$ CFU. Children aged 2-5 years residing in Old Fadama had the highest dose, with an average 1.41 $\log_{10}$ CFU consumed per day. In comparison, we estimate that children aged 0-1 years and 2-5 years in the other three study neighborhoods consumed on average 0.47 $\log_{10}$ CFU and 0.91 $\log_{10}$ CFU of *E. coli* per day, respectively. The distributions of log-transformed *E. coli* exposure for drinking water scenario C, by neighborhood, are presented in Figure 3.6.

Food Exposure Scenarios

The arithmetic means, medians, and 95 percent confidence intervals for *E. coli* doses corresponding with eating a street food vended side salad are presented in Table 3.6. Because a child aged 2-5 years was assumed to eat a salad portion half that of a child aged 5-12 years, and frequency of salad consumption was assumed to be one-third that of a child aged 5-12 years, all exposure estimates were lowest for children aged 2-5 years. For children aged 2-5 years, the estimated average dose of *E. coli* consumed each week via a street food side salad was 3.28 $\log_{10}$ CFU, with estimates ranging from 3.22 $\log_{10}$ CFU to 3.33 $\log_{10}$ CFU. For children aged 5-12 years, the estimated average dose of *E. coli* consumed each week via a street food side salad was 4.05 $\log_{10}$ CFU, with estimates ranging from 4.00 $\log_{10}$ CFU to 4.11 $\log_{10}$ CFU. The distributions of log-transformed *E. coli* exposure for both food scenarios are presented in Figure 3.7.

Comparing Risk of Exposure from Drinking Water and Food Scenarios for Children aged 2-5 years

The arithmetic means, medians, and 95 percent confidence intervals for weekly *E. coli* doses for each drinking water and food exposure scenario are presented in Table 3.7. Children aged 2-5 years were the only age group common across all exposure scenarios and is therefore the only group included in the comparative analysis. Drinking water scenario A (consumption of sachet water) had the lowest *E. coli* exposure with an estimated average dose of 1.62 $\log_{10}$ CFU consumed each week. The greatest *E. coli* exposure was in drinking water scenario B (consumption of stored household water) with an estimated weekly dose of 3.61 $\log_{10}$ CFU. Food scenario B (children aged 2-5 years consuming half a portion of salad one time a week) produced the second highest *E. coli* exposure with an estimated weekly average dose of 3.28 $\log_{10}$ CFU. Drinking water scenario B, which represented the greatest estimated weekly dose of *E. coli,* produced an average weekly *E. coli* dose estimate 97 times larger than that of drinking water scenario A, which represented the lowest estimated weekly dose of *E. coli*. When compared to the second leading weekly *E. coli* dose estimate (food scenario B), drinking water scenario B produced an estimated exposure that was twice that of the food scenario.

D. Discussion

i. Exposure Dose Estimates

To our knowledge, this study represents the first attempt to quantify the exposure of children in low-income urban setting to *E. coli* through either drinking water or consumption of street food side salads. The results of the drinking water scenarios show that scenario B (consumption of stored household water) resulted in the highest exposure to *E. coli*, with an average dose of 2.32 log_{10} CFU consumed per day for children 0-1 year old and an average dose of 2.76 log_{10} CFU consumed per day for children aged 2-5. The lowest exposure was associated with drinking water scenario A (consumption of sachet water), with children aged 0-1 year consuming on average 0.33 log_{10} CFU per day and children aged 2-5 years consuming on average 0.77 log_{10} CFU per day. These results are consistent with a previous QMRA by Machdar et al. (2013), who also found stored household and sachet water to be associated with the highest and lowest exposure, respectively, although he was reporting risk for adult consumers [20]. The food exposure scenarios show that consumption of a street food side salad results in substantial ingestion of *E. coli*. Scenario A, which assumed a child aged 5-12 years consumed a full portion of salad three times per week, was associated with an average *E. coli* dose of 4.05 log_{10} CFU per week while scenario B, which assumed a child aged 2-5 years consumed half a portion of salad once a week, produced an average dose of 3.28 log_{10} CFU per week.

In order to compare the two exposure models, doses for the water model were extrapolated from daily to weekly estimates. Children aged 2-5 years were the only age group in common across all exposure scenarios and are therefore the only group included in the comparative analysis. Once again, drinking water scenario A (consumption of sachet water) had the lowest *E. coli* dose estimate, with an average of 1.62 log_{10} CFU consumed per week, and drinking water scenario B (stored household water) had the largest *E. coli* exposure, with an average dose of 3.61 log_{10} CFU per week. Although food scenario B (children aged 2-5 years) had the second largest weekly dose estimate, with an average *E. coli* dose of 3.28 log_{10} CFU, it is still half the dose estimated for drinking water scenario B.

It is important to note that the development of this risk assessment model required several assumptions and simplifications, both in regards to behavioral characteristics of the study populations and model parameters. First, these exposure dose estimates only reflect on the state of contamination for produce items, as they were at the local market, and do not take into account microbial growth, decay, or reduction by washing before salad preparation. Previous studies by Amoah et al. reported that 90 percent of lettuce-consuming households in Ghana report washing produce before consumption [10]; these results were later confirmed by Barker et al., who reported that 100 percent of survey respondents in Kumasi (the second largest urban city in Ghana) reported some method of vegetable washing [12]. Given this information, it is likely that our exposure dose estimates only reflect the risk from the consumption of a prepared street food salad, where produce washing habits vary by vendor, as opposed to household salad consumption, where produce washing is more likely. Second, since neither side salads nor their vegetable components were collected and weighed, we had to make assumptions about the size of a salad serving and the relative weights of the vegetables comprising the salad. Moreover, we had to make assumptions about frequency of salad consumption by children for both scenarios, given that the frequency of salad consumption in our study was only reported by adult consumers.

ii. Microbial Data and Behavioral Characteristics of the Study Populations: Implications for Microbial Risk Assessment

Except for sachet water, which had only 1 out of 60 samples quantifiable for *E. coli, E. coli* contamination was found consistently in drinking water sources across the AMA. Out of the three drinking water sources tested, stored household water had both the highest frequency and the highest concentration of *E. coli*, with 93.5 percent (n=62) of samples quantifiable and an average detected concentration of 1.78 $\log_{10}$ CFU per 100mL. These concentrations are slightly higher than what Machdar et al. (2013) previously reported for stored household water in Accra (1.11 $\log_{10}$ CFU per 100mL) [20], and Mattioli et al., who reported an average *E. coli* concentration of 1.5 $\log_{10}$ CFU per 100mL for stored household water in urban Tanzania [17]. The increased frequency and magnitude of *E. coli* concentration found in stored household are not surprising – it has been frequently observed that the microbiological quality of stored water is lower than that of water at its source. A meta-analysis by Wright et al. (2004) found that out of 57 studies assessing microbiological contamination between water at its source and point-of-use, approximately half identified significant contamination after collection [21].

E. coli concentrations observed in drinking water sources did not vary by neighborhood except for Old Fadama, where municipal piped water had significantly higher mean levels of *E. coli* (1.08 $\log_{10}$ CFU per 100mL) compared to the other study neighborhoods (0.30 $\log_{10}$ CFU per 100mL) (p-value=0.0003). The higher levels of *E. coli* contamination found in Old Fadama are not surprising given that its residents reported the least access to sanitation facilities and the neighborhood is an illegal squatter settlement built on top of a large e-waste dumpsite [58].

E. coli contamination on produce was widespread, as every produce item tested had quantifiable *E. coli* concentrations. Lettuce showed the highest concentration of *E. coli* with an average of 2.41 $\log_{10}$ CFU per g while the other three produce item ranged from 0.47-0.61 $\log_{10}$ CFU per g. The average *E. coli* concentrations observed on cabbage (0.57 $\log_{10}$ CFU per g) are consistent with those reported in a study of produce contamination in Mexico

(0.86 log_{10} CFU per g) [60]. However, the average *E. coli* concentrations found on tomatoes in this study (0.54 log_{10} CFU per g) are markedly lower than what was previously reported by Shenge et al. (2015) in a similar study of tomatoes in Nigeria (2.66 log_{10} MPN per g) [61].

Given that urban farmers rely on Accra's urban drainage system as their primary source of irrigation water, produce contamination occurs predominantly on the farm [10, 25]. A recent study on irrigation water quality in Accra by Silverman et al. (2013) found that every water sample tested failed to meet the standards set forth by the WHO: all samples had *E. coli* concentrations greater than 1 x 10^4 per 100mL and some had *E. coli* concentrations more than three orders of magnitude larger than this limit [62]. This, combined with the use of over-head watering cans as the chief irrigation technique, deposits any fecal microbes present in irrigation water directly onto produce surfaces [25]. Although this study only examined produce contamination in four low-income neighborhoods, these practices likely affect all produce distributed across the AMA. Therefore, protecting irrigation water from contamination or providing farmers with a reliable, alternative source of water would improve produce safety throughout the AMA.

While the probability of illness was not calculated in this study, differences in neighborhood-level consumer habits and preferences suggest that risk is likely not uniform across the city or settings. These differences were most notable for two study neighborhoods – Old Fadama and Alajo – which represent the two extremes of our study neighborhoods' population and physical characteristics. Agreeing with a previous report by Stoler et al. (2012), who found that sachet-using households were more likely to have lower socioeconomic status [49], Old Fadama residents were more likely to rely on sachet water for their primary drinking water source (p-value<0.001). Given that drinking water scenario A (consumption of sachet water) produced the lowest dose estimates, it is likely that the children in this neighborhood are less exposed to fecal microbes via drinking water exposure. Old Fadama residents were also more likely to rely on vended street food than the other neighborhoods (p-value <0.0001). While this does not affect exposure dose, it does have implications for how frequently residents purchase street food and thus how likely a caregiver is to feed a child portions of a meal that they purchased from a food vendor. If adults are more likely to purchase street food, frequency of child exposure to street food salads may be higher than estimated in this model (food scenario B assumes a frequency of once a week); therefore, our exposure model may underestimate the true risk of exposure for children in this neighborhood. Conversely, residents in Alajo were more likely to never purchase vended food compared to the other study neighborhoods (p-value<0.0001); therefore, the likelihood of a child consuming a portion of salad is small, and thus our exposure model may overestimate the true risk of exposure for children in this neighborhood. Furthermore, Alajo residents were also more likely to report that their child never purchased vended food at school (p-value=0.009), suggesting that the dose estimates produced in food scenario A (children aged 5-12 years) are not as applicable for this study neighborhood.

