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April 21, 2014

Assessment of Exposure to Fecal Contamination at Beaches in Low-Income Neighborhoods in Accra, Ghana

By

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Global Epidemiology

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By

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Faculty Thesis Advisor: Christine Moe, Ph.D.

An abstract of

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

in partial fulfillment of the requirements for the degree of Master of Public Health in Global Epidemiology 2014

## Abstract

Assessment of Exposure to Fecal Contamination at Beaches in Low-Income Neighborhoods in Accra, Ghana

### By Amanda Santander

**Background:** In developing countries, there are many pathways by which children may be exposed to fecal contamination, especially in urban areas where overcrowding is common and water and sanitation systems are poor. The purpose of this study was to identify and quantify risk of exposure to fecal contamination at beaches and in marine water in low-income neighborhoods in Accra, Ghana.

**Methods:** This study used household surveys, environmental samples, and structured observations of children at beaches to characterize marine water and sand as exposure pathways to fecal contamination. Two coastal neighborhoods in Accra were selected for the study, and eight exposure scenarios were identified in order to estimate exposure dose of selected indicator microorganisms.

**Results:** The geometric mean *E. coli* concentration in marine water samples was 3.98 cfu  $\log_{10}/100$ ml and the geometric mean concentration in sand samples was 2.53 cfu  $\log_{10}/100$ g. This concentration did not differ significantly by neighborhood. There was a significant difference in the concentration of *E. coli* and coliphage in sand versus water samples (p < 0.02). The exposure scenario resulting in the highest dose of fecal contamination was direct contact with sand on the beach, which resulted in a mean *E. coli* dose of 1339 cfu/event for children under five years and 1749 cfu/event for children 5-12 years. Pair-wise comparisons of exposure dose distributions found no significant different in dose for children under five years compared with children ages 5-12 years. However, comparisons of exposure activity (direct contact with sand, contact with object, head submerged in water, or head not submerged in water) showed significant differences in dose.

**Conclusions:** Both water and sand at beaches in Accra are highly contaminated due to runoff from fecal sludge discharge sites and from open defecation by beachgoers. Previous studies may have overestimated the risk of exposure to pathogens in the marine water by assuming a greater volume of water ingested during swimming events. The use of stochastic models in this study helped to control some of the variability and uncertainty in the exposure scenarios that were not accounted for previously.

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# Abbreviations

AMA	Accra Metropolitan Area
AUWS	Accra Urban Water System
cfu	Colony forming units
DALY	Disability-adjusted life year
E. coli	Escherichia coli
EPA	United States Environmental Protection Agency
MDG	Millennium Development Goal
pfu	Plaque forming units
QMRA	Quantitative microbial risk assessment
UN	United Nations
UNEP	United Nations Environment Programme
UNICEF	United Nations International Children's Emergency Fund
WASH	Water, sanitation, and hygiene
WHO	World Health Organization
WRI	Water Research Institute
WSMP	Water and Sanitation Monitoring Platform

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#### I. Background

#### A. The Burden of Diarrheal Disease in Developing Countries

Diarrheal disease is the second leading cause of death in children in Sub-Saharan Africa, resulting in an estimated 760,000 child deaths per year (1). While mortality due to diarrhea has shown a steady decline in developing countries from 4.6 million in the 1980s to 2.5 million in the 2000s, morbidity has remained unchanged throughout the decades at 2-3 diarrheal episodes per child per year (1, 2). In Sub-Saharan Africa, morbidity reaches as high as 7.3 diarrheal episodes per child per year, mainly due to lack of clean water and proper sanitation (2). In 2002, Pruss *et al* estimated the global burden of diarrheal disease due to poor water, sanitation and hygiene (WASH) to be 4.0% of all deaths (3). This is likely to be an underestimate because not all diarrheal diseases and exposure pathways were measured. They also estimated that the WASH-related health burden of disease on children under five in Africa is up to 240 times higher than the burden to children in high-income countries (3).

The global burden of diarrheal disease is so great that the Millennium Development Goals (MDGs) have called for a reduction by two-thirds in the under-five mortality rate and to reduce by half the proportion of the population lacking access to improved drinking water and basic sanitation (4). However, according to a 2010 report from the United Nations, while child mortality is decreasing worldwide, it is not falling fast enough to meet the goal by 2015. Among the 67 countries with high child mortality rates (over 40 deaths per 1,000 live births) only 10 are on track to meet their target (4). Most child deaths, especially those from diarrheal illness, are preventable through improved sanitation and access to safe water. While the MDG target for improved drinking water has been met as of 2010, it is unlikely that the world will meet the MDG sanitation target by 2015 (5). From 1990 to 2010, the proportion of the population in Sub-Saharan Africa with improved sanitation facilities only increased from 26% to 30% (5).

### **B.** Sanitation Challenges in Urban Environments

It is estimated that nearly 300 million people in sub-Saharan Africa do not have access to clean drinking water and 440 million do not have access to basic sanitation (6). Increased urbanization in many parts of the world, including sub-Saharan Africa, has accelerated the deterioration of urban environments, leading to high outdoor air and water pollution and improper waste disposal systems. Child morbidity and mortality is typically four times higher in poorer urban areas (slums) than in richer areas (7). Many communicable diseases can be found in areas with little clean water and poor sanitation; overcrowding and unsanitary environmental conditions only serve to intensify their transmission.

Diseases related to unsafe water and poor sanitation affect people of all ages, but children, the elderly, and the immunocompromised have a higher risk for such illnesses. Urban children living in slums in Sub-Saharan Africa have mortality and morbidity rates greater than those in rural areas (8). With over half of the urban population in Sub-Saharan Africa still living in slums, the need for improved water and sanitation services is great (9).

## C. Sanitation and Fecal Sludge Disposal in Accra, Ghana

As of 2010, the country of Ghana had a total population of about 25 million people (10). Between 2000 and 2010, the urban population of Ghana grew from 44% to

50%; in Accra alone, and more than 60% of the residents live in over-crowded areas (11, 12). According to the 2012 update from the World Health Organization (WHO) on the progress on clean drinking water and improved sanitation, Ghana has the highest proportion of shared sanitation facilities (58%) in the world. Shared facilities are defined as those that are used by more than two households, including public toilets, and are not considered improved, according to the United Nations International Children's Emergency Fund (UNICEF) (13). Nine percent of facilities are unimproved, and 19% of the population report that they regularly practice open defecation (5).

A report by the Water and Sanitation Monitoring Platform (WSMP) of Ghana in 2008 found that, although Greater Accra improved the least in access to safe drinking water compared to nine other regions in Ghana (61%), it had the greatest increase in improved sanitation facilities (25%) from 1990 to 2008 (14). Sanitation coverage (access to toilet facilities) remains low across the country with 8.2% of rural homes and 17.8% of urban homes having improved sanitation in 2008 (14). Even at the current rate of increase in improved sanitation, Ghana is not on track to meet its 2015 target for this Millennium Development Goal. In fact, the WSMP estimates that by 2015, only 15% of the population in Ghana will be using improved sanitation facilities, which falls far short of the MDG target of 53% (14).

In Ghana, 12% of children under 5 years of age are reported to die from diarrheal disease (12). This can be attributed in part to the lack of sanitation infrastructure and clean water in urban areas, such as Accra. There is no functional municipal wastewater treatment plant in Accra. Shared latrines and septic tanks are emptied by privately owned vacuum tankers, which discharge their contents onto the beach at Lavender Hill (15).

About 700  $\text{m}^3$ /day of fecal sludge is disposed of at Lavender Hill, most of which ends up in the sea (15). During the rainy season the runoff likely causes an even higher level of fecal contamination in recreational waters (16).

#### D. Recreational Water, Beaches, and Health

Recreational waters and beaches can become contaminated with human feces through the dumping of sewage, discharge from rivers that contain sewage, and from bather contamination. The most frequent adverse health outcome from exposure to fecalcontaminated beaches is enteric illness, but skin, eye, and ear irritations have also been reported (17). Multiple epidemiological studies have shown an association between swimming in fecal-contaminated water and gastrointestinal illness (18, 19). For example, a study in the Great Lakes region of the United States showed a range of fecal contamination from -1.75  $\log_{10}/100$  ml to 4.17  $\log_{10}/100$ ml, using *Enterococcus* as an indicator of fecal contamination (19). For swimmers immersing their bodies, they observed a 1.43 increase in the odds of gastrointestinal illness for a  $\log_{10}$  increase in *Enterococcus* levels (19).

In 2004, the WHO provided a reference level for the "tolerable risk to human health" from pathogenic enteric viruses, bacteria and protozoa of 10<sup>-6</sup> DALYs per person per year (20). A DALY expresses the years of life lost due to premature death and years lived with disability due to disease (*i.e.* one DALY equals one lost year of healthy life compared to the average life expectancy in a given country) (17, 21). These guidelines assume that 20-50 ml of water is swallowed per hour of swimming-related activity (17, 22). Additional studies have found that children only swallow 30-50 ml of water per swimming event, depending on age and water type, so this value may be an over- or underestimation (22, 23).

