Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

Laura de Mondesert

Date

Quantifying Urban Sanitation Coverage and its Association with Fecal Contamination in Open Drain Systems in Accra, Ghana

By

Laura M. de Mondesert Master of Public Health

Global Epidemiology

Dr. Amy E. Kirby Committee Co-Chair

Dr. David M. Berendes Committee Member

Quantifying Urban Sanitation Coverage and its Association with Fecal Contamination in Open Drain Systems in Accra, Ghana

By

Laura M. de Mondesert Bachelor of Science Saint Louis University 2013

Faculty Thesis Advisor: Amy E. Kirby, 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 2017

Abstract

Quantifying Urban Sanitation Coverage and its Association with Fecal Contamination in Open Drain Systems in Accra, Ghana By Laura M. de Mondesert

Introduction: Poor sanitation coverage is a common issue in most low and middle income countries around the world today and is often associated with higher levels of fecal contamination in the environment. This study aims to analyze whether associations exist between sanitation coverage at the household level and fecal contamination in open drain systems in Accra, Ghana.

Methods: Household surveys (n = 750) and drain water samples (n = 163) were collected in 5 neighborhoods of Accra. Kulldorff's Bernoulli spatial scans were used to identify most-likely clusters of surveyed household sanitation characteristics (level of toilet containment, shared vs. unshared toilet, and frequency of public toilet use). Toilet containment refers to the safe disposal and physical separation of excreta from human contact. *E. coli* concentrations were quantified in drain water samples and regressed against sanitation characteristics, controlling for neighborhood, drain infrastructure, and rainfall.

Results: Compared to all other study neighborhoods, Ringway — the neighborhood with the highest coverage of private sanitation use (vs. public sanitation use) — had significantly lower *E. coli* concentrations in drain samples. Drain sample sites within 50 meters of clusters of high coverage of contained sanitation had, on average, 1.08 log_{10} CFU/100mL lower *E. coli* concentrations than drains located outside of these clustered areas (p=0.003). Drains within 100 meters of clusters of low coverage of households that did not use public toilets had, on average, 1.19 log_{10} CFU/100mL higher *E. coli* concentrations located outside of these clustered areas (p=0.007).

Discussion: The results from this study provide some of the first evidence suggesting that clustering of contained sanitation and private toilet use, as opposed to public toilet use, may be associated with lower levels of fecal contamination in open drain systems, and conceivably the remaining proximal environment.

Quantifying Urban Sanitation Coverage and its Association with Fecal Contamination in Open Drain Systems in Accra, Ghana

By

Laura M. de Mondesert

Bachelor of Science Saint Louis University 2013

Thesis Committee Chair: Amy E. Kirby, Ph.D.

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 2017

Acknowledgements

I would like to thank my advisors, Dr. Amy Kirby and Dr. David Berendes, for their support and mentorship. The influence that you both have had on my personal and professional development is immeasurable. I am especially appreciative of your time and dedication to my advisement. You have pushed me to create a research paper that I never thought I was capable of writing.

Lastly, I would like to thank my family, and above all my parents Eduardo and Rosalba de Mondesert. Words are not sufficient in expressing my gratitude. My accomplishments would have been impossible without your unconditional love and support.

Table of Contents

| I. | Background/Literature Review | 1 |
|-----|---|----|
| | A. Setting Up the Context | 1 |
| | i. Diarrheal Disease | 1 |
| | ii. WASH & Diarrheal Disease | 2 |
| | iii. Sanitation in Low- and Middle-Income Countries (LMICs) | 4 |
| | iv. Diarrheal Disease & Sanitation in Context to Africa | 4 |
| | v. Urbanization & Sanitation | 5 |
| | vi. Urbanization and Sanitation Coverage in Accra | 6 |
| | B. Fecal Sludge Management (FSM) | 8 |
| | i. Fecal Waste Management & the Sustainable Development Goa | ls |
| | (SGDs) | 8 |
| | ii. FSM & Open Drain Systems | 9 |
| | C. Evaluation of Fecal Contamination | 11 |
| | i. Fecal Indicator Organisms | 11 |
| | ii. <i>E. coli</i> as a Contaminant | 11 |
| | D. Fecal Contamination Exposure Pathways | 12 |
| | i. Fecal-Oral Transmission Pathway | 12 |
| | ii. Uncontained Toilets as an Environmental Exposure | 13 |
| | iii. Shared Sanitation as an Environmental Exposure | 14 |
| | iv. Fecal Exposure Through Open Drain Systems in Accra | 16 |
| | E. Impacts of Understanding Fecal Exposure Pathways | 17 |
| II. | Manuscript | 19 |

| | A. | Abstra | ct | 19 |
|------|----|---------|--|----|
| | B. | Introd | uction | 20 |
| | C. | Metho | ds | 24 |
| | | i. | Study Area | 24 |
| | | ii. | Household Surveys | 25 |
| | | iii. | Drain Sample Collection | 26 |
| | | iv. | Sanitation Coverage Quantification | 27 |
| | | V. | Regression Modeling | 29 |
| | | vi. | Assumptions | 30 |
| | | vii. | Analysis | 30 |
| | D. | Result | S | 32 |
| | | i. | Household Demographics & Environmental Characteristics | 32 |
| | | ii. | Spatial Sanitation Cluster Coverage | 34 |
| | | iii. | Variation in E. coli Concentration in Drain Water by | |
| | | | Neighborhood, Drain Infrastructure, & Rainfall | 35 |
| | | iv. | Variation in <i>E. coli</i> Contamination in Drain Water | 36 |
| | E. | Discus | ssion | 38 |
| | F. | Streng | ths/Limitations | 44 |
| | G. | Refere | ences | 47 |
| | H. | Tables | 5 | 62 |
| | I. | Figure | S | 69 |
| | J. | Glossa | ıry | 73 |
| III. | Fu | ture Di | rections | 75 |

| IV. | Appendix | 76 |
|-----|---|----|
| | A. IRB Approval | 76 |
| | B. Household Survey | 78 |
| | C. Drain Water: Environmental Drain Water and Processing Form | 82 |

List of Tables

- Average rainfall across Accra and days of precipitation by month, March July 2016
- Sub-neighborhood household demographics and environmental characteristics of Accra, Ghana
- E. coli concentrations (in log₁₀CFU/100mL) in public domain samples, by neighborhood
- Differences in *E. coli* concentrations (in log₁₀CFU/100mL) of sample drain sites by drain infrastructure and seasonality
- Differences in *E. coli* concentrations (in log₁₀CFU/100mL) of sample drain sites by neighborhood
- E. coli concentrations (in log₁₀CFU/100mL) in sample drain sites by sanitation coverage cluster

List of Figures

 Sub-neighborhood sanitation containment coverage and drain sample sites – Accra, Ghana

- Sub-neighborhood shared sanitation coverage and drain sample sites Accra, Ghana
- Sub-neighborhood public sanitation use coverage and drain sample sites Accra, Ghana
- 4. Causal relationship between sanitation indicator variables and fecal contamination in open drains

I. BACKGROUND/LITERATURE REVIEW

A review of the recent literature suggests that there are large gaps in the described associations between sanitation and fecal contamination. Many studies describe the effect of sanitation on diarrheal disease incidence, ignoring the intermediate outcomes that may contribute to this causal pathway (1-6). A targeted effort to identify the missing links to these pathways is described throughout this report. This paper reviews the impact of water, sanitation, and hygiene (WASH) activities on health and environmental outcomes, paying particular attention to the existing pathways of fecal contamination. The analysis provides evidence to support the relationship between spatial heterogeneity in sanitation coverage and fecal contamination of the proximal environment. Understanding how these mechanisms function, especially in low-resource settings, is integral to the success of future interventions that aim to reduce the global burden of sanitation-related consequences.

A. Setting Up the Context

Diarrheal Disease

Diarrheal disease is one of the largest contributors to morbidity and mortality, accounting for 4% of all deaths around the world (7, 8). Lack of access to appropriate water, sanitation, and hygiene (WASH) infrastructure is believed to be the main cause of the 4 billion reported cases of diarrhea per year, and 1.8 million deaths (9-11). Diarrhea, defined as 3 or more uncommonly loose stools within the past 24 hours, is the second leading cause of death in children under 5 years of age, killing an estimated 1 in 9 children every year (10, 12, 13). While annual childhood mortality has decreased from 4.6 million deaths in the late 1980's to approximately 2.5 million in recent years due to increased use of pit latrines, diarrheal diseases are still considered to be the third leading cause of morbidity behind respiratory infections and HIV/AIDS (14, 15). The burden of disease is most felt in low- and middle-income countries (LMICs), where diarrhea is responsible for 7.2% of all disability-adjusted life years (DALYs), or years of healthy life lost due to disease, and the average child experiences 2-3 episodes of acute diarrhea per year (15, 16).

Diarrhea is clinically classified as a symptom, typically of an enteric infection, caused by the ingestion of viral, parasitic, or bacterial organisms (12-14). While most episodes of diarrhea result from some form of enteric infection, not all enteric infections lead to diarrhea (17). Some of the most common agents responsible for these types of infections include pathogenic *E. coli*, norovirus, *Cryptosporidium* spp., *Shigella* spp., and rotavirus (13, 18). People residing in low- and middle-income countries (LMICs) are primarily exposed to enteric pathogens through poor food sources, contaminated drinking water, and poor sanitation infrastructure (19).

WASH & Diarrheal Disease

Water, sanitation, and hygiene (WASH) comprises three separate, yet interdependent, themes that are used collectively to describe issues surrounding sanitary behaviors along with water access, quality, and sustainability (20). These three themes include water quality and access, access to basic sanitation* facilities that safely separate excreta from human contact, and the practice of good hygiene behaviors across entire populations (20, 21). WASH activities function as barriers that act upon the F-diagram, which illustrates how the routes of fecal contamination flow in a stream-like manner with sanitation – and its role in containment – upstream of water, flies, fingers, food, and fields (22). The careful and safe separation of feces creates a physical barrier against the contamination of liquids, the environment and insects through piped toilet systems, interrupting major fecal contamination exposure pathways in both humans and their environment (22, 23). This serves as a first line of defense against the introduction of harmful pathogens to water systems and foods (24, 25).

Much of the WASH literature uses incidence of diarrhea as a primary outcome measure. Impact assessments of these interventions in the developed and developing world alike have reported a 30-50% decrease in new cases of diarrheal disease (26). Meta-analyses of different WASH-related studies have found that the most effective strategies addressed a combination of WASH components, such as improved sanitation and water quality together (4, 26). Those with improved sanitation alone had a 27% reduced risk of diarrheal disease compared to those with unimproved sanitation. However, this effect was even greater (79% reduced risk), when improved sanitation was

^{*}The WHO defines sanitation as the "provision of facilities and services for safe management and disposal of human urine and feces" (WHO, 2015). The terms "improved" and "unimproved" sanitation do not indicate a certain level of cleanliness, but rather are indicative of the quality of the toilet and its ability to efficiently separate excreta (WHO, 2001). The terms "improved sanitation" and "quality of sanitation" are often used interchangeably and incorrectly in the literature. However, it is important to note their distinction, especially when using them as measures to quantify levels of fecal contamination. "Improved sanitation" refers to the degree of exposure to contaminants (Exley, 2015).

combined with other WASH interventions (e.g., piped water access) (4). These results demonstrate the need for a collaborative effort towards more comprehensive WASH-interventions to reduce incidence of diarrheal disease at the community level.

Sanitation in Low- & Middle-Income Countries (LMICs)

Poor sanitation coverage, or lack of access to improved sanitation facilities and fecal management infrastructure, is a common issue in most low and middle income countries (LMICs) around the world today and is often associated with poorer health outcomes (9). Unimproved sanitation is responsible for over 126,300 deaths globally, 88% of which occur in LMICs in Africa and Southeast Asia (27). Nearly a third of the world's population resides in homes that lack basic sanitation, leaving them particularly vulnerable to disease through contact with human feces (14, 22, 23, 28, 29). Of these 2.6 billion people living with poor sanitation, over 90% live in LMICs (15, 30). Access to sanitation is low primarily because those who reside in LMICs lack the financial resources to pay for these services (31). Even health facilities in LMICs suffer from poor WASH, as an estimated 19% lack improved sanitation and over a third (38%) do not have access to piped water systems (32). The WHO estimates that improving global sanitation alone could lead to 1.8 billion fewer cases of diarrheal disease annually, an impact that would be felt most in LMICs (15).

Diarrheal Disease and Sanitation in Context to Africa

Diarrheal disease is the second leading cause of child mortality in Sub-Saharan Africa next to respiratory infections, responsible for over 750,000 deaths in children under 5 per year (33). A disproportionate number of deaths due to diarrheal disease worldwide (43%) is concentrated in the continent of Africa (16, 34). The burden of disease is also disproportionately felt in Africa, as the average child has 7 episodes of diarrhea a year, compared to global averages of 2-3 episodes per child per year (15, 35). While WHO disease updates report a global decrease in mortality rates in the past three decades, Africa has witnessed marginal reductions since the 1980's (36). This consistently high incidence of disease has been linked to limited access to improved water and sanitation infrastructure across the continent (23, 30, 36, 37).

Poor WASH conditions are responsible for 90% of all diarrhea-related deaths in Africa (27, 38, 39). In 2015, around 32% of people living in Sub-Saharan Africa did not have access to safe water for consumption. Ingestion of contaminated water from these sources alone was responsible for an estimated 229,000 diarrhea-related deaths (40). Access to improved sanitation, or a toilet that is capable of hygienically separating excreta from human contact, is restricted to 31% of the African population, leaving 535 million people exposed to unsafe sanitation practices, and subsequent environmental fecal contamination (9, 37).

Urbanization & Sanitation

The world is currently experiencing its largest surge in urban population to date. For the first time in history, the majority of the world's population (54%) resides in urban settlements (41). Urban populations have been growing at a rate of 1.84% per year, with 180,000 people moving to already-dense cities every day (9, 41). The United Nations (UN) projects that by 2050, the world's population will increase by 2.5 billion people in urban settlements alone (41). Among some of the largest drivers of urbanization are economic development, poverty, and hopes for better access to health and sanitation services. Yet, many upon arrival are faced with the grim reality of water and sanitation infrastructures that are unable to support the growing population (11, 42).