Additionally, there were several limitations in this study, but the most salient included the use of the fecal indicator bacteria *E. coli* and the sampling methods used to collect environmental samples. Measuring pathogens in environmental samples is a complex process that requires large amounts of personnel and financial capital and results may be undependable. While this study attempted to measure pathogens, concentration estimates were highly unreliable; therefore, the fecal indicator organisms *E. coli,* was used as a proxy for other enteric pathogens. Fecal indicator organisms, such as *E. coli,* are indigenous to the intestines of both humans and warm-blooded animals and can provide evidence for the potential presence of other pathogenic organisms surviving under similar environmental conditions [26]. However, their validity as an indicator for health risks has been questioned. Several studies have reported an association between levels of *E. coli* in drinking water with childhood diarrhea [63, 64] while others, including a meta-analysis by Gundry et al. (2004), could not find any relationship between the indicator organism concentration and incidence of diarrhea [65, 66]. Thus, *E. coli* dose estimates reported here should be used to illustrate fecal exposure pathways, as opposed to making direct inferences about health risks. Moreover, environmental samples were only collected from households that were selected for participation via a community liaison, which could limit the generalizability of our results.

E. Conclusions

- With the lowest frequency of contamination, sachet water was the safest water source in the AMA. It also represents both the lowest daily and weekly dose to *E. coli* across all scenarios (food and water). The majority of residents across the AMA used sachets as their primary source for drinking water, and residents of Old Fadama relied on them almost exclusively, suggesting that exposure to fecal contaminants through consumption of sachet water is minimal.
- Municipal water had varying degrees of *E. coli* contamination across the AMA, and Old Fadama had significantly higher concentrations than the other three study neighborhoods. Although consumption of municipal tap water does not produce the largest daily or weekly dose estimate, nursery schools, primary schools, and a

sizeable proportion of the AMA population rely on this source for their drinking water needs; therefore, it is imperative to address possible sources of fecal contamination and protect the water supply.

- Stored household water had the greatest frequency and the highest average *E. coli* concentration among the water sources examined. Stored household water also represents both the largest daily and weekly dose of *E. coli* across all exposure routes (food and water). The frequency of *E. coli* contamination observed in stored household water suggests that contamination may be due to: inadequately protected water storage containers, unsanitary dispensing methods, possible exposure to vectors, or lack of hygienic cleaning of storage containers. Fixing the intermittent municipal water supply, which is a large driver of household water storage practices in Accra [20], may be prohibitively expensive. However, as an alternative solution, educating households about proper water storage techniques and ensuring proper disinfection prior to consumption can protect citizens (and their children) from the health risks associated with consuming fecal-contaminated water sources. Convincing residents to habitually treat their water, however, may prove challenging.
- Produce in the AMA had significant fecal contamination: every produce item tested had quantifiable *E. coli* concentrations. Lettuce had the highest average observed *E. coli* concentration while the other three produce items had smaller, but consistent levels. Therefore, consuming raw produce items constitutes a public health risk and measures should be taken to mitigate the risk of exposure for this route. This can be achieved in the short term by urging consumers to rinse raw produce items in a bleach solution prior to consumption. Considering residents in the AMA already

routinely cleanse their vegetables before consumption, altering this behavior to a safer and more effective version should be met with low resistance.

- A general lack of sanitation, specifically household latrines and municipal sewage treatment, combined with residents' propensity to dispose of sewage into the urban drainage system creates an environment in which both food and water sources become readily contaminated with fecal matter. In order to reduce these exposures, residents should be educated about safe water storage practices and urged to disinfect both produce and drinking water prior to consumption. While drinking water exposures may be mitigated through education, in order to improve the safety of produce, the microbiological quality of irrigation water needs to be improved. This can be achieved by either preventing fecal contamination of the urban drainage system, via improving sanitation coverage in the AMA and encouraging the cessation of waste dumping in drains, or increasing farmers' access to a reliable, safe alternative water source for crop irrigation.

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G. Tables

Table 2.1 Population and physical characteristics of study neighborhoods

Neighborhood	**Population and Physical Characteristics**				
	Predominant Religion	Inland vs. Coastal	Density	Older vs. Newer	Squatter Settlement
Alajo	Christian	Inland	Medium	Older	No
Bukom	Christian	Coastal	High	Older	No
Old Fadama	Muslim	Inland	High	Newer	Yes
Shiabu	Christian	Coastal	High	Newer	Yes

Table 2.2 Model parameters used to estimate food exposure dose distributions

Variable	Description	Units	Parameter	Source	Key Assumptions
	Produce Contamination		(Mean, Std. Dev)		
C_F[1]	*E. coli*	CFU[2] per g	Log_{10} Units		Pathogens are evenly distributed and concentrations are constant across time.
	Lettuce		(2.48, 1.03)	This study	
	Cabbage		(0.50, 1.22)	This study	
	Tomato		(-0.04, 1.58)	This study	
	Spring Onion		(0.24, 0.91)	This study	
I	Mass of Salad Consumed	g per salad			
	Full portion		Point Estimate: 20	Fung et. al	Salad portion is 20g
	Half portion		Point Estimate: 10	Assumption	Half a portion of salad is 10g
F	Frequency of Consumption	salad per week			
	Children aged 5-12 years		Point Estimate: 3	Assumption	Salad is consumed 3x a week
	Children aged 2-5 years		Point Estimate: 1	Assumption	Salad is consumed 1x a week
	Mass of Lettuce Head	g	Point Estimate: 83	This study	Lettuce heads are 83 g
	Mass of Cabbage Head	g	Point Estimate: 312	This study	Cabbage heads are 312g
	Mass of Tomato	g	Point Estimate: 91	USDA[3]	Tomatoes are 91g
	Mass of Spring Onion	g	Point Estimate: 5	USDA[3]	Spring Onions are 5g

[1] Monte Carlo simulations used to create parameter estimates

[2] CFU denotes Colony Forming Units

[3] USDA denotes United States Department of Agriculture

Table 2.3 Model parameters used to estimate drinking water exposure dose distributions

Variable	Description	Units	Parameter	Source	Key Assumptions
	Drinking Water Contamination		(Mean, Std. Dev)		
C_W[1]	*E. coli*	CFU[2] per 100mL	Log_{10} Units		
	Sachet		(-0.33, 0.11)	This study	Pathogens are evenly distributed and concentrations are constant across time.
	Stored Household		(1.68, 1.09)	This study	
	Municipal Piped[3]		(-0.18, 0.36)	This study	
	Fadama piped[4]		(0.25, 0.88)	This study	
L[1]	Child Water Consumption	L per day			
	0-1 years old		(0.56, 0.39)	This study	Parent reported intake volumes are accurate.
	1-2 years old		(1.07, 0.69)	This study	
	2-5 years old		(1.49, 0.66)	This study	

[1] Monte Carlo simulations used to create parameter estimates
[2] CFU denotes Colony Forming Units
[3] Estimates apply only to Alajo, Bukom, and Shiabu neighborhoods
[4] Estimates apply only to the Old Fadama neighborhood

Table 3.1 Reported demographics by neighborhood, Accra, Ghana, 2012

	Alajo	**Bukom**	**Old Fadama**	**Shiabu**
Population Characteristics	**n (%) n=200**	**n (%) n=200**	**n (%) n=200**	**n (%) n=200**
Homeowner	103 (51.5)	160 (80.0)	129 (64.5)	109 (54.5)
Religion				
Christian	158 (79.0)	176 (88.0)	75 (37.5)	193 (96.5)
Muslim	44 (21.0)	16 (8.0)	121 (60.5)	5 (2.5)
Other	0	8 (4.0)	4 (2.0)	2 (1.0)
HH[1] with no formal education	25 (12.5)	27 (13.5)	87 (43.5)	17 (8.5)
HH[1] with children under 5	116 (58.0)	110 (55.0)	90 (45.0)	110 (55.0)
HH[1] with sanitation facility in compound	116 (58.0)	12 (6.0)	2 (1.0)	94 (47.0)
HH[1] with bathing facility in compound	182 (91.0)	118 (59.0)	6 (3.0)	168 (84.0)
Household Characteristics (±SD)	**n=200**	**n=200**	**n=200**	**n=200**
Average Size	5.2 (±3.6)	5.8 (±4.9)	4.1 (±3.0)	4.5 (±2.0)
Average # of children under 5 years	0.6 (±0.8)	0.7 (±1.0)	0.7 (±0.7)	0.6 (±0.8)
Average # of children 5-12 years	0.8 (±1.0)	1.1 (±1.2)	0.5 (±0.1)	0.7 (±0.9)
Diarrhea Prevalence	**n=81**	**n=115**	**n=115**	**n=89**
Child with diarrhea in past two weeks	10 (12.3)	20 (17.4)	29 (25.2)	9 (10.1)

[1] HH denotes household

Table 3.2 Reported primary drinking water sources by neighborhood, Accra, Ghana, 2012

Primary drinking water source	**Alajo**	**Bukom**	**Old Fadama**	**Shiabu**
	n=200 (%)	**n=200 (%)**	**n=200 (%)**	**n=200 (%)**
Sachet	153 (76.5)	144 (72.0)	186 (93.0)[3]	150 (75.0)
Tap from pipe[1]	46 (23.0)	56 (28.0)	13 (6.5)	48 (24.0)
Tap from tank[2]	1 (0.5)	0	1 (0.5)	2 (1.0)

[1] Tap from pipe refers to the municipal piped water supply

[2] Tap from tank refers to municipal piped water stored in a polytank

[3] Old Fadama residents were more likely to rely on sachet water than other study neighborhoods (p-value<0.001)

