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Maria Julia Bianca Garcia Corpuz Date

Identifying Enteric Pathogens and Assessing Their Health Risks in Urban Environments of Dhaka, Bangladesh

By

Maria Julia Bianca Garcia Corpuz
Master of Public Health

Global Epidemiology

____

Christine Moe
Committee Chair

____

Pengbo Liu
Committee Member

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By

Maria Julia Bianca Garcia Corpuz

Bachelor of Science
University of Maryland, Baltimore County
2016

Thesis Committee Chair:
Christine Moe, M.S., Ph.D.

Thesis Committee Member:
Pengbo Liu, MPH, Ph.D.

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Abstract

Identifying Enteric Pathogens and Assessing Their Health Risks in Urban Environments of Dhaka, Bangladesh
By Maria Julia Bianca Garcia Corpuz

In densely populated urban environments such as Dhaka, Bangladesh, frequent exposure to fecal contamination may cause a high risk of enteric infections and diarrheal diseases. Children and adults are frequently exposed to ubiquitous open drains and canals in their immediate environment, which serve as outlets for wastewater and feces disposal in Dhaka city. A cross-sectional study was conducted to detect and quantify pathogens in open drains and canals and estimate monthly risks of infection from exposure to these hazards. This study was conducted in two densely populated areas of Mirpur, Dhaka from April 2019 to October 2019. Selected enteric pathogens, Norovirus Genogroup II (GII), *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi, were quantified in environmental samples using ultrafiltration, polyethylene glycol precipitation (PEG), total nucleic acid (TNA) extraction, and polymerase chain reaction (PCR) methods. Monthly risks of pathogen-specific infections in adults and children from open drain and canal exposure were estimated by quantitative microbial risk assessment (QMRA) using Monte Carlo simulations. The average concentration of pathogens ranged from 0.54 $\log_{10}$ genomic copies/100 mL of *Giardia* in canal samples to 4.16 $\log_{10}$ genomic copies/100 mL of *Shigella* in drain samples. The highest monthly risks of infection from exposure to open drains in adults and children were associated with Norovirus GII and the lowest with *Salmonella* Typhi. The probability of pathogen-specific infection from canal exposure was highest for adults and children for *Vibrio cholerae* and the lowest for *Giardia*. Because of the high concentrations of pathogens detected in both open drain and canal water samples, any contact with open drains or canals will likely result in a high level of exposure to fecal contamination and therefore an increased risk of infection by enteric pathogens. The results from this study should be used to guide public health communications and the development of targeted interventions to reduce exposure to fecal contamination in open drains and canals in Dhaka, Bangladesh.

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TABLE OF CONTENTS

CHAPTER I: BACKGROUND 1

A. THE GLOBAL BURDEN OF DIARRHEAL DISEASE 1
B. THE BURDEN OF DIARRHEAL DISEASE CAUSED BY ENTERIC PATHOGENS IN BANGLADESH 7
C. SANITATION CHALLENGES IN URBAN ENVIRONMENTS 13
D. SANITATION IN DHAKA, BANGLADESH: THE ROLE OF CANALS AND OPEN DRAINS 15
E. DETECTION METHODS FOR ENVIRONMENTAL ENTERIC PATHOGENS 17
F. ENTERIC PATHOGENS IN THE ENVIRONMENT 19
G. QUANTITATIVE MICROBIAL RISK ASSESSMENT 25
H. STUDY OBJECTIVES 28

CHAPTER II: MANUSCRIPT 30

A. TITLE, AUTHORS, ABSTRACT 30
B. INTRODUCTION 30
C. METHODS 33
i. Study Site Selection 34
ii. Environmental Sample Collection 34
iii. Data Collection 35
iv. Laboratory Methods 37
v. Statistical Methods 40
vi. Exposure Assessment 41
D. RESULTS 46
i. Enteric Pathogen Positive Rates in Open Drains and Canals 46
ii. Enteric Pathogen Concentrations in Open Drains and Canals 48
iii. Exposure Assessment 50
E. DISCUSSION 52
i. Enteric Pathogen Concentrations in Ultrafiltration and Grab Samples: Implications for Detection Methods 52
ii. Open Drain and Canal Exposure Models 55
iii. Open Drain and Canal Exposure Data: Implications for Quantitative Microbial Risk Assessment 60
iv. Strengths and Limitations 62
F. REFERENCES 66
G. TABLES 82
H. FIGURES 93

CHAPTER III: SUMMARY, PUBLIC HEALTH IMPLICATIONS, POSSIBLE FUTURE DIRECTIONS 99

A. SUMMARY AND PUBLIC HEALTH IMPLICATIONS 99
B. RECOMMENDATIONS 100
C. FUTURE DIRECTIONS 101

TABLES

Table 2.1. Primers and Probes Profiles Used in the qPCR Assays in this Study 82
Table 2.2. Pathogen Concentrations (log_{10} genomic copies/100mL)[a] in Environmental Samples from Urban Neighborhoods, Dhaka, Bangladesh 2019 83
Table 2.3. Survey Data Used to Estimate Monthly Exposure to Canals and Drains 84
Table 2.4. Dose Response Parameters Used to Translate Dose into Infection Risk 85
Table 3.1 Enteric Pathogen Positive Rates in Environmental Samples from an Urban Neighbourhood, Dhaka, Bangladesh 86
Table 3.2. Comparison Between Enteric Pathogen Positive Rates Between Drain Samples and Canal Samples 87
Table 3.3. Comparison Between Enteric Pathogen Positive Rates Between Grab Samples and Ultrafiltration Samples 88
Table 3.4. Comparison Between Pathogen Concentrations (log_{10} genomic copies/100 mL)[a] Between Drain Samples and Canal Samples 89
Table 3.5. Comparison Between Pathogen Concentrations (log_{10} genomic copies/100 mL)[a] Between Grab Samples and Ultrafiltration Samples 90
Table 3.6. Estimated Monthly Risks of Pathogen Infection for Children from Canal and Drain Exposures 91
Table 3.7. Estimated Monthly Risks of Pathogen Infection for Adults from Canal and Drain Exposures 92

FIGURES

Figure 2.1. Sampling Locations in Urban Neighborhoods 93
Figure 2.2. Flow Diagrams for Environmental Sample Processing Procedures for Large Volume (20 L) and Small Volume (400 mL) Samples 94
Figure 3.1. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Adults from Open Drain Exposure 95
Figure 3.2. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Children from Open Drain Exposure 96
Figure 3.3. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Adults from Canal Exposure 97
Figure 3.4. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Children from Canal Exposure 98

CHAPTER I: BACKGROUND

A. The Global Burden of Diarrheal Disease

Diarrhea is the eighth leading cause of death among all ages, accounting for over 1.6 million deaths worldwide. Ninety percent of these deaths are geographically concentrated in South Asia and Sub-Saharan Africa. Diarrhea accounts for 446,000 deaths per year in children younger than five years of age, making diarrhea the fifth leading cause of death for this age group. Those who reside in areas with poor or no access to health care, safe water, and sanitation are disproportionately affected by diarrheal disease (1). Low-income countries lack the resources and infrastructures to manage the burden of diarrheal diseases. As a result, low-income countries experience inequitable proportions of morbidity and mortality due to diarrhea (2). It is estimated that the access to safe water can reduce child mortality by 8 deaths per 1,000 children. Technologies such as household water connections, flush toilets, and safely-managed sanitation systems can lower child mortality further by 25 deaths per 1,000 children (3). Many types of enteric pathogens, including bacteria, viruses, and parasites, are major contributors to the global burden of diarrheal disease. In this literature review, *Shigella,* Norovirus*, Salmonella* Typhi, *Giardia*, and *Vibrio cholera* will be discussed in more detail.

Shigella. Shigellosis is caused by *Shigellae spp.* which are gram-negative bacteria transmitted via the fecal-oral route (4). Clinical characteristics of shigellosis include diarrhea, bloody stool, mucoid stool, fever, abdominal cramps, and tenesmus. Shigellosis does not require a high infectious dose for infection, 10 to 100 organisms can cause infection and possibly disease. *Shigella sonnei, Shigella boydii, Shigella flexneri,* and

Shigella dysenteriae are the four serogroups of *Shigella* which can cause shigellosis (5). *Shigella dysenteriae and Shigella flexneri* are frequently reported in low-income countries (6). Infections caused by *Shigella boydii* are not typically seen outside of India, though they can present in low-income countries (7). *Shigella sonnei* and *Shigella flexneri* are the serogroups currently associated with most shigella outbreaks and epidemics.

Approximately 125 million diarrheal episodes and 160,000 deaths are caused by *Shigella* each year. The global burden of *Shigella* is mostly experienced in low and middle-income countries where clean water access and proper sanitation are limited (7). Overcrowding and sewage-contaminated water supplies have been associated with high infection rates of *Shigella* (8). Of the 164.7 million cases of shigellosis estimated to occur each year, an estimated 163.2 million of those cases will occur in low-income countries (9). As the second leading cause of mortality due to diarrhea in 2016, *Shigella* was responsible for 212,438 deaths and 13.2% of all diarrhea-related deaths. An estimated 63,713 (30%) cases were experienced by children less than five years of age. For individuals over the age of 70, shigella was the leading cause of death (10). Though illness caused by *Shigella* can typically resolve without treatment within seven to ten days in healthy persons, it can be deadly in infants, children, and immunocompromised persons (4,7).

Norovirus. Norovirus (NoV) is a non-enveloped, single-stranded RNA virus responsible for 18% of all acute gastroenteritis cases globally (11,12). Clinical characteristics of NoV infection include nausea, vomiting, diarrhea, stomach pains, low-grade fever, and muscle

pain. While symptoms typically last for one to three days, NoV can be shed in feces for up to two weeks after recovery (13). For the elderly, immunocompromised, and children, NoV infection can cause malnutrition, severe dehydration, and death (13). An infectious dose as low as 18 viral particles, persistent shedding, environmental stability, survival in heating and freezing temperatures, resistance to common disinfectants, and multiple transmission pathways are characteristics that contribute to the spread and persistence of NoV in the environment. Since NoV are transmitted via the fecal-oral route, high viral loads of NoV can be detected in sewage waters (14,15). NoVs are categorized in five genogroups, GI to GV. The GI, GII, and GIV strains of NoV can cause disease in humans. The GII genogroup is the predominant strain of NoV found in sewage waters (14).

In both low- and high-income countries, NoV is one of the major causes of gastroenteritis (16). Gastroenteritis associated with NoV in low-income countries is generally more severe than in high-income countries and often requires medical attention (15). In high-income countries, approximately 900,000 episodes of gastroenteritis and 64,000 hospitalizations among children less than five years of age are associated with NoV. In low-income countries, approximately 200,000 child deaths are associated with NoV annually (17). The mortality associated with NoV infection among children is higher in low-income countries and is the leading cause of diarrhea in children aged less than five years worldwide (18,19). The most severe cases of NoV occur in children less than five years, immunocompromised persons, and persons over 65 years (20).

Giardia. *Giardia* protozoa, including *Giardia lamblia, Giardia intestinalis,* and *Giardia duodenalis,* are zoonotic parasites which causes giardiasis. Giardiasis is an infection with a clinical description of diarrhea, cramps, bloating, and malabsorption. Intestinal inflammation has been reported to occur, and those symptoms can remain for several years. However, individuals with giardiasis may not always experience symptoms (21). *Giardia* can be transmitted via fecal-oral, person-to-person, and animal-to-person routes with infection only requiring ingestion of one to ten oocysts (22). *Giardia* has eight assemblages, however assemblages A and B are the only assemblages associated with human infections (22). Symptoms of giardiasis are associated with assemblage A, and asymptomatic giardiasis is associated with assemblage B (21).

Globally, giardiasis is the most prevalent intestinal parasitic infection and the third most common cause of diarrheal disease in both children and adults (22,23). Giardiasis is common in low-income countries where there is a lack of safe water and sanitation facilities. The prevalence of *Giardia* ranges from 4 to 43% in low-income countries, with the rate of infection highest in children less than five years. In high-income countries, the prevalence ranges from 1% to 7%. Many cases of *Giardia* remain undetected, so reported prevalence rates of *Giardia* are highly underestimated (22). Infection rates of giardiasis are lower in high-income countries than in low-income countries. However, poor regions of high-income countries can still report high infection rates of *Giardia* (21).

Vibrio cholera. Cholera is a diarrheal disease caused by toxigenic *Vibrio cholerae* which colonizes and produces a cholera toxin in the small intestine which leads to severe watery

diarrhea (24). Cholera is often mild and does not typically present symptoms. However, 10% of persons infected with cholera have severe symptoms such as watery diarrhea, vomiting, and leg cramps. In individuals where symptoms are severe, dehydration, shock, and death may occur if treatment is not obtained (25).

In regions endemic to cholera, such as southern Asia, parts of Africa, and Latin America, outbreaks occur seasonally and are associated with poor sanitation, contaminated water and food, and poverty (24). Depending on the location within endemic regions, large outbreaks of cholera may occur once or twice a year (26). It is estimated that 2.8 million cholera cases occur each year in endemic countries. In countries endemic to cholera, the annual incidence rate is 2.0 cases per 1,000 people. In non-endemic countries, the annual incidence rate is 1.15 cases for 1,000 people. In endemic countries, it is estimated that cholera is responsible for 91,000 deaths annually, with approximately half of them occurring in children who are less than five years of age. In non-endemic countries, it is estimated that 2,500 deaths are caused by cholera annually (27). There are two major serogroups of *Vibrio cholerae* which are known to cause epidemics, O1 and O139. There are two biotypes for serogroup O1, classical and El Tor, and each of these biotypes has two major serotypes, Ogawa and Inaba (28). However, there are Ogawa and Inaba strains that are not associated with cholera epidemics (24). Cholera has been studied for two centuries, but there are still some features of cholera outbreaks that are not yet understood. One of cholera's epidemiological features includes simultaneous outbreaks in distinct regions. Researchers hypothesize that the concurrent outbreaks are a product of environmental triggers. A high infectious dose (10^3-10^6 organisms) is required for cholera

infection, and this dose is not likely to be present in the natural environment. This raises a question of how cholera epidemics become explosive (26).