Increased urbanization along with growing urban populations have contributed to the challenges of maintaining an adequate global WASH landscape by adding stress to already poor sanitation infrastructure (42, 43). Since 1990, 146 million Africans have gained access to improved sanitation. At this same time, however, the urban population in Africa also grew by 213 million, leaving an additional 159 million people without access to basic sanitation (9, 41, 44). A total of 863 million people worldwide currently live in urban slums, many with no access to toilets (11). Public toilets in slums often do not have sufficient capacity to serve the local population, and thus an estimated 25 million people living in Sub-Saharan Africa must resort to open defecation, or the convention of defecating in open spaces instead of toilets, in or around their communities (28, 30, 45-47). Thus, as population growth and urbanization continue to drive large numbers of people into already crowded areas, fecal waste management is expected to become more scarce (48).

Urbanization & Sanitation Coverage in Accra

Among cities in Africa experiencing rapid urban population growth is Accra, Ghana (49). The Greater Accra Metropolitan Area (GAMA) is the most densely populated region (2,154 people per km²) in the country of Ghana with an estimated population of 2.7 million people (50, 51). While the city as a whole has experienced mass expansions in both population and geographic size, the United Nations Centre for Human Settlements indicated that peri-urban populations are growing at twice the rate of the city's center (52, 53). About 60% of the GAMA population is concentrated in urban slums and neighborhoods, with the most population-dense settlements positioned along the city's coast (49). Population redistribution to these urban areas is largely driven by rural dwellers escaping their more remotely located homes in search of infrastructural development and the provision of better services. However, a 38% increase in population density over the past 10 years has started to make these resources less accessible to Ghana's poorer citizens (51, 54).

The growing population in Accra has pushed residents out to poorer areas within the city where adequate sanitation infrastructure and piped water supply systems are either scarce or inaccessible (51, 55, 56). In Accra, only 28.8% of households have piped indoor plumbing or equivalent forms of improved sanitation (57). Of the households that report ownership of a toilet, 79.5% of these are considered to be improved. Access to adequate sanitation, however, varies immensely by region. For example, roughly 70% of people residing in West Accra have no access to a toilet and report practicing open defecation, while the majority (93.7%) of those who live closer to the center of the city have access to some form of a toilet. Among those who do not have a toilet in the home, over a third (34.8%) report using nearby public latrines (51). Many of the remaining citizens rely on pit latrines, defecate in the open, or manage their own fecal sludge by depositing it in nearby drainage channels (51, 57). Depending on the season as well as the size and infrastructural composition of the drains, these channels are prone to overflowing of flood and other contaminated wastewater, exposing the environment to hazardous waste. Pools of nearby standing water are commonly seen around homes and compounds belonging to poorer communities in Accra and are presumed to be the result of these flooding incidences (58, 59). The proportion of people in Accra with access to piped water systems has decreased by 3.4%, with a third of the city's population reliant on potentially contaminated water sources (51).

Residents of Accra have already begun to see the effects that urbanization on the population's health, as the sanitation infrastructure in place has not been able to support the city's growing sanitation needs (60). In 2013, diarrheal disease alone was responsible for 12% of all deaths in children under 5 in Ghana. Many of these deaths were linked to low sanitation coverage in large cities like Accra (61). An observational study conducted in Accra that aimed to describe the associations between diarrheal disease and household sanitation coverage found that 24.5% of diarrheal cases in their study population resided in homes where the practice of open defecation was common. Among other sanitation characteristics considered, toilets shared with 5 or more households were associated with increased incidence of diarrheal disease (57).

B. Fecal Sludge Management (FSM)

Fecal Waste Management & the Sustainable Development Goals (SDG)

While the Millennium Development Goals (MDG) successfully expanded coverage of improved sanitation facilities, the final fate of fecal waste was not measured (62, 63). Because fecal waste management has often been viewed as a temporary solution to insufficient sewerage coverage, little attention was paid to its impact on sanitation. Many global sanitation assessments and MDG reports measured improvements in sanitation by using the Joint Monitoring Program's (JMP) classifications of "improved" and "unimproved" sanitation (23, 62, 64). These definitions did not explicitly consider the final fate of the fecal waste, likely due to the difficultly of measurement (65). Creation of the Sustainable Development Goals (SDG) have since shifted the focus of sanitation goals to ensure safe management of feces along the entire sanitation chain.

Sustainable development goal (SDG) 6 aims to "ensure availability and sustainable management of water and sanitation for all by the year 2030"(66). Sub-goals that serve as intermediate results of the final aim of SDG 6 involve water system quality improvement, prioritization of vulnerable populations, and equitable access to safely managed sanitation services (23). The term "safely managed services" encompasses the provision of private, contained toilets that allow for the safe disposal of excreta, with access to appropriate fecal waste treatment services (67). Achievement of this goal would help to reduce exposure to fecal waste in the 1.8 billion people in LMICs who lack fecal sludge management (FSM) services (68). Experts believe that this shift in focus will allow for a more comprehensive approach to sanitation expansion, better accommodating to the demands of the growing global population (23).

FSM & Open Drain Systems

An estimated 2.7 billion people in LMICs are reliant on the sanitation services provided by onsite FSM technologies (62). FSM refers to the management of untreated excreta from onsite systems, including the emptying, safe transport, and eventual treatment of fecal sludge (23, 62, 64). These technologies are estimated to serve 65-100% of unsewered toilets, both private and public in urban African settlements (64). FSM technologies must often adapt to the funds available in the population and the environment they are serving, making it difficult to devise a standard solution applicable to all LMICs. However, countries like Tanzania have implemented the use of mobile sludge management companies that send tankers with desludging equipment to the homes of people who do not have access to fecal waste management treatment centers (46). This method along with other FSM models provide an effective alternative for sewer-based systems and sustainable solution for solving ecological sanitation issues.

The issue with FSM technologies, specifically in Africa, is that most communities lack the appropriate infrastructure to support safely-managed sanitation, often leading to spillover of residual waste and the manual emptying of fecal waste into open drain systems (62, 69). Infrequent services along with small or too few septic tanks are not able to keep up with the volume of fecal sludge that requires FSM services. With nowhere else to store excess sludge, many resort to dumping excreta into open drain systems. Many illegal and legal FSM providers, alike, also directly empty fecal waste into open drains, where an accumulation of fecal waste goes largely untreated (69, 70). In the case of Accra, only one privately owned fecal sludge treatment facility serves the entire city, capable of collecting a no more than of 700 m^3 of sludge per day. The majority of the waste that is unable to reach this facility often ends up contaminating the nearby coastal waters and the proximal environment (70). A study evaluating microbial risks by sampling different environmental structures in Accra found that open drains had the highest concentrations of fecal indicator bacteria and other harmful pathogens, demonstrating the harmful effects of these practices and of insufficient resources to support successful FSM (56).

C. Evaluation of Fecal Contamination

Fecal Indicator Organisms

Indicators of fecal contamination have been suggested and used in the past due to their presence in feces at concentrations much higher than those of pathogens (65). Humans expel between 100-400 billion coliform bacteria per day, including over 50 types of pathogenic organisms such as pathogenic *E. coli*, *Cryptosporidium* spp., and *Giardia* (71). While fecal indicator bacteria (FIB) generally do not cause disease, their detection indicates that disease-causing fecal pathogens may be present. Thus, they are useful in assessing the safety and quality of recreational waters, and in determining whether water is suitable for human consumption (72). Ideal indicators that have strong disease predictability due to their correlation with pathogenic organisms (73). Results of a systematic review on studies assessing water quality indicators found that enterococci and enteric viruses are generally strong indicators for gastrointestinal illnesses, while *E. coli* is a strong indicator for the presence of fecal matter in a recreational water source (72, 73).

E. coli as a Contaminant

E. coli has been one of the most popularly used indicator organisms for fecal contamination in varied water sources because it is naturally abundant in fecal matter, it is a large contributor to overall contamination, and is easily detectable (74-76). *E. coli* largely exists and originates from the intestinal tracts of mammals and is expelled into the

environment through fecal waste (77). The bacteria, however, is a non-specific indicator of fecal contamination because it can originate from other sources outside of the gastrointestinal tract (78). Bacterial biomass, including *E. coli* and other coliform bacteria, accounts for 25-54% of solid organic material in feces (79). Pathogenic *E. coli* are a small fraction of the total *E. coli* strains found in fecal matter (25, 65, 72, 80). While the majority of *E. coli* is non-pathogenic, it is significantly associated with a 54% increase in risk of diarrheal disease. This finding suggests that the presence of *E. coli* is likely consistent with the presence of diarrhea-causing pathogens (76). *E. coli*'s presence is easily quantified using standard culture-based methods in routine microbiological analyses (73, 81). Furthermore, *E. coli* is considered the best indicator of fecal contamination by the WHO's Guidelines for Drinking-Water Quality (GDWQ) and is the Environmental Protection Agency's (EPA) recommended FIB for all recreational water quality assessments (76).

D. Fecal Contamination Exposure Pathways

Fecal-Oral Transmission Pathway

Fecal-oral exposure pathways are best represented by the F-diagram, which is a useful tool for visualizing environmental transmission of enteric pathogens (14, 22). As the phrase "fecal-oral exposure" implies, enteric pathogens present in the feces of some host infect another host through the ingestion of these microorganisms. These pathogens are spread through their interaction with other hosts and/or the proximal environment by way of one of the "five f's" – fluids, flies, food, fingers, or fields (18, 22). The literature often describes water, or fluids, as one of the most effective fecal exposure pathways, as

there are many ways that people can interact with untreated water (e.g., consumption, recreational purposes, handwashing, etc.) on a daily basis (2, 82). Enteric pathogens interact with fluids both at the source (e.g., lakes, oceans, and other recreational waters) and through poor water containment infrastructure (62, 73, 83). Fecal matter is often introduced in the environment, or fields, directly through open defecation (18, 84). Pathogens can interact directly with flies or with hands directly following defecation, which threaten the contamination of foods (84, 85). While these transmission pathways are well-defended in the literature, actual contamination depends on the frequency and the magnitude of exposure to enteric pathogens (14, 18, 22, 25).

Uncontained Toilets as an Environmental Exposure

Many observational studies consider toilet ownership as an important predictor of exposure to fecal contaminants, as different types of types of toilets pose different levels of risk of enteric infection (86). Uncontained toilets pose the largest risk, as they cannot safely separate fecal waste from human contact or the environment (87, 88). Because of this, uncontained toilets, such as pans or buckets are much more difficult to clean (86). Those without access to contained toilets may also be required to manage or dispose of their own waste, exposing them to potentially harmful microorganisms. Enteric pathogens, thus, are able to interact with and potentially contaminate the surrounding environment, the hands of those who may touch the waste, and any animals or insects in the area through direct contact (89). Hand contamination can lead to the direct transmission of pathogens through hand-to-mouth contact, or can lead to indirect transmission by contaminating foods or water that are later ingested by a host (18).

Previous studies found that ownership of contained toilets is associated with significantly lower concentrations of *E. coli* in the proximal environment (15, 35, 86, 90). A 63% reduction in *E. coli* concentrations was observed in a performance evaluation of newly installed contained toilets in Ghana (90). Homes with contained toilets were also found to have significantly lower levels of *E. coli* concentrations (-1.18 $log_{10}CFU/900cm^2$) compared to homes with uncontained toilets (35). Still, the magnitude of exposure is dependent on the type of toilet used, as lower levels of toilet containment functionality was associated with higher concentrations of *E. coli* (86).

Many observational studies that consider sanitation coverage as an exposure variable aim to determine its effect on the risk of diarrhea (1, 2, 4). In many children residing in areas of low sanitation coverage, fecal contamination by way of the environment has been linked to enteric infections caused by the direct ingestion of fecal matter (89). The results of a meta-analysis considering the impact of toilet containment on diarrheal disease risk found that people who use contained toilets had a 27% reduced risk compared to those who reported using uncontained toilets (4). A similar study found that uncontained toilet use was associated with a 33% increase in diarrheal disease (1).

Shared Sanitation as an Environmental Exposure

Shared sanitation has widely been used as an environmental exposure variable in observational studies, which have described clear trends between increased sharing practices and increased incidence of diarrhea (91-93). This mechanism is likely driven by the fact that shared toilets tend to be dirtier than private ones and may also be prone to over-filling (28). This mechanism, furthermore, presents a high risk of direct contact with

enteric pathogens for all those who use the shared facility (18). Regions where shared sanitation coverage is high have often been associated with higher prevalence of diarrhearelated diseases compared to low shared sanitation coverage areas (91). This is likely due to the fact that a larger proportion of the population is exposed to fecal waste through direct contact with dirty toilets or indirect means of transmission through other elements of the F-diagram (i.e., fields, fluids, and/or foods that interact with the contaminated hands of an exposed person) (22). Issues with toilet cleanliness are exacerbated by the growing population in regions of high shared sanitation coverage (28).

Overpopulation and increased migration to urban areas have outpaced the expansion of sanitation infrastructure in LMICs, often requiring households to share a single toilet with many other community members (94). The proportion of people who use shared toilets is highest in Sub-Saharan Africa, where 19% of the population reports sharing a toilet with one or more households (23, 95). Many households in these areas, however, often report sharing a toilet or latrine with more than five other households (94). Inability to afford private sanitation and issues with land ownership prevent people from building sanitation facilities of their own, thus driving the proportion of people who share up as rapid urban growth continues (95).

While causality has yet to be confirmed, study results have generally described a link between use of shared sanitation and diarrheal disease prevalence (91-93). An analysis of surveys describing sanitation characteristics across 51 countries observed a 9% increase in prevalence of diarrhea among people who shared toilets with other households, compared to those who do not share a toilet. This association was markedly higher in Ghana, where shared toilet users had 35% higher prevalence of disease compared to private toilet users (92). Higher frequencies of sharing, larger number of people or households sharing a toilet, and uncontained shared toilets (vs. contained toilets) were all related to an increased prevalence of diarrheal disease (91).

Fecal Exposure Through Open Drain Systems in Accra

Similar to toilet ownership and shared sanitation status, contact with open drains has been described as an important source of fecal contamination in the literature (29, 56). Quantitative Microbial Risk Assessments (QMRA) – models built using a risk-based scenario – recently conducted in Uganda found that exposure to open drain systems was the largest contributor to the burden of waterborne disease (29). A similar study found that exposure to open drain systems contributed 62% to the total number of DALY's per year (56). Exposure to harmful enteric pathogens through open drains can be described in the context of the F-diagram. In Accra, different drain channels lead to larger drains which often lead to recreational water sources such as rivers, streams, and the ocean (56, 96). As fecal waste is discharged into these drains, either directly through open defecation and illegal emptying of waste by waste treatment agencies, enteric pathogens interact with much of the proximal environment (56, 62, 69, 97). Contamination of fluids and fields, thus, increases the risk of human fecal contamination for those who interact with the contaminated environment (22, 73).