The most common ways in which beaches and recreational waters become contaminated in Accra is through the direct dumping of fecal sludge at Lavender Hill or the flow of sewage from open drains into seawater. A study by Labite *et al.* in 2010 showed that the major routes of exposure to enteric pathogens in Accra, Ghana are through open drains, followed by recreational water. They measured fecal indicators in open drains, sand, and marine water and used the disability-adjusted life year (DALY) as a measure of disease burden in the population. The ingestion of contaminated water by children playing near open drains contributed 64% of total DALYs from the Accra Urban Water System while the ingestion of polluted seawater or beach sand contributed 26% of DALYs (6). The microbial pollution of the seawater is not surprising since untreated wastewater from open drains (the highest source of pollution) flows into the Odaw River and then discharges into the sea (6). A similar study by Lulani et al. in 2008 predicted that recreational ocean swimming contributed 91% of all Campylobacter cases in the study area and nearly as many cases of *Rotavirus* as open drain, based on the DALYs that each pathway contributes and the estimated duration and severity of each illness (24). This study used assumptions and values from prior literature for all calculations.

Children playing on beaches and in the water have several possible routes of exposure to pathogens. Some of these include accidental ingestion of seawater and swimming in shallow water (dermal exposure) or in small pockets of stagnant water near the coastline (25). Children are also more susceptible to exposure to fecal contamination in recreational water than adults because they spend more time in the water, submerge their heads more often and swallow more water than adults (23). Additionally, hot weather, high water temperatures, and large numbers of bathers in a small area provide ideal conditions for pathogen exposure (25).

When assessing the risks of enteric illness in children due to recreational water exposure, it is important to also consider exposure to beach sand. Young children generally spend more time at the edge of the water and may spend more time on the sand than in the water (26). Pathogenic bacteria, viruses, fungi and parasites have all been isolated from samples of beach sand, sometimes at higher concentrations than seawater. At a beach in the Gaza Strip, *Salmonella* and *Vibrio* were isolated from sand samples more frequently than from water samples despite the fact that only 10g of sand was collected, while 1L of water was sampled (27). Fecal indicator bacteria have also been found in sand at densities 2 to 38 times higher than in water (26, 28), which indicates the possible presence of pathogenic microorganisms in the sand as well. Heaney *et al* (2012) found fecal contamination in wet sand to be positively associated with enteric illness among both children digging in sand and those being buried in sand. This is important because beachgoers may spend more time on the sand than in the water, and young children especially have a lot of contact with beach sand.

#### **E. Indicator Organisms of Fecal Contamination**

Enteric pathogens are normally found in very small numbers in the environment and can be difficult to detect. For this reason, microbial indicator organisms, such as *Escherichia coli* (*E. coli*), are routinely used to test for the possible presence of fecal contamination in water (29). There are several criteria that a good indicator should meet, but there is not one indicator organism that fulfills all of these criteria. Ideally, the indicator should be present when a fecal pathogen is present and absent when the pathogen is absent. The persistence and growth characteristics of the indicator should be similar to those of the pathogen. The pathogen and the indicator should be present in a constant ratio in order to estimate the concentration of pathogen present. Finally, the indicator should be present in much higher concentrations than the pathogen so that it is easier to detect (30).

*E. coli* is the most widely used fecal indicator to test for the presence of fecal pollution from humans and other warm-blooded animals (31). It does not survive long in seawater, so when *E. coli* is present in marine water samples, this is an indication of recent fecal pollution. One study determined that *E. coli* had a decay rate of -2.9 cfu/100ml per day in marine water, which was significantly faster than in freshwater (32). *E. coli* is also a preferred indicator because there are several rapid, sensitive, and specific methods of detecting the microbe in water and sand samples (33). However, *E. coli* is not an ideal indicator for enteric viruses and protozoa because these pathogens survive for much longer in marine waters than *E. coli* (34). The World Health Organization and the United Nations Environment Programme (WHO/UNEP) have set guidelines for *E. coli* indicator levels in recreational marine water. *E. coli* counts are not to exceed 100 cfu/100 ml in 50% of samples taken in a season and 1,000 cfu/100ml in 90% of samples (35).

Coliphage are viruses that infect coliform bacteria and are not pathogenic to humans (36). Coliphage are important indicators because, unlike *E. coli*, they can serve as an indicator of viral pathogens. Laboratory experiments with coliphage have shown that their survival in the environment is similar to that of enteric viruses (31, 34).

#### F. Quantitative Microbial Risk Assessment of Recreational Water Exposures

Quantitative microbial risk assessment (QMRA) is a method used to quantify the risk of exposure to pathogens in food and water and other environmental matrices. It can be used to quantify risk from pathogens in various scenarios associated with exposure to fecal contamination at recreational sites (12, 37). QMRA can be preferable to epidemiologic studies of risk when there are many possible routes of exposure, which is true for most pathogens that infect humans through the fecal-oral transmission route (Figure 1) (3, 38). Two advantages of QMRA over epidemiologic studies is that it allows for the assessment of attributable risk from separate pathways and can be used to estimate low risks that may be hard to measure in epidemiologic studies (17).

There are four steps involved in conducting a QMRA: hazard identification, exposure assessment, dose-response analysis, and risk characterization (38, 39). In our study, hazard identification involved a qualitative step to characterize the beach locations and a quantitative step to measure the magnitude of fecal contamination at beaches and the frequency that households reported going to the beach. Exposure assessment involves calculating the dose that a person may be exposed to in a given scenario. The dose-response analysis describes the probability that anyone who is exposed to a given pathogen will become infected. Each pathogen has its own dose-response relationship. Finally, risk characterization is a calculation of the risk of illness given the concentration and dose of specific pathogens in the given exposure scenario (39).

QMRA is routinely used to quantify the human health risk of exposure to pathogens in drinking water, wastewater, irrigation water, and food (40, 41). Researchers have also used QMRA to determine the risk to swimmers in recreational waters, but the majority of these studies have been in industrialized countries (16, 37, 42-44). Shibata *et al.* used the EPA reference level of risk for swimming in marine water of  $1.9 \times 10^{-2}$  to estimate the reference pathogen levels (RPLs) in sand that would be necessary to result to illness in children. For enterovirus, the estimated range was 5-500 MPN/g, and for *Cryptosporidium*, the estimated range was 10-1000 oocysts/g (44). Few studies have measured the risks associated with recreational waters in developing countries. Diallo *et al.* (2008) used QMRA with Monte Carlo simulations to calculate the risk of exposure to diarrheal pathogens in canal networks in Thailand, where canal waters are used for irrigation and recreation. The most contaminated canal had *E. coli* contamination levels ranging from 4.1 - 5.0 cfu log<sub>10</sub>/100ml. They estimated the annual infection risks from *Cryptosporidium* and *E. coli* to be 0.66 and 0.61 per person per year, respectively, from swimming in the most contaminated canal. These risks were up to 10,000 times greater than WHO references levels (45).

*Previous QMRA studies in Accra, Ghana.* Two QMRA studies have been conducted in Accra previously to evaluate the Accra Urban Water System (AUWS). Lulani *et al.* (2008) estimated that recreational swimming accounted for 91% of total *Campylobacter* cases and nearly as many *Rotavirus* cases as open drains in the study population (24). However, since over half of fecal sludge from drains was dumped into the ocean or disposed of at the seashore, they concluded that the greatest risk actually originated from open drains and the poor state of the sanitation system in Accra. They predicted that the disease burden from the AUWS was 28,531 DALYs per year; 9% of DALYs were contributed from the water pathway and 91% from the sanitation pathway (24). Similarly, Labite *et al.* (2010) predicted that the disease burden from the AUWS was 36,329 DALYs per year, of which 12% were caused by the water supply and 88% were caused by inappropriate sanitation (6). Both studies show a need for improved sanitation systems in Accra, especially with regard to open drains and drainage systems that impact waters that people may use recreationally.

*Limitations of dose-response and exposure assessment.* A successful QMRA requires information on the concentration of pathogens in the water or on the correlation of indicator bacteria and pathogens in the water, and on the exposure of the population to these pathogens (41). The Lulani study used many assumptions to estimate doseresponse relationships and exposure assessment. The exposed population was estimated through field surveys and census data to determine where people lived in relation to possible exposure points; ingestion volume and dose-response relationships were defined from previous literature. The assumed volume of water ingested during recreational swimming was 100 ml per swim with 7 swims per person per year (24). The Labite study did field surveys in two densely populated neighborhoods to determine the exposed population and the major routes of exposure to diarrheal pathogens among beachgoers. They assumed that swimmers ingested 75 ml of water per swim with only 2 swims or beach visits per year (6). Both studies used *E.coli* : pathogen ratios to estimate the concentrations of Cryptosporidium, Rotavirus, Campylobacter, and Ascaris through the exposure routes (6, 24). Ratio assumptions from studies conducted in high-income countries or in rural areas may not be applicable to low-income, urban settings such as Accra. Additionally, ratios determined in a laboratory setting may not correlate with ratios in the environment where differential die-off of indicators and pathogens can occur.

*Common parameters used for recreational water QMRA studies.* Studies of exposure to fecal contamination at beaches often describe scenarios in which the exposed populations accidentally ingest contaminants in water or sand. For these scenarios, assumptions need to be made about the amount of water or sand that comes in contact with the hands, the amount of water or sand that then adheres to the hands, the frequency of hand-mouthing events, and the proportion of the hand that enters the mouth. It is not always possible to collect data on these parameters in the field, but numerous studies have attempted to quantify each of these parameters (46-49). For example, AuYeung *et al.* did multiple studies examining hand-to-object and hand-to-mouth contacts in young children. They videotaped children to determined the number of hand contacts and also calculated distributions for the fractional surface area of the hand that came in contact with objects and with the mouth (46, 47).

## **G. Study Objectives**

Previous studies in Accra, Ghana have determined that open drains and their contamination of recreational water are the most important exposure sources of fecal contamination (6, 24). These studies have relied on numerous assumptions to calculate the risk of disease.