***Salmonella* Typhi.** Typhoid fever is caused by a gram-negative bacteria, *Salmonella* Typhi. Typhoid fever, a systemic infection, can result in fevers, constipation, diarrhea, malaise, abdominal, and other multiple complications (29). The lack of safe water and sanitation facilities are risk factors for typhoid fever, and the disease is typically contracted from the consumption of contaminated food or water (30). The reservoir for *Salmonella* Typhi is humans, and the bacteria has limited capacity to multiply outside of the human host (29). *Salmonella* Typhi can survive in groundwater and pondwater for days to weeks and in sewage for less than a week (31). For children less than 12 years old and adults older than 65 years, the average id50 for *Salmonella* Typhi infection is 1.30 CFU and the average id50 for illness is 54.0 CFU. For those who are at least 12 years old and no older than 65, the average id50 for infection is 1.82 CFU and the average id50 for illness is 54.0 CFU (32). For infection to occur, *Salmonella* Typhi has to survive the gastric acid barrier. With effective treatment, the case fatality risk of *Salmonella* Typhi is less than 1% (33).

Globally, it is estimated that per year, typhoid fever is responsible for 11 million to 20 million sicknesses and 128, 000 to 161,000 deaths (34). In 2017, an estimated 10.9 million cases of typhoid fever were reported with an estimated global case-fatality rate of 0.95% (35). Although typhoid fever is a bacterial infection that affects the global population, the burden of disease may be underestimated because of poor diagnostic tests,

and many areas in low-income countries do not have access to bacteriology laboratories that can reliably diagnose *Salmonella* Typhi (29). Typhoid fever places an enormous burden on those living in low- and middle-income countries. It is estimated that in low- and middle-income countries, 17.8 million cases of typhoid fever occur each year (36). South Asia had the highest incidence rate of typhoid fever at 549 cases per 100,000 person years (35).

B. The Burden of Diarrheal Disease Caused by Enteric Pathogens in Bangladesh

Shigella. Shigellosis is a major diarrheal disease that is endemic to Bangladesh and accounts for 20% of diarrheal deaths in children (6). Based on data from the International Center for Diarrheal Disease Research, Bangladesh (icddr,b), there are approximately 1,960 deaths caused by shigellosis per year. The number of deaths per year caused by shigellosis in Bangladesh ranges from 1,347 to 2,595 (37). The true number of shigellosis-associated deaths in Bangladesh may actually be 6 to 8 times higher than deaths recorded in hospital records (38). Thus, the number of deaths caused by *Shigella* may be higher across all age groups per year. The case-fatality rate for the 0-11 month age group is the highest among all age groups at 2.73 and decreases as age increases (37).

One study obtained 5,112 isolates of *Shigella* from patients receiving treatment from the icddr,b from 1999 to 2002. *S. flexneri* was the predominant species with isolation rates of 57.4%, 53.6%, 65.7%, and 62.5% in 1999, 2000, 2001, and 2002 respectively. *S. flexneri* was followed by *S. boydii, S. dysenteriae,* and *S. sonnei* (6). Another study obtained 10,827 isolates of *Shigella* from patients from 2001 and 2011. Similar to the previous

study, *S. flexneri* was the most prevalent species throughout the study period with isolation rates of 65.7% in 2001 and 47% in 2011. However, *S. sonnei* was the second most prevalent species and replaced the dominance by *S. boydii* and *S. dysenteriae* observed in earlier years. Multi-drug resistant strains of *Shigella* are increasing rapidly, and in this study 94% of the strains were multi-drug resistant (39). With the rise of multi-drug resistant strains of *Shigella,* it may be in the best interest of the global community to develop a vaccine to prevent future cases of *Shigella*. Further, it is important to monitor *Shigella* in the environment, particularly where individuals are most exposed, to control and reduce rates of infection caused by *Shigella*.

Norovirus (NoV). In Bangladesh, NoV is responsible for viral gastroenteritis in both adults and children. NoV is the most common cause of gastroenteritis outbreaks in Bangladesh (19). Studies have reported high proportions of NoV infections observed in both adults and children in Bangladesh (20,40).

The first epidemiological study of Norovirus in Bangladesh took place from October 2004 to September 2005 in the city of Dhaka. A total of 917 fecal samples were collected from infants and children with acute gastroenteritis. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to detect Norovirus and found 41 (4.47%) positive samples. The NoV sequences belonged only to Genogroup II (19). Another study was conducted from 2010 to 2014 to assess the prevalence and genotypes of NoV. A total of 953 fecal samples were collected from children and adults who were receiving treatment for diarrheal disease in the cities of Dhaka and Matlab, and 239 (25.08%) fecal

samples tested positive for NoV. Norovirus Genogroup I, Norovirus Genogroup II, Norovirus Genogroup IV, and Norovirus mixed Genogroup I/II/IV were all detected by PCR with Norovirus Genogroup II being the most prevalent genogroup (40). With new genotypes of NoVs being found, NoV surveillance may be beneficial to identify trends in genotypes responsible for NoV infections in Bangladesh.

Giardia. *Giardia* is a highly infectious parasite and a common cause of diarrheal disease in Bangladesh (41). Age-specific prevalence rates of *Giardia lamblia* infections have been reported in various studies. However, larger scale studies need to be conducted to obtain a more accurate estimate of the overall prevalence of *Giardia* infections in Bangladesh (41,42).

A study conducted in Mirpur, Dhaka, Bangladesh collected diarrheal samples from 289 pre-school children between January 1999 and July 2002. Microscopy was used to identify the presence of *Giardia lamblia. Giardia lamblia* was found in 11% of the stool samples (42). Another study observed the prevalence of *Giardia lamblia* in children less than five years of age. A total of 266 fecal samples were collected from children who were admitted and hospitalized in Dhaka from January 2011 to May 2012. Children between 24 and 60 months of age exhibited the highest prevalence of *Giardia lamblia* at 8.7%. The overall prevalence of infection by *Giardia lamblia* was 3.8% (41). Though both studies were conducted in Dhaka, the study conducted by Suman et al. took place in a specialized hospital for diarrheal patients, whereas the study conducted by Haque et al. was conducted in urban slum neighborhoods (36,37). The higher prevalence exhibited in

children living in the urban slum environments may have been affected by overcrowding, lack of sanitation, and contaminated water. Monitoring *Giardia* in the environment, particularly where individuals are most exposed, may aid in the control or reduction of infections caused by *Giardia*.

Vibrio cholerae. *Vibrio cholerae* thrives in densely populated environments and areas with poor water and sanitation facilities, such as slum environments. Cholera is endemic in Bangladesh. Due to the frequent and extensive flooding in Bangladesh, the World Health Organization considers the entire population of Bangladesh at risk for cholera infection (27). The city of Dhaka is a geographical region in Bangladesh with moderate to high prevalence of cholera where 2% of all cases of diarrhea test positive for *Vibrio cholera* (43). Though all age groups are affected by cholera, children under five years of age experience the highest mortality rates. Each year, Bangladesh experiences at least 100,0000 cases and 4,500 deaths from cholera. The incidence rate of cholera in Bangladesh is 1.64 per 1,000 people (43). Cholera incidence has seasonal peaks in Bangladesh in the spring and fall (44). Since Bangladesh lacks a population-based surveillance system of diarrheal disease, the true burden of cholera is unknown (45). Cholera is highly underreported in Bangladesh due to lack of surveillance and laboratory diagnostic capacity (43).

One study estimated the incidence of diarrheal disease caused by *Vibrio cholerae* using hospital surveillance data collected from March 2010 and February 2011. The hospitals selected for this study were the six hospitals in Bangladesh with diarrheal disease

surveillance. They found the incidence of diarrheal disease caused by *Vibrio cholerae* in the hospital catchment areas to be 2.6, 4.9, 1.1, 0.3, 3.7, and 1.8 per 1,000 people (45). Another study conducted surveillance of *Vibrio cholerae* in eight Divisions in Bangladesh from May 2014 to June 2018. Of all divisions, the Dhaka division consistently had the highest number of cholera cases. The Chittagong and Dhaka divisions had the highest prevalence of cholera at 18.3% and 13.9%, respectively. Specimens from 25,958 person were collected and cultured for *Vibrio cholerae* detection. *Vibrio cholerae* was detected in 1,604 (6.18%) specimens, where 70% were Inaba serotype. Among those who were less than five years and those older than five, 2.1% and 9.5% of stool samples tested positive for *Vibrio cholerae*, respectively (43). Monitoring *Vibrio cholerae* in the environment, particularly where individuals are most exposed, may aid in the control or reduction of cholera. Further, it could help address the issue of underreporting cholera cases in Bangladesh.

***Salmonella* Typhi.** Typhoid fever is highly endemic and a common bloodstream infection in low-income countries, such as Bangladesh. The burden of enteric illness in Bangladesh caused by *Salmonella* Typhi is attributed to several factors including the lack of safe water, sanitation facilities, and overcrowding (46). Estimating the exact number of typhoid cases in Bangladesh is not possible because there is no regular surveillance, though studies have provided estimates (46). The overall incidence of typhoid fever in Bangladesh is estimated to be 200 cases per 100,000 person years (47).

One study conducted surveillance of typhoid fever in Bangladesh from 2004 to 2016 in three hospitals located in Dhaka. Using culture methods, they found 7,072 cases of typhoid cases, 12% of these cases were among children who were less than two years, and 46% of these cases were in children who were less than five years (47). This study suggested typhoid fever is common in Dhaka, especially in children less than five years. Another study observed the distribution of typhoid fever cases in Dhaka from 2005 to 2009. Data on typhoid fever cases were obtained from 11 major hospitals in Dhaka. Young children were found to be disproportionately affected by typhoid fever. The 0-4 years age group exhibited the highest incidence rate at 277 cases per 100,000 people (0.28%), followed by the 60+ years age group (55 cases, 0.06%), the 15-17 years age group (45 cases, 0.05%), the 18-34 age group (37 cases, 0.04%). The 35-39 years age group (34 cases, 0.03%), and lastly, the 10-14 years age group (11 cases, 0.01%). The study also found a statistically significant inverse association between the incidence of typhoid and distance to water sources (48). Another study examined the prevalence of typhoid fever in semi-urban Bangladesh from June 2009 to May 2010 among children of low-income groups. A total of 96 children who were receiving treatment for fevers from the Dhaka Medical College Hospital were enrolled in the study, and 84 (87.5%) patients tested were suffering from typhoid fever. The children were divided into three age groups, preschool, school, and adolescence; age ranges for each age group were not identified. School-aged children had the highest prevalence of typhoid fever (66.67%), followed by adolescent children (18.75%) and preschool-aged children (14.58%) (49). Monitoring *Salmonella* Typhi in the environment, particularly where children are most

exposed, may provide additional information about the burden of infection in specific geographic areas.

C. Sanitation Challenges in Urban Environments

There has been a dramatic rise in urbanization in the global community. As the global population continues to rise, it is estimated that 70% of the global population will be urbanized by 2050 (50). As of 2018, about 55% of urban populations live in urban slums, where one third of them are children (51). In Bangladesh, it is projected that by 2030, 40% of the population will reside in urban settings. This is a drastic increase from 1974 when only 8.8% of the population was urbanized. In Dhaka, the slum population increased from 250,0000 in 1974 to 2,840,000 in 2005. Dhaka is home to more than 3,400 slum developments (52).

The exponential growth of urbanization adds pressure to strained sanitation systems which are essential to protect the health of urban community residents (50). The governments of low-income countries do not have enough resources or funding to create adequate sanitation services (53). Globally, the safe disposal of fecal matter in rural and urban settings remains a major challenge (54). Globally, it is estimated that 2.5 billion lack access to basic sanitation services. Several reasons for this include the lack of infrastructure, space to build infrastructure, and finances for safe feces management and treatment (53). Studies have found that in dense, urban environments, exposure to fecal contamination considerably contributes to the risk of enteric infections (55). The lack of access to safe, sanitation services is one of the world's most urgent challenges, especially

in high-density informal settings such as slum areas (56). Urban cities with piped water, sanitation structures, adequate waste management, and proper drainage experience mortality rates of 10 per 1,000 live births. Urban cities without piped water, sanitation structures, adequate waste management, or proper drainage can experience a 10 to 20-fold increase in child mortality (50). The lack of basic water and sanitation infrastructure, in addition to residing in low-income, urban environments, creates various fecal exposure pathways which residents may experience frequently (53). Identifying the fecal pathways and populations who have frequent exposure to them could help local government administrators, local NGOs, and policy makers prioritize future sanitation investments and develop targeted interventions.

In the urban areas of Bangladesh, with the exception of Dhaka, sewage disposal and treatment do not exist. Though Dhaka has a sewage system, only 18% of the city's population has access to it. Pour-flush pit latrines are the most common sanitation facilities found in Bangladesh. However, most latrines are not made for children to use, only one in four are hygienic, and most do not have lids to separate the feces from the environment (54). Children have reported to be afraid of using latrines due to the smell, fear of the dark, and falling inside. As a result, latrine usage is low, and open defecation practices are high among children (57). Although 84% of schools in Bangladesh have toilets, only 24% are functional and 45% are unlocked. Due to these factors, it is common for young children in Bangladesh to defecate in the living environment which increases the risk of disease transmission (54).