Wastewater disposal into open drain systems increases the risk of fecal contamination for nearby populations, among which children are most affected. An observational study analyzing environmental health disparities in Accra found that approximately 51% of households in the city admit to discarding wastewater into open

drains near their homes (57). Those that are most vulnerable to contamination from these drains are children residing in poor neighborhoods near open drain systems, as they are often seen playing near and at times in the drains (59). Children generally tend to be more vulnerable to enteric/waterborne diseases caused by contact with fecal contamination than adults living in the same communities, as they are more likely to play and come in contact with the contaminated environment and generally have poorer hygiene behaviors. A team of researchers working in Accra found that the sequence of these behaviors leads to different pathways of exposure to fecal contamination (59). For example, if a child plays in an open drain then eats, his or her likelihood of oral exposure to fecal contaminants is much higher than if they would have eaten first then played in or near the same drain.

E. Impacts of Understanding Fecal Exposure Pathways

If we hope to meet the aims set by SDG 6 by 2030, public health must place greater emphasis on the study of how fecal exposure at the environmental level affects environmental infrastructure in low-resource communities (23). Understanding pathways of fecal contamination exposure by way of the environment could potentially lead to policy changes to improve sanitation conditions for the poor. The health interventions currently in place could be improved by prioritizing environmental goals through sanitation-based activities. Census projections indicate that populations will continue to get more congested with increased urbanization (15, 41). Thus, it is important for policy makers and public health professionals alike to recognize the interconnected relationships between the environment, the population, and safely-managed sanitation for the sake of their populations and the quality of the environment.

Much of the literature has focused on the associations between sanitation and diarrhea, with little examination of fecal contamination as either an intermediate outcome or as an exposure (1-6). Thus, there is a need to understand how sanitation conditions can lead to improvements in environmental conditions through reduced levels of fecal contamination in the public domain. The goal of this analysis is to study the relationship between varied levels of household sanitation on environmental contamination. This analysis attempts to measure this directly instead of examining the more distal, and potentially confounded, outcome of diarrhea. Improved understanding of this pathway will allow greater success towards achieving the goals that apply to SDG 6 by 2030 and can lead to dramatic improvements in urban environmental conditions.

II. MANUSCRIPT

A. Abstract

Introduction: Poor sanitation coverage is a common issue in most low and middle income countries around the world today and is often associated with higher levels of fecal contamination in the environment. This study aims to analyze whether associations exist between sanitation coverage at the household level and fecal contamination in open drain systems in Accra, Ghana.

Methods: Household surveys (n = 750) and drain water samples (n = 163) were collected in 5 neighborhoods of Accra. Kulldorff's Bernoulli spatial scans were used to identify most-likely clusters of surveyed household sanitation characteristics (level of toilet containment, shared vs. unshared toilet, and frequency of public toilet use). Toilet containment refers to the safe disposal and physical separation of excreta from human contact. *E. coli* concentrations were quantified in drain water samples and regressed against sanitation characteristics, controlling for neighborhood, drain infrastructure, and rainfall.

Results: Compared to all other study neighborhoods, Ringway — the neighborhood with the highest coverage of private sanitation use (vs. public sanitation use) — had significantly lower *E. coli* concentrations in drain samples. Drain sample sites within 50 meters of clusters of high coverage of contained sanitation had, on average, 1.08 log_{10} CFU/100mL lower *E. coli* concentrations than drains located outside of these clustered areas (p=0.003). Drains within 100 meters of clusters of low coverage of households that did not use public toilets had, on average, 1.19 log_{10} CFU/100mL higher *E. coli* concentrations than all other drains located outside of these clustered areas (p=0.007).

Discussion: The results from this study provide some of the first evidence suggesting that clustering of contained sanitation and private toilet use, as opposed to public toilet use, may be associated with lower levels of fecal contamination in open drain systems, and conceivably the remaining proximal environment.

B. Introduction

Diarrheal disease is one of the largest contributors to morbidity and mortality, accounting for 4% of all deaths around the world (7, 8). Lack of access to appropriate water, sanitation, and hygiene (WASH) infrastructure is believed to be the main cause of the 4 billion cases of diarrhea per year, and 1.8 million deaths (9-11). The burden of disease is most felt in low- and middle-income countries (LMICs), where diarrhea is responsible for 7.2% of all disability-adjusted life years (DALYs), or years of healthy life lost due to disease, and the average child experiences 2-3 episodes of acute diarrhea per year (15, 16).

Poor sanitation coverage, or lack of access to improved sanitation facilities and fecal management infrastructure, is a common issue in most LMICs around the world today and is often associated with poorer health outcomes (9). Nearly a third of the world's population resides in homes that lack basic sanitation, leaving them particularly vulnerable to disease through contact with human feces (14, 22, 23, 28, 29). Of these 2.6 billion people living with poor sanitation, over 90% live in LMICs (15, 30). The WHO

20

estimates that improving global sanitation alone could lead to 1.8 billion fewer cases of diarrheal disease annually, an impact that would be felt most in LMICs (15).

Most communities in LMICs lack the appropriate infrastructure to support safelymanaged sanitation, often leading to spillover of residual waste and the manual emptying of fecal waste into open drain systems (62, 69). Infrequent services along with small or too few septic tanks are not able to keep up with the volume of fecal sludge that requires fecal sludge management (FSM) services. FSM refers to the management of untreated excreta from onsite systems, including the emptying, safe transport, and eventual treatment of fecal sludge (23, 62, 64). With nowhere else to store excess sludge, many resort to dumping excreta into open drain systems. Many illegal and legal FSM providers, alike, also directly empty fecal waste into open drains, where an accumulation of fecal waste goes largely untreated (69, 70).

Increased urbanization along with growing urban populations have contributed to the challenges of maintaining an adequate global WASH landscape by adding stress to already poor sanitation infrastructure (42, 43). As is, access to improved sanitation, or a toilet that is capable of hygienically separating excreta from human contact, is restricted to 31% of the African population, leaving 535 million people exposed to unsafe sanitation practices, and subsequent environmental fecal contamination (9, 37). Since 1990, 146 million Africans have gained access to improved sanitation. At this same time, however, the urban population in Africa also grew by 213 million, leaving an additional 159 million people without access to basic sanitation (9, 41, 44).

Residents of Accra have already begun to see the effects that urbanization on the population's health, as the sanitation infrastructure in place has not been able to support

the city's growing sanitation needs (60). In 2013, diarrheal disease alone was responsible for 12% of all deaths in children under 5 in Ghana. Many of these deaths were linked to low sanitation coverage in large cities like Accra (61). An observational study conducted in Accra that aimed to describe the associations between diarrheal disease and household sanitation coverage found that 24.5% of diarrheal cases in their study population resided in homes where the practice of open defecation was common. Among other sanitation characteristics considered, toilets shared with 5 or more households were associated with increased incidence of diarrheal disease (57).

Previous studies have found that ownership of contained toilets is associated with significantly lower concentrations of *E. coli* in the proximal environment (15, 35, 86, 90). A 63% reduction in *E. coli* concentrations was observed in a performance evaluation of newly installed contained toilets in Ghana (90). Homes with contained toilets were also found to have significantly lower levels of *E. coli* concentrations (-1.18 $log_{10}CFU/900cm^2$) compared to homes with uncontained toilets (35). Still, the magnitude of exposure is dependent on the type of toilet used, as lower levels of toilet containment functionality was associated with higher concentrations of *E. coli* (86).

Shared sanitation has also been widely used as an environmental exposure variable in observational studies, which have described clear trends between increased sharing practices and increased incidence of diarrhea (91-93). This mechanism is likely driven by the fact that shared toilets tend to be dirtier than private ones and may also be prone to over-filling (28). This mechanism, furthermore, presents a high risk of direct contact with enteric pathogens for all those who use the shared facility (18). Regions where shared sanitation coverage is high have often been associated with higher

prevalence of diarrhea-related diseases compared to low shared sanitation coverage areas (91). This is likely due to the fact that a larger proportion of the population is exposed to fecal waste through direct contact with dirty toilets or indirect means of transmission through other elements of the F-diagram (i.e., fields, fluids, and/or foods that interact with the contaminated hands of an exposed person) (22). The F-diagram is a tool used to visualize environmental transmission of enteric pathogens (14, 22).

Similar to toilet ownership and shared sanitation status, contact with open drains has been described as an important source of fecal contamination in the literature (29, 56). A study evaluating microbial risks by sampling different environmental structures in Accra found that open drains had the highest concentrations of fecal indicator bacteria and other harmful pathogens (56). In Accra, different drain channels lead to larger drains which often lead to recreational water sources such as rivers, streams, and the ocean (56, 96). As fecal waste is discharged into these drains, either directly through open defecation and illegal emptying of waste by waste treatment agencies, enteric pathogens interact with much of the proximal environment (56, 62, 69, 97). Contamination of fluids and fields, thus, increases the risk of human fecal contamination for those who interact with the contaminated environment (22, 73).

Much of the literature has focused on the associations between sanitation and diarrhea, with little examination of fecal contamination as either an intermediate outcome or as an exposure (1-6). Thus, there is a need to understand how sanitation conditions can lead to improvements in environmental conditions through reduced levels of fecal contamination in the public domain. The goal of this analysis is to study the relationship between varied levels of household sanitation on environmental contamination. This

analysis attempts to measure this directly instead of examining the more distal, and potentially confounded, outcome of diarrhea. Improved understanding of this pathway will allow greater success towards achieving the goals that apply to SDG 6 by 2030 and can lead to dramatic improvements in urban environmental conditions.

C. Methods

i. Study Area

This cross-sectional study was conducted in five neighborhoods (Adabraka, Chorkor, Kokomlemle, Ringway, and Shiabu) of Accra, Ghana, in collaboration with the Water Research Institute of the Center for Scientific and Industrial Research Institute, Ghana (WRI). Neighborhoods were purposively selected for variation in levels of sanitation coverage, population density, and socioeconomic status per the 2010 Ghana census (96). The data used in this analysis were collected from March-July 2016 as part of a SaniPath rapid assessment,[†] which seeks to characterize the environmental pathways of exposure to fecal contamination in urban neighborhoods using both behavioral and microbiological data (98). The dataset consisted of geo-coded household surveys and environmental samples from soil and open drains in each neighborhood. All study protocols and documents were approved by Emory University's Institutional Review Board (IRB).

Accra receives approximately 760 mm of rainfall each year, with March-July as the wettest months (Table 1) (99). Accra has two rainy seasons – the first in March-July

[†] SaniPath's (<u>www.sanipath.org</u>) rapid assessment tool collects information on households' sanitation practices, hygiene behaviors, and their level of interaction with the environment to characterize the potential environmental pathways of exposure to fecal contamination.

and the second August-October, and has a year-round mean temperature of $20^{\circ} - 30^{\circ}$ C (45). Ghana's most recent census report (2010) indicated that the average household size within the Greater Accra Metropolitan Area (GAMA) was approximately 3.7 persons per household (51).

ii. Household Surveys

Within a neighborhood, 200 households were surveyed using purposive sampling methods. A purposive approach was used to ensure heterogeneity within sub-populations and even representation of the sanitation characteristics of the residents. Households were defined as any living space shared by a group of two or more people. Each neighborhood was divided into 10x10m sub-section grids using ArcGIS version 10.2 mapping software (ESRI, Redlands, CA, USA) and all households within a subsection were enumerated. Subsequently, households were systematically sampled within a sub-section, with total subsection sample size proportional to the estimated number of households during enumeration. A random starting location was chosen and an interval of eight households was used for the systematic sampling.

Enumerators worked in pairs to administer the survey. In cases where the randomly selected household did not wish to participate in the survey, the nearest household was selected until the survey was successfully conducted. Enumerators collected GPS coordinates at each household using a Garmin eTrex Venture HC device (Garmin Ltd., Olathe, KS, USA).

Surveys were administered to female household heads or female adult members of the residence regarding their sanitation practices for the purposes of characterizing the potential environmental pathways of exposure to fecal contamination. If a household toilet was reported, enumerators observed the toilet and classified it as a pour flush/flush toilet, Kumasi ventilated improved pit (KVIP) latrine, ventilated improved pit (VIP) latrine, traditional pit latrine with a slab, bucket/pan, or other (see Glossary for definitions). Households containing at least one toilet were also asked whether they shared their toilet with any other households, and if so, how many.

iii. Drain Sample Collection

Drain sample sites within each study neighborhood were selected spatially at random *a priori* using a spatial grid of each neighborhood. Each study neighborhood was divided into 10x10m square grids in ArcGIS version 10.2, of which 30 squares were randomly selected. Sample collection teams collected 500 mL of drain water at a random site within the grid and recorded observations of drain conditions at the time of collection (e.g. rainfall at the time of collection, size of the drain, and other conditions). If no drains were present within a selected grid, the team sampled from the drain located nearest to the grid. GPS coordinates were collected at the drain sample site using a Garmin eTrex Venture HC device.

Drain samples were collected using a sterile bailer or Sludge Nabber (Nasco, Fort Atkinson, WI, USA) to scoop drain water into sterile 500 mL Whirl-Pack bags (Nasco, Fort Atkinson, WI, USA). Whirl-Pack bags were sealed and transported in coolers with ice packs to the WRI laboratory within 8 hours of collection. Samples were stored at 4°C until analysis. *Escherichia coli* (*E. coli*) was chosen as an indicator of fecal contamination for the purposes of this analysis because of its natural abundance, its
specificity to fecal sources of contamination, it is a large contributor to overall contamination, and it is universally used as an indicator for fecal contamination (74-76). Samples were analyzed by membrane filtration for *E. coli* and total coliforms according to United States Environmental Protection Agency (USEPA) Method 1604 (100). Three dilutions of $1:10^{-5}$, $1:10^{-6}$, and $1:10^{-7}$ were performed on each sample.