This study examines exposure to fecal contamination at two beaches in Accra, Ghana by:

 Characterizing behavior of beachgoers in low-income neighborhoods in Accra, Ghana, paying special attention to children's behavior. 11

- 2. Determining if the location of the beach with respect to the dumping of untreated fecal sludge affects the level of fecal contamination in the water and sand and the magnitude of exposure of children playing in the sand or water.
- 3. Estimating the exposure dose of children to fecal contamination from recreational water and sand using a stochastic modeling method.

#### **II. Manuscript**

### A. Abstract

**Background:** In developing countries, there are many pathways by which children may be exposed to fecal contamination, especially in urban areas where overcrowding is common and water and sanitation systems are poor. The purpose of this study was to identify and quantify risk of exposure to fecal contamination at beaches and in marine water in low-income neighborhoods in Accra, Ghana.

**Methods:** This study used household surveys, environmental samples, and structured observations of children at beaches to characterize marine water and sand as exposure pathways to fecal contamination. Two coastal neighborhoods in Accra were selected for the study, and eight exposure scenarios were identified in order to estimate exposure dose of selected indicator microorganisms.

**Results:** The geometric mean *E. coli* concentration in marine water samples was 3.98 cfu  $\log_{10}/100$ ml and the geometric mean concentration in sand samples was 2.53 cfu  $\log_{10}/100$ g. This concentration did not differ significantly by neighborhood. There was a significant difference in the concentration of *E. coli* and coliphage in sand versus water samples (p < 0.02). The exposure scenario resulting in the highest dose of fecal contamination was direct contact with sand on the beach, which resulted in a mean *E. coli* dose of 1339 cfu/event for children under five years and 1749 cfu/event for children 5-12 years. Pair-wise comparisons of exposure dose distributions found no significant different in dose for children under five years compared with children ages 5-12 years. However, comparisons of exposure activity (direct contact with sand, contact with object,

head submerged in water, or head not submerged in water) showed significant differences in dose.

**Conclusions:** Both water and sand at beaches in Accra are highly contaminated due to runoff from fecal sludge discharge sites and from open defecation by beachgoers. Previous studies may have overestimated the risk of exposure to pathogens in the marine water by assuming a greater volume of water ingested during swimming events. The use of stochastic models in this study helped to control some of the variability and uncertainty in the exposure scenarios that were not accounted for previously.

#### **B.** Introduction

It is estimated that nearly 300 million people in sub-Saharan Africa do not have access to clean drinking water and 440 million do not have access to basic sanitation (6). Many diseases can be found in areas with little water and poor sanitation; overcrowding and unsanitary environmental conditions only serve to intensify their transmission. Increased urbanization has accelerated the deterioration of urban environments, leading to high outdoor air and water pollution and improper waste disposal systems. Additionally, child morbidity and mortality is typically four times higher in poorer urban areas (slums) than in richer areas (7).

Diseases related to unsafe water and poor sanitation affect people of all ages, but children, the elderly, and the immunocompromised have the highest risk for such illnesses. Urban children living in slums in Sub-Saharan Africa have mortality and morbidity rates greater than those in rural areas (8). With over half of the urban population in Sub-Saharan Africa still living in slums, the need for improved water and sanitation services is great.

Between 2000 and 2010, the urban population of Ghana grew from 44% to 50%; in Accra alone, more than 60% of the residents live in over-crowded areas (11, 12). In Ghana, 12% of children under 5 are reported to die from diarrheal disease (12). This can be attributed in part to the lack of sanitation infrastructure and clean water in urban areas, such as Accra. There is no functional municipal wastewater treatment plant in Accra. Shared latrines and septic tanks are emptied by privately owned vacuum tankers, which discharge their contents onto the beach at Lavender Hill (15). About 700 m<sup>3</sup>/day of fecal sludge is disposed of at Lavender Hill, most of which ends up in the sea (15). During the rainy season the runoff likely causes an even higher level of fecal contamination in recreational waters (16).

The most common way in which beaches and recreational waters become contaminated in Accra is through the direct dumping of fecal sludge or the flow of sewage from open drains into seawater. Children playing on beaches and in the water have several possible routes of exposure to pathogens, including accidental ingestion of seawater and swimming in shallow water (dermal exposure) or in small pockets of stagnant water near the coastline (25). Children are also more susceptible to exposure to fecal contamination in recreational water than adults because they spend more time in the water, submerge their heads more often, and swallow more water than adults (23).

Quantitative microbial risk assessment (QMRA) is a method that can be used to quantify risk in various scenarios that may lead to exposure to fecal contamination at recreational sites (12, 37). Previous studies in Accra, Ghana have determined that open

drains and their contamination of recreational water are the most risky exposures to fecalcontaminated water. Because of the lack of previous research, these studies have relied on numerous assumptions to calculate the risk of disease associated with these exposures.

This study examines exposure to fecal contamination at two beaches in Accra, Ghana by:

- Characterizing behavior of beachgoers in low-income neighborhoods in Accra, Ghana, paying special attention to children's behavior.
- Determining if the location of the beach with respect to the dumping of untreated fecal sludge affects the level of fecal contamination in the water and sand and the magnitude of exposure of children playing in the sand or water.
- 3. Estimating the exposure dose of children to fecal contamination from recreational water and sand using a stochastic modeling method.

## C. Methods

## i. Site Selection

This study was conducted as part of a larger study called SaniPath, to assess fecal exposure pathways in low-income neighborhoods in Accra, Ghana. Four communities were selected for the study to represent diverse population and physical characteristics, such as predominant religion, income, inland or coastal location and formal or informal settlements. The four study communities were Alajo, Bukom, Shiabu, and Old Fadama.

### ii. Data Collection

Household surveys

Household surveys were conducted in each of the four neighborhoods. Surveys topics included household demographics, WASH conditions and practices, and weekly activities including beach activities. Responses were recorded on the Household Description and Condition Structured Observation Form (Appendix B).

#### Environmental samples

Environmental samples were collected from sand and marine water in Shiabu and Bukom between August and October 2012. Samples were collected from marine water in 20L sterile containers and returned to the Water Research Institute (WRI) along with the Large Volume Water Environmental Sample Collection Form (Appendix C). Sand samples were collected using sterile procedures in 250 ml and 500 ml Whirl-Pak bags. A Particulate Environmental Sample Collection Form was also completed at the time of sampling (Appendix D). Each bag was massaged and rotated several times to ensure that the sample was homogenous and free of large pieces. All samples, with the exception of the 500 ml Whirl-Pak bags, were stored at 4°C at the WRI until they were ready for processing.

## Structured observations

Structured observations were conducted at beaches in the neighborhoods of Shiabu and Bukom. Observations were conducted at least once a week in each neighborhood from 6am to 10am. Observers were instructed to choose a vantage point that prioritized the most heavily populated areas, particularly by children and defecators. Open defecation was observed and totaled for the entire 4-hour period. In the second hour of observation, the observer was instructed to walk along the coast for 10 minutes to the left and record the number of children in the water, eating, etc. In the third hour, the observer was instructed to record the duration and characteristics of 3 children in the water. In the final hour, the observer was instructed to walk along the coast for 10 minutes to the right and record the number of children in the water, eating, etc. A child was defined as being "in the water" if he or she was on the ocean side of the beach as the observer was walking by. Children playing football were counted as children coming into contact with each other. All observations were recorded on the Beach Description and Conditions Structured Observations Form (Appendix E).

#### iii. Laboratory Methods

The WRI processed samples immediately after arrival at the laboratory. Marine water samples were concentrated to 100ml by ultrafiltration. Dilutions of  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  were test for *E. coli* and coliphage. For marine water and samples, *E. coli* was analyzed by membrane filtration using MI agar according to EPA Method 1604 (50). Samples were analyzed for coliphage by the standard single-agar layer method according to EPA Method 1602 (51). Samples were also tested for norovirus and adenovirus using quantitative real-time polymerase chain reaction (PCR).

#### iv. Data Management

All paper surveys and environmental sample forms completed in the field were entered into a Microsoft Access database. Twenty-five percent of all forms were double entered to ensure data quality.

#### v. Statistical Methods

Descriptive statistics were performed in SAS 9.3 (Cary, NC). The concentration of *E. coli* and coliphage was determined for each environmental sample. Samples below the limit of detection were assigned a value of negative square root two. The final

microbe concentrations were log-transformed, and the mean, standard deviation, minimum, and maximum were calculated. Two sample t-tests were performed on *E. coli* and coliphage concentrations to determine if there was a difference in concentration by coastal neighborhood or by location in sand or water. All statistical tests were performed at a significance level of  $\alpha = 0.05$ . *E. coli* and coliphage concentrations were plotted using kernel density methods for sand and water samples in Bukom and Shiabu.

The rate of children observed per hour on beaches was calculated for the following activities: children in water, children eating, children defecating in the water, and children defecating on the beach. Each recorded count from the structured observations was assumed to represent a unique child. Poisson regression models for each activity listed above and stratified by neighborhood and age group were fitted to determine if there were differences in the rates of children performing each activity.

#### vi. Exposure Assessment

#### Exposure scenarios

Exposure scenarios were identified based on structured observations of children under five years and children ages 5-12 years at beaches in Bukom and Shiabu. Exposure scenario A involved children under 5 years coming in direct contact with sand. Scenario B involved children under 5 years playing with an object, such as a soccer ball, that came in contact with sand. Scenario C involved children under 5 years entering the water and submerging their heads, and scenario D involved children under 5 years entering the water but not submerging their heads. Scenarios E-H were the same as scenarios A-D but for children ages 5-12 years.