D. Sanitation in Dhaka, Bangladesh: The Role of Canals and Open Drains

Bangladesh's rapid urbanization has created a sanitation crisis due to poor waste management and high frequencies of exposure to fecal contamination (58). Environmental contamination of enteric pathogens is a widespread health challenge and environmental hazard in Bangladesh (59). Dhaka is home to 1,500 miles of open drains which are often directly in front of homes. At least 36% of residents in Dhaka lack access to sanitation facilities. As a result, it is common for residents of Dhaka to use roadside drains to defecate. The drains contain high concentrations of fecal sludge and pose a household, public health, and environmental hazard. It is common for children to play around and in the open drains, which increases their risk of exposure to fecal matter and contracting enteric infections (60).

The canals in Dhaka were initially meant to serve as a natural drainage system, water reservoir, and river route (61). The rapid urbanization and growth in population experienced by Dhaka have destroyed the ability for canals to serve as an effective drainage system. As a result, Dhaka experiences devastating flooding after heavy rainfall and drainage congestion (62). As of 2007, there are 57 kilometers of open canals in Dhaka, and their current infrastructure cannot adequately manage floodwater (63). Similar to drains, canals contain high concentrations of fecal sludge. The open canals serve as popular dumping grounds for fecal matter and garbage. The buildup of fecal matter and garbage has aided in the shrinkage of the canals (62). A study conducted in 2003 found 3% of Dhaka slum households drank water from drainage canals (64).

Children are also found frequently playing in canals which increases their risk of exposure to fecal matter and contracting enteric infections.

Though there are pit latrines constructed in Dhaka, Bangladesh, only one in four are found to be hygienic, and most do not have lids to separate the feces from the environment (54). Pit latrines often empty directly into drains which flow throughout the city in both slum and non-slum areas (60). In Dhaka, there are also hanging latrines which extend over bodies of water, such as rivers and canals (60). They are constructed with nonmetallic material such as wood, bricks, and concrete which are susceptible to the leakage of feces (65). Sludge from latrines pollutes the drains, rivers, and canals in the city (65). It is estimated that more than 80% of fecal sludge from latrines is not safely managed (60). Fecal sludge management is a challenge in Dhaka, Bangladesh due to nonexistent or inadequate sewage systems and lack of fecal sludge treatment plants (65). Improvements in fecal sludge management practices should be implemented to maintain effective drainage and protect the health of urban community members.

Most buildings in Dhaka are not connected to a sanitation system, and the discharge of fecal sludge often ends up in canals, lakes, and rivers (66). Though uncommon, some areas in Bangladesh, including Dhaka, have decentralized sanitation facilities in the form of septic tanks and anaerobic baffled reactors. In Dhaka, wastewater from septic tanks and anaerobic baffled reactors is discharged to open drains and canals. The limited removal of microbial pathogens by septic tanks and anaerobic baffled reactors can be

examined by quantifying pathogenic microorganisms in the effluent that is discharged into the environment.

Bangladesh experiences a high prevalence of enteric disease year-round, yet there is limited data available on environmental fecal contamination associated with the different exposure pathways such as canals and drains in urban Dhaka (58). A recent, cross-sectional study was conducted to assess the scale of fecal contamination in urban Dhaka by collecting ten different types of environmental samples in ten neighborhoods. The results of this study found high levels of *E. coli* fecal indicator bacteria in numerous environmental samples, including those from open drains, surface waters, and flood waters (58). Although there have been a few studies which have quantified the levels of fecal contamination in food items, there are even fewer studies quantifying the levels of fecal contamination in samples collected from open drains and canals (58,67). Monitoring enteric pathogens in fecal pathways, particularly where individuals are most exposed, may aid in the control or reduction of diarrheal diseases in Bangladesh. Identifying the fecal pathways and populations who have frequent exposure to them can aid in the development of targeted interventions to reduce their exposures to fecal contaminants and inform decisions about sanitation investments.

E. Detection Methods for Environmental Enteric Pathogens

Detecting enteric pathogens in the environment where individuals may be exposed can inform efforts to prevent transmission of enteric infections. Though there are numerous

methods to detect and quantify enteric pathogens in environmental samples, this literature review will discuss microscopy and polymerase chain reaction methodologies.

Microscopy is considered the gold standard in parasite detection as it can detect both cysts and trophozoites. However, to confirm the presence or quantify the pathogen that is present in the sample, microscopy is often supplemented with another detection method such as PCR. While microscopy is a relatively simple method and effective in parasite detection, training in microscopy is needed and microscopes can be costly (68). Microscopy has been used to detect *Giardia* in stool and water samples (69).

Polymerase Chain Reaction (PCR) is a method used to amplify a specific DNA segment within a target genome. PCR is able to detect the presence or absence of specific DNA in a sample – either a clinical specimen or an environmental sample. The materials required for PCR are primers, deoxynucleotide triphosphates, magnesium chloride, template DNA, an appropriate reaction buffer, DNA polymerase, a 96-well plate, and a PCR machine. There are three steps to PCR: denaturation, annealing, and extension. During the first step, DNA is denatured at high temperatures which range from 90-97 degrees Celsius. Afterwards, primers anneal to the DNA template at temperatures which range from 50-60 degrees Celsius. During the last step, extension takes place at the end of the annealed primers to replicate the DNA segment at approximately 72 degrees Celsius (70). PCR is advantageous because it is a simple technique to understand, produces results in approximately 2 hours, can be very sensitive and specific, and can be used to detect various pathogens (71). However, it is prone to contamination, can only identify known

pathogens, requires sequence data for PCR primer design, and gel electrophoresis must be performed to observe the amplified DNA fragments (72). Conventional PCR is able to detect the presence or absence of the target pathogen DNA in a sample, however, it does not have the ability to quantify the DNA in the sample. Real-Time PCR, also known as Quantitative Real-Time PCR (qPCR) can measure how much initial DNA is present in a sample during amplification (72). To quantify the DNA from a sample, qPCR results must be compared to the signal from a standard, known amount of DNA. Real-Time PCR does not require subsequent gel electrophoresis. Instead, fluorescent dyes are used to react with the amplified DNA while being simultaneously measured. Since gel electrophoresis is not required, qPCR provides more rapid results (70). Unlike the previous methods described, PCR has been used to detect and quantify nucleic acid from *Salmonella* Typhi, *Vibrio cholerae, Shigella,* Norovirus, and *Giardia* in environmental samples (15,73–76).

F. Enteric Pathogens in the Environment

Shigella. *Shigella* has been detected in wastewater, rivers, recreational water, sewage, surface water, and drinking water (77–82) An outbreak of shigellosis associated with a recreational spray fountain occurred in 1997. Of the seven cases, 4 (57.1%) tested positive for *Shigella sonnei* by pulse field gel electrophoresis, but *Shigella* was not detected in any of the water samples by culture methods (79). In 1985, there was an outbreak of shigellosis associated with a recreational lake. Of the 68 people involved in the outbreak, 29 (42.6%) cases tested positive for *Shigella sonnei* and 4 (5.9%) cases tested positive for *Shigella boydii*. Similar to the study in 1997, water samples tested positive for *E. coli* by culture methods, but shigella was not detected (82). In Northern

Israel, there was an outbreak of shigellosis due to the contamination of the community's drinking water supply. A sewage pipe broke and leaked into a well and contaminated their drinking water supply. Though *Shigella* was not detected in the water samples, *Shigella* were identified in stool samples from several cases using culture methods (77). Attempts to detect *Shigella* in environmental samples have not only been reported in association with outbreak investigations, but also in environmental studies. One study analyzed 40 water samples from the Narmada River in India for *Shigella*. They found 23 (57.5%) samples to be positive for *Shigella flexneri,* 10 (25%) for *Shigella sonnei,* and 7 (17.5%) for *Shigella dysenteriae* (81) by multiplex PCR. A study conducted in China collected and analyzed 35 sewage samples to detect *Shigella*. Using PCR, 23 (65.8%) raw sewage samples tested positive for *Shigella* (80). Another study collected a total of 128 surface water samples and analyzed them for *Shigella* in Dhaka, Bangladesh by PCR. Of the 86 river water and 42 lake water samples collected, 10 (11.6%) river water and 4 (9.5%) lake water samples tested positive for *Shigella* (78).

Norovirus. NoVs have been detected in surface waters, sewage waters, estuarine environments, and wastewater (15,83–85). A study in Singapore collected urban surface water samples from rivers and canals which were receiving stormwater runoff. A total of 60 water samples were analyzed using semi-nested RT-PCR for the presence of NoVs. A total of 43 of the 60 samples (71.7%) tested positive for NoV. Of these positive samples, 4 (9.3%) contained only GI strains, 16 (37.2%) contained only GII strains, and the remaining 23 (53.5%) contained both GI and GII strains (83). In central Italy, a total of 97 sewage samples (inflow and outflow) were collected from wastewater plants. Nested

RT-PCR was conducted to detect the presence of NoV in the sewage samples. Overall, 88 samples tested positive for NoV, 62 (96.9%) were influent samples and 26 (78.7%) were effluent samples. Of the positive samples, 19 (19.6%) contained only GI strains, 2 (2.1%) contained only GII strains, and 67 (69.1%) samples tested positive for both genogroups (15). A study was conducted to observe the distribution of NoV in estuarine environments. Water samples were obtained from two estuaries, Wassaw and Sapelo, off the coast of Georgia, USA. Of the 72 water samples collected, 6 tested positive for human NoV (8.3%) by RT-PCR (84). The average concentration of NoV genome copies was $1.9X10^4$ ml^{-1}. Genogroup GI NoV were detected at the highest concentration of viral genomes at $1.4*10^6$ ml^{-1}. A study in Sweden collected wastewater samples every month for one year at eight different sites to study the concentrations of NoVs GI and GII by RT-PCR. The average NoV GI concentration for the year was $3.2*10^5$ genome equivalent (g.e.) per liter H_2O, whereas the average NoV GII concentration for the year was $4.1*10^5$ g.e. per liter H_2O. Higher concentrations of GII NoVs were detected during the winter months, and higher concentrations of GI NoVs were detected during the summer months. Most notably, NoVs were detected from samples collected during the whole year, underscoring the persistence of NoV throughout the year (85).

***Salmonella* Typhi.** *Salmonella* Typhi has been detected in wastewater, brackish water, surface water, and municipal drinking water. A study conducted in the Yamanashi Prefecture, Japan reported the detection of *Salmonella* isolates in wastewater. Of the 157 *Salmonella* isolates obtained from wastewater, real-time PCR results indicated that one isolate tested positive for *Salmonella* Typhi (0.64%) (86). This finding suggests

wastewater should undergo further treatment to ensure *Salmonella* Typhi microorganisms are eliminated before being discharged into the environment. One study observed the survival of *Salmonella* Typhi in brackish waters. The culture method was used to detect *Salmonella* Typhi in environmental samples, and linear regression was used to estimate T_{90} values (time for 90% of the *Salmonella* Typhi microorganisms in the sample to die off) to assess persistence. *Salmonella* Typhi was found to survive in brackish waters year-round with T_{90} values ranging from 20 to 165 hours (87). *Salmonella* Typhi has also been detected in surface waters in coastal communities in the Bayelsa State of Nigeria by culture methods. Approximately 75% of river water samples tested positive for *Salmonella* Typhi by culture methods (88). These data suggest that surface waters in the Bayelsa State are highly contaminated and should not be used for drinking purposes or recreational use. In Kathmandu, Nepal, *Salmonella* Typhi was detected in multiple municipal drinking water sources by quantitative RT-PCR. Of the 432 water samples analyzed, 333 (77%) tested positive for *Salmonella* Typhi DNA. The median number of genome copies per reaction was estimated to be 208 (73). This study concluded that municipal water may be an important vehicle for *Salmonella* Typhi transmission in Kathmandu.

Giardia. *Giardia* has been detected in sewage, surface water, wastewater, recreational water, and drinking water sources (89–93). One study conducted in Quebec, Canada collected six sewage samples from wastewater treatment plants. In all six samples, *Giardia* (assemblages A and B) was detected by qPCR (90). In the Galicia community of Spain, 116 river water samples were collected from 29 sampling sites during each season.

Giardia cysts were found in 78 (67%) samples and 29 (100%) sampling sites by PCR. In the spring and summer seasons, there was a higher prevalence of *Giardia* cysts in the samples than in the fall and winter seasons (91). One study reviewed 25 studies which used either RT-PCR, light microscopy, or immune fluorescence to detect *Giardia* in wastewater samples. A total of 21 (85%) of these studies reported detection of *Giardia* in wastewater samples from 17 different countries. The detection rates of *Giardia* in wastewater samples from South America, the Caribbean, the Middle East, and Southeast Asia ranged between 8 to 40%. The detection rates of *Giardia* in wastewater samples from countries in Western Europe, North America, Australia, and New Zealand ranged from 2 to 7% (92). In Selangor, Malaysia, *Giardia* was detected in recreational lakes. Recreational water samples were collected from a total of 13 stations. Water samples from 9 (69%) stations tested positive for *Giardia.* A range of 0.2 to 10.4 *Giardia* cysts per liter were detected by immunomagnetic separation (93). In Sao Paulo State, Brazil, *Giardia* was detected in drinking water. One study collected 206 samples of drinking water and found that 102 (49.5%) samples tested positive for *Giardia* using immunomagnetic separation-immunofluorescence assay. The concentrations of *Giardia* in the drinking water samples ranged from 0.1 cyst/L to 97 cysts/L (89).