The lower limit of detection (LLOD), or the lowest observable quantity of *E. coli* colonies found in an agar plate, was 10^{-5} CFU/100mL. If a sample did not have any detectable colonies following standard dilutions (above), sequential ten-fold dilutions were performed (up to $1:10^{-3}$). If at least one colony was detected in these dilutions, that result was used in place of the standard dilution results. If no colonies were detected up to a $1:10^{-3}$ dilution, a random number between zero and 10^{3} CFU was generated using SAS version 9.4 (Cary, NC) as a replacement for the LLOD. All final coliform concentrations were \log_{10} transformed to and calculated per 100mL.

iv. Sanitation Coverage Quantification

Within each neighborhood, sanitation coverage was quantified using SaTScan version 9.4.4 (101) to identify most-likely clusters of sanitation characteristics, including a) presence/absence of a toilet, b) quality of containment of the toilet, c) shared sanitation coverage, and d) public toilet use (Glossary). Contained sanitation was defined as a toilet that allowed for the safe disposal and physical separation of excreta from human contact (Glossary). Shared sanitation was defined as any toilet that was shared or used by two or more households (Glossary). Private toilet use was defined by households who reported never using public toilets vs. households who reported any weekly use of public toilets

(Glossary). Kulldorff's Bernoulli spatial scan was used to identify clusters of binary measures of household sanitation, while the ordinal spatial scan was used to identify clusters of the ordered categorical variable describing toilet type (contained toilet vs. uncontained toilet vs. no toilet). The Bernoulli model detected the degree of non-random clustering of binary 0/1 values of group-level data in space using a spatial probability model. This model identified areas of high and low coverage of selected sanitation characteristics, or areas where an excess of households with a certain sanitation characteristic was present and areas where this characteristic was sparse. Conversely, the ordinal model detected non-random clustering of ordered categorical data in space using a spatial probability model. This model detected statistically significant high clusters, or spatial areas where an excess of high-valued categories were present. Spatial scans were conducted within each neighborhood given the spatial discontinuity of neighborhoods and elliptical cluster shapes were identified.

Private sanitation was defined as a binary variable to address the issue of uncertainty in the frequency of public toilet use. This variable was created by defining whether people in the surveyed household ever use public toilets or if they exclusively use the private toilets belonging to a home. For interpretation purposes, Bernoulli spatial scan "cases" (or "1"s) were defined as people who reported never using a public toilet, while "controls" (or "0"s) were defined as people who reported using a public toilet at least once per week. Thus, when trying to identify clusters of private sanitation use coverage (as opposed to public sanitation coverage) using the log likelihood ratio generated by SaTScan output, test statistic values greater than one indicated clusters of high coverage of private sanitation. In other words, clusters with a log likelihood ratio >1 described clustered areas of high density of households who reported never using public toilets in any given week.

Statistically significant ($\alpha = 0.10$) clusters of households with high or low sanitation coverage were transferred to ArcGIS 10.4 mapping software (Figures 1-3). Buffers of 50 and 100 meters around GPS data points of drain sample sites were created as catchment areas to match household clusters to drain sample sites for regression modeling. In the event that drain sample site buffers overlapped, household clusters were assigned to closest the drain by Euclidean (straight-line) distance.

v. Regression Modeling

A literature search was conducted to identify potential confounders of the relationship between sanitation and fecal contamination, and a directed acyclic graph (DAG) was subsequently constructed to identify causal and non-causal associations (Figure 4). This method allowed for consideration of multiple confounders at once. While some potential confounders identified in the DAG were not measured during the data collection phase of the study, proxy variables were identified and included in regression models. According to the DAG, we suspected that contaminated runoff water may be a confounder of the association between sanitation coverage and fecal contamination. Since it is difficult to measure contaminated runoff water that flows into open drain systems, data collected on rainfall and the presence of rain the day before collection served as suitable representations of the confounder. This method illustrated the need to control for neighborhood, drain infrastructure, and rainfall (45, 102-104). Two variables were used to estimate rainfall: whether or not it rained the day before drain site sample collection

(observed by enumerators onsite) and millimeters of rainfall in Accra on the day of sample collection (collected through an online database for global weather and climate reports) (105). Final models were adjusted for neighborhood, drain size, drain lining composition (cement, stone, dirt, or mixed), presence of rain the day before sampling (binary), and millimeters of rain recorded in the GAMA on the day of drain site sample collection (continuous).

vi. Assumptions

The assumptions of linearity, independence, homoscedasticity, and normality were verified by analyzing the partial and residual plots of the selected final model. Residual plots were generated and correlation coefficients assessed to confirm the presence of a linear relationship between *E. coli* concentration and the independent variables in question. Residual plots also served as a tool to assess homogeneity of variance. Since samples and households were sampled randomly, it is appropriate to assume that there was no violation of the assumption of independence.

vii. Analysis

An exploratory data analysis was initially performed to investigate the validity of the assumptions and to describe environmental and sanitation characteristic distributions. Tests of association were performed to assess the relationships between the risk factors of interest. A two-sample t-test was chosen when comparing one continuous predictor to a binary predictor. In a two sample t-test, pooled results were reported when the equality of variances had a p-value <0.05, indicating that the variances between the two categorical

groups were similar. Conversely, unpooled results were reported when the p-value of the equality of variances was >0.05. Assessments of proportional differences between two categorical variables were estimated using the Chi square test of association.

Multiple linear regression models were estimated using SAS version 9.4 (Cary, NC). Test statistic p-values <0.05 were considered statistically significant. Using the prespecified potential confounders identified in Figure 4, multiple linear regression models, adjusting for neighborhood, were used to test whether a particular variable was significant. The three sanitation exposure variables along with the selected covariates were regressed, individually, against *E. coli* concentration to determine which factor had the highest correlation with the outcome and explained the largest amount of variability in the model.

Three sets of linear regression models were performed on five predictor variables to determine differences in *E. coli* concentrations across different sanitation characteristics. One set of models included shared sanitation status as the exposure variable of interest, another considered inclusion in contained versus uncontained clusters, and the last considered reported usage of public toilets per week. All three sets of models controlled for neighborhood, drain infrastructure, and rainfall conditions (i.e, millimeters of rainfall on the day of sample collection and whether or not it rained the day before sample collection). Effect modification of sanitation characteristics was tested in each model.

Assumptions of linearity, independence, homoscedasticity, and normality were verified by analyzing the partial and residual plots of the selected final model. Residual plots were generated and correlation coefficients assessed to confirm the presence of a linear relationship between level of fecal contamination in open drains and the predictors in question. Residual plots also served as a tool to assess homogeneity of variance.

D. RESULTS

i. Household Demographics & Environmental Characteristics

A total of 843 households within the 5 study neighborhoods – Adabraka, Chorkor, Kokomlemle, Ringway, and Shiabu – were surveyed without replacement. From this, 65 households were missing GPS coordinates and 28 households had GPS coordinates that were outside of the city limits of Accra. Therefore, a total of 750 households were used in this analysis. Demographics and sanitation characteristics of surveyed households were compared across five neighborhoods in Accra, Ghana to assess heterogeneity and spatial clustering of sanitation.

Most (70.3%) of the surveyed households had a toilet (Table 2). Household toilet coverage, as defined by the presence of any type of toilet, varied significantly between the different neighborhoods (X^2 =204.7, p<.0001). By observation, 69.7% of surveyed households had contained toilets, mostly in Ringway (96.5%), Kokomlemle (92.0%), and Adabraka (85.8%). In Shiabu and Chorkor, only about 44% of surveyed households had contained toilets. Of those who had a toilet in their home, nearly half (47.2%) also reported sharing their toilet with other households. Exactly 40% of all surveyed households reported using a public toilet at least once per week. Most contained toilets (88.4%) were flush toilets. Over half of all households in Chorkor (55.6%) and Shiabu (50.3%) reported that they did not have a toilet in the household and would instead defecate in a bush or field whenever necessary. All household sanitation characteristics,

including toilet type and toilet sharing practices, varied significantly across neighborhoods (p < 0.05).

A total of 175 drain sites were sampled. Five of these samples had duplicate sample ID numbers, therefore one of each duplicate was excluded in the analysis. From this, 7 drain samples were not processed or counted in a laboratory, and thus were also excluded from the analysis. The remaining 163 drains – 35 in Adabraka, 48 in Chorkor, 37 in Kokomlemle, 27 in Ringway, and 16 in Shiabu – were included in the analysis (Table 2).

Drain characteristics and their surrounding conditions, such as whether or not it rained the day before sample collection, were significantly different across neighborhoods (X^2 =71.1, p<.0001). The average *E. coli* concentration across all sampled drains sites was 4.6 ± 1.3 log₁₀CFU/100mL. Drains in Chorkor had the highest average concentration of *E. coli*, with a mean concentration that was 1.0 log₁₀CFU/100mL higher than the overall average concentration. Average *E. coli* concentrations in Adabraka (4.3 ± 1.0 log₁₀CFU/100mL) and Kokomlemle (4.2 ± 1.4 log₁₀CFU/100mL) were similar to the average concentration across all neighborhoods. Mean *E. coli* concentrations in sampled drain sites was lowest in Ringway (3.4 ± 0.7 log₁₀CFU/100mL). Most of the sampled drains (64.4%) were less than 0.5 meters wide at the sampled location. Additionally, most of the sampled drains (89.0%) were lined with cement. As with drain size, drain lining composition also varied significantly across neighborhoods (X^2 =30.9, p<.0001). Rain during (1.2%) or the day before (16.0%) drain sample collection was rare. The month of May 2016 experienced the most rainfall, on average, across all of Accra (218.9 mm) during the time of the study, while March 2016 experienced the most days of precipitation (27 days) (Table 1).

ii. Spatial Sanitation Cluster Coverage

Four sanitation characteristics -a) presence/absence of a toilet in the household, b) presence of a contained versus an uncontained toilet, c) whether an individual's toilet was shared with other households or not, and d) reported public toilet use (vs. exclusive private toilet use) – were assessed for spatial clustering. Separation of clusters of toilet presence from clusters of contained toilets was not possible within the study population. Thus, clusters of "contained sanitation" were comparing households with contained sanitation to households without sanitation facilities, and only the Bernoulli model was used to identify in subsequent analyses. Across measured sanitation characteristics, significant heterogeneity in coverage—identification of both clusters of high and clusters of low coverage in a single neighborhood—was most often observed in Ringway and Shiabu. Only single clusters of either low or high sanitation coverage were observed in other neighborhoods for each of the sanitation characteristics (Figures 1-3). Multiple clusters of high (71.4% and 92.9%) and low (0%, 3.4%, and 13.2%) coverage of contained sanitation were identified in Shiabu (Figures 1 & 3), while clusters of high (96.4%) and low (0%) coverage of private sanitation use (vs. public sanitation use) were identified in Ringway. Among all neighborhoods, areas of high versus low sanitation were consistently on opposite sides of the neighborhoods (e.g. high coverage of shared sanitation in northern Adabraka versus low coverage of shared sanitation in southern Adabraka).

Average E. coli concentrations were compared for each type of cluster across each of the 5 study neighborhoods (Table 3). A total of 27 drain sample collection sites (16.6%) were within 50 meters of clusters of high coverage of any type of measured sanitation. Of drain sample sites within 50 meters of clusters of high coverage of unshared sanitation, the average E. coli concentration was lowest in Ringway (4.0 ± 0.8) \log_{10} CFU/100mL). Only two drains, one in Ringway and one in Shiabu, were within 50 meters of clusters of high coverage of private sanitation (indicating a high density of households who reported never using a public toilet, thus exclusively using their own private toilet). None of the sampled drain sites were within 50 nor 100 meters of any clusters of high sanitation coverage in Kokomlemle. Of all drain sample sites, 37 (22.7%) were within 100 meters of clusters of high coverage of any type of measure sanitation. Average E. coli concentration was highest in drain sample sites within 100 meters of clusters high coverage of contained sanitation $(5.5 \pm 1.0 \log_{10} CFU/100 mL)$ in Chorkor. The average *E. coli* concentration of drains within 100 meters of clusters of high coverage of contained sanitation was 25% higher in Chorkor (5.5 ± 1.0 \log_{10} CFU/100mL) than in Shiabu (4.4 ± 0.6 \log_{10} CFU/100mL). Only one drain in Ringway was identified within 100 meters of clusters of high coverage of private sanitation use (vs. public sanitation use).

iii. Variation in E. coli *Concentration in Drain Water by Neighborhood, Drain Infrastructure, & Rainfall*

Multiple linear regression was used to examine the effect of drain infrastructure and rainfall conditions (including millimeters of measured rainfall on the day of sample collection and whether or not it rained the day prior to sample collection) on fecal contamination, adjusting for neighborhood (Table 4). Drain sample locations that had received rain in the previous 24 hours, on average, had significantly lower *E. coli* concentrations (-0.61 \pm 0.30 log₁₀CFU/100mL) than other drain sites (p=0.043). The presence of rainfall in the previous day explained 38% of the variation in *E. coli* concentration (R²=0.38). No significant associations between drain size and *E. coli* were observed: drain *E. coli* concentrations varied by 0.08-0.13 log₁₀CFU/100mL by size. *E. coli* concentration was not significantly associated with drain lining composition or millimeters of rainfall on the day of sampling. The model assessing impact of drain size on *E. coli* concentration explained 37% of the variation in the outcome variable, *E. coli* concentration, while models that considered rainfall conditions explained 37 – 38% of the variation in concentration.

Linear regression was also used to examine the differences in *E. coli* concentrations of sample drain sites between different study neighborhoods (Table 5). The largest difference noted was between Ringway and Chorkor: drain sample sites in Ringway were, on average, 2.20 log₁₀CFU/100mL lower than drain sites in Chorkor (p<.0001). Compared to all other study neighborhoods, Ringway drain sample sites had significantly lower *E. coli* concentrations (p<0.05). Average *E. coli* concentrations in drain samples from Shiabu and Chorkor did not vary significantly (p=0.224). Two other adjacent neighborhoods—Adabraka and Kokomlemle—also did not have a significant difference in mean *E. coli* concentrations (p=0.622).

Variation in E. coli Contamination in Drain Water

Relationships between sanitation characteristics (presence of a contained versus an uncontained toilet, whether an individual's toilet was shared with other households or not, and private - vs. public - sanitation use) and fecal contamination (measured by E. *coli* concentration) were assessed using multiple linear regression models, adjusting for neighborhood, drain infrastructure, and rainfall conditions (Table 6). Drains within 50 meters of clusters of high coverage of contained sanitation had 1.08 log₁₀CFU/100mL lower E. coli concentrations than drains located outside of these clustered areas (p=0.003). Conversely, drains within 50 meters of clusters of low coverage of contained sanitation had an average of 0.43 log₁₀CFU/100mL higher *E. coli* concentrations than drains outside of these clustered areas, though this difference was not significant (p=0.148). This model explained 46.9% of the total variability in E. coli concentration $(R^2=0.469)$. A similar, though not significant, trend was observed in drains within 100 meters of clusters of high coverage of contained sanitation. Drains within 100 meters of clusters of high coverage had, on average, 0.56 log₁₀CFU/100mL lower *E. coli* concentration than all other drains (p=0.078). Drains within 100 meters of clusters of low coverage of contained sanitation had, on average, 0.16 log₁₀CFU/100mL higher E. coli concentration, compared to drains outside of these clustered areas (p=0.549). Drains within 100 meters of clusters of low coverage of private sanitation use (vs. public sanitation use) had 1.19 log₁₀CFU/100mL higher E. coli concentrations than all other drains located outside of these clustered areas (p=0.007). Use of shared sanitation was not significantly associated with E. coli concentrations in drains, adjusting for neighborhood, drain infrastructure, and rainfall conditions. E. coli concentrations of drains near clusters of high coverage of private sanitation use (vs. public sanitation use) showed similar

results. No significant interactions were observed between sanitation characteristics (data not shown).