Model parameters

Model parameters and their assumptions are listed in Table 1 for the hand and object contamination parameters and Table 2 for the dose parameters. Model parameters were based on prior literature and were calculated using Microsoft Excel and the @Risk 6.2 add-on (Palisade, Newfield).

Point estimates from the literature were used to describe the transfer efficiency of microbes from objects to hands and from hands to the mouth (52). The remaining parameters were assigned a distribution by conducting 10,000 Monte Carlo simulations for each parameter. A log-normal distribution was used to describe the concentration of *E. coli* and coliphage in water and sand. To describe the frequency of contact of hands and objects with water or sand, Poisson distributions were fitted with a value of 1. Three parameters were used to describe the amount of water or sand loaded onto hands: the surface area of the hands, the proportion of the object contacted, and the water film thickness or sand adherence on the hands. The surface area of the hands was described by a uniform distribution with upper and lower bounds from the 5<sup>th</sup> and 95<sup>th</sup> percentile of hand surface area for children in each age group (53). The proportion of the hand that contacted the sand or water was assumed to follow a uniform distribution described previously by AuYeung et al. 2008 (54). The water film thickness was assumed to follow a uniform distribution with lower and upper bounds representing partial and full wipes of the hand after immersion in water (55), and the sand adherence factor was assumed to follow a log-normal distribution described by Shoaf et al. 2005 (56). These were assumed to be the same for both hands and non-porous objects, such as a ball.

Parameters used to describe the transfer of microbes from the hand to the mouth included the proportion of the hand that entered the mouth, the frequency of hand

mouthing, the time to hand washing, and the amount of water ingested for children in the water (Table 2). The proportion of the hand that entered the mouth was assumed to follow a uniform distribution described by AuYeung *et al.* 2007 (57). The frequency of hand-mouthing for children under five years was assumed to follow a log-normal distribution based on structured observations of hand-mouthing behaviors of children under five years. The frequency of hand-mouthing for children to follow a the study area. The frequency of hand-mouthing for children 5 to 12 years was assumed to follow a Weibull distribution based on observation of hand-mouthing behaviors (58). The time until hand washing was assumed to follow a uniform distribution with an upper bound of 16 hours, based on the observation that children who are awake for 16 hours wash their hands an average of one time per day. Finally, the amount of water ingested was assumed to follow a gamma distribution with parameters defined previously (23)

#### Model Equations

Three equations were used to describe the contamination of hands, either directly or through food or an object, and the transfer of microbes from the hands to the mouth to determine the dose that a child in each scenario could be exposed to. The equations are based on previous equations derived to describe dermal exposure to chemicals (59).

Direct hand contamination (Equation 1) was determined by the concentration of microbes in the sand or water ( $C_{XY}$ ), the frequency of hand contact with sand or water ( $F_Y$ ), the surface area of the hand that comes in contact with the sand or water ( $A_Z*S_{HY}$ ), and the water thickness (V) or adherence of sand (D).

[1] 
$$E_x = \begin{bmatrix} C_{ES} \\ C_{EW} \\ C_{CS} \\ C_{CW} \end{bmatrix} \times \begin{bmatrix} F_S \\ F_W \end{bmatrix} \times \begin{bmatrix} A_5 \\ A_{12} \end{bmatrix} \times \begin{bmatrix} S_{HS} \\ S_{HW} \end{bmatrix} \times \begin{bmatrix} V \\ D \end{bmatrix}$$

Hand contamination through an object (Equation 2) was determined by the concentration of microbes in the sand or water ( $C_{XY}$ ), the frequency of object contact with sand ( $F_0$ ), the surface area of the object that comes in contact with the sand ( $A_0*S_0$ ), the adherence of sand to the object (D), the proportion of the object that comes in contact with the hand (P), and the transfer efficiency of microbes from the object to the hand for each organism.

$$[2] E_{X} = \begin{bmatrix} C_{ES} \\ C_{EW} \\ C_{CS} \\ C_{CW} \end{bmatrix} \times F_{O} \times A_{O} \times S_{O} \times D \times P \times \begin{bmatrix} TE_{OE} \\ TE_{OC} \end{bmatrix}$$

The dose distribution (equation 3) was determined by the contamination on the hands as determined from Equations 1 and 2 ( $E_X$ ), the frequency of hand mouthing ( $M_X$ ), the proportion of the hand that enters the mouth ( $S_M$ ), the transfer efficiency of microbes from the hand to the mouth ( $T_{MY}$ ), and the time until hands are washed ( $T_{HW}$ ).

$$[3] D_X = \begin{bmatrix} E_{AE} & E_{EE} \\ E_{AC} & E_{EC} \\ E_{BE} & E_{FE} \\ E_{BC} & E_{FC} \\ E_{CE} & E_{GE} \\ E_{CC} & E_{GC} \\ E_{DE} & E_{HE} \\ E_{DC} & E_{HC} \end{bmatrix} \times \begin{bmatrix} M_5 \\ M_{12} \end{bmatrix} \times S_M \times \begin{bmatrix} TE_{ME} \\ TE_{MC} \end{bmatrix} \times T_{HW}$$

For scenarios that took place on the beach (scenarios A, B, E, and F), the final dose was calculated by Equation 3. For scenarios that took place in the water (C, D, G, and H), children also ingested some amount of water. Their additional dose (equation 4) was calculated from the initial concentration of microbes in the water ( $C_{XW}$ ) and the volume of water ingested ( $I_X$ ). The final dose for these scenarios was calculated by adding together the doses from equations 3 and 4.

$$[4] A_X = \begin{bmatrix} C_{EW} \\ C_{CW} \end{bmatrix} \times \begin{bmatrix} I_U \\ I_O \end{bmatrix}$$

#### Comparisons of Exposure Dose Concentrations

Pair-wise comparisons were performed to determine the difference in mean concentrations between different exposure scenarios. Comparisons were made between *E. coli* and coliphage concentrations, water and sand exposures, age groups, direct contact with the sand versus contact through an object, and having the head submerged versus not submerged while swimming.

## **D.** Results

## i. Neighborhood characteristics

Two hundred household surveys were completed in each of the four study neighborhoods (Figure 2). Information on household size, health outcomes, and beach visits is shown in Table 3. Bukom and Shiabu had the highest average household sizes at seven and five people per household, respectively. They also had the highest numbers of children under 5 years old at two to three per household. A high percentage of households in every neighborhood reported that a child in the household had diarrhea in the past two weeks; the highest proportion was in Old Fadama (25.2%). Over 90% of respondents in every neighborhood reported that, in a regular week, their youngest child never visited the beach. In the coastal neighborhoods of Bukom and Shiabu, respondents visited the beach much more frequently than in the inland neighborhoods of Alajo and Old Fadama. Eight percent of respondents and 5.6% of youngest children in Bukom and 4.5% of respondents and 3.6% of youngest children in Shiabu visited the beach every day. However, in Alajo and Old Fadama, no one who completed the survey visited the beach every day.

#### ii. Microbial concentrations at beaches

The initial concentration of *E. coli* and coliphage was measured in sand and water samples from Bukom and Shiabu. Concentrations by neighborhood are shown in Table 4 for both sand and marine water samples. Seventy-five samples (100%) were positive for *E. coli*, 38 from the sand and 37 from the water. The geometric mean concentration of *E. coli* in the sand was 339 cfu/100g, and the geometric mean concentration of *E. coli* in the water was 9,550 cfu/100ml. There was a statistically significant difference in mean concentration between water and sand samples (p < 0.01). There were 38 *E. coli* samples from Bukom and 37 from Shiabu. The difference in geometric mean *E. coli* concentrations between neighborhoods was not statistically significant for either sand (p=0.91) or water samples (p=0.09).

There were six positive sand samples and 15 positive water samples for coliphage in the study. The geometric mean concentration of coliphage in the sand was 23 pfu/100g and the geometric mean concentration in the water was 6,166 pfu/100ml. The difference in geometric mean concentrations between sand and water samples was statistically significant (p = 0.02). There were 14 coliphage samples from Bukom and seven from Shiabu. The difference in mean coliphage concentrations between neighborhoods was not statistically significant for sand samples (p = 0.52) but it was statistically significant for water samples (p<0.01).

Figure 3 shows the kernel density of *E. coli* and coliphage in sand samples at Bukom and Shiabu. The peak concentration of coliphage for both Bukom and Shiabu

was at about 1 pfu/100g. The peak concentration of *E. coli* for both neighborhoods was about 100 cfu/100g. Figure 4 shows the kernel density of *E. coli* and coliphage in marine water samples at Bukom and Shiabu. The peak concentrations of *E. coli* and coliphage in Shiabu were at about  $10^{3.5}$  cfu/100ml and  $10^{3.5}$  pfu/100ml, respectively. The peak concentration of *E. coli* in Bukom was around  $10^{4.3}$  cfu/100ml, and the peak concentration of coliphage in Bukom was around  $10^{3.9}$  pfu/100ml.