Table 3.3 Reported dietary habits by neighborhood, Accra, Ghana, 2012

Consumer Group	Frequency	Alajo	Bukom	Old Fadama	Shiabu
Adults	Purchase vended food	**n=200 (%)**	**n=199 (%)**	**n=199 (%)**	**n=200 (%)**
	Everyday	41 (20.5)	60 (30.2)	101 (50.8)[1]	34 (17.0)
	Few times per week	82 (41.0)	102 (51.3)	73 (36.7)	98 (49.0)
	Once a week	15 (7.5)	10 (5.0)	5 (2.5)	15 (7.5)
	Never	62 (31.0)1	27 (13.6)	20 (10.1)	53 (26.5)
	Eat raw produce				
	Everyday	69 (34.5)	73 (36.9)	68 (34.2)	69 (34.5)
	Few times per week	91 (45.5)	77 (38.9)	98 (49.3)	99 (49.5)
	Once a week	10 (5.0)	8 (4.0)	7 (3.5)	15 (7.5)
	Never	30 (15.0)	26 (13.1)	26 (13.1)	17 (8.5)
Children	Purchase vended food (at school)	**n=50 (%)**	**n=51 (%)**	**n=47 (%)**	**n=62 (%)**
	Every time	1 (2.0)	17 (33.3)	14 (29.8)	15 (24.2)
	Sometimes	1 (2.0)	7 (13.7)	2 (4.3)	5 (8.1)
	Never	48 (96.0)[2]	27 (52.9)	30 (63.8)	41 (66.1)
	No response	0	0	1 (2.1)	1 (1.6)

[1] Residents of Alajo were more likely to never purchase vended food while residents of Old Fadama were more likely to purchase vended food everyday compared to the other study neighborhoods (p-value<0.0001)

[2] Children in Alajo were more likely to never purchase vended food at school compared to children in the other study neighborhoods (p-value=0.009)

Table 3.4 Estimated *E. coli* concentrations for environmental samples from four study neighborhoods in Accra, Ghana, 2011-2012

E. coli **concentration**	**Total**	**Quantifiable Samples**					**Non-quantifiable Samples**	
Log$_{10}$ units	**N**	**N (%)**	**Mean**	**Median**	**Min**	**Max**	**N (%)**	**Assigned Value**[4]
Water Samples (CFU[1] per 100mL)								
Sachet	61	1 (1.6)	0.44	0.44	0.44	0.44	60 (98.4)	
Stored household	62	58 (93.5)	1.78	1.75	0	4.30	4 (6.5)	-0.35
Municipal Piped[2]	86	21 (24.4)	0.30	0	0	1.51	65 (75.6)	
Fadama Piped[3]	31	15 (48.4)	1.08	1.00	0	3.03	16 (51.6)	
Food Samples (CFU[1] per g)								
Lettuce	149	149 (100)	2.41	2.57	-0.57	4.08	0	NA[5]
Cabbage	62	62 (100)	0.61	0.57	-1.74	3.50	0	NA[5]
Tomato	18	18 (100)	0.54	0.32	-2.00	2.64	0	NA[5]
Spring onion	11	11 (100)	0.47	0.30	-1.11	2.29	0	NA[5]

[1] CFU denotes Colony Forming Units
[2] Estimates only apply to Alajo, Bukom, and Shiabu neighborhoods
[3] Estimates only apply to the Old Fadama neighborhood
[4] Non-quantifiable samples were assigned a value of half the limit of detection
[5] NA denotes Not Applicable

Table 3.5 Monte Carlo simulated *E. coli* dose estimates for three drinking water scenarios

Exposure Scenario	*E. coli* **CFU[1] consumed per day**		
Log_{10} units	**Mean**	**Median**	**95% Confidence Interval**
Scenario A (Sachet)			
Children 0-1 Years Old	0.33	0.35	(0.31, 0.34)
Children 1-2 Years Old	0.60	0.65	(0.58, 0.62)
Children 2-5 Years Old	0.77	0.83	(0.76, 0.79)
Scenario B (Stored Household)			
Children 0-1 Years Old	2.32	2.31	(2.25, 2.39)
Children 1-2 Years Old	2.59	2.57	(2.52, 2.66)
Children 2-5 Years Old	2.76	2.77	(2.69, 2.83)
Scenario C (Municipal Piped)[2]			
Children 0-1 Years Old	0.47	0.35	(0.44, 0.50)
Children 1-2 Years Old	0.74	0.65	(0.71, 0.77)
Children 2-5 Years Old	0.91	0.83	(0.88, 0.94)
Scenario C (Fadama Piped)[3]			
Children 0-1 Years Old	0.97	0.70	(0.90, 1.03)
Children 1-2 Years Old	1.24	0.95	(1.17, 1.30)
Children 2-5 Years Old	1.41	1.05	(1.34, 1.47)

[1] CFU denotes Colony Forming Units

[2] Estimates apply only to Alajo, Bukom, and Shiabu neighborhoods

[3] Estimates apply only to the Old Fadama neighborhood

Table 3.6 Monte Carlo simulated *E. coli* weekly dose estimates for two food scenarios

Exposure Scenario Log$_{10}$ units	*E. coli* **CFU[1] consumed per week**		
	Mean	**Median**	**95% Confidence Interval**
Scenario A[2] (Children 5-12 years)	4.05	4.14	(4.00, 4.11)
Scenario B[3] (Children 2-5 years)	3.28	3.36	(3.22, 3.33)

[1] CFU denotes Colony Forming Units
[2] Scenario A was based on a child aged 5-12 years consuming a full serving of salad, three times per week
[3] Scenario B was based on a child aged 2-5 years consuming half a salad, one time per week

Table 3.7 Monte Carlo simulated *E. coli* weekly dose estimates for all scenarios

Exposure Scenario Log$_{10}$ units	*E. coli* **CFU**[1] **consumed per week**		
	Mean	**Median**	**95% Confidence Interval**
Water			
Scenario A (Sachet)			
Children 0-1 Years Old	1.18	1.20	(1.16, 1.20)
Children 1-2 Years Old	1.45	1.50	(1.43, 1.47)
Children 2-5 Years Old	1.62	1.67	(1.60, 1.64)
Scenario B (Stored Household)			
Children 0-1 Years Old	3.16	3.15	(3.09, 3.24)
Children 1-2 Years Old	3.43	3.42	(3.37, 3.50)
Children 2-5 Years Old	3.61	3.61	(3.54, 3.67)
Scenario C (Municipal Piped)[2]			
Children 0-1 Years Old	1.32	1.20	(1.29, 1.34)
Children 1-2 Years Old	1.59	1.50	(1.56, 1.61)
Children 2-5 Years Old	1.76	1.67	(1.73, 1.78)
Scenario C (Fadama Piped)[3]			
Children 0-1 Years Old	1.81	1.54	(1.75, 1.87)
Children 1-2 Years Old	2.08	1.80	(2.02, 2.14)
Children 2-5 Years Old	2.25	1.90	(2.19, 2.31)
Food			
Scenario A (Children 5-12 years)	4.05	4.14	(4.00, 4.11)
Scenario B (Children 2-5 years)	3.28	3.36	(3.22, 3.33)

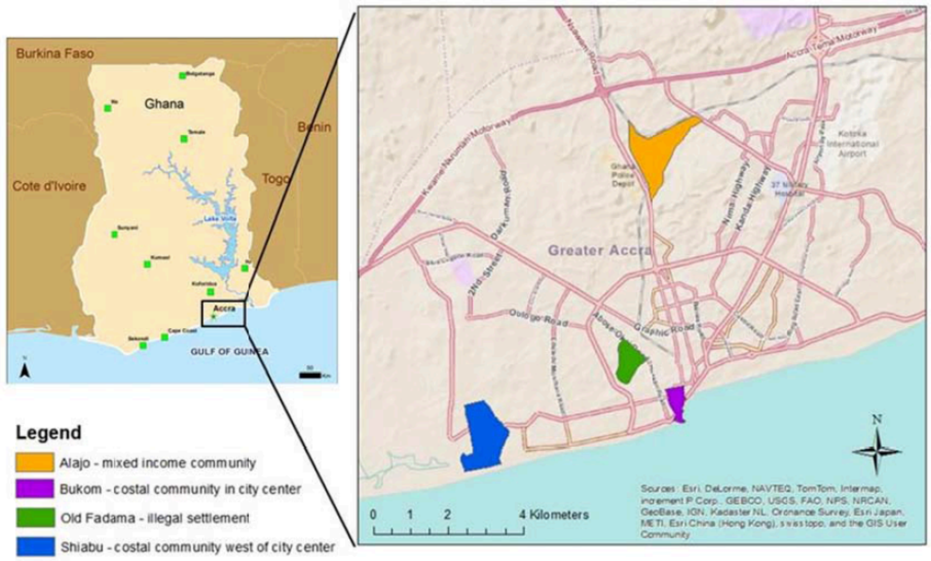
[1] CFU denotes Colony Forming Units

[2] Estimates apply only to Alajo, Bukom, and Shiabu neighborhoods

[3] Estimates apply only to the Old Fadama neighborhood

H. Figures

Figure 2.1 Study site areas: four low-resource urban neighborhoods in Accra, Ghana