Vibrio cholera. *Vibrio cholerae* has been detected in a wide range of environmental samples, including village tube wells, ponds, rivers, coastal waters, seas, estuarine waters, ground water, sewage water, tap water, and surface water (75,94–98). Water samples were collected from village tube wells, ponds, and a river in the endemic region of Matlab, Bangladesh. The river and ponds where the water samples were collected were

used by the community to bathe, wash, cook, and drink. Six river water samples and seven pond water samples were collected and analyzed using culture methods which included the most-probable-number index. The most-probable-number estimates for all of the river and pond water samples were <0.3 *V. cholerae* O1 cells per 100 ml except for one river water sample which had 2.0 *V. cholerae* O1 cells per 100 ml (94). Over the course of two years, 50 coastal water samples were collected in Peru and tested for *Vibrio cholerae, V. cholerae* O1, and *V. cholerae* O139 by PCR. The investigators reported that 33 (66%) of the water samples tested positive for *Vibrio cholerae*, 32 (62.5%) had *V. cholerae* O1 and none tested positive for *V. cholerae* O139 (75). From the Pearl River in the Guangzhou area, 146 estuarine water samples were collected over four months. The study reported that 29 (19.9%) of the samples contained *V. cholerae* by an Immunofluorescent-Aggregation Assay, and 43 (29.5%) samples were positive by PCR (95). In Isfahan, Iran, a study collected 144 tap and 304 bottled drinking water samples. The drinking water source for the study site was river water. The investigators found that only 3 (2.08%) tap water samples tested positive for *V. cholerae* and no bottled drinking water samples tested positive for *V. cholerae* by PCR (96). In Nkonkobe, South Africa, one study determined the presence of *V. cholerae* in drinking water by collecting and analyzing surface and groundwater samples by both culture methods and PCR. Toxigenic *V. cholerae* was detected in the 100% of the surface and 25% of the groundwater samples. (97). One study collected ground, sewer, and tap water samples in Gwalior, India and seawater from Marina Beach in Chennai, India which were then spiked with *V. cholerae* O1. Immunological biosensors to detect *V. cholerae* O1 in the samples and

found the limit of detection for sewer and tap water was 80 CFU/ml and 8 CFU/ml for tap and seawater (98).

G. Quantitative Microbial Risk Assessment

Quantitative microbial risk assessment (QMRA) is a methodology which integrates information on human behaviors, pathogen occurrence, and pathogen infectivity to estimate health risks associated with exposure. This approach has been used to estimate health risks attributable to contaminated water supplies (99). QMRA can be used to examine health risks associated with exposure to various environmental hazards, such as direct contact with contaminated drain and canal waters, and health risks. In this study, the QMRA framework consisted of four parts: 1) hazard identification, 2) dose response analysis, 3) exposure assessment, and 4) risk characterization. Hazard identification was the process of identifying pathogens associated with human illness. Dose response analysis was the process of observing the relationship between specific levels of pathogen exposure and the likelihood of infection. Exposure assessment was the process of identifying the affected populations, the relevant exposure pathways, amount of exposure, and distribution of doses associated with various exposures. Finally, risk characterization integrated the previous steps to estimate the magnitude of the health risks associated with exposures (100).

QMRA is beneficial because it is evidence-based and considers all components needed to efficiently estimate health risk. It can estimate low and high levels of risk and disease for various exposure routes in outbreak and non-outbreak conditions. QMRA can provide a

scientific basis for evaluating risk management and control strategies at a low cost (100). However, QMRA does have limitations because it relies on availability of data. One limitation is the lack of data on pathogen occurrence and fate and transport that is currently available. Since QMRA is dependent on its model inputs, without reliable data, QMRA will need to rely on default assumptions. Default assumptions use conservative data and estimates which may overestimate the true risk associated with exposure (101). Despite limited data however, QMRA can still provide valuable information for risk management (102).

There have been QMRAs that have estimated the burden of diarrheal diseases associated with exposure to surface and drain waters. In Kampala, Uganda, a QMRA was conducted to estimate the daily and annual risk of infection from *Salmonella* spp. from surface water. Since the study did not collect behavioral data, the study assumed that each individual ingested 10 mL of surface water and did not differentiate risk of infection between adults and children. The average concentration of *Salmonella* spp. in the surface water was $1.9*10^5$ CFU per 100 mL. The highest concentrations of *Salmonella* spp. were found in surface water collected from a storm water drain. The daily probability of infection by *Salmonella* ranged from $9.39*10^{-2}$ to $2.17*10^{-1}$ and the yearly probability of infection range from $4.04*10^{-1}$ to $4.47*10^{-1}$ (103). Future QMRAs assessing the burden of diarrheal disease associated with exposure to surface water should conduct behavioral studies to determine how much contact an individual has with surface water in the specific study area. Instead of using arbitrary ingestion volumes, incorporating specific ingestion volumes by exposure source can be useful for creating more accurate dose-

response models. Lastly, future assessments can differentiate risks between adults and children.

In Côte d'lvoire, West Africa, a QMRA estimated the yearly probability of infection from *Giardia lamblia.* The study examined whether there was a link between giardiasis and consuming wastewater-contaminated foods and farming with wastewater irrigation. Researchers found green salad to be watered using contaminated wastewater by farmers. This study conducted surveys to obtain information on the frequency of exposure to wastewater and the quantity of green salad consumed daily. Wastewater from drains was found to be the biggest source of contamination. The yearly probability of *Giardia lamblia* infection associated with food consumption ranged from 0 to 0.0151. The yearly probability of *Giardia lamblia* infection associated with farming ranged from 0.0096 to 0.0397 (104). This study provided an estimate of risk of *Giardia lamblia* associated with consuming wastewater-contaminated foods and farming with wastewater irrigation. Future QMRAs on this topic could examine the risk of infection by *Giardia lamblia* separately for adults and children. Incorporating different types of exposures to open drains and canals can be useful in determining the risk of infection by *Giardia.*

A QMRA was conducted in Bangladesh to estimate the burden of cholera associated with urban flood waters. The study examined the association between urban flooding and cholera from direct contact with flood water. The behaviors included in the study were collected from those who lived in slum areas and areas comprised of middle-class and poor individuals. The average intake volumes used for adults and children were 3.5 mL

and 37 mL, respectively (105). The study found children in the slum environments to be in contact with flood waters daily as it was often involved in their play activities. Adults from the slum environments and poor areas were found to have had contact with flood waters for at least one hour per day during their walks to work. *Vibrio cholera* concentrations ranged from 10^3 to 10^5 organisms per 100 mL of flood water. This QMRA estimated that the daily risk of illness from cholera to range from $5.2*10^{-5}$ to $2.2*10^{-3}$, with the highest risk attributed to children in the living in the slum and the lowest risk attributed to middle-class adults (99). The intake volumes used for this QMRA were obtained from a study in the Netherlands. Since the environment of a high-income setting is different from a low-income setting, future QMRAs conducted in Dhaka could utilize exposure parameters and ingestion volumes for low-income settings to obtain a more accurate estimate of health risks. Future QMRAs conducted in the Dhaka could also assess the risks of enteric infections associated with open drain and canal exposure routes in children and adults who live in the slum areas. Lastly, to assess the risks of enteric infections and diarrheal diseases in Dhaka, different QMRA models could be conducted to assess the risk of infection for numerous enteric pathogens.

H. Study Objectives

In low-income urban settings such as Bangladesh, canals and open drains have been identified as fecal exposure pathways. The limitations of estimating the concentrations of pathogens in these fecal exposure pathways and their associated health risks motivated this study. The goal of the study was threefold:

1) Examine the presence of the selected pathogens, Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi, in open drains and canals and quantify their concentrations
2) Determine if the occurrence and concentrations of these selected pathogens differ between open drains and canals
3) Estimate the health risks of exposure to fecal contamination via canals and open drains in children and adults

The results of this study will provide useful evidence to effectively prioritize future investments in sanitation infrastructure. The results can also be used by local government administrators, local NGOs, and policy makers to inform the development of targeted interventions to reduce exposure to fecal contamination in open drains and canals. This study's findings can also be used to develop public health communications about the health risks associated with canals and open drains for urban community members.

CHAPTER II: MANUSCRIPT

A. Title, Authors, Abstract

Identifying Enteric Pathogens and Assessing Their Health Risks in Urban Environments of Dhaka, Bangladesh

By Maria Julia Bianca Garcia Corpuz

In densely populated urban environments such as Dhaka, Bangladesh, frequent exposure to fecal contamination may cause a high risk of enteric infections and diarrheal diseases. Children and adults are frequently exposed to ubiquitous open drains and canals in their immediate environment, which serve as outlets for wastewater and feces disposal in Dhaka city. A cross-sectional study was conducted to detect and quantify pathogens in open drains and canals and estimate monthly risks of infection from exposure to these hazards. This study was conducted in two densely populated areas of Mirpur, Dhaka from April 2019 to October 2019. Selected enteric pathogens, Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi, were quantified in environmental samples using ultrafiltration, polyethylene glycol precipitation (PEG), total nucleic acid (TNA) extraction, and polymerase chain reaction (PCR) methods. Monthly risks of pathogen-specific infections in adults and children from open drain and canal exposure were estimated by quantitative microbial risk assessment (QMRA) using Monte Carlo simulations. The average concentration of pathogens ranged from 0.54 $\log_{10}$ genomic copies/100 mL of *Giardia* in canal samples to 4.16 $\log_{10}$ genomic copies/100 mL of *Shigella* in drain samples. The highest monthly risks of infection from exposure to open drains in adults and children were associated with Norovirus GII and the lowest with *Salmonella* Typhi. The probability of pathogen-specific infection from canal exposure was highest for adults and children for *Vibrio cholerae* and the lowest for *Giardia*. Because of the high concentrations of pathogens detected in both open drain and canal water samples, any contact with open drains or canals will likely result in a high level of exposure to fecal contamination and therefore an increased risk of infection by enteric pathogens. The results from this study should be used to guide public health communications and the development of targeted interventions to reduce exposure to fecal contamination in open drains and canals in Dhaka, Bangladesh.

B. Introduction

As the population continues to rise, it is estimated that 70% of the global population will be urbanized by 2050 (50). As of 2018, about 55% of urban populations have been reported to live in urban slums, one third of them being children (51). In Bangladesh, it is

projected that by 2030, 40% of the population will reside in urban settings. This is a drastic increase from 1974 when only 8.8% of the population was urbanized (52). Bangladesh's rapid urbanization has created sanitation crises due to poor waste management and high levels of exposure to fecal contamination (58). In dense, urban environments, exposure to fecal contamination contributes to the risk of enteric infections and diarrheal diseases (55). Environmental contamination with enteric pathogens is a widespread health and environmental hazard challenge in Bangladesh (59). Identifying pathways of fecal exposure and populations who are normally exposed to those pathways will provide useful evidence to inform the development of targeted interventions to reduce exposure to those pathways and effectively prioritize future investments in sanitation infrastructure.

Dhaka is home to 1,500 miles of open drains which are often built directly in front of homes. More than 36% of Dhaka residents lack access to sanitation facilities. As a result, it is common for residents of Dhaka to use roadside drains for defecation (60). As of 2007, there are 57 kilometers of open canals in Dhaka, and they serve as popular dumping grounds for fecal matter (62,63). Though uncommon, some areas in Bangladesh, such as Dhaka, have decentralized sanitation in the form of septic tanks and anaerobic baffled reactors. In Dhaka, effluent from septic tanks and anaerobic baffled reactors are often discharged to open drains and canals (106). Drains and canals contain high amounts of fecal sludge and can be an environmental hazard for households ad the community. Residents of Dhaka have frequent contact with open drains and canals, and

children are often playing around, or in, these fecal exposure pathways which may put them at risk of ingesting fecal matter (60).

Bangladesh experiences a high prevalence of enteric disease year-round, yet there is limited data available on the fecal contamination associated with the different environmental exposure pathways, such as canals and drains, in urban Dhaka (58). Detecting and quantifying enteric pathogens in the environment, such as *Shigella,* Norovirus*, Salmonella* Typhi, *Giardia*, and *Vibrio cholera* which cause enteric and systemic diseases, can aid inform strategies for reducing the risk of exposure to these pathogens. Molecular methods, such as Quantitative Real-Time PCR (qPCR), can detect and quantify how much of the specific pathogen DNA (or RNA when using reverse transcription PCR) is present in a given environmental sample (72). These data are useful for dose inputs in quantitative microbial risk assessment (QMRA).