#### iii. Exposure assessment

Eight exposure scenarios were developed to assess the dose of E. coli and coliphage that children may be exposed to at beaches. The geometric mean, median, and 95% range of the final microbe exposure doses are shown in Table 5. Scenarios in which children had direct contact with the sand (A and E) resulted in the greatest exposure to E. *coli* and coliphage. Children under five years had a geometric mean dose of 1339 cfu E. coli and 663 pfu coliphage per exposure event and children 5-12 years had a geometric mean dose of 1749 cfu E. coli and 865 pfu coliphage per exposure event. Scenarios involving children having contact with an object on the sand (B and F) resulted in the least exposure to microbes. Children under five years had a geometric mean dose of 201 cfu E. coli and 171 pfu coliphage per exposure event, and children 5-12 years had a geometric mean dose of 140 cfu E. coli and 119 pfu coliphage per exposure event. There was no significant difference in the ingested dose of microbes between children under 5 and children ages 5-12 (Table 6). In general, there was no significant difference in the dose from activities involving water contact and from activities involving sand contact (Table 6). There was, however, a statistically significant difference between exposure doses for both E. coli and coliphage that were associated with touching the sand directly

versus playing with an object on the sand (p = 0.02), and between submerging one's head in the water versus not submerging the head (p = 0.001).

## E. Discussion

#### i. Exposure Model for Recreational Water and Sand

To our knowledge, this is the first study to attempt to quantify children's exposure to fecal contamination at beaches in Accra, Ghana that uses a stochastic model. The stochastic model is generally considered to be superior to the deterministic model because it incorporates uncertainty and variability into each model parameter by assigning a probability density function to each parameter. Deterministic models are often still used for simplicity of analysis. We modeled four exposure scenarios based on observations of children's activities at beaches: 1) direct contact with the sand, 2) contact with an object on the sand, 3) entering the water and submerging the head, and 4) entering the water without submerging the head. Each scenario was modeled separately for children under five and for children aged five to 12 for a total of eight scenarios. These age groups were modeled separately to account for differences in hand sizes and in the frequency of hand-mouthing for children of different ages.

A deterministic model of each exposure scenario, where each parameter in the model is represented by its mean value, yields microbe dose concentrations up to two orders of magnitude greater than the stochastic model (Appendix F). This difference was also seen in a study by Hamilton and Stagnatti (2008), who found that the deterministic approach for modeling the risk associated with wastewater irrigation of food crops differed from the stochastic approach by one to two orders of magnitude, depending on the uncertainty of the parameter in the model (60). Given the uncertainty surrounding the parameter estimates in our model, the stochastic approach seems to offer the most accurate measures of exposure to fecal pathogens for each scenario. These comparisons also suggest that previous studies that used deterministic models may have overestimated the risk of exposure to fecal contamination at beaches in Accra.

### ii. Limitations of the Exposure Dose Model

Environmental samples were tested for norovirus and adenovirus by quantitative real-time PCR, but all the samples were negative, despite about 16.4% and 28.1% of diarrheal infections in Ghana by attributed to norovirus and adenovirus, respectively (61, 62). This negative finding may be due to our poor limit of virus detecting in sand. Therefore, the data used for exposure dose estimations in this study was based on indicator organisms, not pathogens. Norovirus and adenovirus may still be present in the environment, but at levels below the limit of detection. In addition, norovirus infection shows a distinct seasonality, peaking during the dry season months of October-May and nearly disappearing during the wet season (61). Environmental samples in our study were collected between August and October, when norovirus may have been shed in very low levels.

The development of this model required several simplifications and assumptions regarding exposure to fecal contamination at beaches. Structured observations of children at beaches did not record the frequency or duration of sand and water contact, so reasonable estimations were used for the exposure assessment. Measurements of these frequencies and durations would be useful for future studies. Additionally, each exposure scenario assumed that a child performed only one activity at a time. It is possible that
when a child goes to the beach he or she may have multiple contacts with both sand and water. In these cases, the child would be exposed to additional doses of fecal contamination based on each activity, and this was not accounted for in this study. So, our estimates of exposure may be conservative. Future microbial assessments could be improved by including additional parameters in the models that were not considered in this study, such as inactivation of microbes in the environment over time.

# iii. Implications of Structured Observations and Microbial Concentrations for Microbial Risk Assessment

The WHO determined that recreational waters should have less than 100 *E. coli* cfu per 100 ml in 50% of samples to be considered safe for swimming (35). The geometric mean concentrations of *E. coli* in sand (2.53 cfu  $log_{10}/100$  g) and water (3.97 cfu  $log_{10}/100$  ml) measured in this study indicate that beaches in Accra were highly contaminated. Although few studies have been done on recreational water in developing countries, the microbe concentrations in our study are consistent with those found in other developing countries. Diallo *et al.* (2008) measured *E. coli* concentrations ranging from 2.9-5.2 cfu  $log_{10}/100$ ml in canals in Thailand used for recreational purposes (45). Steyn *et al.* (2004) measured the geometric mean *E. coli* in surface water in South Africa at 4.45 cfu  $log_{10}/100$ ml. Labite *et al.* (2010) measured 4.0  $log_{10}$  cfu *E. coli* /100ml in marine water and 6.0  $log_{10}$  cfu /100g in sand at beaches in two different neighborhoods in Accra (6). While the concentration of *E. coli* in water is very similar to the geometric mean concentration measured in our study, the concentration in sand is over twice as large. If the study done by Labite were to be repeated using the microbe concentrations

calculated in our study, the risk estimates for exposure to marine water would likely remain the same, but the estimates for exposure to sand would decrease dramatically.

The neighborhood of Bukom is located downstream of Lavender Hill, a common site for the dumping of raw fecal sludge. It was expected that the water and sand samples from Bukom would be more contaminated than those from Shiabu, a neighborhood upstream of Lavender Hill. However, we found no significant difference in *E. coli* or coliphage concentrations between the two neighborhoods. The Shiabu beach may be contaminated in other ways, such as from runoff from latrines or from a higher rate of defecation on the beach. The rate of open defecation observed on the beach in Shiabu was three times higher than the rate in Bukom, although this difference was not statistically significant (Appendix G). Additionally, structured observations of children at beaches showed no significant differences between Bukom and Shiabu in the rates of children in the water, children eating at the beach, or children defecating in the water or on the sand (Appendix G). Although infection risk was not calculated in this study, the similarity between microbe concentrations and beach activities suggests that the risk of enteric infection is likely to be comparable between these two coastal neighborhoods.

We also found no significant difference in the dose of fecal contamination between age groups. We expected that children under five years would be exposed to greater fecal contamination because they have a higher frequency of hand-mouthing than children aged five to 12. However, the mean exposure doses for *E. coli* between the two age groups differed by only 0.24 cfu per event. The high initial concentration of *E. coli* in the water and sand may have masked the effect of hand-mouthing frequencies on exposure dose. Our study found no statistically significant difference between the concentration of microbes in water and in sand. Furthermore, exposure scenarios that took place in the water were associated with lower doses of *E. coli* than scenarios involving contact with sand, but the difference in dose was not statistically significant. Studies in industrialized countries have found that sand generally has higher concentrations of fecal indicator organisms than surrounding waters (28). The recreational waters of Accra, unlike those in industrialized countries, are subject to constant addition of fecal contamination from the dumping of sewage and fecal sludge, either directly or through open drains that lead to the ocean, which may explain why the water in Accra was just as contaminated as the sand.

The most risky exposure scenario in this study was direct contact with the sand. The geometric mean dose of *E. coli* for this activity differed from the least risky activity (playing with an object on the sand) by 1,138 cfu per event. It was expected that swimming in the water with the head submerged would provide the highest dose of fecal microbes because submerging this scenarios allows for exposure both by involuntary ingestion of water and by mouthing of contaminated hands. Our study used a probability distribution function to describe the ingestion of water during swimming events with a mean of 51 ml of water swallowed when the head was submerged and 31 ml when the head was not submerged (23), which may have underestimated the risk of exposure to fecal contamination in recreational water. Previous studies used much higher values of 75 ml and 100 ml per swimming event (6, 24).

#### F. Conclusions

- Beaches in Accra, Ghana are highly contaminated. Beaches are contaminated in several ways due to the lack of sanitation infrastructure in Accra. Dumping of fecal sludge at Lavender Hill, runoff from public latrines at beaches, and open defecation all contribute to high levels of fecal contamination in sand and marine water. This is a problem that needs to be addressed since people in coastal neighborhoods, especially children under 12 years, visit beaches multiple times per month.
- Children that visit beaches can be exposed fecal contamination through multiple pathways. Several pathways were assessed in this study, including swimming in marine water, direct contact with sand, and playing with an object on the sand. All of these scenarios resulted in high doses of fecal microbes.
- The most risky exposure activity was direct contact with sand on the beach. According to our structured observations of children at beaches, 9/28 (32%) children observed on the beach touched the sand with their hands, indicating that the scenario is a likely event. Structured observations also showed that 8/29 children (27.6%) had contact with both sand and water during the four hour observation period. It is important to note that the exposure doses presented here are additive for each contact with water or sand. Therefore, the dose that a child is exposed to each time they visit the beach is likely to be quite high.