Reproduced with permission from Stephanie Gretsch

Figure 3.1 Household survey locations

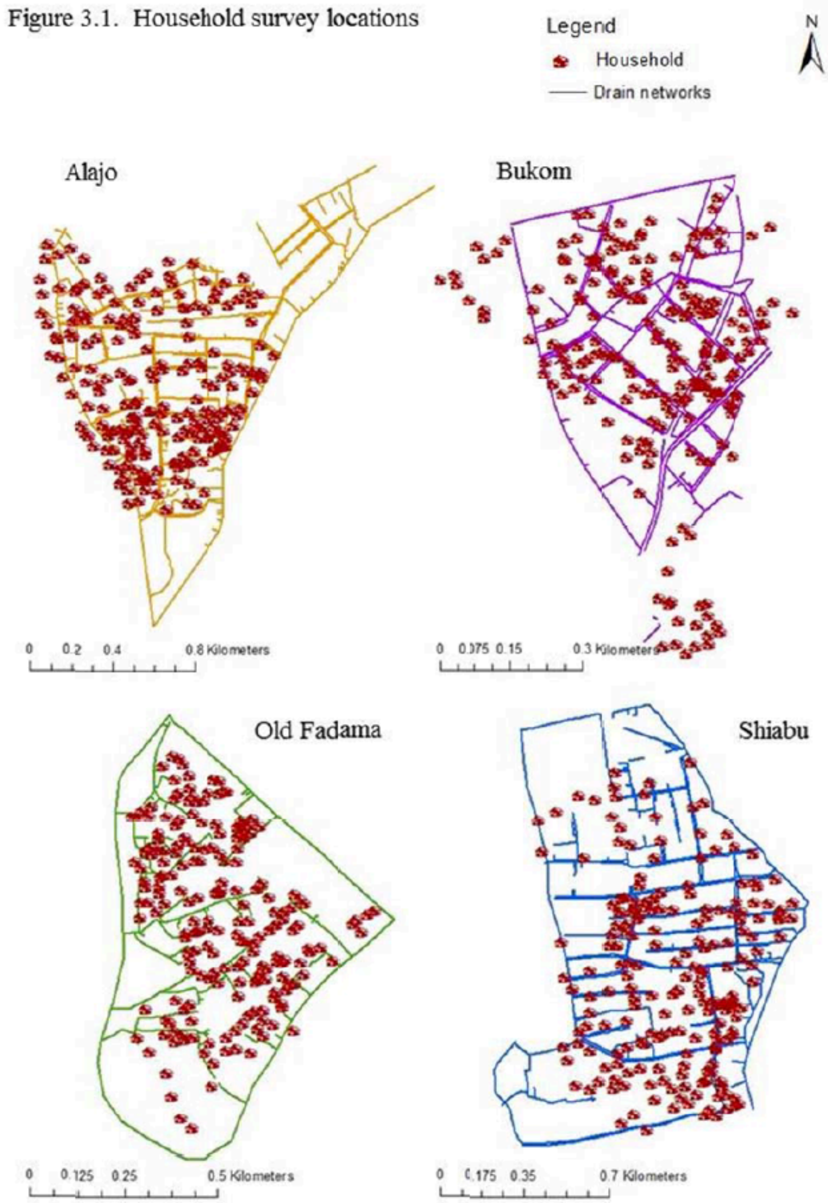
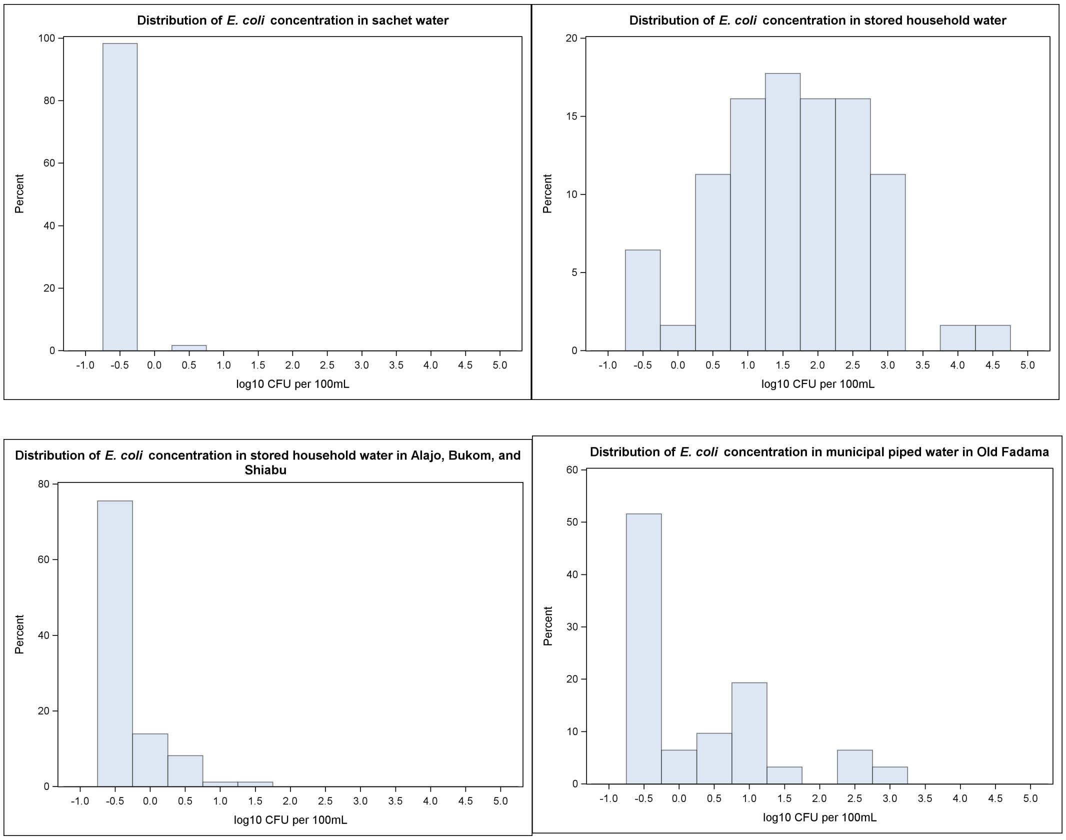
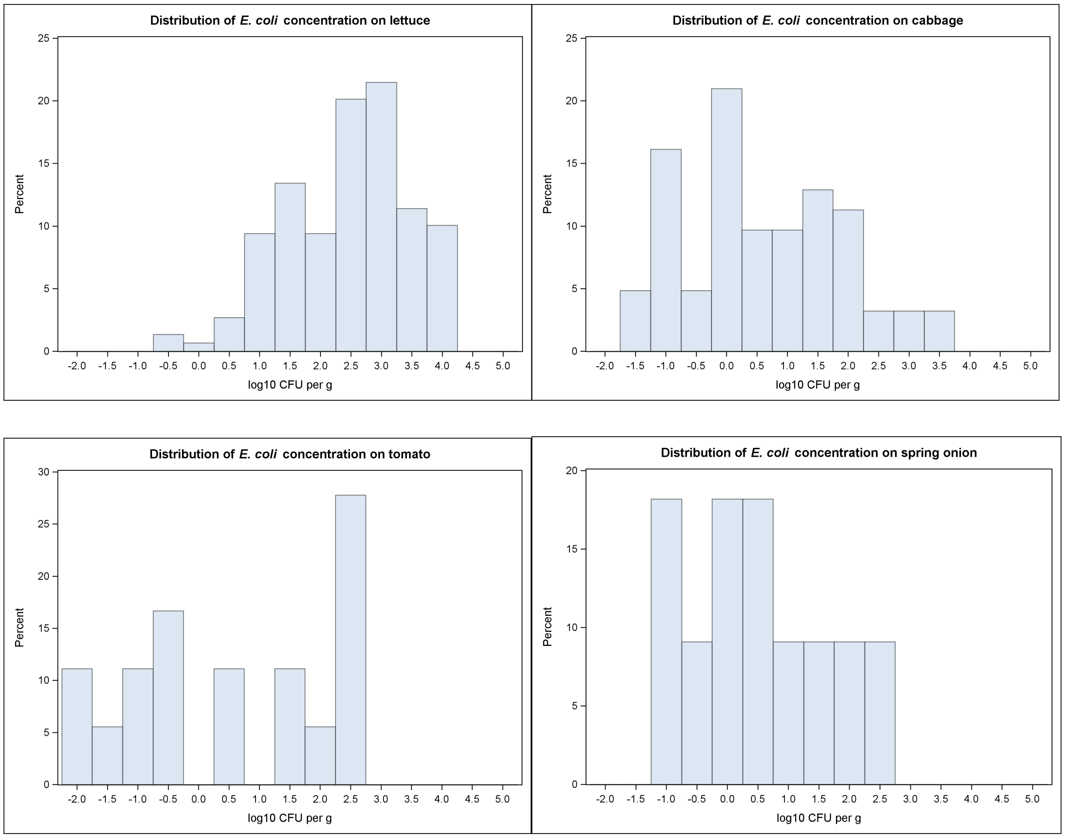


Figure 3.2 Distributions of log-transformed *E. coli* concentration in drinking water samples



CFU denotes Colony Forming Units

Figure 3.3 Distributions of log-transformed *E. coli* concentration on produce items



CFU denotes Colony Forming Units

Figure 3.4 Distribution of the reported daily volume of water consumed by age group