QMRA is a methodology which integrates information on human behaviors, pathogen occurrence, and pathogen infectivity to estimate health risks associated with exposures to specific pathogens (99). A QMRA was conducted in Bangladesh to estimate the risk of cholera associated with urban flood waters. This QMRA estimated the daily risk of illness from cholera to range from $5.2*10^{-5}$ to $2.2*10^{-3}$, with the highest risk attributed to children living in the slum areas who had the most exposure to flood waters and the lowest risk attributed to middle-class adults who had the lease exposure (99). This study provided a solid foundation for the estimate of the risk of cholera associated with flood waters. Building on the work of this study, we propose to conduct QMRAs to estimate

the risks of infection and disease for children and adults to a range of viral, bacterial and protozoan pathogens associated with exposure to open drains and canals in Dhaka. These investigations can provide evidence to improve human fecal management and reduce exposure to enteric pathogens in densely populated, urban populations. The goals of the study include:

1) Examine the presence of the selected pathogens, Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi, in open drains and canals and quantify their concentrations
2) Determine if the occurrence and concentrations of these selected pathogens differ between open drains and canals
3) Estimate the health risks of exposure to fecal contamination via canals and open drains in children and adults

C. Methods

The study was conducted from April 2019 to October 2019 in the Duaripara and Bauniabadh areas of Mirpur, Dhaka. The study includes the following laboratory and data analysis: 1) ultrafiltration of 20 L water samples followed by polyethylene glycol (PEG) precipitation or PEG precipitation alone for small volume grab samples, 2) total nucleic acid (TNA) extraction, 4) Singleplex quantitative real-time polymerase chain reaction (PCR) for *Shigella, Salmonella* Typhi, *Giardia*, and *Vibrio cholera* [and reverse transcription-quantitative real-time PCR for detection of noroviruses] , 5) two-sample t-tests, 6) Fisher's exact tests, and 7) Quantitative microbial risk assessment (QMRA).

i. Study Site Selection

Before selecting study sites, collaborators from the International Center for Diarrheal Disease Research, Bangladesh (icddr,b) held informal meetings with Water and Sanitation for the Urban Poor (WSUP), Dhaka Water Supply and Sewerage Authority (DWASA), Dhaka North City Corporation, and numerous international NGOs to develop the selection criteria based on the sanitation context in urban Dhaka. The following population and physical characteristic criteria were considered: 1) high population density, 2) low-income status, 3) sanitation technology variation, 4) high exposure through drainage and surface waters, and 5) simple topography with uncomplicated hydraulic and drainage characteristics. Study sites were also chosen based on results from the SaniPath fecal exposure assessment protocol conducted in Dhaka previously (107). In Duaripara, four out of nine roads were selected for the study: Roads #1, 6, 7, and 8. The roads contained an average of 46 household compounds each and 10 septic tanks/containment tank/latrine pit coverage. In Bauniabadh, anaerobic baffled reactors constructed by NGOs supported by WSUP were selected for the study.

ii. Environmental Sample Collection

In Duaripara, 400 mL (grab samples) and 20 L (ultrafiltration samples) of drain, canal, flood, and septic tank effluent, supernatant, and sludge were collected. 20 L volume samples were collected so that the pathogens could be concentrated from a large volume to increase the likelihood of pathogen detection when conducting ultrafiltration. 200 grams of sediment samples were collected from drains, but not from other locations. 400 mL volume samples of effluent, supernatant, and sludge were collected from septic tanks.

In Bauniabadh, 400 mL volume samples of effluent, supernatant, and sludge were collected from anaerobic baffled reactors (ABRs). All drain water samples and grab samples collected from canals were collected at the end of the drains. The ultrafiltration samples collected from canals were collected from the middle of the canal. Sediment samples were collected from the middle of each drain. The floodwater samples were collected within 24 hours of rain from the overflow of drains. The sludge and supernatant samples obtained from ABR and septic tanks were collected from the first chambers, and the effluent samples were collected from the last chamber outlets near the drains (Figure 2.1).

iii. Data Collection

All data collection was facilitated by fieldworkers who had intimate knowledge of the neighborhoods and helped guide the selection of sampling sites. All paper forms completed in the field were entered into a Microsoft Excel sheet (Microsoft, Redmond, WA) managed by the study team in icddr,b. The Microsoft Excel sheet (Microsoft, Redmond, WA) was used to record the sample type collected and the time, date, and location of sample collection.

Pathogen Selection

Environmental sample analysis consisted of two phases: 1) a pilot phase, and 2) an implementation phase. Pathogen suitability, sample size, and analytical methods were assessed during the pilot phase. TaqMan Array Card (TAC) analysis and qPCR were conducted to assess pathogen suitability. Enteric pathogens that had been found to be

detectable with good sensitivity and specificity using quantitative PCR were chosen to be candidate target pathogens for the study. Candidate pathogens were examined in eight environmental samples using TaqMan Array Card (TAC) analysis and qPCR detection (108). Based on the results, six target pathogens were selected for the implementation phase. The following pathogens were selected for environmental sample analyses: 1) *Shigella*, 2) *Vibrio cholerae*, 3) Norovirus GII, 4) *Giardia*, and 5) *Salmonella* Typhi. Other factors that contributed to pathogen selection included: 1) high prevalence of infection in Dhaka among adults and children, 2) availability of environmental transport data, 3) prolonged persistence in the environment, 4) availability of qPCR methods to detect specific pathogens in environmental samples, 5) human specificity, 6) availability of dose-response data, 7) availability of data on duration and magnitude of fecal shedding, and 8) importance of sanitation for control.

Sample Collection

Samples were collected each month from April 2019 to October 2019 by icddr,b study staff. On average, samples were collected nine days each month totaling 154 collected samples. Seven different sample types were collected for the study. The environmental samples collected for this study were: drain water, drain sediment, flood water, and canal water. The sanitation samples collected for this study were: sludge, effluent, and supernatant from ABRs and septic tanks. In each sampling round, each environmental sample type was collected using sterile technique in 500 mL or 2 L Whirl-Pak® (Nasco, Fort Atkinson, WI) or sterile buckets. The environmental samples were collected from

drains and canals where children were seen playing or where people were seen to have had contact.

Environmental Sample Processing

The 400 mL volume samples were concentrated using PEG precipitation, centrifugation, and TNA extraction as shown in Figure 2.2. The 20 L volume samples were concentrated using ultrafiltration methods also shown in Figure 2.2 followed by PEG precipitation, and TNA. Drain sediment samples were concentrated using TNA extraction.

iv. Laboratory Methods

Ultrafiltration (UF) Methods

Tangential flow ultrafiltration was conducted to concentrate the 20 L volume samples collected from drain and canal waters within 1-4 hours of sampling. A dispersant which composed of 10 mg per liter of sodium polyphosphate (NaPP) (Sigma-Aldrich, St. Louis, MO, USA) was added to the sample before ultrafiltration began. The apparatus used for UF was set up as described by Liu et al. (109). A Polynephron Synthetic Hemodialyzer ultrafilter (NIPRA, Medical Corporation, FL, USA) was connected to a pressure gauge, flow meter, and peristaltic pump (Cole Parmer Instrument Co., Vernon Hills, IL, USA) using silicon tubing (Masterflex; Cole-Parmer Instrument Co.). The first step in the ultrafiltration process was concentrating the 20 L volume samples to a retentive volume of approximately 100 ml. An elution step of removing pathogens that were adsorbed onto the silicon tubing and ultrafilter using an elution solution followed. The elution solution was composed of 0.01% Tween 80, 0.01% NaPP, and 0.001% antifoam A Y-30 emulsion

in 1X PBS. The elution solution was pumped at 2000 ml per minute until approximately 100 ml of elution solution was collected. The elution step was followed by a back flushing procedure. The back flushing procedure releases the pathogens from the ultrafilter's inner surface using a backflush solution. The backflush solution was composed of 0.05% Tween 80, 0.01% NaPP, and 0.001% antifoam A Y-30 emulsion. After pumping the backflush solution at 650 ml per minute through the permeate port of the ultrafilter, approximately 200 ml of backflush solution was collected. The solutions collected from the concentration, elution, and back flush steps were combined, and the combined total volume of approximately 400 ml was recorded. The combined solution was collected for PEG precipitation.

Pathogen Concentration Using Polyethylene Glycol (PEG) Precipitation

Pathogens present in the combined solution from the ultrafiltration procedure were precipitated by adding 12% polyethylene glycol 8000 (PEG 8000) (Sigma, St. Louis, MO, USA), 0.9 mol sodium chloride, 1% bovine serum albumin (Sigma). This mixture stirred overnight using a magnetic stir bar. The next day, the mixture was centrifuged at 13,000 g for 30 minutes. The pelleted material was resuspended in 1 ml InhibitEX buffer obtained from the QIAmp East DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) for subsequent TNA extraction.

Pathogen Total Nucleic Acid (TNA) Extraction

For all samples and extraction controls, approximately 370 mg of beads (Sigma, Minnesota, USA) were poured into a 2 ml microcentrifuge tube. 1 ml of the sample

suspension from the PEG precipitation procedure was added to the same 2 ml microcentrifuge tube. After vortexing the microcentrifuge tube for one minute, the solution was mixed at maximum speed for 2-3 minutes. The suspension was incubated at 95°C for 5 minutes and then followed by full speed centrifugation for 15 seconds to pellet the sample particles. 600 μl of the supernatant, 25 μl of proteinase K, and 600 μl of buffer AL were transferred to a 2 ml microcentrifuge tube. After vortexing the microcentrifuge tube for 15 seconds, it was incubated at 95°C for 5 minutes. Subsequently, 600 μl of 100% ethanol was added to the lysate, and 600 μl of the lysate and ethanol solution was transferred to a QIAamp Mini column. The column was centrifuged at full speed for one minute. This procedure was repeated until all lysate was transferred to the same column. With 150 μl of supplied elution butter, TNA was eluted from the column and then aliquoted and stored at -80°C for subsequent PCR analysis.

Pathogen Detection Using TaqMan Singleplex Real-Time Polymerase Chain Reaction (PCR)

To detect DNA or RNA pathogens, quantitative Singleplex Real-Time PCR assays were performed on all extracted TNA using a Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories, Inc., Berkeley, CA). PCR primers and probes used for this study are shown in Table 2.1. Pathogen-specific protocols used in this study are described in detail elsewhere: Norovirus GII as described by Zhang et al. (110), *Vibrio cholera* as described by Huang et al. (111), *Salmonella* Typhi as described by Karkey et al. (73), *Giardia* by Narayanan et al. (in press), and *Shigella* by Thiem et al. (112). For Norovirus GII detection, reverse transcription and amplification reactions were performed using the

Qiagen OneStep RT-PCR Kit (Qiagen, Valencia, CA, USA). For *Vibrio cholera, Salmonella* Typhi, *Giardia,* and *Shigella* detection, a 25 µl reaction mix which contained 2X Bio-Rad iQ power mix buffer, dNTPs, 12 mM $MgCl_2$, iTaq DNA polymerase, 400 nM target pathogen and inhibition primer pairs, 200 nM pathogen and inhibition probes for each amplicon, and 5 µl of template TNA or negative control was used. The amplification procedure was as follows: 1) denature at 95°C for 3 minutes 2) anneal at 40-45 cycles of 95°C for 15 seconds, and 3) extend at 55°C-60°C for 30-45 seconds. To quantitatively estimate genomic copies of Norovirus GII, *Vibro cholerae, Shigella*, and *Giardia* in each sample, standards that were developed or purchased were serially diluted and included in the assays of samples determined to be positive. To quantitatively estimate genomic copies of *Salmonella* Typhi in each sample, we first ran PCR assays without standards and re-ran the positive samples with the standard to quantify the pathogen's DNA. To enumerate pathogens, Norovirus GII, *Vibrio cholera, Salmonella* Typhi, *Giardia,* and *Shigella*, in environmental samples, the standard curve method with a dilution series of a known concentration was used. DNA standards for *Vibrio cholera*, *Shigella*, and *Giardia* were obtained from Vircell Inc. (Parque Tecnológico de la Salud, Spain). The RNA standard for Norovirus and DNA standard for *Salmonella* Typhi were developed by Dr. Pengbo Liu at Emory University in the laboratory of Dr. Christine Moe. For all environmental water samples, absolute quantification was expressed as $\log_{10}$ genome equivalent copy numbers per 100 ml.

v. Statistical Methods

Statistical analyses were conducted in SAS 9.4 (SAS, Cary, NC). All statistical tests were evaluated at an alpha level of 0.05. The prevalence, mean, and 95% confidence intervals of pathogen-specific concentrations in environmental samples were calculated using univariate analysis. Fisher's exacts tests were used to determine if there were significant differences in pathogen-specific detection rates between canal and drain samples. Fisher's exacts tests were also used to determine if there were significant differences in pathogen-specific detection rates between ultrafiltration and grab samples. Two-sample t-tests were used to determine if there were significant differences in mean pathogen concentrations between drain and canal samples. Two-sample t-tests were also conducted to determine if there were significant differences in mean pathogen concentrations between ultrafiltration and grab samples.

Modeling and statistical analyses were conducted in R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). To cover all combinations of groups, pathogens, and reservoirs, 20 models were created and used to perform quantitative microbial risk assessment. Monte Carlo simulations were generated to estimate the daily risks of pathogen-specific infections and monthly risks of pathogen-specific infections in adults and children from drain and canal exposures in Dhaka, Bangladesh.

vi. Exposure Assessment

Quantitative Microbial Risk Assessment (QMRA) was conducted to determine pathogen-specific infection risk in four steps: 1) hazard identification, 2) exposure assessment, 3) dose-response, and 4) risk characterization. Models were created for this study's QMRA

for each target pathogen (Norovirus GII, *Vibrio cholera, Salmonella* Typhi, *Giardia,* or *Shigella*), reservoir (drain or canal), and group (children or adults). For each risk model, random samples of ten thousand were generated to describe the distribution of each variable.

Exposure Scenarios

Based on household survey data from a SaniPath exposure assessment study conducted in Dhaka in 2017, exposures to drain and surface water were identified. Exposure scenarios were defined by the environmental reservoirs (drain and canal) and the group who had contact with the reservoirs (children and adults). For this study, we used surface water as a proxy for canal water.

Exposure scenario A involved whether children had access to canal water, and if so, how many times per month they came into contact with the canal. Exposure B involved whether adults had access to canal water, and if so, how many times per month they had contact with the canal. Exposure C involved whether children had access to drain water, and if so, how many times per month they had contact with drains. Exposure D involved whether adults had access to drain water, and if so, how many times per month they had contact with drains. Each exposure scenario is detailed in Table 2.3.