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# H. Tables

# Table 1. Hand and Object Contamination Parameters

Variable	Symbol	Distribution (Parameters)	Source	Assumptions
Microbe concentration, Sand				Microbes are distributed homogenously in sand.
<i>E. coli</i> (cfu $\log_{10}/100$ ml)	C <sub>ES</sub>	Log-normal (2.53, 1.11)	This study	Samples are representative of all beaches.
Coliphage (pfu log <sub>10</sub> /100 ml)	C <sub>CS</sub>	Log-normal (1.36, 1.68)	This study	Measured concentration of microbes is representative of that type and location.
Microbe concentration, Water				Microbes are distributed homogeneously in
<i>E. coli</i> (cfu log <sub>10</sub> /100 ml)	$C_{\text{EW}}$	Log-normal (3.98, 0.85)	This study	water. Samples are representative of all recreational water sources. Measured
Coliphage (pfu log <sub>10</sub> /100 ml)	$C_{CW}$	Log-normal (3.79, 0.33)	This study	concentration of microbes is representative of that type and location.
Frequency of contact				
Hand to sand	Fs	Poisson (1)	Assumption	
Hand to water	$F_{W}$	Poisson (1)	Assumption	Children had an average of one contact per
Object to sand	Fo	Poisson (1)	Assumption	exposure event.
Area of surface, $cm^2$				
Hand, child under 5	$A_5$	Uniform (244.4, 329.0)	EPA 1985	Children 2 to <3 represent the average age.
Hand, child age 5-12	A <sub>12</sub>	Uniform (380.7, 695.6)	EPA 1985	Children 6 to <11 represent the average age.
Object	A <sub>0</sub>	Uniform (378.8, 1.515)	FIFA, Assumption	A ball ranging from 34.5 to 69.0 cm in circumference represents the average object played with on the beach.
Object contacted, %				
Hand in sand	$S_{HS}$	Uniform (0.13, 1.00)	AuYeung 2008	Contact is equal for all children.

Hand in water	$S_{\rm HW}$	Uniform (0.08, 1.00)	AuYeung 2008	Contact is equal for all children.
Object in sand	So	Uniform (0.08, 0.75)	Assumption	No more than 75% of the object comes in contact with the sand.
Hand on object	Р	Uniform (0.08, 0.27)	AuYeung 2008	Contact is equal for all children.
Water film thickness, cm	V	Uniform (0.00241, 0.00499)	EPA 1987	Equal for hand or non-porous objects. Ranges from partial wipe to no wipe after full immersion in water.
Sand Adherence, mg/cm <sup>3</sup>	D	Log-normal (0.49, 8.2)	Shoaf 2005	Equal for hand or non-porous object.
Transfer efficiency, object to h	and			
E. coli	TE <sub>OE</sub>	38.47%	Rusin 2002	Transfer efficiency for <i>Serratia rubidea</i> (gram- negative bacteria) and <i>E. coli</i> are equal
Coliphage	TE <sub>OC</sub>	65.80%	Rusin 2002	Transfer efficiency for Phage PRD-1 and Coliphage are equal.

Table 2. Exposure Dose Parameters

Variable	Symbol	Parameter	Source	Assumptions
Object contacted (%)				
Hand in mouth	$S_M$	Uniform (0.06, 0.33)	AuYeung 2006	Equal for children of all ages and locations.
Frequency of hand mouthing (#/hr)				Based on outdoor hand-mouthing frequencies.
Child under 5	$M_5$	Log-normal (0.92, 0.98)	This study	Based on household structured observations of children under 5 years.
Child, age 5-12	M <sub>12</sub>	Weibull (0.49, 1.47)	Xue 2007	Children 6-<11 represent the average age.
Time to hand washing, hr	$\mathrm{T}_{\mathrm{HW}}$	Uniform (0,16)	This study	Children are awake for 16 hours and wash once per day.
Transfer efficiency, hand to mouth (%	<i>5)</i>			
E. coli	TE <sub>ME</sub>	33.90%	Rusin 2002	Transfer efficiency for <i>Serratia rubidea</i> (gram-negative bacteria) and <i>E. coli</i> are equal
Coliphage	TE <sub>MC</sub>	33.97%	Rusin 2002	Transfer efficiency for Phage PRD-1 and Coliphage are equal.
Water Ingestion (ml)				Representive of all children in the study area.
Head not submerged	Io	Gamma (0.58, 55)	Schets 2011	Equal for children of all ages and locations.
Head submerged	$I_U$	Gamma (0.64, 58)	Schets 2011	Equal for children of all ages and locations.

		Neighbo	orhood	
	Alajo	Bukom	Old Fadama	Shiabu
	(n=200)	(n=200)	(n=200)	(n=200)
Household Size				
Total	4.7 (2.1)	6.8 (3.7)	4.1 (1.8)	5.0 (1.6)
# of children under 5 years	1.3 (0.7)	1.7 (1.0)	1.4 (1.1)	1.5 (0.7)
# of children 5-12 years	1.6 (2.8)	1.6 (1.6)	0.7 (1.0)	1.0 (1.0)
Health				
Child had diarrhea in the past 2 weeks	$10(12.4\%^{a})$	20 (17.2% <sup>a</sup> )	29 (25.2% <sup>a</sup> )	9 (10.1% <sup>a</sup> )
Visits to the Beach, Respondent				
Everyday	-	16 (8.0%)	-	9 (4.5%)
5-10 times per month	2 (1.0%)	6 (3.0%)	-	3 (1.5%)
1-4 times per month	22 (11.1)	46 (23.0%)	14 (7.0%)	24 (12.0%)
Never	175 (87.9)	131 (65.5%)	185 (92.5%)	164 (82.0%)
Visits to the Beach, Youngest Child				
Everyday	-	11 (5.6%)	-	7 (3.6%)
Once a week	1 (0.6%)	4 (2.0%)	1 (0.5%)	3 (1.6%)
Twice a week	-	-	-	2 (1.0%)
None	177 (99.4%)	181 (90.5%)	183 (99.5%)	181 (93.8%)

### Table 3. Descriptive Statistics from Household Surveys

<sup>a</sup>Proportion of children in surveyed households

		Buk	om	Shia	abu
		<i>E. coli</i> (cfu $\log_{10}/100 \text{ ml})^{a}$	Coliphage (pfu log <sub>10</sub> /100 ml) <sup>b</sup>	<i>E. coli</i> (cfu log <sub>10</sub> /100 ml)	Coliphage (pfu log <sub>10</sub> /100 ml)
	N (%) <sup>c</sup>	19 (100%)	10 (100%)	18 (100%)	5 (83.3%)
	Mean	4.20	3.95	3.73	3.46
Water	SD	0.74	0.26	0.92	0.16
	Min	2.78	3.66	2.30	3.30
	Max	5.27	4.43	5.69	3.68
	N (%) <sup>c</sup>	19 (100%)	4 (57.1%)	19 (100%)	2 (50%)
	Mean	2.51	0.73	2.55	2.60
Sand	SD	0.99	0.68	1.25	2.83
	Min	1.30	0.30	0.90	0.60
	Max	4.58	1.73	4.60	4.60

Table 4. E. coli and coliphage concentrations in sand and marine water samples by neighborhood

<sup>a</sup>cfu denotes colony-forming unit

<sup>b</sup>pfu denotes plaque-forming unit

<sup>c</sup>% = number positive/number tested

		E	E. <i>coli</i> (cfu	/event)		С	oliphage (j	pfu/event)	
		Geometric				Geometric			
]	Exposure Scenario	Mean	Median	95% I	Range	Mean	Median	95% H	Range
	(A) Direct contact, sand <sup>a</sup>	1339.07	490.56	2169.70	2617.89	662.62	233.80	1145.51	1400.18
Children	(B) Object contact, sand <sup>b</sup>	201.45	68.21	365.14	437.95	171.14	55.56	333.33	437.53
Under 5 Years	<ul> <li>(C) Head submerged,</li> <li>water<sup>c</sup></li> <li>(D) Head not submerged</li> </ul>	649.12	585.91	710.12	724.99	621.00	555.53	676.32	690.15
	water <sup>d</sup>	442.25	394.87	504.57	517.52	423.10	375.82	480.23	492.31
	(E) Direct contact, sand <sup>a</sup>	1749.00	568.36	3725.61	4720.79	865.46	275.25	1953.82	2466.96
Children	(F) Object contact, sand <sup>b</sup>	140.32	42.76	313.33	392.03	119.20	35.49	285.45	368.88
Ages 5- 12 Years	<ul> <li>(G) Head submerged,</li> <li>water<sup>c</sup></li> <li>(H) Head not submerged.</li> </ul>	658.46	596.54	720.92	736.03	629.95	566.53	686.68	700.75
	water <sup>d</sup>	450.85	405.16	515.36	528.59	431.34	386.57	490.57	502.93

Table 5. Exposure Scenario Results

cfu denotes colony-forming units; pfu denotes plaque-forming units

() indicates exposure scenario

<sup>a</sup>Describes a scenario in which a child's hands are directly contaminated by contact with the sand, then the hands enter the mouth.

<sup>b</sup>Describes a scenario in which a child is playing with an object on the sand, the hands become contaminated by the object and the hands then enter the mouth.

<sup>c</sup>Describes a scenario in which a child swims in the water, submerges the head and is therefore exposed through direct contact and ingestion of water.

<sup>d</sup>Describes a scenario in which a child swims in the water but does not submerge the head. The child is exposed through direct contact and ingestion of water.

Tuble 0. Tull wibe comparise	nis of exposure doses of detivity, a	50 group, and interove	
Pairwis	se Comparisons	Geometric Mean	n-value
Α	В	Difference (A-B)	p vulue
E. coli concentration	Coliphage concentration	213.34	0.344
E. coli (cfu)			
Water	Sand	-307.29	0.482
Under 5 Years	Ages 5-12 Years	-91.69	0.837
Direct contact, sand <sup>a</sup>	Contact with object, sand <sup>b</sup>	1373.15	0.022
Head submerged, water <sup>c</sup>	Head not submerged, water <sup>d</sup>	207.24	0.001
Coliphage (pfu)			
Water	Sand	71.74	0.722
Under 5 Years	Ages 5-12 Years	-42.02	0.836
Direct contact, sand <sup>a</sup>	Contact with object, sand <sup>b</sup>	618.87	0.027
Head submerged, water <sup>c</sup>	Head not submerged, water <sup>d</sup>	198.26	0.001

Table 6. Pairwise	comparisons of	of exposure of	loses by a	activity, age	group, and	microb	)e
	1	1	-		U I '		

cfu denotes colony-forming units; pfu denotes plaque-forming units

<sup>a</sup>Describes a scenario in which a child's hands are directly contaminated by contact with the sand, then the hands enter the mouth.