E. DISCUSSION

The purpose of this study was to examine whether associations exist between sanitation coverage at the household level and fecal contamination in open drain systems in urban environments. Sanitation coverage was measured by statistically significant nonrandom spatial clusters within five neighborhoods of Accra that varied in SES, while fecal contamination was quantified using E. coli concentrations in sampled drain water. When comparing clusters of 1) contained vs. uncontained sanitation coverage, 2) shared vs. unshared sanitation coverage; and 3) private toilet vs. public toilet use, drains within 50 meters of clusters of high coverage of contained sanitation had $1.08 \log_{10}$ CFU/100mL lower E. coli concentrations then drains in the rest of the study area. This finding suggests that higher coverage of toilets capable of efficiently separating human excreta may yield improved environmental quality, with the potential for community-level benefits. These benefits include a lower level of fecal contamination in the proximal environment. At the neighborhood level, average E. coli concentrations in sampled drain sites were significantly inversely associated with neighborhood socioeconomic status and sanitation coverage. This association may be because neighborhoods of higher SES are able to afford better sanitation conditions and have better access to sanitation services than households of lower SES, thus leading to lower levels of contamination in the environment. Finally, drains within 100 meters of clusters of low coverage of private sanitation had 1.19 log₁₀CFU/100mL higher E. coli concentrations than drains in the rest

of the study area, indicating that available public toilets may be insufficient in containing large volumes of excreta in areas where private household sanitation coverage is low. There were no significant associations between shared sanitation coverage and fecal contamination in drains.

This study is one of the first to analyze the associations between spatial heterogeneity in sanitation coverage and fecal contamination in drains. Many other observational studies that have considered sanitation coverage as an exposure variable aimed to determine its effect on the risk of disease transmission, namely diarrheal disease (1, 2, 4). The results of a meta-analysis considering the impact of toilet containment on diarrheal disease risk found that people who use contained toilets had a 27% reduced risk compared to those who reported using uncontained toilets (4). A similar study found that uncontained toilet use was associated with a 33% increase in diarrheal disease (1). Since contained toilets are generally less contaminated with E. coli (106), E. coli is a strong indicator of fecal contamination in the environment (65). Temporal analyses support strong correlations between high concentrations of E. coli in the environment and increasing incidence of diarrheal disease (107). Thus, it is likely that fecal contamination as indicated by the presence of *E. coli* is mediating these researched associations. This connection shows that studying the effect of structural sanitation on diarrheal disease transmission alone disregards the role that contamination of the environment plays as an intermediate outcome on this mechanism. The results from our study suggest that the level of contamination in the environment may contribute to this under-researched mechanism and presents new evidence that illustrates the relationship between spatial heterogeneity of clusters of fecal contamination.

The association between lower concentrations of *E. coli* and drains near clusters of high coverage of contained sanitation is likely explained by the functional separation of human waste through contained toilets, leading to community-wide environmental benefits (86, 108, 109). Toilets classified as "contained" were those designed to ensure the physical and hygienic separation of human waste to avoid human and environmental contamination, including flush toilets, pour flush toilets, Ventilated Improved Pit (KVIP) latrines, Ventilated Improved Pit (VIP) latrines, traditional pit latrines with a slab, and other facilities connected to either pipes or a septic tank (110). Previous studies have confirmed the functionality of this design, as ownership of various types of contained toilets has been linked to significantly lower concentrations of E. coli in the surrounding environment (5, 9, 90, 106). Another study observed broad reductions in levels of fecal contamination of the environment in communities that had high levels of latrine ownership (65%). Conversely, communities that had low latrine ownership (0%) were associated with significantly higher *E. coli* concentrations and twice the number of cases of diarrhea compared to communities with high coverage of contained sanitation (5). This association was likely due to common open defecation practices in the proximal environment (5, 23, 111). Households with uncontained toilets have also reported defecating in or near open drain systems, many of which connect to open water sources such as the ocean, rivers, ponds, etc. (23, 112). Drains near clusters of low coverage of contained sanitation in our study, however, were not significantly associated with higher concentrations of *E. coli*. While the literature has focused on the differences between the two sanitation extremes (contained vs. uncontained toilets) (5, 26, 86, 89, 109), these associations are expected to vary with differing levels of the functional quality in

contained toilets. Although we would have expected to see significantly higher *E. coli* concentrations in clusters of low coverage of contained sanitation, this association was likely moderated by households' ability to access contained toilets outside of their homes. For instance, if a household that does not have a contained toilet uses the contained toilet of a nearby household, this area would conceivably have lower levels *E. coli* concentration in the environment. This diminished effect would thus underestimate the effect of clusters of low coverage of contained sanitation on fecal contamination.

Households in higher SES neighborhoods may have lower levels of fecal contamination than their lower SES counterparts, as they likely have access to better fecal containment and treatment services leading to improved environmental outcomes. Results showed significant differences in average *E. coli* concentrations in sampled drain sites, with a clear division between Ringway and the other study neighborhoods. Furthermore, drains in Adabraka and Kokomlemle along with drains in Chorkor and Shiabu had comparable average *E. coli* concentrations. Ringway drains were between 0.79 - 2.20log₁₀ CFU/100mL lower than the drains from the other study neighborhoods. In their most recent Human Development Report (2006), the United Nations Development Programme (UNDP) described that wealth, or higher SES, is directly related to lower levels of fecal contamination in the public environment (30). We also found that drains in Adabraka and Kokomlemle along with drains in Chorkor and Shiabu had comparable average E. coli concentrations, thus we were able to detect large-scale differences in fecal contamination between neighborhoods, but not finer scale differences. These results may have been confounded by spatial proximity in addition to the similar neighborhood SES. An observational study in Kenya that described the spatial distribution of sanitation

coverage reported similar results, as nearby neighborhoods of similar SES also had similar sanitation coverage rates (113). Much of the clustering of areas of high and low sanitation coverage can, in part, be explained by the sanitation infrastructure in place. Access to improved sanitation and adequate FSM services are often reserved for those who can afford them (28). As a result, poorer households are often grouped together in remote areas where FSM access is limited, piped sewer or sanitation systems are absent, and sanitation infrastructure is lacking (30, 113-115). The disparity of sanitation coverage among different SES groups has been well described in the literature, which also reported patterns of lower E. coli concentrations in the environment in wealthier neighborhoods (113). Thus, we would expect that areas with high sanitation coverage are likely to have better access to FSM services than areas of low coverage of contained sanitation. These improved sanitation services often lead to better health outcomes and thus, improved environmental conditions. Conversely, clusters of low sanitation coverage are often positioned in areas of low SES where plumbing or other type of fecal sludge management infrastructure are scarce (9, 116). These associations support the finding that Ringway (the neighborhood with the highest SES of all study neighborhoods) had the lowest concentration of E. coli across all study neighborhoods. Adabraka and Kokomlemle, and similarly Chorkor and Shiabu, were found to be of similar SES, thus explaining why levels of fecal contamination in these drains were similar (51).

While public latrines have long been considered a successful alternative to large gaps in sanitation coverage, the results from this study suggest that use of public toilets, especially in population-dense regions, may still be associated with environmental contamination (117). Our results showed that spatial areas of low coverage of private sanitation use were associated with much higher (1.19 log₁₀CFU/100mL) E. coli concentrations in nearby open drains. A systematic review of health outcomes associated with different types of shared sanitation confirmed this higher presence of fecal contaminants in public toilets and found that they are traditionally less hygienic than private toilets. Public toilets were also much more likely to have feces on or near the facility than private toilets (91). These associations can largely be explained by overuse of public toilets and low levels of functional containment, indicating that public toilets were not as efficient in separating excreta from human contact than other types of private improved sanitation (28, 49, 91). Lack of access to public toilets in Accra means that public latrines often serve many households, leading to long lines, especially during busy hours of the day. For many of those (29.3%) who cannot wait in long queues, neighborhood open defecation has become a common alternative (94). As mentioned in previously described mechanisms of drain contamination, improper containment of feces can lead to poor environmental outcomes through direct contact with fecal waste or by way of runoff wastewater that empties fecal sludge into nearby drains (23, 56, 57, 116). The wastewater flowing in open drain systems in Accra are rarely treated, thus leaving people who reside nearby vulnerable to the effects of exposure to harmful fecal contaminants (29, 45, 56).

While some drains had lower *E. coli* concentrations than others, it should be noted that none of the five neighborhoods had low concentrations of *E. coli* on an absolute scale. Ringway had an average *E. coli* concentration of 3.4 log₁₀CFU/100mL, which is still well above the Environmental Protection Agency's (EPA) criteria for safe freshwater

(Table 1) (118). These criteria are developed based on the highest acceptable risk of exposure to waterborne pathogens from wastewater sources (118-120).

As diarrheal disease continues to be one of the leading causes of death around the world today, a better understanding of the causal pathways of exposure to fecal contaminants may lead to improvements in environmental conditions (10, 20). The findings presented in this study suggest that better spatial coverage of contained sanitation and increased coverage of private toilet use (vs. public toilet use) may reduce environmental fecal contamination, a known risk factor for diarrheal disease (1-4, 18, 107). Furthermore, the evidence provided in this study supports the importance of extending household sanitation coverage and access to basic sanitation services to reduce risks associated with exposure to fecal contaminants in the proximal environment.

F. STRENGTHS/LIMITATIONS

The largest limitation to this study was the relatively small sample size, as this increases the potential for random error in parameter estimates, contributes to low statistical power, and may cause collinearity issues between different sanitation characteristics. Purposive sampling of surveyed households provides another limitation in terms of generalizability of study results. However, random sampling of spatial grids attempts to resolve generalizability issues by allowing for much geographic variation in the data. A meaningful amount of missing household data (11.0%) also contributes to systematic error in the study results. However, a very small amount of drain sample site data was missing (6.9%), meaning that missingness in drain laboratory and collection data probably did not have a large effect on the results data.

Information regarding shared toilet status and frequency of public toilet use was self-reported and thus subject to recall bias. Specifically, household surveys collected data on the frequency of both sanitation characteristics and it is likely that some respondents misreported some of these characteristics. Potential recall bias, thus, may have increased the potential for random error. The methods used in this study's analysis addressed the issue of uncertainty in the frequency of public sanitation use by creating a binary variable, defining whether people in the surveyed household ever use public sanitation or if they exclusively use the private toilets in their homes. Still, analyzing variables at two ends of the extreme (i.e., contained toilets vs. uncontained toilets) may be influencing the strength in the observed effect, as there is no middle group to help identify finer scaled differences. Lastly, household surveys were designed to capture the general behaviors of all members of each household, however only one person was asked to respond on behalf of the household. Therefore, some reported behaviors may not be representative of all members of said household.

A strength in this study involved the verification of some of the information reported in the household survey. While respondents were asked whether or not they had a toilet in their respective household, people administering the questionnaire were asked to verify this and note the type of toilet present, if applicable. Another strength included the collection of behavioral and microbial data in parallel.

Some researchers argue that zero-replacement of quantified bacterial counts is inappropriate when modeling pathogen concentrations, as it may bias result estimates (121, 122). Thus, some may consider this a limitation to the study. However, given the study's moderately small sample size, it seemed essential to include these observations in the construction of the model. These observations contribute valuable data to the overall analysis and help to improve the study's statistical power.

G. REFERENCES

- Lim SS VT, Flaxman AD et al. . A comparative riskassessment of burden of disease and injury attributable to 67risk factors and risk factor clusters in 21 regions, 1990–2010:a systematic analysis for the Global Burden of Disease Study2010. *The Lancet* 2012;380:2224-60.
- Esrey SA PJ, Roberts L, & Shiff C. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. *Bulletin of the World Health Organization* 1991;69:609-21.
- Genser B, Strina A, Teles CA, et al. Risk Factors for Childhood Diarrhea Incidence Dynamic Analysis of a Longitudinal Study. *Epidemiology* 2006;17:658-67.
- Wolf J, Prüss-Ustün, A., Cumming, O., et al. Assessing the impact of drinking water and sanitation on diarrhoeal disease in low- and middle-income settings:systematic review and meta-regression. *Tropical Medicine and International Health* 2014;19(8):928-42.
- 5. Root G. Sanitation, community environments, andchildhood diarrhoea in rural Zimbabwe. *Journal of Health, Population and Nutrition* 2001;19(2):73-82.
- Prüss-Ustün A, Bartram J, Clasen T, et al. Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: A retrospective analysis of data from 145 countries. *Tropical Medicine and International Health* 2014.

- Prüss-Ustün A KD, Fewtrell L, & Bartram J Estimating the burden of disease from water, sanitation and hygiene at a global level. *Environmental Health Perspectives* 2002;110(5):537-42.
- Black RE CS, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R, Eisele T, Liu L, Mathers C. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *The Lancet* 2010;375:1969–87.
- World Health Organization (WHO). Global water supply and sanitation assessment 2000 report. In: (JMP). WaUJMPfWSaS, ed. Geneva, Switzerland, 2001.
- World Health Organization. Water, sanitation and hygiene links to health: facts and figures. 2004:2.
- 11. UNICEF. Water, sanitation and hygiene [electronic article].
- Clasen T, Schmidt, Wolf-Peter, Rabie, Tamer, Roberts, Ian, & Cairncross, Sandy. Interventions to improve water quality for preventing diarrhoea: systematic review and meta-analysis. *British Medical Journal* 2007;334(7597):782.
- World Health Organization (WHO). Diarrhoeal disease factsheet. In: Organization WH, ed, 2013.
- Clasen TF BK, Scmidt WP, Boisson S, Fung ICH, Jenkins MW, Scott B, Sugden S, & Cairncross S. Interventions to improve disposal of human excreta forpreventing diarrhoea (Review). *Cochrane Database of Systematic Reviews* 2010(6).