Model Parameters

Pathogen concentrations (log10/100 mL) in environmental samples were obtained from the qPCR results from 8 samples for canals and 64 samples for drains (Table 2.2). Model

parameters used to estimate monthly exposure to canals and drains in children and adults and dose response parameters (to translate exposure dose into infection risk) are shown in Table 2.3 and Table 2.4, respectively. Exposure doses for drain water used in this study assumed every child directly ingested 1 mL of drain water and every adult ingested 0.06 mL of drain water per exposure event from SaniPath Phase 1 data. Exposure doses for canal water used in this study assumed every child directly ingested 49 mL of drain water and every adult ingested 3.7 mL of canal water per exposure event as assumed in previous studies (113,114).

Modeling

Each model was stratified by pathogen, reservoir, and group resulting in the creation of 20 separate models. Pathogen-specific concentrations were calculated from the qPCR data, stratified by reservoir. Then, a distribution was fitted to these data to simulate possible concentrations by random sampling. To allow for the high proportion of negative (no pathogen detection) samples, a two-step process was performed: 1) estimate the probability of having a pathogen concentration of 0 organisms/100 mL, and 2) estimate parameters of a lognormal distribution of nonzero pathogens concentrations. The Nelder-Mead method was used to obtain maximum likelihood estimates for the log normal parameters. Monte Carlo simulations were conducted to randomly sample 10,000 concentrations from the constructed (log normal with added zeros) distribution function.

To determine the frequency of exposure by group, behavior results from SaniPath Phase 1 data were integrated into the model. The questionnaire data categorized frequencies of

exposure into the following four groups: 1) more than 10 times in the past month, 2) 6 to 10 times in the past month, 3) 5 times or less in the past month, and 4) never. For prediction of exposure, a two-step random process was constructed. A categorical distribution was used to choose any of the 4 categories, using the collected responses. For the selected category, this was followed by a random sample using a uniform distribution using the frequency bounds from the questionnaire. Thus, a random sample of 10,000 was generated of daily contact frequencies for drain or canal waters for children or adults. The equation used to calculate the exposure is as follows:

$$exp = C * F * V$$

where exp is the exposure to the specific enteric pathogen, C is the concentration of the enteric pathogen in the selected reservoir, F is the frequency of contact with the selected reservoir, and V is the intake volume. The dose-response function was used to estimate the risk of infection upon exposure to a specific dose.

An exponential dose-response model was used to characterize risk of infection from *Giardia*. The exponential model used the following equation:

$$P_{dinf} = 1 - e^{-N*k}$$

where P_{dinf} is the probability of daily risk of infection, N is the exposure dose, and k is the probability of *Giardia* surviving to initiate infection. The value used for k is 0.0199 (115).

A fractional poisson dose-response model was used to characterize risk of infection from Norovirus GII. The fractional poisson model used the following equation:

$$P_{dinf} = p * (1 - e^{-N})$$

where P_{dinf} is the probability of daily risk of infection, N is the exposure dose, and p is the probability of nonzero exposure. The value used for p is 0.722 (116).

A beta poisson dose-response model was used to characterize risk of infection from *Vibrio cholerae, Shigella,* and *Salmonella* Typhi. The beta poisson model used the following equation:

$$P_{dinf} = 1 - \frac{\left(1 + N * \left(2^{\frac{1}{\alpha}-1}\right) - 1\right)^{-\alpha}}{n_{50}}$$

where P_{dinf} is the probability of daily risk of infection, N is the exposure dose, a is a maximum likelihood estimate, and n_{50} is the dose at which 50% of the study population is expected to be infected. The a values for *Vibrio cholerae, Shigella,* and *Salmonella* Typhi were 0.25, 0.265, and 0.175 respectively. The n_{50} values for *Vibrio cholerae, Shigella,* and *Salmonella* Typhi were 243, 1480, and 1,110,000 respectively (117–119).

To translate the probability of being infected within a month from the probability of being infected in a day, the following equation was used for each pathogen:

$$P_{m,inf} = 1 - \prod_{d=1}^{30}\left(1 - P_{d,inf}\right)$$

where P_{minf} is the pathogen-specific monthly probability of infection and P_{dinf} is the pathogen-specific probability of infection on day *d.*

D. Results

i. Enteric Pathogen Positive Rates in Open Drains and Canals

A total of 72 open drain and canal water samples were tested for the presence of Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi (Table 3.1). There were 64 water samples from open drains and 8 water samples from canals. In drain water samples, the pathogen detected most frequently was *Vibrio cholerae* at a detection rate of 92.2% (59/64), and the pathogen detected least frequently was *Salmonella* Typhi at a detection rate 26.6% (17/64) (Table 3.2). In canal water samples, the pathogen detected most frequently was *Shigella* at a detection rate of 100.0% (8/8), and the pathogen detected least frequently was *Giardia* at a detection rate 12.5% (1/8) (Table 3.2).

To assess for statistically significant differences in the pathogen detection rates between open drain and canal water samples, Fishers exact tests were conducted at an alpha level of 0.05 (Table 3.2). There were no statistically significant differences in the detection rates of Norovirus GII, *Vibrio cholerae, Giardia,* and *Shigella,* between open drains and canals. *Salmonella* Typhi was detected more frequently in canal water samples compared to drain water samples (p-value: 0.011).

Of the 64 drain water samples, there were 31 ultrafiltration samples (20 L) and 33 grab samples (400 mL). The ultrafiltration samples and grab samples were not matched samples but instead were collected from different drains on different dates. Microorganisms in the 31 ultrafiltration samples were concentrated by tangential flow

ultrafiltration before PEG precipitation, TNA extraction and qPCR analysis. Microorganisms in the 33 grab samples were only concentrated by PEG precipitation. The detection rates for all pathogens from the ultrafiltration samples collected from open drains were consistently higher than the detection rates for grab samples collected from open drains (Table 3.1). Norovirus GII*, Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi were detected in 83.9% (26/31), 93.6% (29/31), 51.6% (16/31), 100.0% (31/31), and 29.0% (9/31) of the ultrafiltration samples collected from open drains, respectively. Norovirus GII*, Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi were detected in 51.5% (17/33), 90.9% (30/33). 45.5% (15/33), 90.9% (30/33), and 24.2% (8/33) of the grab samples collected from open drains, respectively.

Of the eight canal water samples, there were four ultrafiltration samples and four grab samples. The detection rates for all pathogens from the ultrafiltration samples collected from canals were consistently higher than detection rates from the grab samples collected from canals, except for *Shigella* (Table 3.1). When ultrafiltration methods were used, Norovirus GII*, Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi were detected in100.0% (4/4), 100.0% (4/4), 25.0% (1/4), 100.0% (4/4), and 100.0% (4/4) of samples collected from canals, respectively. In contrast, Norovirus GII*, Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi were detected in 0.0% (0/4), 75.0% (3/4), 0.0%, 100.00% (4/4), and 50.0% (2/4) of the grab samples collected from canals, respectively.

To assess for statistically significant differences in pathogen detection rates from of open drain and canal waters between ultrafiltration and grab samples, Fishers exact tests were

conducted at an alpha level of 0.05 (Table 3.3). There were no statistically significant differences in detection rates of *Vibrio cholerae, Giardia, Salmonella* Typhi, and *Shigella,* between ultrafiltration and grab samples. Norovirus GII was detected more frequently in ultrafiltration samples compared to grab samples (p-value: 0.005).

ii. Enteric Pathogen Concentrations in Open Drains and Canals

A total of 72 open drain and canal water samples were tested to measure concentrations of Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi. There were 64 water samples from open drains and 8 water samples from canals. Pathogen concentrations were calculated only for samples which tested positive. The mean concentrations for Norovirus GII, *Vibrio cholerae, Giardia,* and *Shigella* were higher in drain samples than canal samples, whereas the mean concentration for *Salmonella* Typhi was higher in canal samples than drain samples (Table 3.4). In open drain water samples, the pathogen detected in the highest concentration was *Shigella* with a mean concentration of 4.16 $\log_{10}$ genomic copies/100 mL (SD: 1.19) and the pathogen detected in the lowest concentration was *Salmonella* Typhi with a mean concentration of 1.54 $\log_{10}$ genomic copies/100 mL (SD: 1.12). In canal water samples, the pathogen detected in the highest concentration was *Shigella* with a mean concentration of 3.71 $\log_{10}$ genomic copies/100 mL (SD: 1.05) and the pathogen with the lowest concentration was *Giardia* 0.54 $\log_{10}$ genomic copies/100 mL.

To assess for statistically significant differences in pathogen concentrations between open drain and canal water samples between ultrafiltration and grab samples, two-sample t-

tests were conducted at an alpha level of 0.05 (Table 3.4). There were no statistically significant differences in the concentrations of any of the target pathogens between open drain and canal samples.

Of the 64 drain water samples, there were 31 ultrafiltration samples and 33 grab samples. Of the 8 canal water samples, there were 4 ultrafiltration samples and 4 grab samples. Overall, the mean pathogen concentrations for grab samples were consistently higher than the mean pathogen concentrations for ultrafiltration samples (Table 3.5). In ultrafiltration samples, the pathogen detected in the highest concentration was *Shigella* with a mean concentration of 3.50 $\log_{10}$ genomic copies/100 mL (SD: 0.95) and the pathogen detected in the lowest concentration was *Salmonella* Typhi with a mean concentration of 0.94 $\log_{10}$ genomic copies/100 mL (SD: 0.69) (Table 3.5). In grab samples, the pathogen detected in the highest concentration was *Shigella* with a mean concentration of 4.73 $\log_{10}$ genomic copies/100 mL (SD: 1.05) and the pathogen with the lowest concentration was *Salmonella* Typhi 2.52 $\log_{10}$ genomic copies/100 mL (SD: 0.67) (Table 3.5).

To assess for statistically significant differences in pathogen concentrations between ultrafiltration and grab samples, two sample t-tests were conducted at an alpha level of 0.05 (Table 3.5). The mean concentration of Norovirus GII was higher in grab samples compared to ultrafiltration samples (p-value: 0.0023). The mean concentration of *Vibrio cholerae* was higher in grab samples compared to ultrafiltration samples (p-value: <0.0001). The mean concentration of *Shigella* was higher in grab samples compared to

ultrafiltration samples (p-value: <0.0001). The mean concentration of *Giardia* was higher in grab samples compared to ultrafiltration samples (p-value: <0.0001). The mean concentration of *Salmonella* Typhi was higher in grab samples compared to ultrafiltration samples (p-value: <0.0001).

iii. Exposure Assessment

The monthly risks of infection by Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi were estimated for open drain and canal exposures among children and adults in Dhaka, Bangladesh. All estimated pathogen-specific monthly risks of infection correspond to adults and children who reside in the study neighborhoods in Dhaka, Bangladesh. On average, the monthly risks of infection by Norovirus GII, *Vibrio cholerae, Giardia,* and *Shigella* from exposure to drain waters among adults and children were higher than the corresponding risks of infection from exposure to canal waters. In contrast, the monthly risks of infection by *Salmonella* Typhi from exposure to canal waters among adults and children were higher than the corresponding risks of infection from exposure to drain waters. The mean and 95% credible interval for the risks of pathogen-specific infections from drain exposure in children are presented in Table 3.6 and in adults are presented in Table 3.7.

The highest average monthly risks of infection from exposure to open drains in adults and children were for Norovirus GII and the lowest risks were for *Salmonella* Typhi. The average monthly risk of Norovirus GII infection among those who had exposure to open drains was 0.94 (95% credible interval: 0.72, 1.00) for adults (Table 3.7) and 0.99 (95%

credible interval: 0.92, 1.00) for children (Table 3.6). The average monthly risk of *Salmonella* Typhi infection among those who had exposure to open drains was 0.00003 (95% credible interval: 0.00001, 0.00011) for adults and 0.0024 (95% credible interval: 0.0004, 0.0180) for children. The average monthly risk of infection by *Vibrio cholerae* among adults who had exposure to open drains was 0.81 (95% credible interval: 0.47, 0.98). The average monthly risk of infection by *Vibrio cholera* among children who had exposure to open drains was 0.98 (95% credible interval: 0.88, 1.00). The average monthly risk of infection by *Giardia* among adults who had exposure to open drains was 0.29 (95% credible interval: 0.01, 0.89). The average monthly risk of infection by *Giardia* among children who had exposure to open drains was 0.75 (95% credible interval: 0.16, 1.00). The average monthly risk of infection by *Shigella* among adults who had exposure to open drains was 0.55 (95% credible interval: 0.20, 0.85). The average monthly risk of infection by *Shigella* among children who had exposure to open drains was 0.92 (95% credible interval: 0.70, 1.00). The distribution of monthly risks of pathogen-specific infections among those who had exposure to open drains are displayed in Figure 3.1 for adults and Figure 3.2 for children.

We also examined the probability of pathogen-specific infection from canal exposure for the five target pathogens. The highest average monthly risks of infection associated with canal exposure for adults and children was for *Vibrio cholera* and the lowest was for *Giardia*. The average monthly risk of *Vibrio cholera* infection among those who had exposure to canals was 0.61 (95% credible interval: 0.16, 0.93) for adults and 0.76 (95% credible interval: 0.24, 0.99) for children. The average monthly risk of *Giardia* infection

among those who had exposure to canals was 0.0008 (95% credible interval: 0.0000000293, 0.00909) for adults and 0.00009 (95% credible interval: 0.00000292, 0.009) for children. The average monthly risk of infection by Norovirus GII among adults who had exposure to canals was 0.59 (95% credible interval: 0.04, 0.96). The average monthly risk of infection by Norovirus GII among children who had exposure to canals was 0.61 (95% credible interval: 0.02, 0.74). The average monthly risk of infection by *Shigella* among adults who had exposure to canals was 0.49 (95% credible interval: 0.09, 0.87). The average monthly risk of infection by *Shigella* among children who had exposure to canals was 0.74 (95% credible interval: 0.27, 0.98). The average monthly risk of infection by *Salmonella* Typhi among adults who had exposure to canals was 0.0019 (95% credible interval: 0.0008, 0.0046). The average monthly risk of infection by *Salmonella* Typhi among children who had exposure to canals was 0.02 (95% credible interval: 0.01, 0.05). The distribution of monthly risks of pathogen-specific infections among those who had exposure to canals are displayed in Figure 3.3 for adults and Figure 3.4 for children.