<sup>b</sup>Describes a scenario in which a child is playing with an object on the sand, the hands become contaminated by the object, and the hands then enter the mouth.

<sup>c</sup>Describes a scenario in which a child swims in the water, submerges the head and is therefore exposed through direct contact and ingestion of water.

<sup>d</sup>Describes a scenario in which a child swims in the water but does not submerge the head. The child is exposed through direct contact and ingestion of water.

### I. Figures



Figure 1. Transmission pathways of fecal-oral diseases. Adapted from Pruss (2002).



Figure 2. Study area: four low-resource neighborhoods in Accra, Ghana.



Figure 3. Kernel density plot of microbe concentrations in beach sand.



Figure 4. Kernel density plot of microbe concentrations in marine water.

#### **III. Implications, and Future Study**

### **A. Public Health Implications**

- As the population of Ghana becomes increasingly urban, we expect levels of fecal contamination at beaches and children's exposure to fecal contamination in sand and marine water to remain the same as levels observed in this study or to increase. In order to eliminate fecal contamination at beaches, fecal sludge should no longer be dumped into the ocean. Open drains should also be covered to prevent the disposal of feces in drains.
- Since Accra does not have a functioning wastewater treatment plant and few people have access to improved sanitation, people should be educated on the proper disposal of feces. Additionally, parents should be educated about the danger of contact with beach sand and water and should be encouraged to keep their children away from contaminated beaches.

### **B.** Future Studies

- Using exposure dose estimates and data on frequency of beach visits from this study, the possible risk of gastrointestinal swimming-related illness in the exposed population could be estimated.
- A sensitivity analysis may be useful for selected exposure parameters to examine how uncertainty in these parameters impact the dose estimates. In particular, the transfer efficiency parameters that were considered point estimates in the models and to time until hand washing, which had a large range of values.
- Future exposure assessments may want to consider additional parameters such as: the rates at which microbes are inactivated on objects and hands before entering

the mouth, inefficiency of hand washing for removing 100 percent of microbes on hands, and the decay in the number of microbes present on the hands after each mouthing event.

• Our exposure model assumed that the number of microbes on hands or objects was cumulative after each contact with water and/or sand. Other models may assume that some saturation point may be reached, at which point addition water and sand contact does not matter, or that additional contacts actually reduce the number of microbes by detaching them from hands or objects.

### **IV. Appendix**

#### A. IRB Approval



Institutional Review Board

TO: Christine Moe, PhD Principal Investigator Global Health

DATE: January 31, 2014

RE: Notification of Amendment Approval AM5\_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 1/31/2014. This amendment includes the following:

Personnel Change only: Adding Amanda Santander and Han-Hsyan Tsai 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: <u>http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-129.html</u>

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

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 Administrative Assistant This letter has been digitally signed

CC Peprah Dorothy Global Health

Raj	Suraja	Public Health
Robb	Katharine	Financial Aid - Cdc
Yakubu	Habib	Global Health
Null	Alex	Global Health

Emory University IRB 1599 Clifton Road, 5th Floor - Atlanta, Georgia 30322 Tel: 404.712.0720 - Fax: 404.727.1358 - Email: irb@emory.edu - Web: <u>http://www.irb.emory.edu/</u> An equal opportunity, affirmative action university

# **B.** Household Survey

Locatio	on 10 OP5 longtus	de WODD. Dista
	GPS latitud	de 1905.
.0-Gene	eral Information	
Ques a	Description	
301	Should I describe you as a head of a household Female head of household Female household member III Male hou	ir household member? (A household is people sharing a cooking pot) I of household sehold member
302	Tenincystatus	
102	How long have you lived in this household?	meethylyears
104	What is your highest level of education?	
	No formal education	Higher than secondary
	B Some primary	No mucore
	Covaleted originary	
	III Some secondary	
	Completed secondary	
	What is used within 2	
105	mat a your range av	
	E Christian III No te	gonae
	Moders .	
	Traditional/Spiritualist	
	Chart	
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	UII Famto	
	US Hausa	
907	h a haniness our from this compound/household	47 Ves
		III No.
		Ho response
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	F so, what and of business?	
	C Other	
109	How many households are in this compound cooking pot.	67 A household is people sharing a
_		1
110	Hew many people like is this compound?	

111	How many people are in your household?	
112	How many male adults (18 and alider) live in year household?	
113	How many female adults (18 and older) live in your household?	
114	How many children under 5 live in your household?	
115	How many children ages 5 -12 live in your household?	
116	How many young people ages 13-17 live in your household?	
117	Davis your household keys electricity?	III. tres III. No III. No response
118	Does your household have a radio?	<ul> <li>Wes</li> <li>No response</li> </ul>
139	Does your household have a tolevision?	III Ves III No III No response
120	Ones your household have a refrigerator?	III Yes III No III No response
121	Dave your household have a bicycle?	III Nes III No III No tesponee
122	Does your household have a motorcycle?	III Yes III No III No response
123	Does your household have a car?	E Ym E No F No response
124	Does your household have a donestic worker not related to the head of the insusehold	III Yes III No

301.	What is your primary source of drinking water?  Sachet/Water bottle  Tap from pipe network  Tap form pilytank  Harveoted Balroweter	Cother Specify other
202	How often do you replenish this primary source of drinking we	dati P
	III Fave times per vereik III Osce per vereik or less	
203	Fine times per verek     Gace per verek or less     How reach water does your child drink every day?	Caups 🖂 Sachets

	we summer see represe	S. 1.			
301	Hew many labrines are on	this compound?			if none -> 305
502	How many households alo	you shore a lattine with?			
808	Where do shidten (ages 5-	tilt in your household type	cally defecate?		
	()) Corepound Istrine	III Chamber pet	E Other Specify	athe	
	Public latrim	E Outside	Don't know		
	III. In a bagyflying tollet:	CBeach	Na eesponse		
304	If single incussions, then a	Aig) is this the same for the	other children in this compo	cand?	
14.6	E Yes D	Don't know			
	E nu E	Nanepunse			
305	(If respondent has a child u	oder 51 The last Droc years	compost child delecated, wh	ere did they defe	cate?
	Compound latrice	III Ongr	pund/inside compound		
- 1	E Public lattine	🗏 in dra	in/putter		
	III tria potty	III Don't	know		
	III to a daper/suppr	E Nore	sponse		
- 1	🗄 On ground/outside car	repard			
	(If resortent does not ha	we child under 5, skip to 30	46		
		-			Internet Name
300	What is the age of this chik	17			Searcher Texts
807	The tax time your sounder	t child defecated, how did	reu dispase of feues/	2632522	
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	E Las Leves were report	ET Washed de	continential and a second second	No recommend	
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31.2	How much did members of your including showers?	heasehold spend on public	attines yestextay, not	Cools/Pesevice
813	Where did you last bathe?			
	III in compound	III Other Specify	athe	
	Public bath	No response		
	Beach			
334	How much did members of your household spend on bathing facilities yesterday? Cada/0			
315	How much did you spend on dri	aking water yestenday for t	your tamily?	Codis/Presswar
316	Now much did you speed on with	er (for all purposes) yeste	rday for your family?	Cedis/Pesewas
817	How does your household prima	rily dispase of rubbish?		
	📃 Dispetal pit in compound	Drain Drain	Coher Specify other	
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	Private local collection	B Burned	III No response	

ie,

401	Hes this child had dianhoes in the past two weeks? (Dianhoes is 3 or more loose or		
	watery stools within 24 hours)		
	Yes Don't know -> 403		
	⊡ No → 488 ⊡ No response → 488		
602	Was this bloods diampoint?		
	B tes Don't know		
- 3	No Ma No Maporoe		
409	Do you ever de-worm your child?		
	E ym Don't knew		
	II No response		
404	When did you last de worm him/liver?		days/months/years
5.0 Was	th facilities on compound		
501	what kind of latrine do you have in this compound?		
	E No faritite/Seek/Tidd E VP(single) E Other		
	Traditional pit latrice		
	E Pour fish		
	11 Plash tailet		
502	Do you have a tink where you wash your hands after defection?		
	III Yes		
	II No *		
	III No response		
503	Do you have a container where you store drinking water?:		
	B Ns		
	B No -> 506		
	B No response > 506		
504	if an one take planes down this to par?		
	(What type of denking water container do they have?)		
	🗍 Namova marath (clicm)		
	Wide mouth (Hern)		
	The stor while to user		
1.75	the second s		
300	NAME AND ADDRESS OF A DESCRIPTION OF A D		
	Covered		
	- Not coverso		,
	- Not able to see -> skip tes		10.00
506	(Observe, don't ask() Are from observed around compound grounds?:	Tes .	110 100
507	(Observe, don't ask!) Are animals observed roaming around compound grounds?:	🗏 yes	Wite skip:

508	(Observe, don't axid) If you, which animals are observed? (check all that apply):
	Contra II Dave
	E radius E con
	I Pigs II Other
509	(diserve, don't asil) vie feed wate observed of compound grounds: tes III iso
.0 Week	dy Activities Questions
601	The plot week, new there units on you for a point and inter-
	Derryday Naver
- 1	No response
	This and such have even these did as on to the station
602	The past vector, new many times are por go to the manuel.
	Everyday Newor
	A new bines a week
	LL USCE & WEEK
603	This part week, how many times did you est new produce?:
	Excryday III News
	🖂 A few times a week 📃 Re response
	Chice a week
604	This past week, how many times did you buy food from a vendor?.
	Everyday Never
	A few times a week
	Chice a week
2.10.000	This cast month, how many times did you go to the beach? [This is for any reason,
201	including reprovidented, buying, selling, etch
	Cveryday
	S to 10 times
	1 to 4 times
	The New York Contract of Contr
	[]] No response
702	In a regular week, when there are no restrictions on going to the beach, how often
	does your sourcest child go to the beach?:
- 1	- Everyday
	Date a week
	Twice a weak
	III. Rene
	iii offier
703	Do you have a job that pails you in contact with eas reader?:
	Tan't know
	No Kereiparse
	these previous other than your in your branchold have a just that puts them in constant
204	with sea water?
	E Don't know
	E No E Noresponse
705	How often do you get into sea water when you go to the boach:
	Eurytime News
	E Semetimes III No response

.0 Scha 901	Yes. Don't know No	
0 Scha 901	I No I no children, End of Survey	
.0 Scha 901	ook [If no children, End of Survey	
901		
	Do any children in your household attend numery school 7:	
	The Yes Even't know (find Survey)	
	Mp (End Survey) In No response (End Survey)	
902	if yos, how many of them?:	
909	If yes, how many days per week? (If more than one child, ask this question for the wanneys of them):	
904	If yes, how many hours per day? (If many than one child, ask this question for the youngest of them):	
905	How often doen your child purchase fixed at school	
	III Formitie	
	Servetimes	
	Mayer	
	Moresponse Skip to 906	
906	France who's	
	a recently receipt.	
	Faad carried from home	
	School feeting program	
	Examinator code	
	Data entry code	

# C. Large Volume Water Environmental Sample Collection Form

Large Volume Water Environmental Sample Collection F	Form
Batcode	Date Time
1. GPS latitude NOS. GPS longitude W000.	Location Desc
2. Location ID:	
Select the neighborhood:     Alalo     Did Fadama	
Bukom Shabu	
4. What type of water sample was collected?	
Drinking Water Ocean Water	
5. If ocean water, complete the following (select one):	
Open water	
If driving water, complete the following:	
b. It is in the second se	
Public tap	Check box If pay-per-use
Compound/Private tap	Price (Pesewas)/Volume(L) /
Hand-dug well	
Tube Well/Borehole	
Tanker Truck	
Other Specify:	
7. If "Yes", check box:	
Within 3m of trash? Field	Notes:
Within Jm of feces 7	
defection area?	
8. Physiochemical Characteristics	
Turbidity Lab	Notesc
Total chlorine residual	
Sainty	
Temperature	
y. At IAC: Starting Volume(1) Post Ultrafilt	ration Volume:
Collector Data 8	Intry Code:

Particu Enviror	ilate nmental Sample Co	ollection Form	I
Barcode			Date
L	GPS latitude NOS. GPS longitude W000.	Locatio Descrip	on pition
2.	Location ID:	]	
3.	Select the neighborhood:	-	
	Alajo O	id Fadama	
	Bukom S	haibu	
4	If "Yes" for any sample check box:		
	Within 3m of feces?		
	Within 30m of latrine or defecation area?		
5.	Check box for Sample Type		
	Sedment		
	L Sal		
	Sand Sand		
	Sa. If sand, check location:		
	E wear open water		
	Cother H"other," specifi		
	Chark where samples for composite	sample wate collected	
	area entrance		play area one
	structure entrance		
	WC/latrine		
	Cooking area	Corner three	play area three
	water area	comer four	
	Handara	- center	
	In version area	Notes	1
7.	AT LAD:		
	Weight (g)		
Collector		Data Entry Code:	

**D.** Particulate Environmental Sample Collection Form

### E. Beach Description and Conditions Structured Observations Form

Beach Description an	d Conditions	page 1/3
Structured Observati	on	
GPS longitude GPS latitude	Observation start time  Observation end time	Date / /

Arrive at the beach by 6 am and observe until 10am. Find a vantage point from which to observe. Vantage point selection should prioritize heavy traffic, particularly from children and defecators. Tally observations of open defecation for the entire 4 hour period. In the second hour of observation, walk to the left of your vantage point along the coast for 10 minutes while tallying the number of children in water, eating, etc. After ten minutes, walk back to your original vantage point. Take the next hour to observe the characteristics and duration of 3 children in the water. In the last hour of observation, walk 10 minutes to the right of your vantage roler should be water the coast of the sector of observation.

Please take note of areas where kids are swimming for microbial collection of water and soil samples. A child is defined as being "in water" if they are on the ocean side of the beach as you are walking by. They can be standing, sitting, swimming or doing any other activity. Children who are eating can be eating on either side of you—either sea side or land side. Kids playing football on the beach count as all kids coming into contact with each other.

Please do not welk into risky areas. If you are unable to walk 10 minutes in a certain direction, please just note the number of minutes you were able to walk.

Enurmerator code		ക്കരിലാണ്ട
Location ID		sourchean

# Beach Description and Conditions Structured Observation

CITILO COSET VEDOTIS	Child 1	Child 2	Child 3
Age	Under 5 years 🔲	Under 5 years 🔲	Under Syears 🔲
	5-12 years	5-12 years	5-12 years
Start time			
End time			
Head underwater			
Number of other children in physical contact with target			
Playing with sand (prolonged hand contact)			
Toy contact with cand			
Food contact with sand			

### **Child Observations**

#### **General Observations**

Adults	Tale	Total
Defecation over water		
Defecation on beach		
Children 5-12 years		
in water		
Eating		
Defecation over water		
Defecation on beach		
Children under 5 years		
In water		
Exting		
Defecation over water		
Defecation on beach		

Enurmerator code	andreater
Location ID	searcheann

page 2/3

## Beach Description and Conditions Structured Observation

#### **General Observations**

Animala	Total	Total
Pigs		
Chickens		
Dogs		
Goats		
Other		
Latrines		
Public		
Household		

Enurmerator code

ANPATH

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## F. Table 7. Exposure Scenario Results for a Deterministic Model

		<i>E. coli</i> (cfu/event)	Coliphage (pfu/event)
	Exposure Scenario	· · · ·	· · · · ·
Children Under 5 Years	(A) Direct contact, sand <sup>a</sup>	97.70	52.63
	(B) Object contact, sand <sup>b</sup>	15.96	14.70
	(C) Head submerged, water <sup>c</sup>	204.09	194.35
	(D) Head not submerged, water <sup>d</sup>	124.49	118.55
Children Ages 5- 12 Years	(E) Direct contact, sand <sup>a</sup>	518.28	279.18
	(F) Object contact, sand <sup>b</sup>	45.09	41.55
	(G) Head submerged, water <sup>c</sup>	208.86	198.90
	(H) Head not submerged, water <sup>d</sup>	129.26	123.10

Table 7. Exposure Scenario Results for a Deterministic Model

cfu denotes colony-forming units; pfu denotes plaque-forming units

() indicates exposure scenario

<sup>a</sup>Describes a scenario in which a child's hands are directly contaminated by contact with the sand, then the hands enter the mouth.

<sup>b</sup>Describes a scenario in which a child is playing with an object on the sand, the hands become contaminated by the object, and the hands then enter the mouth.

<sup>c</sup>Describes a scenario in which a child swims in the water, submerges the head and is therefore exposed through direct contact and ingestion of water.

<sup>d</sup>Describes a scenario in which a child swims in the water but does not submerge the head. The child is exposed through direct contact and ingestion of water.

## G. Tables 8 and 9. Structured observations for children at beaches.

	Children in Water <sup>b</sup>				Children Eating <sup>b</sup>				Defecating in Water <sup>c</sup>				Defecating on Beach <sup>c</sup>			
	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value
Neighborhood																
Bukom	20	20	1.00	0.192	12	20	0.60	0 0.993 1	0	20	0.00	N/A <sup>e</sup>	3	20	0.15	0.250
Shiabu	98	23	4.26		14	23	0.61		0	23	0.00		72	23	3.13	

Table 8. Structured observations for children under 5 years<sup>a</sup>

<sup>a</sup>Age was estimated by the observer.

<sup>b</sup>Each observation period lasted for 2 hours. It was assumed that each recorded count represented a unique child.

<sup>c</sup>Each observation period lasted for 4 hours. It was assumed that each recorded count represented a unique child.

<sup>d</sup>Total hours of observation.

<sup>e</sup>Unable to run analysis because no children were observed doing this activity.

	Children in Water <sup>b</sup>				Children Eating <sup>b</sup>				Defecating in Water <sup>c</sup>				Defecating on Beach <sup>c</sup>			
	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value	No. children observed	Hrs <sup>d</sup>	Rate (children /hr)	p- value
Neighborhood																
Bukom	75	20	3.75	0.145	21	20	1.05	0.972	17	20	0.85	0.178	6	20	0.30	0.179
Shiabu	211	23	9.17		23	23	1.00	00	0	23	0.00		88	23	3.83	

## Table 9. Structured observations for children 5-12 years<sup>a</sup>

<sup>a</sup>Age was estimated by the observer.

<sup>b</sup>Each observation period lasted for 2 hours. It was assumed that each recorded count represented a unique child.

<sup>c</sup>Each observation period lasted for 4 hours. It was assumed that each recorded count represented a unique child.

<sup>d</sup>Total hours of observation.