- World Health Organization (WHO). The World Health Report 2002: Reducing Risks, Promoting Healthy Life. In: Organization WH, ed, 2002.
- 16. World Health Organization (WHO). The global burden of disease: 2004 update.In: Organization WH, ed. Switzerland, 2008.
- Schorling J.B. GRL. Diarrhoea and catch-up growth. *The Lancet* 1990;335:599-600.
- Julian TR. Environmental transmission of diarrheal pathogens in low and middle income countries. *Environmental Science: Processes & Impacts* 2016;18:944-55.
- Ashbolt N. Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology* 2004;198:229-38.
- UNICEF. Water, Sanitation and Hygiene: About WASH. 2016.
 (https://www.unicef.org/wash/3942_3952.html). (Accessed).
- 21. Centers for Disease Control and Prevention (CDC). Hygiene challenges and resources in lower income countries [electronic article].
- Wagner EG, & Lanoix, J. N. Excreta disposal for rural areas and small communities. In: Organization WH, ed. Geneva, 1958.
- World Health Organization (WHO) U. WASH post-2015: proposed indicators for drinking water, sanitation and hygiene. In: Organization WH, ed, 2015:1-8.
- 24. United Nations Millennium Project. Health, dignity, and development: What will it take?, 2005.
- World Health Organization. Protecting surface water for health: identifying, assessing and managing drinking-water quality risks in surface-water catchments.
 In: Rickert B, Chorus, I., & Schmoll, O., ed, 2016:196.

- Fewtrell L, Kaufmann, R. B., Kay, D., Enanoria, W., Haller, L., & Colford, J. M. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *Lancet Infectious Diseases* 2005;5:42-52.
- 27. World Health Organization (WHO). Preventing diarrhoea through better water, sanitation and hygiene: exposures and impacts in low- and middle-income countries. Switzerland: World Health Organization, 2014.
- UNICEF W. Progress on sanitation and drinking water: 2010 update. In: Sanitation WUJMPfWSa, ed, 2010.
- 29. Katukiza AY, Ronteltap M, van der Steen P, et al. Quantification of microbial risks to human health caused by waterborne viruses and bacteria in an urban slum. *Journal of applied microbiology* 2014;116(2):447-63.
- United Nations Development Program (UNDP). Human Development Report:
 2006. 2006.
- WSSCC (Water Supply and Sanitation Collaborative Council). The Campaign: WASH Facts and Figures. WSSCC, 2004.
- 32. World Health Organization (WHO). Water, sanitation and hygiene in health care facilities: status in low and middle income countries and way forward. Geneva, Switzerland: World Health Organization, 2015.
- 33. Boschi-Pinto C LC, Mendoza W, et al. Diarrheal Diseases. *Disease and Mortality in Sub-Saharan Africa*. Washington, DC: The International Bank for Reconstruction and Development / The World Bank, 2006.

- Bryce J, Boschi-Pinto, C., Shibuya, K., Black, R. E. WHO estimates of the causes of death in children. *The Lancet* 2005;365(9465):11471152.
- 35. Kosek M BC, & Guerrant RL. . The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization* 2003;81(3):197-204.
- Victora C. G. BJ, Fontaine O., Monasch R. Reducing Deaths from Diarrhea Through Oral Rehydration Therapy. *Bulletin of the World Health Organization* 2000;78:1246-55.
- 37. UNEP. Improving the Quantity: Quality and Use of Africa's Water. 2006:123-74.
- Pengpid S, Peltzer K. Hygiene behaviour and health attitudes in African countries. *Current Opinion in Psychiatry* 2012;25(2):149-54.
- Mukama T, Ndejjo R, Musoke D, et al. Practices, Concerns, and Willingness to Participate in Solid Waste Management in Two Urban Slums in Central Uganda. *J Environ Public Health* 2016:1-7.
- World Health Organization (WHO) U, & JMP. Progress on sanitation and drinking water – 2015 update and MDG assessment. . Switzerland: World Health Organization, 2015.
- 41. United Nations. World Urbanization Prospects: The 2014 Revision. Department of Economic and Social Affairs, Population Division, 2014.
- 42. Ooi GL PK. Urbanization and Slum Formation. *Journal of Urban Health* 2007;84(Suppl 1):27-34.
- Obuobie E, Keraita B, Danso G, et al. Irrigated Urban Vegetable Production in Ghana Characteristics, Benefits and Risks. 2006.

- 44. United Nations Environment Programme (UNEP). *Improving the quantity, quality and use of Africa's water*. Nairobi, Kenya; 2010.
- 45. Gretsch SR, Ampofo JA, Baker KK, et al. Quantification of exposure to fecal contamination in open drains in four neighborhoods in Accra, Ghana. *J Water Health* 2016;14(2):255-66.
- 46. Hawkins P, Blackett I, Heymans C, et al. Poor-Inclusive Urban Sanitation: An Overview. *Water and Sanitation Program (WSP)* 2013:1-24.
- 47. United Nations Population Fund (UNFPA). State of the world population 2009:
 Facing a changing world: women, population and climate. In: UNFPA, ed. New York, 2009.
- World Health Organization (WHO). Urban health: major opportunities for improving global health outcomes, despite persistent health inequities. In: Organization WH, ed, 2016.
- 49. Adank M DB, Moriarty P, Osei-Tutu H, Assan D, & van Rooijen D. Towards integrated urban water management in the Greater Accra Metropolitan Area Current status and strategic directions for the future. Accra, Ghana: SWITCH/RCN, 2011.
- 50. Twum-Baah KA. Population growth of Mega-Accra: emerging issues. *Visions of the City, Accra in the 21st Century*. Accra, Ghana: Woeli Publishing, 2002.
- 51. Ghana Statistical Service. Population & housing census of 2010: district analytical report [electronic article].
- United Nations Human Settlements Program (UNHSP). The Challenge of Slums [electronic article].

- United Nations Centre for Human Settlement (UNCHS). Habitat: the state of the world's cities. United Nations, 2001.
- 54. Ghana Statistical Service (GSS). 2000 population and housing census: summary report of final results. Accra, Ghana: Ghana Statistical Service, 2002.
- 55. Barten F SV, Rongo L, Varillas W, & Pakasi TA. Contextualising workers' health and safety in urban settings: the need for global perspective and an integrated approach. *Habitat International* 2008;32:223-36.
- 56. Labite H, Lunani I, van der Steen P, et al. Quantitative Microbial Risk Analysis to evaluate health effects of interventions in the urban water system of Accra, Ghana. *J Water Health* 2010;8(3):417-30.
- 57. Owusu Boadi K, Kuitunen M. Environment, wealth, inequality and the burden of disease in the Accra metropolitan area, Ghana. *International Journal of Environmental Health Research* 2005;15(3):193-206.
- Peprah DI BK, Moe C, Robb K, Wellington N, Yakubu H, & Null C. Public toilets and their customers in low-income, urban Accra, Ghana. *Environmental Urbanization* 2015;27:589-604.
- 59. Teunis PFM, Reese HE, Null C, et al. Quantifying contact with the environment: Behaviors of young children in Accra, Ghana. *American Journal of Tropical Medicine and Hygiene* 2016;94(4):920-31.
- 60. Seidu R, Heistad A, Amoah P, et al. Quantification of the health risk associated with wastewater reuse in Accra, Ghana: a contribution toward local guidelines. *Journal of Water and Health* 2008;6(4):461-71.

- Machdar E vdSN, Raschid-Sally L, & Lens PN. Application of Quantitative Microbial Risk Assessment to analyze the public health risk from drinking water quality in a low income area in Accra, Ghana. *Science of the Total Environment* 2013;449:134-42.
- 62. Strande L. Faecal Sludge Management: Systems Approach for Implementation and Operation. In: Strande L RM, & Brdjanovic D, ed: IWA Publishing, 2014.
- 63. Koné D. Making urban excreta and wastewater management contribute to cities ' economic development : a paradigm shift. *Water Policy* 2010;12:602-10.
- Strauss M LS, Heinss U, & Montangero A. Treating Faecal Sludges in Ponds.
 Water Science and Technology 2000;42(10):283–90.
- 65. Feachem RG BD, Garelick H, & Mara DD. Sanitation and Disease. Health aspects of excreta and wastewater management. In: Sons JWa, ed. *World Bank studies in water supply and sanitation*. New York, 1983.
- United Nations (UN). Sustainable Development Goals Report. New York: United Nations, 2016.
- United Nations (UN). Transforming our world: the 2030 Agenda for Sustainable Development. Presented at General Assembly on Sustainable Development Goals2015.
- Berendes DM ST, & Brown JM. Safely managed sanitation for all means fecal sludge management for at least 1.8 billion of the world's poorest people.
 Environmental Science and Technology 2017;Epub ahead of print 2017.

- Thye YP TM, & Ali M. A Critical Review of Technologies for Pit Latrine Emptying in Developing Countries. *Critical Reviews in Environmental Science* and Technology 2011;41:1793–819.
- 70. Boot NLD SR. Faecal sludge management in Accra, Ghana: strengthening links in the chain. *33rd WEDC International Conference*. Accra, Ghana, 2008.
- Safe Water International (SWI). Information about water quality parameters relevant to the CDC SWS program. 2003.
- 72. Environmental Protection Agency (EPA). Recreational Water Quality Criteria.
 Health and Ecological Criteria Division, Office of Science and Technology,
 United States (U.S.) Environmental Protection Agency (EPA), 2015.
- 73. Wade TJ PN, Eisenberg JNS, & Colford JM. Do U.S. Environmental Protection Agency Water Quality Guidelines for Recreational Waters Prevent Gastrointestinal Illness? A Systematic Review and Meta-Analysis. *Environmental Health Perspectives* 2003;111(8):1102-9.
- Tallon P, Magajna, B., Lofranco, C., & Leung, K. T. Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution* 2005;166:139-66.
- World Health Organization (WHO). Guidelines for drinking-water quality.Geneva: World Health Organization, 2011.
- 76. Gruber JS, Ercumen A, Colford JM. Coliform bacteria as indicators of diarrheal risk in household drinking water: Systematic review and meta-analysis. *PLoS ONE* 2014;9(9).

- 77. Centers for Disease Control and Prevention (CDC). E.coli (Escherichia coli).
 Division of Foodborne, Waterborne, and Environmental Diseases (DFWED),
 Centers for Disease Control and Prevention, 2015.
- 78. Kravitz JD NM, Mandel R, & Petersen E. Quantitative bacterial examination of domestic water supplies in the Lesotho Highlands: water quality, sanitation, and village health. *Bulletin of the World Health Organization* 1999;77(10):829-36.
- Stephen AC, JH. The microbial contribution to human faecal mass. *Journal of Medical Microbiology* 1980;13(1):45-56.
- Evans D, & Evans, DG. Escherichia Coli in Diarrheal Disease. In: Baron S, ed. *Medical Microbiology*. Galveston, TX: University of Texas Medical Branch at Galveston, 1991.
- Cabral JPS. Water Microbiology. Bacterial Pathogens and Water. *Int J Environ Res Public Health* 2010;7(10):3657–703.
- Geldreich E. Microbial Quality of Water Supply in Distribution Systems. In: Publishers L, ed. New York, 1996.
- Rufener S MD, Mosler HJ, & Weingartner R. Quality of drinking-water at source and point-of-consumption--drinking cup as a high potential recontamination risk: a field study in Bolivia. *Journal of Health, Population and Nutrition* 2010;28(1):34-41.
- Rochon K LT, & Selinger LB. Persistence of Escherichia coli in immature house fly and stable fly (Diptera: Muscidae) in relation to larval growth and survival.
 Journal of Medical Entomology 2004;41(6):1082-9.

- 85. Pickering AJ JT, Mamuya S, Boehm AB, & Davis J. Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. *Tropical Medicine and International Health* 2011;16:233–9.
- Exley JL LB, Cumming O, & Ensink JH. The sanitation ladder, what constitutes an improved form of sanitation? *Environmental Science and Technology* 2015;49(2):1086-0194.
- Blackett I HP, & Heymans C. The missing link in sanitation service delivery: a review of fecal sludge management in 12 cities. In: Brief W-WBR, ed.
 Washington, DC, 2014.
- Kwiringira J AP, Niwagaba C et al. Descending the sanitation ladder in urban Uganda: evidence from Kampala Slums. *BMC Public Health* 2014;14:624.
- Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. *The Lancet* 2009;374(9694):1032-5.
- 90. Amoah P GNE, Schrecongost A. Performance evaluation of biofil toilet waste digester technologies in Ghana: the efficacy of effluent treatment options. *Environmental Technology* 2016;37(23):3002-13.
- 91. Heijnen M, Routray P, Torondel B, et al. Shared sanitation versus individual household latrines in urban slums: A cross-sectional study in orissa, India. *American Journal of Tropical Medicine and Hygiene* 2015.
- 92. Fuller JA, Clase, T., Heijnen, M., & Eisenberg, J. N. S. Shared sanitation and the prevalence of diarrhea in young children: evidence from 51 countries, 2001-2011. *American Journal of Tropical Medicine and Hygiene* 2014;91(1):173-80.

- Baker KK ORC, Levine MM, Kotloff KL, Nataro JP, Ayers TL, Farag TH, Nasrin D, Blackwelder WC, Wu Y, Alonso PL, Breiman RF, Omore R, Faruque AS, Das SK, Ahmed S, Saha D, Sow SO, Sur D, Zaidi AK, Quadri F, & Mintz ED. Sanitation and Hygiene-Specific Risk Factors for Moderate-to-Severe Diarrhea in Young Children in the Global Enteric Multicenter Study, 2007-2011: Case-Control Study. *PLoS ONE* 2016;13(5):e1002010.
- 94. Owusu Boadi K. Environmental and Health Impacts of Household Solid Waste Handling. *Journal of Environmental Health* 2005;68(4).
- 95. Rheinländer T ea. Redefining shared sanitation. *Bulletin of the World Health Organization* 2015;93:509–10.
- Ghana Statistical Service (GSS). Ghana Poverty Mapping Report. Ghana: Ghana Statistical Service, 2015.
- 97. Boot NLD SR. Faecal sludge management in Accra, Ghana: strengthening links in the chain. *Water Science and Technology* 2009;60:623–31.
- Sanipath Project Website. Assessing Public Health Risks from Unsafe Fecal Sludge Management in Poor Urban Neighborhoods. 2015.
- 99. Adank M, Darteh B, Moriarty P, et al. Towards integrated urban water management in the Greater Accra Metropolitan Area Current status and strategic directions for the future. 2011.
- 100. USEPA. Method 1604: total coliforms and escherichia coli in water by membran filtration using a simultaneous detection medium (MI medium). 2002.
- Kulldorff M. A spatial scan statistic. Communications in Statistics: Theory and Methods 1997;26(6):1481-96.