E. Discussion

i. Enteric Pathogen Concentrations in Ultrafiltration and Grab Samples: Implications for Detection Methods

In Dhaka, Bangladesh, drains and canals are often used as dumping sites for fecal matter (60). This study quantified the levels of contamination of five enteric pathogens in water samples collected from open drains and canals. PEG precipitation, TNA extraction, and PCR methods were used to concentrate and quantitatively detect the target pathogens in

each grab and ultrafiltration sample. For the 20 L volume samples, ultrafiltration methods were used before PEG precipitation, TNA extraction and PCR so that the pathogens could be concentrated from a large volume to increase the likelihood of pathogen detection (120). This study found that drain water samples that were concentrated by ultrafiltration methods had higher detection rates of pathogens than 400 mL grab samples of drain water samples. Moreover, canal water samples that were concentrated by ultrafiltration methods had higher pathogen detection rates than grab samples of canal water (Table 3.1). This data suggests that ultrafiltration methods were successful in concentrating low levels of the target pathogens in the samples to a concentration where their nucleic acid was more likely to be detected by PCR.

Ultrafiltration methods were used in this study to concentrate large volumes to increase microbial concentration (120). One of the important strengths of these methods is that they simultaneously concentrate viruses, bacteria and protozoa with good efficiency (121). Liu et al., 2012 found that the use of a non-blocked ultrafilter for UF followed by elution using a surfactant-based solution achieved recovery efficiencies greater than 50% recovery of viruses, bacteria, and protozoa (121). Though ultrafiltration methods are not usually used to concentrate microorganisms in wastewater, there have been studies that have used ultrafiltration methods to concentrate microorganisms from groundwater, surface, and recreational waters. In these studies, the concentrations of microorganisms in the final product of the ultrafiltration methods were compared to the concentrations of microorganisms in grab samples that did not undergo ultrafiltration (122,123). In one study, the concentrations of *E. coli* and enterococci in ultrafiltration and grab samples

collected from surface water were compared. The investigators found the concentration of *E. coli* was 120-436 times higher in the ultrafiltration concentrate compared to the grab samples, and the concentration of enterococci was 127-257 times higher in the ultrafiltration concentrate than in the grab samples (122). Another study reported that the concentration of enterococci was 400 times higher in the final ultrafiltration concentrate than in grab samples (123). These studies suggest ultrafiltration is effective in concentrating enteric bacteria in water samples. However, in this study, the estimated concentrations of the target microorganisms in the grab samples were consistently significantly higher than the estimated concentrations of the target microorganisms in the ultrafiltration samples suggesting there may be uncertainty in the concentration estimates for these pathogens.

The 20 L samples were processed by ultrafiltration, followed by PEG precipitation, TNA extraction, whereas the 400 mL grab samples were processed only by PEG precipitation, and TNA extraction. This study found the detection rates for all five target pathogens were always higher in the concentrates from the ultrafiltration samples, but the estimates of the pathogen concentrations were consistently and significantly higher in the grab samples. The reason for lower concentration estimates in the ultrafiltration samples is most likely due to: 1) some losses during the ultrafiltration process due to adsorption of the microbes to the filter and incomplete elution off the filter, and 2) inefficiency of TNA extraction methods used in this study. The pelleted material obtained from ultrafiltration samples were larger in volume than the pelleted material obtained from grab samples. Despite this, the TNA extraction protocol was not adjusted for processing ultrafiltration

samples, and all samples followed the same TNA extraction procedure. By not adjusting the TNA extraction protocol to process different sizes of pelleted material, there may have been more TNA loss from the larger pellets from the ultrafiltration samples compared to the pellets from the grab samples. The actual pathogen concentrations in the ultrafiltration samples are likely to be higher than the reported values. The higher detection rates for all pathogens in ultrafiltration samples compared to grab samples suggest that the ultrafiltration methods were useful for concentrating the five target pathogens in the canal and open drain samples.

ii. Open Drain and Canal Exposure Models

In this study, the monthly risks of infection by Norovirus GII*, Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi from open drain and canal exposures in children and adults were estimated using a mathematical modeling approach, QMRA. This study pioneers estimation of health risks for adults and children from open drain and canal exposures in Dhaka, Bangladesh using QMRA. By detecting the presence and estimating the concentrations of the five target enteric pathogens in open drain and canals, a data-driven estimation can be made of monthly risks of infections from these pathogens. Accounting for all combinations of exposed age groups (adults and children), pathogens (Norovirus GII*, Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi), and fecal exposure pathways (open drains and canals) resulted in risk estimates for 20 exposure scenarios.

Our results indicate that there was a greater risk of infection by Norovirus GII, *Vibrio cholerae, Giardia,* and *Shigella* from drain exposure compared to canal exposure in children and adults. There was a greater risk of infection by *Salmonella* Typhi from canal exposure compared to drain exposure in children and adults. The average monthly risks of pathogen infections by Norovirus GII, *Vibrio cholerae, Giardia,* and *Shigella* were higher for children than for adults regardless of exposure.

The pathogen concentrations in drain and canal waters were not statistically significantly different from one another. However, our results suggest that children and adults had the highest risk of infection by Norovirus GII from drain exposures and *Vibrio cholerae* from canal exposures. The high estimated monthly risk of infection by Norovirus GII in children and adults from drain exposure may be explained by the dose-response parameters, dose-response relationship, frequency of exposure, and concentration of Norovirus GII. The dose-response parameter, p=0.722, suggests that there is a high probability of exposure to Norovirus. Furthermore, based on the Norovirus dose-response relationship, the probability of infection when ingesting 1 organism is 0.46. We also observed a high mean concentration of Norovirus GII in drain samples (3.15 $\log_{10}$ genomic copies/100mL). Finally, data of self-reported monthly exposures to surface and drain waters by adults and children from a SaniPath exposure assessment study in Dhaka conducted in 2017 demonstrated that a majority of 319 children (66.7%) and 337 adults (67.3 %) that had any exposure to drain water reported to have contact more than ten times a month (Table 2.3). Taken together, this body of evidence provides support for the high estimated monthly risks of infection. Moreover, our results suggest that even a low

intake volume of drain water will produce a high risk of infection, a conclusion that is supported by the assumed intake volume for adults and children of 1 mL and 0.06 mL, respectively.

The estimated monthly risks of infection in adults and children by *Vibrio cholerae* from canal exposure may be explained by the dose-response parameters, dose-response relationship, frequency of exposure, concentration of *Vibrio cholerae*, and intake volumes. When examining the dose-response relationship for *Vibrio cholerae*, the probability of infection when ingesting 1 organism is 0.015. Further, the dose-response parameter, n50, used to translate dose into infection risk for *Vibrio cholerae* is 243, which lower than that of *Shigella* and *Salmonella* Typhi (Table 2.4). The n50 parameter is the dose at which 50% of the study population is expected to be infected. While the probability of infection when ingesting 1 organism is low, our QMRA model assumed children ingested 49 mL and adults ingested 3.70 mL of canal water each time they are in contact with canals, and the mean concentration of *Vibrio cholerae* for canal samples was 3.59 $\log_{10}$ genomic copies/100mL (SD: 0.69) (Tables 2.3 and 3.4). From data of self-reported monthly exposures to surface and drain waters by adults and children from a SaniPath exposure assessment study in Dhaka conducted in 2017, 32.7% of children (237) and 32.4% of adults (289) that had any contact with canal water reported having contact more than 10 times per month (Table 2.3). Since there were a lot of people who reported having and not having contact, that makes sense why the 95% credible interval ranges from 0.16 to 0.93 in adults and 0.24-0.99 in children (Tables 3.6 and 3.7). Since

adults have a lower intake volume, this makes sense why their range of infection is lower than that of children.

A previous study conducted in Dhaka, Bangladesh estimated the daily infection risk of *Vibrio cholerae* in children and adults from floodwater exposure using QMRA. The study estimated the daily risk of illness from cholera to range from $5.2*10^{-5}$ to $2.2*10^{-3}$, with the highest risk attributed to children living in slum areas (99). In our study, we estimated the monthly infection risks of *Vibrio cholerae* in children and adults from exposures to open drains and canals. The estimated monthly infection risks of *Vibrio cholerae* from open drain exposure was high and the 95% probability of infection was within the range of 0.88 to 1.00 (Table 3.6) and 0.47 to 0.98 (Table 3.7) in children and adults, respectively. The monthly infection risk of *Vibrio cholerae* in children and adults from canal exposure was between 0.24 to 0.99 (Table 3.6) and 0.16 to 0.92 (Table 3.7), respectively. These monthly infections risks were estimated from the daily risks of infection. To compare the daily risks of infection of *Vibrio cholerae* between floodwaters and canal waters and floodwaters and open drain waters, daily risks of infection need to be compared. The daily infection risks of *Vibrio cholerae* in children and adults from open drain exposure ranged from 0.00 to 0.85 and 0.00 to 0.70, respectively. The daily infection risks of *Vibrio cholerae* in children and adults from canal exposure ranged from 0.00 to 0.85 and 0.00 to 0.71, respectively. Our results indicate that the risks of *Vibrio cholerae* infection were highly variable, but canal and drain exposures were associated with higher risks of infection than exposure to flood waters.

The greatest infection risk from exposure to open drains for both children and adults were from Norovirus GII and the lowest risk of infection was from *Salmonella* Typhi. For exposure to canals, the highest risk of infection for children and adults was for *Vibrio cholerae* and the lowest risk of infection was for *Giardia* (Table 3.6 and Table 3.7). This suggests that the infection risks by pathogen vary based on the different dose-response relationships as well as the frequency and duration of contact adults and children have with the fecal exposure sources.

The development of this study's models required a number of assumptions and informed choices for model parameters. Since surface waters include canal waters, this study used surface water as a proxy for canal water and used data on contact frequencies and intake volumes that have been measured in studies of surface water. A previous study conducted in Bangladesh studied pollution in surface waters by assessing the quality of canal waters (124). In a SaniPath exposure assessment study in Dhaka conducted in 2017, data on self-reported monthly exposures to surface and drain waters by adults and children were collected through household surveys in ten neighborhoods. These data were incorporated into the models for the current study. Observational data on contacts of children and adults with open drains and canals were not collected in this study. Future studies could incorporate observational data to supplement household surveys to better understand the frequency and types of behaviors that adults and children have with open drains and canal waters, and this may provide better estimates of health risks from this fecal exposure pathway.

Intake volumes calculated for open drains were utilized in our exposure models for both children and adults from a SaniPath exposure assessment study in Dhaka conducted in 2017 (Table 2.3). Previous studies have attempted to quantify the exposures to surface waters in children and adults. These studies assumed each child directly ingested 49 mL (114) and each adult ingested 3.70 mL of surface water each time they were in contact with surface waters (113) (Table 2.3). However, these studies were conducted in the United States and focused on recreational water (113,114). The activities people engage in when in contact with open drain and canal waters in Bangladesh may differ from the activities people engage in when in contact with recreational water. Therefore, the ingestion volumes from these studies may differ from that of this study's target population. Since the environment of a high-income country is different from a low-income setting such as Dhaka, Bangladesh, future QMRAs conducted in Dhaka could utilize exposure parameters from low-income settings as they become available to obtain more accurate estimates of health risks.

iii. Open Drain and Canal Exposure Data: Implications for Quantitative Microbial Risk Assessment

A previous study used QMRA to estimate the disease burden of cholera in children and adults from exposure to urban flood waters in Dhaka, Bangladesh (102). While that study assessed health risks associated with floodwaters, there are other important fecal exposure pathways that individuals in Dhaka often come into contact with that should be further examined. In this study, drain and canal water samples were analyzed for five

enteric pathogens so that the risks of infection associated with exposure to open drains and canals in low-income neighborhoods in Dhaka could be assessed.

Both canal and drain waters were found to have high concentrations of DNA/RNA of the five target enteric pathogens. This indicates that there were high concentrations of these pathogens in the fecal waste from the study communities that was entering the canal and open drains and corresponding high rates of infections with these pathogens in these study communities. Our results suggest that any contact with open drain and canals in Dhaka, Bangladesh would likely result in a high-level exposure to the enteric pathogens and a high risk of infection. The greatest risks of pathogen infection from drain exposure were from Norovirus GII, *Vibrio cholerae, Giardia,* and *Shigella.* Our study found the average monthly risks of pathogen infection by Norovirus GII, *Vibrio cholerae, Giardia,* and *Shigella* for children and adults to range from 0.75 to 0.99 from drain exposure. The greatest risks of pathogen infection from canal exposure is by Norovirus GII, *Vibrio cholerae,* and *Shigella.* The average monthly risks of pathogen infection by Norovirus GII, *Vibrio cholerae,* and *Shigella* for children and adults to range from 0.61 to 0.76 from canal exposure.