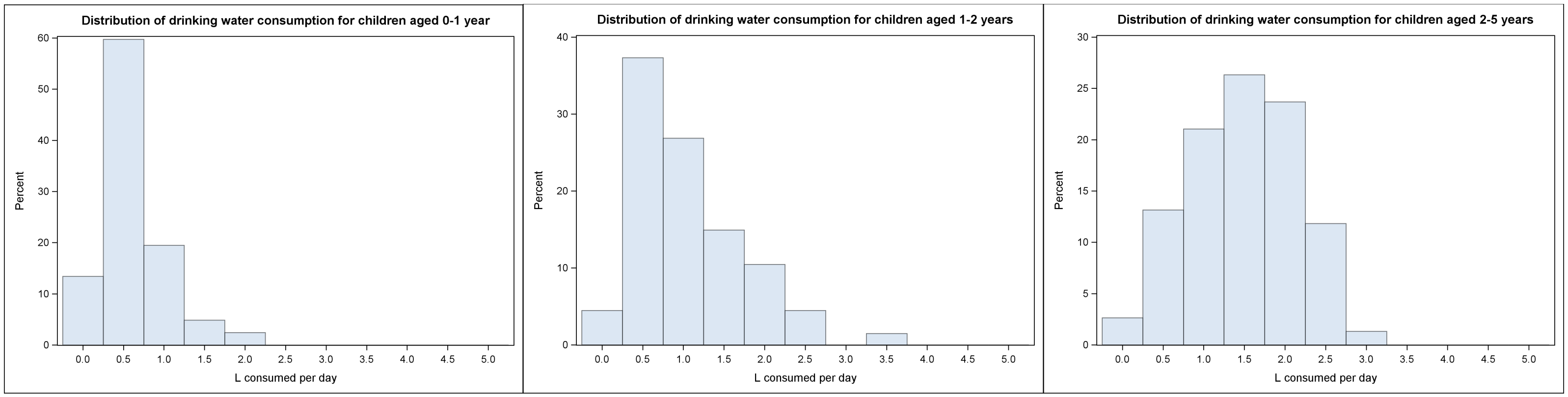
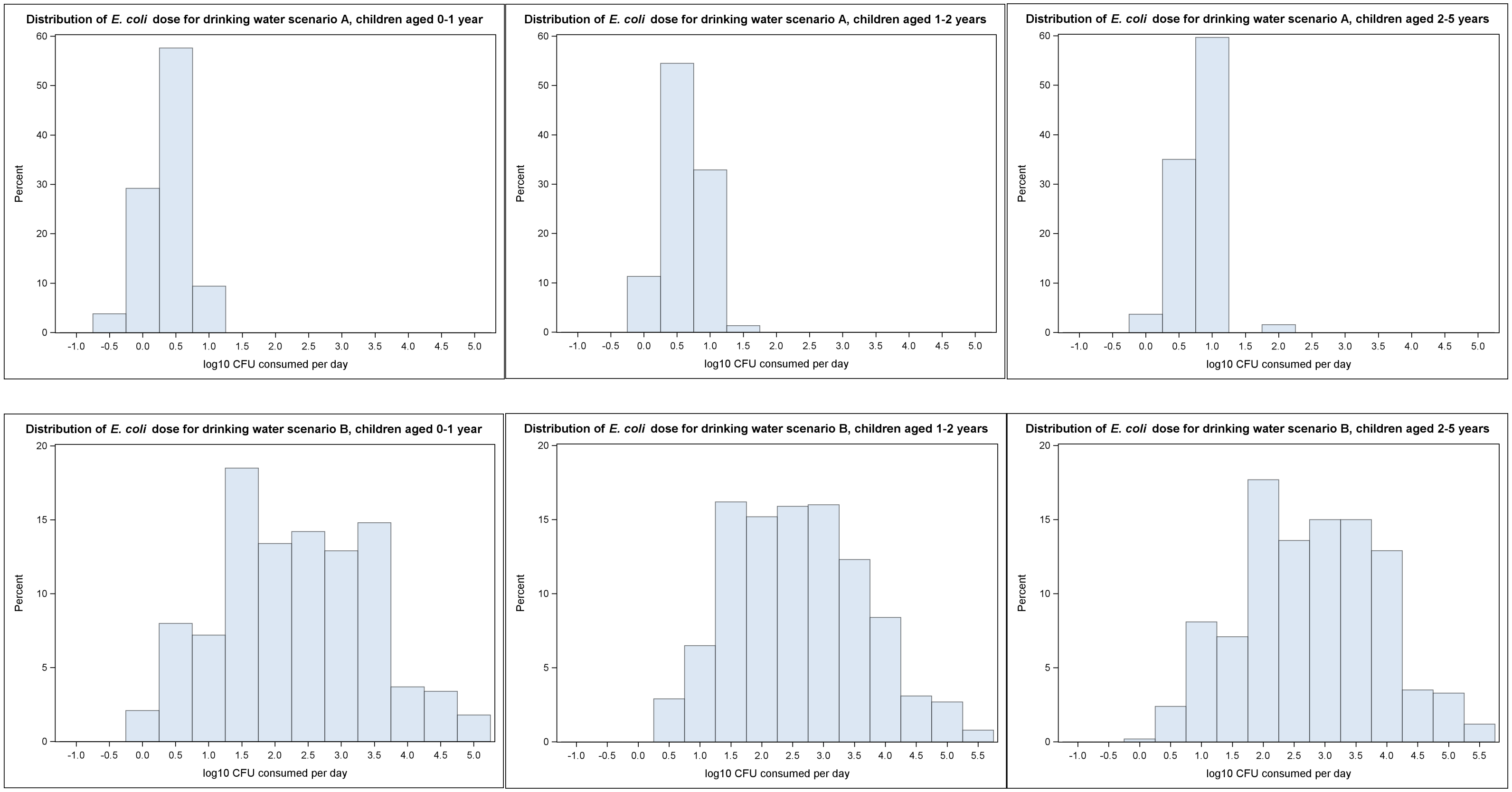
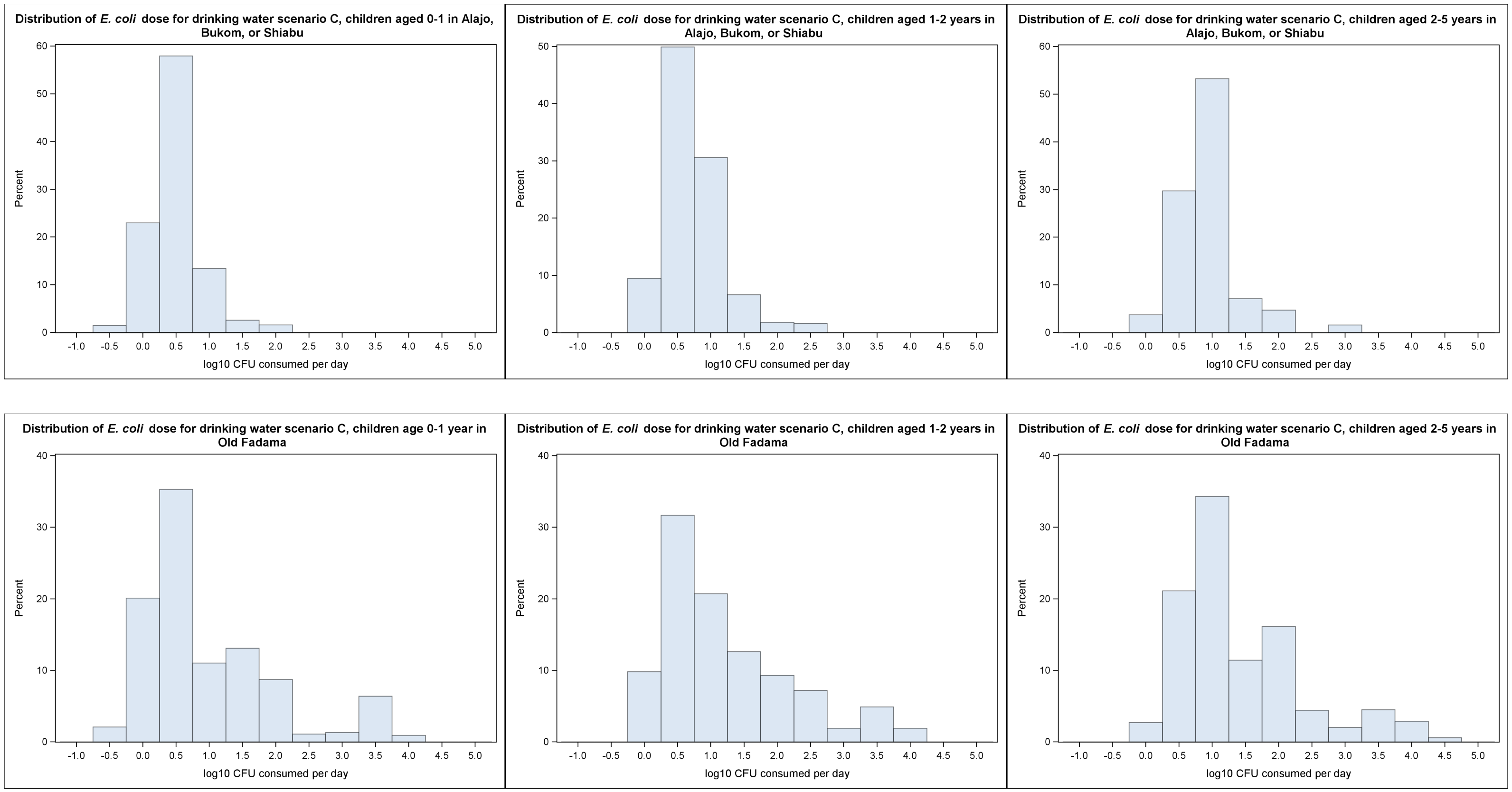


Figure 3.5 Distributions of log-transformed *E. coli* dose for drinking water scenario A (sachet water) and scenario B (stored household water)



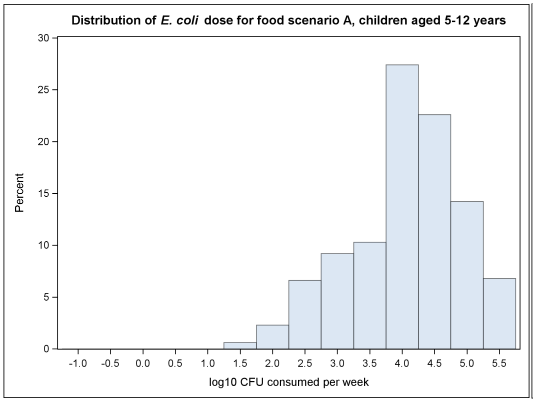
CFU denotes Colony Forming Units

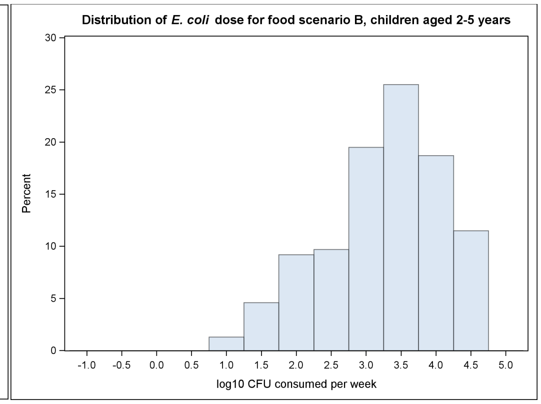
Figure 3.6 Distributions of log-transformed *E. coli* dose for drinking water scenario C (municipal piped water) by neighborhood