- Hathi P, Haque S, Pant L, et al. Place and Child Health The Interaction of Population Density and Sanitation in Developing Countries. *Demography* 2014;54:337-60.
- Marinetti C, Nadi EM, Lexy M, et al. Methodology Urban Flood Risk Assessment. 2016.
- 104. Kwiringira J, Atekyereza P, Niwagaba C, et al. Seasonal variations and shared latrine cleaning practices in the slums of Kampala city, Uganda. *BMC Public Health* 2016;16.
- 105. World Weather Online. Accra, Greater Accra Monthly Climate Average, Ghana.2016.
- Exley JLR, Liseka B, Cumming O, et al. The sanitation ladder, what constitutes an improved form of sanitation? *Environmental Science and Technology* 2015;49:1086-94.
- 107. National Bureau of Statistics (NBS) [Tanzania] aIM. Tanzania Demographic and Health Survey 2010. In: Macro NaI, ed. Dar es Salaam, Tanzania, 2011.
- 108. Peal A EB, Blackett I, Hawkins P, & Heymans C. Fecal sludge management (FSM): analytical tools for assessing FSM in cities. *Journal of Water, Sanitation* and Hygiene Development 2014;4(3):371.
- 109. Exum NG OtM, Yori PP, Davis MF, Heaney CD, Kosek M, and Schwab KJ. Floors and Toilets: Association of Floors and Sanitation Practices with Fecal Contamination in Peruvian Amazon Peri-Urban Households. *Environmental Science and Technology* 2016;50:7373–81.
- 110. World Health Organization (WHO). 2015 Update and MDG assessment. 2015.

- 111. World Health Organization (WHO). A Snapshot of Drinking Water and Sanitation in Africa – 2012 Update A regional perspective based on new data from the WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation. 2008:1-24.
- 112. Unicef WHO. WASH Post-2015: Proposed indicators for drinking water, sanitation and hygiene. *World Health Organization* 2015:1-8.
- 113. Jia P AJ, Leitner M, & Rheingans R. High-Resolution Spatial Distribution and Estimation of Access to Improved Sanitation in Kenya. *PLoS ONE* 2016;11(7):e0158490.
- 114. Rheingans R CO, Anderson J, & Showalter J. Estimating inequities in sanitation-related disease burden and estimating the potential impacts of pro-poor targeting.
 London: SHARE: Sanitation and Hygiene Applied Research for Equity, 2012, 2012.
- 115. Xiao L LT, Chen S, Zhang G, Ye Z, & Yu Z. Characterizing Urban Household Waste Generation and Metabolism Considering Community Stratification in a Rapid Urbanizing Area of China. *PLoS ONE* 2015;10(12):e0145405.
- 116. World Bank. State of the Wastewater and Sanitation at the World Bank. World Bank Water Supply and Sanitation Forum, 2000.
- 117. Barreto ML, Genser, B., Strina, A., et al. Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: assessment by two cohort studies *The Lancet* 2007;370:1622-8.
- U.S. Environmental Protection Agency (USEPA). Ambient Water Quality
 Criteria for Bacteria—1986. In: Agency USEP, ed. Washington, DC: EPA, 1986.

- Sanders EC, Yuan, Y., & Pitchford, A. Fecal Coliform and E. coli Concentrations in Effluent-Dominated Streams of the Upper Santa Cruz Watershed. *Water* 2013;5:243-61.
- American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. In: Association APH, ed. Washington, DC, 1992.
- 121. Jaidi K. BB, Carriere A, Desjardins R., & Prevost M. . Including operational data in QMRA model: development and impact of model inputs. *Journal of Water and Health* 2009;7(1):77-95.
- 122. Smeets PW, van Dijk, J. C., Stanfield, G., Rietveld, L. C., & Medema, G. J. How can the UK statutory Cryptosporidium monitoring be used for quantitative risk assessment of Cryptosporidium in drinking water? *Journal of Water and Health* 2007;5:107-18.

H. TABLES

| Table 1. Average rainfall across Accra and days of | |
|--|--|
| precipitation by month, March – July 2016 | |

| precipitation by month, March – July 2016 | | | |
|---|--------------------------|-----------------------|--|
| Month | Average rainfall (in mm) | Days of precipitation | |
| March | 73.9 | 27 | |
| April | 64.3 | 18 | |
| May | 218.9 | 24 | |
| June | 116.1 | 23 | |
| July | 59.5 | 26 | |

Source: World Weather Online. Accra: Greater Accra monthly climate average, Ghana.
| Table 2. Sub-neighborhood household demographics and environmental characteristics of Acc | ı Ghana |
|---|---------|

| | Adabraka | Chorkor | Kokomlemle | Ringway | Shiabu | Total |
|---|-----------------------|-----------------------|------------------------|-----------------------|----------------------|------------------------|
| Households | 148 | 171 | 150 | 114 | 167 | 750 |
| No. Households with a toilet (%) | 129 (87.2) | 75 (43.9) | 139 (92.7) | 110 (96.5) | 74 (44.3) | 527 (70.3) |
| No. HH's with at least 1 contained toilet $(\%)^{**}$ | 127 (85.8) | 75 (43.9) | 138 (92.0) | 110 (96.5) | 73 (43.7) | 523 (69.7) |
| No. who report sharing toilet with ≥ 1 household/compound $(\%)^{\dagger}$ | 64 (43.2) | 23 (13.5) | 84 (56.0) | 44 (38.6) | 34 (20.4) | 249 (33.2) |
| No. of Households who report using public toilets at least once per month | 82 (55.4) | 45 (26.3) | 67 (44.7) | 63 (55.3) | 43 (25.7) | 300 (40.0) |
| Type of toilet/latrine in Household/Compound [†] | | | | | | |
| Flush toilet | 119 (80.4) | 61 (35.7) | 131 (87.3) | 109 (95.6) | 46 (27.5) | 466 (62.1) |
| Pour flush | 2 (1.4) | 5 (2.9) | 1 (0.7) | 0 | 6 (3.6) | 14 (1.9) |
| Kumasi Ventilated-Improved Pit (KVIP) | 4 (2.7) | 3 (1.8) | 2 (1.3) | 0 | 5 (3.0) | 14 (1.9) |
| Ventilated Improved Pit (VIP) | 2 (1.4) | 5 (2.9) | 4 (2.7) | 0 | 11 (6.6) | 22 (2.9) |
| Traditional pit latrine | 0 | 1 (0.6) | 0 | 1 (0.9) | 5 (3.0) | 7 (0.9) |
| Bucket/Pan | 2 (1.4) | 0 | 0 | 0 | 1 (0.6) | 3 (0.4) |
| No facility/bush/field | 15 (10.1) | 95 (55.6) | 11 (7.3) | 4 (3.5) | 84 (50.3) | 209 (27.9) |
| Other | 0 | 1 (0.6) | 1 (0.7) | 0 | 0 | 2 (0.3) |
| Drain/Environment | | | | | | |
| Sample drain sites | 35 | 48 | 37 | 27 | 16 | 163 |
| Average <i>E. coli</i> Concentration in Sampled Drain Locations (log ₁₀ CFU/100mL) | 4.3 (1.0) | 5.6 (0.9) | 4.2 (1.4) | 3.4 (0.7) | 5.3 (1.1) | 4.6 (1.3) |
| Average Drain Size | $\mathcal{O}((74.2))$ | 20(01.2) | 16(42.0) | 10 (70.4) | 5 (21.2) | 105 (64 4) |
| Small (<0.5m wide) (%) Medium (0.5 – 1m wide) (%) | 26 (74.3) 7 (20.0) | 39 (81.3) 5 (10.4) | 16 (43.2) 21 (56.8) | 19 (70.4) 6 (13.0) | 5 (31.3) 7 (43.8) | 105 (64.4 46 (28.2) |
| Large (>1m wide) (%) | 2 (5.7) | 4 (8.3) | 0 | 2 (7.4) | 4 (25.0) | 12 (7.4) |
| Primary Drain Lining Composition | 2 (3.7) | т (0.5) | v | 2 (7.7) | + (20.0) | 12(7.7) |
| Cement (%) | 35 (100) | 35 (72.9) | 37 (100) | 26 (96.3) | 12 (75.0) | 145 (89.0 |
| Stones (%) | 0 | 2 (4.2) | 0 | 0 | 0 | 2 (1.3) |
| Dirt (%) | 0 | 6 (12.5) | 0 | 0 | 1 (6.3) | 7 (4.3) |

| Mixed (%) | 0 | 5 (10.4) | 0 | 1 (3.7) | 3 (18.8) | 9 (5.5) |
|---|-----------|----------|---|---------|----------|-----------|
| Rained during drain sample collection | 2 (5.7) | 0 | 0 | 0 | 0 | 2 (1.2) |
| Rained the day before drain sample collection | 20 (57.1) | 0 | 0 | 0 | 6 (37.5) | 26 (16.0) |

*Chi-square tests of significance were significant at the 0.05 level across all predictors. In categorical comparisons where expected cell frequencies were less than 5, Fisher's exact tests were also found to be significant at the 0.05 significance level.

**A total of 13 households who had toilets refused to report shared sanitation habits

| | Adabraka | Chorkor | Kokomlemle | Ringway | Shiabu | Total |
|--|-----------|-----------|------------|-----------|-----------|-----------|
| | (n=35) | (n=48) | (n=37) | (n=27) | (n=16) | (n=163) |
| No. of HH's within 50m of High Sanitation Clusters (%) | 10 (28.6) | 6 (12.5) | 0 | 4 (14.8) | 7 (43.8) | 27 (16.6) |
| High Contained Sanitation Coverage $(\pm SD)^{**}$ | - | 4.8 (0.7) | - | - | 4.2 (0.5) | 4.5 (0.7) |
| High Unshared Sanitation Coverage $(\pm SD)^{\dagger}$ | 4.8 (0.7) | - | - | 4.0 (0.8) | - | 4.6 (0.8) |
| High Private Sanitation Coverage $(\pm SD)^{\ddagger}$ | - | - | - | 3.6* | 4.3* | 4.0 (0.5) |
| No. of HH's within 100m of High Sanitation Clusters (%) | 12 (34.3) | 11 (22.9) | 0 | 6 (22.2) | 8 (50.0) | 37 (22.7) |
| High Contained Sanitation Coverage (±SD) | - | 5.5 (1.0) | - | - | 4.4 (0.6) | 5.0 (1.0) |
| High Unshared Sanitation Coverage (±SD) | 4.9 (0.6) | - | - | 3.7 (0.7) | - | 4.5 (0.8) |
| High Private Sanitation Coverage (±SD) | - | - | - | 3.6* | 4.9 (0.8) | 4.4 (0.9) |

Table 3. E. coli *concentrations (in log₁₀CFU/100mL) in public domain samples, by neighborhood*

*Only one drain was detected within these clusters, thus a standard deviation could not be calculated

**Contained sanitation is defined by a facility's structural capability of safely containing and separating excreta away from human contact. Latrine types that are considered to be contained include flush, pour flush, KVIP, VIP, and traditional pit latrines with slabs. Uncontained toilets include buckets, pans, bush, fields, the absence of a latrine in a HH, any other toilet form.

*†*Unshared sanitation includes any and all HH's who do not report sharing a toilet with any other HH

‡Private sanitation describes HH's who report never using a public toilet

| | Pa | rameter Estima | ate | | |
|--|-------------------------|------------------------|---------|--|--|
| | | (n=162) | | | |
| Main effect of model [*] | β | SE | p-value | | |
| Drain size** | | | | | |
| Small (<0.5 m wide) | -0.08 | 0.34 | 0.8073 | | |
| Medium (0.5 – 1.0m wide) | 0.13 | 0.36 | 0.7233 | | |
| Drain lining ^{\dagger} | | | | | |
| Cement | -0.35 | 0.43 | 0.4225 | | |
| Stones | 0.80 | 0.85 | 0.3505 | | |
| Mixed | -0.15 | 0.54 | 0.7765 | | |
| Rainfall (in mm) | -9.3 x 10 ⁻³ | 1.9 x 10 ⁻² | 0.6206 | | |
| Rain last day | -0.61 | 0.30 | 0.0431 | | |

Table 4. Differences in E. coli *concentrations (in log₁₀CFU/100mL) of* sample drain sites by drain infrastructure and seasonality

*Models are adjusted for neighborhood **Reference group used is "large drain size (>1.0m in width) †Reference group used is "dirt lining" of drains

| | Adabraka | Chorkor | Kokomlemle | Ringway |
|------------|---------------------------------------|-------------------------|-------------------------|-------------------------|
| | β Estimate (p-value) ^{**} | β Estimate (p-value) | β Estimate (p-value) | β Estimate (p-value) |
| Adabraka | | | | |
| Chorkor | 1.29 (<.0001) | | | |
| Kokomlemle | -0.12 (0.6223) | -1.41 (<.0001) | | |
| Ringway | -0.91 (0.0010) | -2.20 (<.0001) | -0.79 (0.0041) | |
| Shiabu | 0.91 (0.0052) | -0.38 (0.2235) | 1.04 (0.0015) | 1.82 (<.0001) |

Table 5. Differences in E. coli concentrations (in $log_{10}CFU/100mL$) of sample drain sites by neighborhood^{*}

*Neighborhoods on the tops of each column describe the neighborhoods that were used as the reference group in simple linear regression models.

**Estimates were found to be significant at or below the 0.05 level of significance

| | Within 5 | 0m of drain sa | mple | Within 100m of drain sam | | |
|---|------------|----------------|--------|--------------------------|---------|---------|
| | (n=162) (n | | | (n=162) | (n=162) | |
| Main effect of model [*] | β | β SE | | β | SE | p-value |
| Contained Household Sanitation | | | | | | |
| High Coverage Cluster | -1.08 | 0.35 | 0.0027 | -0.56 | 0.31 | 0.0780 |
| Low Coverage Cluster | 0.43 | 0.30 | 0.1484 | 0.16 | 0.26 | 0.5494 |
| Shared Household Sanitation** | | | | | | |
| High Coverage Cluster | 0.19 | 0.41 | 0.6501 | 0.35 | 0.35 | 0.3202 |
| Private Household Sanitation Use [†] | | | | | | |
| High Coverage Cluster | -0.95 | 0.79 | 0.2330 | -0.55 | 0.64 | 0.3982 |
| Low Coverage Cluster | 0.91 | 0.63 | 0.1528 | 1.19 | 0.43 | 0.0066 |

Table 6. E. coli concentrations (in log₁₀CFU/100mL) in sample drain sites by sanitation coverage cluster

*Models are adjusted for neighborhood, drain sample site infrastructure, and rainfall or seasonality (total rainfall on the day of sample collection)

**No drain sample sites fell within any of the low coverage clusters in either of the five neighborhoods, thus the association with E. coli concentration cannot be determined

† Areas of high private household sanitation use indicate areas of low density of reported public toilet use

I. FIGURES









Figure 4. Causal relationship between sanitation indicator variables and fecal contamination in open drains



J. GLOSSARY

| Term | Abbreviation (if applicable) | Definition |
|--|------------------------------|---|
| Cluster | - | Areas of statistically significant spatial groupings of households based on a similar characteristic |
| Coliform | - | Also known as "fecal coliform". A class of hard-to-detect pathogenic bacteria that grows in colonies |
| Contained toilet (vs. uncontained) | - | A toilet/latrine/sanitation facility that allows for the safe disposal and physical separation of excreta from human contact (includes flush toilets, pour flush toilets, Kumasi Ventilated-Improved Pit latrines (KVIP), Ventilated Improved Pit latrines (VIP), and traditional latrines) |
| Directed acyclic graph | DAG | A visual tool used to evaluate confounding, selection bias, and information bias. This tool is also helpful for visualizing the different pathways between exposure and outcome variables, along with any variable that potentially influence this association. |
| Enteric infection | - | Infection of the gastrointestinal tract by a pathogenic bacteria. Often associated with diarrhea, nausea, vomiting, abdominal pain and discomfort, among other symptoms |
| High coverage cluster | - | Statistically significant cluster based on high density of high sanitation coverage |
| Household | HH | A person or group of people sharing a living space/area |
| Kumasi Ventilated- Improved Pit latrine | - | Similar to a Ventilated Improved Pit (VIP) latrine, the KVIP is a type of contained toilet/latrine whose design also includes a ventilation pipe, but has the added benefit of using two pits which is useful for reducing the volume and assisting in the degradation of excreta |
| Log ₁₀ CFU/100mL | - | Units for E. coli concentration; the log reduction of colony forming units per 100 mL of liquid |
| Low coverage cluster | - | Statistically significant cluster based on low density of high sanitation coverage |
| Lower limit of detection | LLOD | The lowest observable/measurable quantity of colonies found in an agar plate |

| Private household sanitation | - | Households who report never using public toilets in any given week |
|---|-----|---|
| Public sanitation (vs. private sanitation) use | - | Reported use of a public toilet outside of one's household at least once per week |
| Sanitation characteristics | - | Includes the presence/absence of a toilet, containment classification, shared sanitation coverage, and whether or not people from each respective household report using a public toilet at least once per week |
| Shared sanitation | - | Toilets that are shared with one or more households |
| Spatial heterogeneity | - | A property used to describe the difference(s) of a population within a given geographical region or area in space |
| Toilet | - | Also known as a "latrine" or a "sanitation facility"; a sanitation fixture used for the disposal of human waste such as urine, feces, etc. |
| Ventilated Improved Pit latrine | VIP | A contained toilet/latrine that contains a ventilation pipe which serves to help eliminate odors that attract mosquitos, flies, and other insects to the facility |

III. FUTURE DIRECTIONS

Our models dichotomized all exposure variables used in this analysis to examine whether large scale differences in opposing sanitation conditions led to differences in fecal contamination to begin to understand the under-researched intermediate outcomes that interact with environmental fecal exposure pathways. While future research should continue to investigate these broader relationships, it may be beneficial to combine data regarding the frequency of sanitation-based behaviors to multi-level categories in order to identify finer-scaled differences. For example, it is important to consider different levels of shared sanitation, as it is likely that a house that shares a toilet with one other households is located in an area with meaningfully better sanitation coverage (compared to a house contribute to a better understanding of the pathways that contribute to the risk of fecal contamination in the public). Considering multi-level parameters can also avoid burying important relationships that may interact differently in the described mechanisms of exposure and may also help to avoid under-or over-estimating the associations between fecal contamination of certain sanitation conditions.

IV. APPENDIX

A. IRB APPROVAL



Institutional Review Board

TO: Christine Moe PhD Principal Investigator *SPH: Global Health

DATE: February 14, 2017

RE: Continuing Review Expedited Approval CR5_IRB00051584

IRB00051584 Assessment of Fecal Exposure Pathways in Low-Income Urban Settings

Thank you for submitting a renewal application for this protocol. The Emory IRB reviewed it by the expedited process on **February 14, 2017**, per 45 CFR 46.110, the Federal Register expeditable categories F[3], F[7], and/or 21 CFR 56.110. This reapproval is effective from **February 14, 2017** through <u>February 13, 2018</u>. Thereafter, continuation of human subjects research activities requires the submission of another renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. Please note carefully the following items with respect to this reapproval:

- Protocol Document ver. 3/2/2015
- Consent Documents:
 - Assent schoolchildren 9 3 13 (2).doc
 - <u>Consent_FDGs 10.13.11.doc</u>
 - Consent HH Survey + environ. sampling 10.13.11.doc
 - <u>Consent_Interviews 10.13.11.doc</u>
 - Dhaka Community Consent
 - Dhaka Household Consent
 - <u>Dhaka Parental Consent</u>
 - <u>Dhaka School Assent</u>
 - o Maputo- School Children Parental Consent
 - Mozambique- HH consent
 - Mozambique- School Children Assent
 - <u>SaniPath Atlanta_Consent_Stamped template.doc</u>
 - o School Headmasters 1 17 12.doc
 - <u>School Headmasters_survey 9 11 13.doc</u>

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at <u>www.irb.emory.edu</u>, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, and study design), you must submit an amendment request and secure IRB approval.

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

Sincerely,

Emilie Scheffer IRB Analyst Assistant This letter has been digitally signed

| CC: | Green | Jamie | *SPH: Global Health |
|-----|-------|-----------|---------------------|
| | Raj | Suraja | *SPH: Global Health |
| | Robb | Katharine | *SPH: Global Health |

Emory University 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



Household Survey Form

| Neighborhood | Response date |
|--|---------------|
| □Neighborhood A _□ Neighborhood B | |
| Household ID | GPS latitude |
| | |
| GPS longitude | |
| | |
| | |
| | |

1. Do you have children between the ages of 5-12? (Y/N) ☐ Yes ☐ No

2. Think about whether you go into the ocean. This includes wading, swimming, splashing around, fishing, doing laundry, or to **[defecate]**. How often do you go into the ocean for any of these reasons? (select one option)

□ 2a. I go into the ocean more than 10 times total every month.

□ 2b. I go into the ocean 6-10 times total every month.

□ 2c. I go into the ocean 1-5 times total every month. □ 2d. I

never go into the ocean.

3. Now think about whether your children go into the ocean. This includes wading, swimming, splashing around, fishing, helping with laundry, or to **[defecate]**. How often do your children go into the ocean for any of these reasons? (select one option)

□ 3a. My children go into the ocean more than 10 times total every month.

□ 3b. My children go into the ocean 6 to 10 times total every month.

 \square 3c. My children go into the ocean 1 to 5 times total every month.

□ 3d. My children never go into the ocean.

□ 3e. I do not know how often my children go into the ocean.

4. Think about whether you ever go into [rivers or ponds] in your neighborhood? This includes wading, swimming, splashing around, fishing, doing laundry, or to **[defecate]**. How often do you go into the [rivers or ponds]? (select one option)

□ 4a. I go into the [rivers or ponds] more than 10 times total every month.

4b. I go into the [rivers or ponds] 6 to 10 times total every month.

 \Box 4c. I go into the [rivers or ponds] 1 to 5 times total every month. \Box 4d. I never go into the [rivers or ponds].

5. Think about whether your children ever go into the [rivers or ponds] in your neighborhood: this includes wading, swimming, splashing around, fishing, helping with laundry, or to **[defecate]**. How often do your children go into the [rivers or ponds]? (select one option)

☐ 5a. My children go into the [rivers or ponds] more than 10 times total every month.

□ 5b. My children go into the [rivers or ponds] 6 to 10 times total every month.

☐ 5c. My children go into the [rivers or ponds] 1 to 5 times total every month.

☐ 5d. My children never go into the [rivers or ponds].

□ 5e. I do not know how often my children go into the [rivers or ponds].

6. Think about whether you ever go into open drains. This could include picking up something that fell in there, or having to go through the drain to cross the street. How often do you go into the drains? (select one option)

☐ 6a. I come into contact with drain water more than 10 times total every month.

□ 6b. I come into contact with drain water 6 to 10 times total every month.

☐ 6c. I come into contact with drain water 1 to 5 times total every month. ☐ 6d. I never

come into contact with drain water.

7. Think about whether your children ever go into the drains. This could include picking up something that fell in there, or having to go through the drain to cross the street. How often do your children go into the drains? (select one option)

☐ 7a. My children come into contact with drain water more than 10 times every month.

☐ 7b. My children come into contact with drain water 6 to 10 times total every month.

□ 7c. My children come into contact with drain water 1 to 5 times total every month.

☐ 7d. My children never come into contact with drain water.

Te. I do not know how often my children come into contact with drain water.

8. How often do you come into contact with floodwater during the rainy season? (select one option)

□ 8a. I come into contact with floodwater more than 10 times total every month during the rainy season.

☐ 8b. I come into contact with floodwater 6 to 10 times total every month during the rainy season.

□ 8c. I come into contact with floodwater 1 to 5 times total every month during the rainy season.

□ 8d. I never come into contact with floodwater.

9. How often do your children come into contact with floodwater during the rainy season? (select one option)

- ☐ 9a. My children come into contact with floodwater more than 10 times total every month during the rainy season.
- □ 9b. My children come into contact with floodwater 6 to 10 times total every month during the rainy season.
- □ 9c. My children come into contact with floodwater 1 to 5 times total every month during the rainy season.

🗌 9d. My children never come into contact with floodwater during the rainy season. 🗖 9e. I do not know how

often my children come into contact with floodwater.

-10.How many days a week do you drink municipal water? (select one option) □ 10a. I drink municipal water every day.

 \Box 10b. I drink municipal water 4 to 6 days a week.

 \Box 10c. I drink municipal water 1 to 3 days a week.

□ 10d. I never drink municipal water.

□ 10e. I do not know if I drink municipal water.

11.How many days a week do your children drink municipal water? (select one option) □ 11a. My <u>children drink municipal water every day.</u>

 \Box 11b. My children drink municipal water 4 to 6 days a week.

 \Box 11c. My children drink municipal water 1 to 3 days a week.

 \Box 11d. My children never drink municipal water.

□ 11e. I do not know how often my children drink municipal water.

12. Does your family regularly treat your water by [boiling, adding chlorine, or using a filter] to make it less cloudy or safer to drink? (Y/N)

□ Yes □ No

13. How many days during the week do you eat produce that is raw (uncooked)? For this question, we are referring to any produce that does not grow on a tree, and that does not have a peel or shell. Please think both about produce you eat whole and produce you prepare but eat raw, such as a salad. For example [list types of produce identified in the preliminary assessment]? (select one option)

□ 13a. I eat raw produce every day.

 \square 13b. I eat raw produce 4 to 6 days a week.

□ 13c. I eat raw produce 1 to 3 days a week. □ 13d. I

never eat raw produce.

14. How many days during the week do your children eat produce that is raw (uncooked)? Again, for this question, we are referring to any produce that does not grow on a tree, and that does not have a peel or shell. Please think both about produce you eat whole and produce you prepare but eat raw, such as a salad. For example [list types of produce identified in the preliminary assessment]? (select one option)

□ 14a. My children eat raw produce every day.

□ 14b. My children eat raw produce 4 to 6 days a week.

☐ 14c. My children eat raw produce 1 to 3 days a week.

□ 14d. My children never eat raw produce.

□ 14e. I do not know if my children eat raw produce.

15. How often do you use public latrines - that means a community shared toilet, school toilet, or work toilet? (select one option)

□ 15a. I use a public latrine more than 10 times total every month.

 \square 15b. I use a public latrine 6 to 10 times total every month.

 \square 15c. I use a public latrine 1 to 5 times total every month. \square 15d. I never use a public latrine.

16. How often do your children use public latrines - that means a community shared toilet, school toilet, or work toilet? (select one option)

☐ 16a. My children use public latrines more than 10 times total every month.

 $\hfill\square$ 16b. My children use public latrines 6 to 10 times total every month.

□ 16c. My children use public latrines 1 to 5 times total every month.

☐ 16d. My children never use public latrines.

□ 16e. I do not know how often my children use a public latrine.

17.Do you have a latrine in your **[house/compound]**? (Y/N) □ Yes □ No

.....

18.<u>If</u> you have a latrine in your **[house/compound]**, do you use it? (Y/N) □ Yes □ No

19.<u>If</u> you have a latrine, do you flush it with water? (Y/N) □ Yes □ No

20. If you have a latrine in your **[house/compound]**, how many households do you share a latrine with? Your answer can be a zero or higher. (number)

21. If you are outside of your house and you cannot find a latrine, what do you do? For this question, you may select all that apply.

□ 22a. This never happens to me.

□ 22b. I go back to my home.

 \square 22c. I go in search of a latrine (e.g. at a friend's, or in a public area).

□ 22d. I use a plastic bag.

□ 22e. I [defecate] in the open.

C. DRAIN WATER: ENVIRONMENTAL SAMPLE COLLECTION AND PROCESSING FORM

Drain Water

Environmental Sample Collection and Processing Form

| Sample ID | | | Collection date and tim | ie | |
|----------------------------|------------------|----------|--------------------------|--------------|---------|
| generated by tool-leave | e blank | | | | |
| GPS Latitude (N, S) | GPS Longitude | e (W, E) | Way Point | | |
| |][| |] | | |
| Neighborhood | | | | | |
| ☐ Neighborhood A | ☐ Neighborh B | nood | | | |
| Notes | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Laboratory | | | | | |
| Sample processing date | e and time | | | | |
| Data and time placed in in | auhatar | | Data and time removed fr | am inauhatar | |
| Date and time placed in in | cubator | | Date and time removed fr | om incubator | |
| | | | | | |
| Plate Dilution (m | L) | | E. coli Count | TNTC * | TDTC ** |
| Plate 1 1:1 | 1:10 1:100 | 1:1000 | | | |
| Plate 2 1:1 | 1:10 1:100 | 1:1000 | | | |
| Blank | | | | | |
| Collector Name | | | Lab Operator Name | | |
| | | | | | |

* TNTC = too numerous to count (> 200 E. coli)

** TDTC = too dirty to count