CFU denotes Colony Forming Units

Figure 3.7 Distributions of log-transformed *E. coli* dose for food scenarios





CFU denotes Colony Forming Units

Scenario A was based on a child aged 5-12 years consuming a full serving of salad, three times per week

Scenario B was based on a child aged 2-5 years consuming half a salad, one time per week

III. LESSONS LEARNED AND RECOMMENDATIONS

A. Lessons Learned for Future Analyses

1. The household questionnaire only allowed respondents to give water consumption estimates for one child; given that households in the study areas typically had more than one child in residence, future questionnaires should allow respondents to list water consumption for multiple children, representing multiple age groups. This would increase the data points available to estimate average daily water intake for children and thus increase the precision of the consumption estimates used for this analysis. The increased precision on daily water intake volumes would equate to an increased precision on the dose estimates produced from this risk assessment.
2. Produce items should be analyzed for *E. coli* individually rather than in aggregate. Since laboratory analysis was performed on multiple produce items, the *E. coli* concentration estimate had to be divided by the number of items tested. If instead we analyzed *E. coli* contamination on individual produce items, estimates would be more precise and would thus provide a more accurate representation of the variability in microbial contamination found on each produce item. This would reduce the assumptions used in our analysis and would increase the precision of our dose estimates relating to consumption of raw produce items.
3. Produce items, such as lettuce, cabbage, tomato and spring onion, were analyzed for *E. coli* contamination without being weighed; therefore, in order to standardize *E. coli* concentrations by weight, we had to make assumptions on the average weight of the respective produce item. Considering our dose estimates were calculated per gram of produce, changes in produce weight assumptions would significantly change the dose estimates created by our model.

4. The household questionnaire asked whether children in the household purchased vendor food at school but did not ask about vended-food consumption for children under the care of an adult (those aged 0-5 years). Currently, data regarding child consumption of street food (salad) is unavailable and thus collecting this information would have provided additional validity to our exposure scenarios and reduced the number of assumptions used in our analysis.
5. The household questionnaire should have asked about additional behaviors relating to food and drinking water exposures, specifically produce washing and water treatment practices among residents. Previous studies have shown that 90-100 percent of residents in Accra wash produce prior to consumption [10, 12]; however, those studies did not target residents in low-resource settings and thus there could be socio-cultural differences for this population. Furthermore, the household questionnaire did not ask about water treatment practices. With additional information on the percentage of residents consistently washing their produce and treating their water, a sensitivity analysis could be included that may better reflect of the actual exposure risk given the behavioral characteristics of the study populations.
6. Instead of relying on community liaisons to select households for participation, study participants should have been randomly selected within each neighborhood. This would have improved the internal validity of our study.
7. Future analyses could consider microbial growth or decay on food or in water.

B. Recommendations

1. As safe and reliable water sources become scarcer and more of the world's urban farmers rely on wastewater for irrigation, exposure to fecal contamination associated with consuming raw produce items will likely become more prevalent. In order to

mitigate this exposure, those preparing food should ensure that produce items are washed in a bleach solution prior to consumption; alternatively, consumers could avoid eating raw food items.

2. Children, who are at greater risk of contracting diarrheal diseases, should not eat raw produce or street food side salads.

3. In order to mitigate drinking water exposures, residents should also be prompted to boil all drinking water or treat with a disinfectant, such as chlorine, prior to consumption. Furthermore, educational materials on safe water storage and dispensing practices should be given to residents. This information should cover how to appropriately cover water storage containers, dispense water using sanitary methods, protect stored water from possible exposure to vectors (such as rodents and insects), and address how to keep storage containers hygienic.

4. In order to improve the microbiological safety of produce in the AMA, untreated sewage needs to be prevented from entering the environment in the first place, via improving sanitation coverage in the AMA and encouraging the cessation of waste dumping in drains, and/or increasing farmers' access to a reliable, safe alternative water source for crop irrigation.

IV. APPENDIX

A. IRB Approval

EMORY UNIVERSITY | Institutional Review Board

TO: Christine Moe, PhD
Principal Investigator
Global Health

DATE: April 4, 2014

RE: **Notification of Amendment Approval**
AM6_IRB00051584
IRB00051584
Assessment of Fecal Exposure Pathways in Low-Income Urban Settings

Thank you for submitting an amendment request. The Emory IRB reviewed and approved this amendment under the expedited review process on **4/4/2014**. This amendment includes the following:

Personnel Change Only: Adding Yuke Wang, Michelle Schmitz and Melissa Sizemore as other Emory study staff.

Important note: If this study is NIH-supported, you may need to obtain NIH prior approval for the change(s) contained in this amendment before implementation. Please review the NIH policy directives found at the following links and contact your NIH Program Officer, NIH Grants Management Officer, or the Emory Office of Sponsored Programs if you have questions.

Policy on changes in active awards: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-129.html

Policy on delayed onset awards: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-130.html

In future correspondence with the IRB about this study, please include the IRB file ID, the name of the Principal Investigator and the study title. Thank you.

Sincerely,

Donna Thomas
Analyst Assistant
This letter has been digitally signed

CC Peprah Dorothy Global Health

B. Household Survey

Household Survey

Location ID ______ GPS longitude W000. ______ Date ______

GPS latitude N05. ______

1.0 General Information

Ques #	Description
101	Should I describe you as a head of a household or household member? (A household is people sharing a cooking pot) ☐ Female head of household ☐ Male head of household ☐ Female household member ☐ Male household member
102	Tenancy status ☐ Renter ☐ Owner
103	How long have you lived in this household? ______ months/years
104	What is your highest level of education? ☐ No formal education ☐ Higher than secondary ☐ Some primary ☐ No response ☐ Completed primary ☐ Some secondary ☐ Completed secondary
105	What is your religion? ☐ Christian ☐ No response ☐ Moslem ☐ Traditional/Spiritualist ☐ No religion ☐ Other
106	What is your mother tongue? ☐ Ga ☐ Other ☐ Twi ☐ No response ☐ Fante ☐ Hausa
107	Is a business run from this compound/household? ☐ Yes ☐ No ☐ No response
108	If so, what kind of business? ☐ Food vending ☐ Other
109	How many households are in this compound? A household is people sharing a cooking pot. ______
110	How many people live in this compound? ______

1

111	How many people are in your household?	hhs_111
112	How many male adults (18 and older) live in your household?	hhs_112
113	How many female adults (18 and older) live in your household?	hhs_113
114	How many children under 5 live in your household?	hhs_114
115	How many children ages 5 -12 live in your household?	hhs_115
116	How many young people ages 13-17 live in your household?	hhs_116
117	Does your household have electricity? hhs_117	☐ Yes ☐ No ☐ No response
118	Does your household have a radio? hhs_118	☐ Yes ☐ No ☐ No response
119	Does your household have a television? hhs_119	☐ Yes ☐ No ☐ No response
120	Does your household have a refrigerator? hhs_120	☐ Yes ☐ No ☐ No response
121	Does your household have a bicycle? hhs_121	☐ Yes ☐ No ☐ No response
122	Does your household have a motorcycle? hhs_122	☐ Yes ☐ No ☐ No response
123	Does your household have a car? hhs_123	☐ Yes ☐ No ☐ No response
124	Does your household have a domestic worker not related to the head of the household hhs_124	☐ Yes ☐ No ☐ No response

2.0 Water Supply

201	What is your primary source of drinking water? hhs_201 ☐ Sachet/Water bottle ☐ Well ☐ Other Specify other hhs_201a ☐ Tap from pipe network ☐ Water truck ☐ Don't know ☐ Tap form polytank ☐ Harvested Rainwater ☐ No response
202	How often do you replenish this primary source of drinking water? hhs_202 ☐ Everyday ☐ Few times per week ☐ Once per week or less
203	How much water does your child drink every day? hhs_203 ☐ Cups ☐ Sachets hhs_203a
204	What is your primary sources of water for cooking and hygiene? hhs_204 ☐ Sachet/Water bottle ☐ Well ☐ Other Specify other hhs_204a ☐ Tap from pipe network ☐ Water truck ☐ Don't know ☐ Tap form polytank ☐ Harvested Rainwater ☐ No response

3.0 Sanitation and Hygiene

301 How many latrines are on this compound? hhs_301 If none -> 303

302 How many households do you share a latrine with? hhs_302

303 Where do children (ages 5-12) in your household typically defecate? hhs_303

- ☐ Compound latrine
- ☐ Public latrine
- ☐ In a bag/flying toilet
- ☐ Chamber pot
- ☐ Outside
- ☐ CBeach
- ☐ Other Specify othe hhs_303a
- ☐ Don't know
- ☐ No response

304 (If single household, then skip) Is this the same for the other children in this compound? hhs_304

- ☐ Yes
- ☐ No
- ☐ Don't know
- ☐ No response

305 (If respondent has a child under 5) The last time your youngest child defecated, where did they defecate? hhs_305

- ☐ Compound latrine
- ☐ Public latrine
- ☐ In a potty
- ☐ In a diaper/nappy
- ☐ On ground/outside compound
- ☐ On ground/inside compound
- ☐ In drain/gutter
- ☐ Don't know
- ☐ No response

(If resondent does not have child under 5, skip to 308)

306 What is the age of this child? hhs_306 hhs_306a Months/Years

307 The last time your youngest child defecated, how did you dispose of feces? hhs_307

- ☐ Combined with rubbish
- ☐ Dumped into drain/gutter
- ☐ Dumped in public latrine
- ☐ Dumped in compound latrine -
- ☐ Washed diaper/nappy
- ☐ Left on ground
- ☐ Don't know
- ☐ No response

308 Do other mothers in the compound ever use potties for their children? hhs_308

- ☐ Yes
- ☐ No
- ☐ Don't know
- ☐ No response

309 Are there ever times when other mothers leave child feces on the compound ground? hhs_309

- ☐ Yes
- ☐ No
- ☐ Don't know
- ☐ No response

310 How often do you see children defecating in the drain near your house?: hhs_310

- ☐ Everyday
- ☐ Sometimes
- ☐ Never
- ☐ No drain near house
- ☐ No response

311 How often do you see adults defecating in the drain near your house?: hhs_311

- ☐ Everyday
- ☐ Sometimes
- ☐ Never
- ☐ No drain near house
- ☐ No response

312	How much did members of your household spend on public latrines yesterday, not including showers?	hhs_312	Cedis/Pesewas hhs_312a
313	Where did you last bathe? hhs_313 ☐ In compound ☐ Other Specify othe hhs_313a ☐ Public bath ☐ No response ☐ Beach		
314	How much did members of your household spend on bathing facilities yesterday?	hhs_314	Cedis/Pesewas hhs_314a
315	How much did you spend on drinking water yesterday for your family?	hhs_315	Cedis/Pesewas hhs_315a
316	How much did you spend on water (for all purposes) yesterday for your family?	hhs_316	Cedis/Pesewas hhs_316a
317	How does your household primarily dispose of rubbish? hhs_317 ☐ Disposal pit in compound ☐ Drain ☐ Other Specify other hhs_317a ☐ Private collection service ☐ Public dump site ☐ Don't know ☐ Private local collection ☐ Burned ☐ No response		
318	How much did you spend on rubbish disposal yesterday?	hhs_318	Cedis/Pesewas hhs_318a

4.0 Health

401 Has this child had diarrhoea in the past two weeks? (Diarrhoea is 3 or more loose or watery stools within 24 hours) hhs_401

- ☐ Yes
- ☐ No -> **403**
- ☐ Don't know -> **403**
- ☐ No response -> **403**

402 Was this bloody diarrhoea? hhs_402

- ☐ Yes
- ☐ No
- ☐ Don't know
- ☐ No response

403 Do you ever de-worm your child? hhs_403

- ☐ Yes
- ☐ No
- ☐ Don't know
- ☐ No response

404 When did you last de-worm him/her? hhs_404 ____ days/months/years hhs_404a

5.0 Wash facilities on compound

501 What kind of latrine do you have in this compound? hhs_501

- ☐ No facility/Bush/Field
- ☐ Traditional pit latrine
- ☐ KVIP(double)
- ☐ VIP(single)
- ☐ Bucket/pan
- ☐ Pour flush
- ☐ Flush toilet
- ☐ Other
- ☐ No response

502 Do you have a sink where you wash your hands after defecation? hhs_502

- ☐ Yes
- ☐ No
- ☐ No response

503 Do you have a container where you store drinking water?: hhs_503

- ☐ Yes
- ☐ No -> **506**
- ☐ No response -> **506**

504 If so, can you please show this to me? hhs_504
(What type of drinking water container do they have?)

- ☐ Narrow mouth (<6cm)
- ☐ Wide mouth (>6cm)
- ☐ Not able to see -> **skip 506**

505 Is the container that the drinking water is stored in covered or uncovered?: hhs_505

- ☐ Covered
- ☐ Not covered
- ☐ Not able to see -> **skip 506**

506 (Observe, don't ask!) Are feces observed around compound grounds?: hhs_506 ☐ Yes ☐ No

507 (Observe, don't ask!) Are animals observed roaming around compound grounds?: hhs_507 ☐ Yes ☐ No-> **skip 50**

508 (Observe, don't ask!) If yes, which animals are observed? (check all that apply): hhs_508

- ☐ Goats ☐ Dogs
- ☐ Chickens ☐ Cats
- ☐ Pigs ☐ Other

509 (Observe, don't ask!) Are food waste observed on compound grounds?: hhs_509 ☐ Yes ☐ No

6.0 Weekly Activities Questions

601 This past week, how many times did you use a public latrine?: hhs_601

- ☐ Everyday ☐ Never
- ☐ A few times a week ☐ No response
- ☐ Once a week

602 This past week, how many times did you go to the market: hhs_602

- ☐ Everyday ☐ Never
- ☐ A few times a week ☐ No response
- ☐ Once a week

603 This past week, how many times did you eat raw produce?: hhs_603

- ☐ Everyday ☐ Never
- ☐ A few times a week ☐ No response
- ☐ Once a week

604 This past week, how many times did you buy food from a vendor?: hhs_601

- ☐ Everyday ☐ Never
- ☐ A few times a week ☐ No response
- ☐ Once a week

7.0 Beach

701 This past month, how many times did you go to the beach? (This is for any reason, including recreational, buying, selling, etc): hhs_701

- ☐ Everyday
- ☐ 5 to 10 times
- ☐ 1 to 4 times
- ☐ Never
- ☐ No response

702 In a regular week, when there are no restrictions on going to the beach, how often does your youngest child go to the beach?: hhs_702

- ☐ Everyday
- ☐ Once a week
- ☐ Twice a week
- ☐ None
- ☐ Other

703 Do you have a job that puts you in contact with sea water?: hhs_703

- ☐ Yes ☐ Don't know
- ☐ No ☐ No response

704 Does anyone other than you in your household have a job that puts them in contact with sea water?: hhs_704

- ☐ Yes ☐ Don't know
- ☐ No ☐ No response

705 How often do you get into sea water when you go to the beach: hhs_705

- ☐ Everytime ☐ Never
- ☐ Sometimes ☐ No response

8.0 Flooding

801	In the past year, has your compound flooded?: hhs_801 ☐ Yes ☐ Don't know ☐ No ☐ No response	

9.0 Schools **(If no children, End of Survey**

901	Do any children in your household attend nursery school?: hhs_901 ☐ Yes ☐ Don't know **(End Survey)** ☐ No **(End Survey)** ☐ No response **(End Survey)**	
902	If yes, how many of them?:	hhs_902
903	If yes, how many days per week? (If more than one child, ask this question for the youngest of them):	hhs_903
904	If yes, how many hours per day? (If more than one child, ask this question for the youngest of them):	hhs_904
905	How often does your child purchase food at school: hhs_905 ☐ Everytime ☐ Sometimes ☐ Never ☐ No response **Skip to 906**	
906	If never, why?: hhs_906 ☐ Food carried from home ☐ School feeding program	

Comments hhs_notes

Enumerator code

Data entry code locid

C. Nursery Description Questionnaire

Nursery Description and Conditions Structured Observation

page 2/3

Ques #	Description	Indicators
A	What is the managment type? ns_a	1) Public ☐ 2) Private ☐
B	How many years has this nursery been in operation?	ns_b
C	What is the cost of attending this nursery?	ns_c
D	What hours is the nursery open each day?	ns_dam until ns_dpm
E	Does this nursery have a latrine? ns_e	1) Yes ☐ 2) No ☐ **If 'No' -> G**
F	What days of the week is the latrine open? ns_f	1) Sunday ☐ 5) Thursday ☐ 2) Monday ☐ 6) Friday ☐ 3) Tuesday ☐ 7) Saturday ☐ 4) Wednesday ☐
G	How many children are present today at this nursery? Number of MALE children Number of FEMALE children Age range of children:	 ns_g1 ns_g2 ns_g3a 1) Mo ☐ to ns_g3b 1) Mo ☐ ns_g3a1 2) Yrs ☐ ns_g3b1 2) Yrs ☐
H	How many children are enrolled at this nursery? Number of MALE children Number of FEMALE children Age range of children:	 ns_h1 ns_h2 ns_h3a 1) Mo ☐ to ns_h3b 1) Mo ☐ ns_h3a1 2) Yrs ☐ ns_h3b1 2) Yrs ☐
I	How many teachers work at this nursery? Number of MALE teachers Number of FEMALE teachers	 ns_i1 ns_i2
J	Children's food sources ns_j	1) Brought from home ☐ 2) Purchased from vendors ☐ 3) Provided by the nursery ☐

Nursery Description and Conditions
Structured Observation

Ques #	Description		Indicators
K	Children's drinking water sources	ns_k	1) Sachet/water bottle ☐ 2) Tap from pipe network ☐ 3) Tap from polytank/bucket ☐ 4) Well ☐ 5) Water trucks ☐ 6) Rainwater ☐ 7) Other ☐ 8) Don't know ☐ 9) No response ☐
L	Is there a container in the classroom where drinking water is stored?	ns_l	1) Yes ☐ 2) No ☐ **If 'No' -> M**
	Type of container	ns_l1	1) Narrow mouth (<6cm) ☐ 2) Wide mouth (<6cm) ☐
	Lid present	ns_l2	1) Yes ☐ 2) No ☐
	How is water taken from the stored container?	ns_l3	1) Dip ☐ 2) Pour ☐ 3) Tap ☐ 4) Sachet water used ☐ 5) No water available ☐
M	Latrine options (use latrine condition form to observe conditions)	ns_m	1) Traditional pit latrine ☐ 2) KVIP (double) ☐ 3) VIP (single) ☐ 4) Bucket/pan ☐ 5) Pour flush ☐ 6) Flush toilet ☐
N	Characteristics of physical space (room under observation):		
	Approximate square meters		ns_n1
	Flooring type		ns_n2
	Number of windows		ns_n3
O	List objects that children are able to touch (chairs, toys, etc.)	ns_o	

D. School Description Questionnaire

School Description and Conditions Structured Observations

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Ques #	Description		Indicators
101	Respondent status	sc_101	1) Headmaster ☐ 2) School-based health coordinator ☐ 3) Teacher ☐
102	School status	sc_102	1) Public ☐ 2) Private ☐
103	What are the sources of drinking water for students at this school?	sc_103	1) Sachets ☐ 2) Tap from pipe network ☐ 3) Tap from polytank ☐ 4) Well ☐ 5) Water trucks ☐ 6) Rainwater ☐ 7) Don't know ☐ 8) No response ☐
104	Is drinking water provided for students?	sc_104	1) Yes ☐ 2) No ☐ **If 'No' -> 108**
105	May I see the continer used to store drinking water?	sc_105	1) Yes ☐ 2) No ☐ **If 'No' -> 108**
106	Type of container	sc_106	1) Narrow mouth (≤6cm) ☐ 2) Wide mouth (>6cm) ☐
107	Is container covered or uncovered?	sc_107	1) Covered ☐ 2) Not covered ☐
108	What is the primary source of food for most students while at school?	sc_108	1) Brought from home ☐ 2) Purchased from vendors on school compound ☐ 3) Provided by the school ☐ 4) Purchased from vendors outside school compound ☐ 5) Don't know ☐ 6) No response ☐
109	Is vendor food sold at the school?	sc_109	1) Yes ☐ 2) No ☐
110	How many food vendors are at the school today?		sc_110
111	What percentage of students buy food from these vendors?		sc_111 % DK ☐ sc_111a
112	Does the school have a feeding program?	sc_112	1) Yes ☐ 2) No ☐
113	What percentage of students get food from the feeding program?		sc_113 % DK ☐ sc_113a

School Description and Conditions
Structured Observations

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Ques #	Description	Indicators
114	What is the primary means of solid waste disposal for this school? sc_114	1) Collection service ☐ 2) Disposal in compound ☐ 3) Disposal outside compound ☐ 4) Dump in a river ☐ 5) Burn ☐ 6) Other ☐ 7) Don't know ☐ 8) No response ☐
115	Does the school have a budget specifically for water, sanitation, and hygiene-related things? sc_115	1) Yes ☐ 2) No ☐ 3) Don't know ☐ 4) No response ☐
116	How many latrines/toilets are at this school?	sc_116
117	How many urinals are at this school?	sc_117
118	Is someone paid to clean the school latrines? sc_118	1) Yes ☐ 2) No ☐ 3) Don't know ☐ 4) No response ☐
119	Are there sinks or washbasins at the school for handwashing? sc_119	1) Yes ☐ 2) No ☐
120	Does the school have a budget to purchase soap for handwashing? sc_120	1) Yes ☐ 2) No ☐ 3) Don't know ☐ 4) No response ☐

2.0 Enrollment (Complete table based on official enrollment records for 2011. Write '999' if class does not exist at this school.)

	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8	Total
Girls	sc_g1	sc_g2	sc_g3	sc_g4	sc_g5	sc_g6	sc_g7	sc_g8	sc_gt
Boys	sc_b1	sc_b2	sc_b3	sc_b4	sc_b5	sc_b6	sc_b7	sc_b8	sc_bt

E. Student Self-Report

Student Self Report
Structured Observation

Location ID	locid	GPS longitude	st_long
		GPS latititude	st_lat
Date	st_date		

Ques #	Description	Indicators
101	Total class size	st_101
102	Age range of students	st_102a to st_102b
103	Time upon start of questionnaire	st_103

Warm up 1	Raise your hand if you like football	st_w1
Warm up 2	Raise your hand if you are a boy	st_w2
201	Raise your hand if you drank water from a sachet today	st_201
202	Raise your hand if you drank water from a tippy tap today	st_202
203	Raise your hand if you bought food from a vendor inside of the school grounds today	st_203
204	Raise your hand if you bought food from a vendor outside of the school grounds today	st_204
205	Raise your hand if you ate using your hands today	st_205
206	Raise your hand if you ate kenke today	st_206
207	Raise your hand if you ate ground nuts today	st_207
208	Raise your hand if you ate an orange today	st_208
209	Raise your hand if you ate wache today	st_209
210	Raise your hand if you ate with utensils today	st_210
211	Raise your hand if you used the latrine today	st_211
212	Raise your hand if you used the urinal today	st_212

Enumerator code

Data entry code

F. Food Environmental Sample Collection Form

Food
Environmental Sample Collection Form

Barcode [barcode]

Date [ev_dd]

Time [ev_th]

1. Location ID: [locid]

2. GPS latitude N05. [ev_lat]
   GPS longitude W000. [ev_long]

3. Select neighborhood: neighbor
   - ☐ Alajo ☐ Bukom ☐ Old Fadama ☐ Shaibu

4. Food served by (select one) fo_who
   - ☐ caregiver ☐ school feeding prgram ☐ vendor ☐ other

   4a. If "vendor," select type (select one) fo_mobile
   - ☐ mobile ☐ non-mobile

5. Select the type of food collected: fo_ftype
   - ☐ Lettuce ☐ Oranges ☐ Chili Peppers
   - ☐ Tomatoes ☐ Apples ☐ Spring Onion
   - ☐ Alasa ☐ Mango ☐ Cabbage
   - ☐ Prepared Food
   - ☐ Other

   If "prepared food" or "other," specify the food name: [fo_typ]

6. Enter the number collected: [fo_num]

7. How food placed in whirl-pak bag (select one) fo_serv
   - ☐ Plastic or paper wrap ☐ Hands ☐ Utensil or dish

8. Mark how food was covered before serving: fo_cove
   - ☐ covered ☐ not covered ☐ not observed ☐ Other Specify: [fo_o]

9. Mark if the sample was taken:
   - fo_fec ☐ Within 3m of feces
   - fo_lat ☐ Within 30m of latrine or defecation area
   - fo_fly ☐ With flies on food
   - fo_sw ☐ Within 3m of sewage outfall or open drain

10. AT LAB if prepared food:

    Weight (g) [fo_wgt] OR Volume (mL) [fo_vol]

Notes: [ev_notes]

G. Small Volume Drinking Water Environmental Sample Collection Form

Small Volume Drinking Water Environmental Sample Collection Form

Barcode [barcode]

Date [ev_dd]
Time [ev_th]

1. GPS latitude N05. [ev_lat]
   GPS longitude W000. [ev_long]

Location Description [ev_loc]

2. Location ID: [locid]

3 Select the neighborhood: neighbor
- ☐ Alajo
- ☐ Old Fadama
- ☐ Bukom
- ☐ Shaibu

4. Is the sample from a primary or secondary water source? (select one) sd_primw
- ☐ Primary water source
- ☐ Secondary water source

5. Check box if water sample is a sachet. ☐ sd_s

5a. If sachet, complete section below and skip to Question 7. sd_sach
- Manufactured ☐ Brand [sd_smn]
- Hand-tied ☐

6. Check box if water sample is from stored water (other than sachet). ☐ sd_h

6a. Select the water source (select one): sd_source
- ☐ Public Tap
- ☐ Compound/Private Tap
- ☐ Hand-dug Well
- ☐ Tube Well/Borehole
- ☐ Tanker Truck
- ☐ Other If "other," specify: [sd_primo]

7. ASK: Is this water treated? sd_htx
- ☐ Yes
- ☐ No
- ☐ Don't know
- ☐ No response

7a. If water was treated, ASK: How was the water treated? (check all that apply)
- Cloth ☐ sd_htx1
- Boiled ☐ sd_htx3
- Solar Exposure ☐ sd_htx4
- Chlorine ☐ sd_htx5
- Ceramic of commercial filter ☐ sd_htx2
- Other ☐ sd_htx6 Specify: [sd_hpo]
- Don't know ☒
- No response ☒

7b. If water was treated, ASK: When was water treated? sd_wtreat
- Today ☐
- Yesterday ☐
- Two days ago ☐
- More than two days ago ☐
- Don't know ☐
- No response ☐

8. OBSERVE: Container Characteristics

8a. What is the container made of? sd_made

Earthen ☐
Tile or concrete ☐
Metal ☐
Plastic ☐ If "plastic" is it a PolyTank? sd_hcpp Yes ☐ No ☐
Other ☐ If "other," specify: [ ]

8b. Is the container covered? sd_hc

Yes ☐ No ☐ Not applicable ☐

8c. How is the water removed? sd_rem

Cup used to remove water ☐
Ladle used to remove water ☐
Water poured ☐
Tap ☐
Other ☐ If "other," specify: [ ]

8d. How wide is the container opening? sd_ap

Aperature less than or equal to 6cm ☐
Aperature greater than 6cm ☐

9. ASK if the water is used for any of the following purposes: (check all that apply)

Drinking ☐ sd_hp1
Laundry ☐ sd_hp3
Cooking ☐ sd_hp4
Rinsing food ☐ sd_hp8
Hand-washing ☐ sd_hp6
Adult bathing ☐ sd_hp2
Child bathing ☐ sd_hp5
Irrigation of HH plants ☐ sd_hp10
Other ☐ sd_hp9 If "other," specify: sd_hpo

10. Record physiochemical chacacteristics

Note: for "sachet", measure in lab; for "other stored water", measure in field

Turbidity	sd_turb
Free chlorine residual	sd_clf
Total chlorine residual	sd_clt
Salinity	sd_sal

Notes: ev_notes

H. Large Volume Drinking Water Environmental Sample Collection Form

Large Volume Water Environmental Sample Collection Form

Barcode [barcode]

Date [ev_dd]
Time [ev_th]

1. GPS latitude N05. [ev_lat]
   GPS longitude W000. [ev_long]

Location Descr [ev_loc]

2. Location ID: [locid]
3. Select the neighborhood: neighbor
   - ☐ Alajo
   - ☐ Old Fadama
   - ☐ Bukom
   - ☐ Shaibu
4. What type of water sample was collected? lw_type
   Drinking Water ☐ Ocean Water ☐
5. If ocean water, complete the following (select one): lo_where
   - Open water ☐
   - Inland ☐
   - Other ☐ If "other," specify: [lo_ot]
6. If drinking water, complete the following:

Water Source ld_source	Check box if pay-per-use ☐ ld_pay
☐ Public tap	Price (Pesewas)/Volume(L) [ld_prc] / [ld_vol]
☐ Compound/Private tap	
☐ Hand-dug well	
☐ Tube Well/Borehole	
☐ Tanker Truck	
☐ Other Specfiy: [ld_hsot]	

7. If "Yes", check box:
   - Within 3m of trash? ☐ lw_trs
   - Within 3m of feces? ☐ lw_fec
   - Within 30m of latrine or defecation area? ☐ lw_lat

Field Notes: [ev_notes]

8. Physiochemical Characteristics
   - Turbidity [ld_turb]
   - Free chlorine residual [ld_clf]
   - Total chlorine residual [ld_clt]
   - Salinity [ld_sal]
   - Temperature [ld_temp]

Lab Notes: [ld_lnotes]

9. At lab:
   Starting Volume(L) [ld_tvol] Post Ultrafiltration Volume: [ld_uvol]