The pathogen detection rates for the drain and canal samples were not statistically significantly different for all pathogens, except for *Salmonella* Typhi which was detected more frequently in canal water (p-value: 0.0110) (Table 3.2). The estimated concentrations of pathogen-specific DNA (and Genogroup II norovirus RNA) for nonzero samples were not statistically significantly different between drain and canal

samples for all pathogens tested. However, a two-sample t-test could not be conducted to assess differences in *Giardia* DNA concentrations between drain and canal samples due to insufficient sample size (Table 3.4).

Infection risks for each of the target pathogens will differ between exposure sources, based on the amount of contact adults and children have with the fecal exposure pathways, and by biological characteristics of each pathogen – such as persistence in the environment and infectivity. To determine the frequency of exposure by group, self-reported exposure data from a SaniPath exposure assessment in ten Dhaka neighborhoods in 2017 were used (107). The behavior data were obtained from household surveys that asked about both adult and child exposure to open drains and canals. However, questionnaires can influence the validity of the responses due to self-reporting biases, social desirability and recall bias (125). To adjust for self-reporting bias, in addition to household surveys, future studies could conduct structured observations that will observe the frequency and types of contacts adults and children have with the selected fecal exposure pathways. A study conducted in Accra, Ghana found structured observations to be useful in providing information on exposure behaviors of adults and children and determining locations for environmental sampling (53). Structured observations can also be useful in assessing the validity of the household survey findings (125).

iv. Strengths and Limitations

To our knowledge, this QMRA study presents one of the first attempts to estimate the infection risks from multiple important enteric pathogens associated with exposures to

open drain and canals in Dhaka, Bangladesh. A previous study conducted in Dhaka city utilized QMRA methodologies to assess risks of cholera associated with floodwater exposures in children and adults. They provided a solid foundation for estimating disease risks associated with one pathogen and one fecal exposure pathway (99). Our study estimated infection rates for five enteric pathogens and two fecal exposure pathways. Our results revealed that the highest infection risks for children and adults were for Norovirus GII associated with open drain exposures and for *Vibrio cholerae* associated with exposures to canal water. Further, we found infection risks to have a positive association with the exposure frequency.

This study provided a thorough assessment of five enteric pathogens in open drain and canal waters while introducing ultrafiltration methods. These rare data on pathogen detection in environmental samples in a low-resource setting provide valuable insights on the frequency and magnitude of pathogen contamination in the environment and the high rates of these infections in the study communities.

Several studies have reported that ultrafiltration methods are successful for concentrating pathogens from a large volume samples and increasing the likelihood of pathogen detection (120,122,123). Though the mean concentrations of target pathogens were higher in grab samples compared to ultrafiltration samples, the detection rates of target pathogens were higher in ultrafiltration samples than grab samples. This study applied a sensitive method for concentrating a wide range of enteric pathogens in water samples and demonstrated that ultrafiltration methods could be used successfully in a low-

resource setting. This study found that ultrafiltration methods followed by quantitative PCR can be useful in concentrating and detecting pathogens, Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi, from large volume samples and increase the likelihood of pathogen detection in low-income countries, such as Bangladesh. Ultrafiltration methods allowed us to successfully detect a wide range of pathogens from a variety of environmental samples in a low-income setting. However, ultrafiltration methods are time consuming and may not be efficient or practical for widespread use.

This cross-sectional study allowed monthly risks of infection by enteric pathogens to be estimated. However, our estimates of infections risks can only be generalized for the months of April to October when the canal and drain water samples were collected in our study neighborhoods.

While the model parameters used in our analysis were based on our study location, not all parameters were from the Dhaka context because of data gaps. As mentioned before, this study used ingestion volumes measured in studies of surface water exposure as a proxy for ingestion volumes associated with exposure to canals. The intake volume parameters for children and adults were based on recreational water studies of water ingestion during swimming that were conducted in the United States (113,114). Therefore, it is unclear how appropriate they are for approximating water ingestion during contact with canals in a low-income setting, such as Dhaka, Bangladesh. Future studies should attempt to utilize specific behavioral data and intake volumes that more closely approximate the behavior

and context of Dhaka when these data become available. Additionally, since our exposure models were based on Dhaka city, our results may be generalizable to low-income neighborhoods in Dhaka city, but not generalizable to other low-income countries. However, it is possible that the model parameters used in this study could be used in future QMRA studies as long as appropriate assumptions are made.

Another limitation of this study is that pathogen detection and estimated concentrations were based on nucleic acid detection. This detection method does not indicate whether the DNA/RNA came from viable, infectious microorganisms or dead microorganisms. Therefore, this detection method may inflate the infection risks because the model assumed that all of the detected genome copies were from infectious pathogens.

Another limitation of this study is that the dose-response relationships for all pathogens included in this study (except Norovirus GII) were based on cultured organisms or *Giardia* cysts. In this study, we estimated dose-response relationships for all pathogens based on PCR detection of genome copies. Moreover, the Norovirus dose-response parameters used in this study were for Norovirus GI. In this study, we measured Norovirus GII which may have a different dose-response relationship

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G. TABLES

Table 2.1. Primers and Probes Profiles Used in the qPCR Assays in this Study

Pathogen	Primer/probe	Target Gene	Sequence (5'-3')	Reporter-quencher	Amplicon size (bp)	Reference
Norovirus GII[a]	COG2F	ORF1 and ORF2 junction regions	CARGARBCNATGTTYAGRTGGATGAG		98	Kageyama et al (2003)
	COG2R		TCGACGCCATCTTCATTCACA			
	Ring2-TF		TGGGAGGGCGATCGCAATCT	FAM-TAMRA		
V. cholerae[b]	hlyA-F	hlyA	CGCTTTATTGTTCGATGCGTTA		141	Huang et al. (2009)
	hlyA-R		ACTCGGTTATCGTCAGTTTGG			
	hlyA-P		CCCCGATAATCTTGGGCAATCGCATCGGGG	FAM-TAMRA		
S. Typhi[c]	ST-Frt	STY0201	CGCGAAGTCAGAGTCGACATAG		131	Karkey et al. (2015)
	ST-Rrt		AAGACCTCAACGCCGATCAC			
	ST-P		CATTTGTTCTGGAGCAGGCT GACGG	FAM-TAMRA		
Shigella	ipaH_F	ipaH	CCTTTTCCGCGTTCCTTG		---	Thiem et al. (2004)
	ipaH_R		CGGAATCCGGAGGTATTG			
	ipaH_P		CGC CTT TCC GAT ACC GTC TCT GCA	FAM-TAMRA		
Giardia	JVGF	---	ATCCGGTCGATCCTGCCG		---	Narayanan et al. (2020 in printing)
	JVGR		ACGTCTTGGCGCCGGGTT			
	GIAP		CGGCGGACGGCTCAGGA	FAM-BHQ13		

[a] Norovirus GII denotes Norovirus Genogroup II
[b] *V. cholerae* denotes *Vibrio cholerae*
[c] *S.* Typhi denotes *Salmonella* Typhi

Table 2.2. Pathogen Concentrations (log_{10} genomic copies/100mL)[a] in Environmental Samples from Urban Neighborhoods, Dhaka, Bangladesh 2019

Sample Type	N	Norovirus GII[b]		*Vibrio cholerae*		*Giardia*		*Shigella*		*Salmonella* Typhi	
		Mean (SD[c])	95% CI[d]	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean (SD)	95% CI
Ultrafiltration											
Drain	31	2.21 (1.06)	1.82-2.60	2.94 (1.23)	2.49-3.39	0.93 (1.05)	0.55-1.32	3.48 (0.98)	3.12-3.84	0.21 (0.49)	0.03-0.39
Canal	4	2.80 (0.09)	2.64-2.95	3.71 (0.49)	2.92-4.49	0.13 (0.27)	-0.29-0.56	3.62 (0.75)	2.43-4.82	1.41 (0.46)	0.67-2.15
Total	35	2.27 (1.01)	1.93-2.62	3.03 (1.19)	2.62-3.44	0.84 (1.02)	0.49-1.19	3.50 (0.95)	3.17-3.82	0.35 (0.62)	0.14-0.56
Grab											
Drain	33	2.03 (2.24)	1.23-2.82	4.31 (1.78)	3.68-4.95	1.68 (1.91)	1.00-2.36	4.42 (1.69)	3.82-5.02	0.60 (1.12)	0.20-0.99
Canal	4	-	-	2.58 (1.91)	-0.45-5.61	-	-	3.79 (1.40)	1.56-6.02	1.39 (1.61)	-1.17-3.95
Total	37	1.81 (2.21)	1.07-2.54	4.13 (1.85)	3.51-3.47	1.49 (1.87)	0.87-2.12	4.35 (1.64)	3.80-4.90	0.68 (1.18)	0.29-1.08
Total	72	2.03 (1.74)	1.63-2.44	3.59 (1.65)	3.21-3.98	1.18 (1.54)	0.82-1.54	3.94 (1.41)	3.60-4.27	0.52 (0.96)	0.29-0.75

[a] Pathogen concentrations were calculated including samples with concentrations of <1 genomic copies/100 mL
[b] Norovirus GII denotes Norovirus Genogroup II
[c] SD denotes for standard deviation
[d] CI denotes confidence interval

Table 2.3. Survey Data Used to Estimate Monthly Exposure to Canals and Drains

	Children		*Adults*	
	Drain	Canal	Drain	Canal
Frequency of Contact[a]				
> 10	319	237	337	289
6-10	76	136	88	129
≤ 5	83	352	76	473
0	251	1949	248	2331
Intake Volume (mL)	1.00[a]	49.00[b]	0.06[a]	3.70[c]

[a] Frequency of Contact denotes how much contact an individual has with the reservoir per month; values obtained from a SaniPath exposure assessment study in Dhaka conducted in 2017
[b] value obtained from Dorevitch et al. (2011)
[c] value obtained from Dufour et al. (2006)

Table 2.4. Dose Response Parameters Used to Translate Dose into Infection Risk

Pathogen	Model	Parameters	Reference
Norovirus	Fractional poisson	P=0.722	Messner et al. (2014)
Vibrio cholera	Beta poisson	a=0.25, n50=243	Hornick et al. (1971)
Giardia	Exponential	r=0.0199	Haas et al. (1999)
Shigella	Beta poisson	a=0.265, n50=1480	DuPoint et al. (1972)
Salmonella Typhi	Beta poisson	a=0.175, n50=1110000	Hornick et al. (1970)(1966)

Table 3.1 Enteric Pathogen Detection Rates in Environmental Samples from an Urban Neighbourhood, Dhaka, Bangladesh

Sample Type	N	Norovirus GII[a] Pos[b] (%)	*Vibrio cholerae* Pos (%)	*Giardia* Pos (%)	*Shigella* Pos (%)	*Salmonella* Typhi Pos (%)
Ultrafiltration						
Drain	31	26 (83.9)	29 (93.5)	16 (51.6)	31 (100.0)	9 (29.0)
Canal	4	4 (100.0)	4 (100.0)	1 (25.0)	4 (100.0)	4 (100.0)
Total	35	30 (85.7)	33 (94.3)	17 (48.6)	35 (100.0)	13 (37.7)
Grab						
Drain	33	17 (51.52)	30 (90.91)	15 (45.45)	30 (90.91)	8 (24.24)
Canal	4	0 (0.00)	3 (75.00)	0 (0.00)	4 (100.00)	2 (50.00)
Total	37	17 (45.9)	33 (89.2)	15 (40.5)	34 (91.9)	10 (27.0)
Total	72	47 (65.3)	66 (91.7)	32 (44.4)	69 (95.8)	23 (31.9)

[a] Norovirus GII denotes Norovirus Genogroup II
[b] Pos denotes number of positive samples

Table 3.2. Comparison Between Enteric Pathogen Detection Rates Between Drain Samples and Canal Samples

Pathogen	Sample Type	N	Pos[a] (%)	p-value[b]
Norovirus GII[c]	Drain	64	43 (67.2)	0.4361
	Canal	8	4 (50.0)	
Vibrio cholerae	Drain	64	59 (92.2)	0.5201
	Canal	8	7 (87.5)	
Giardia	Drain	64	31 (48.4)	0.0684
	Canal	8	1 (12.5)	
Shigella	Drain	64	61 (91.0)	1.0000
	Canal	8	8 (100.0)	
Salmonella Typhi	Drain	64	17 (26.6)	0.0110
	Canal	8	6 (75.0)	

[a] Pos denotes number of positive samples
[b] Fisher Exact Tests were conducted to estimate p-values
[c] Norovirus GII denotes Norovirus Genogroup II

Table 3.3. Comparison Between Enteric Pathogen Detection Rates Between Grab Samples and Ultrafiltration Samples for 64 Drain Samples and 8 Canal Samples

Pathogen	Collection Type	N	Pos[a] (%)	p-value[b]
Norovirus GII[c]	Grab	37	17 (46.0)	0.0005
	Ultrafiltration	35	30 (85.7)	
Vibrio cholerae	Grab	37	33 (89.2)	0.6745
	Ultrafiltration	35	33 (94.3)	
Giardia	Grab	37	15 (40.5)	0.6356
	Ultrafiltration	35	17 (48.6)	
Shigella	Grab	37	34 (91.9)	0.2400
	Ultrafiltration	35	35 (100.0)	
Salmonella Typhi	Grab	37	10 (27.0)	0.4503
	Ultrafiltration	35	13 (37.1)	

[a] Pos denotes number of positive samples
[b] Fisher Exact Tests were conducted to estimate p-values
[c] Norovirus GII denotes Norovirus Genogroup II

Table 3.4. Comparison Between Pathogen Concentrations (log_{10} genomic copies/100 mL)[a] Between Drain Samples and Canal Samples

Pathogen	Sample Type	N	Mean (SD[b])	Min, Max[c]	Mean Difference	95% CI Difference[d]	t-value[e]	p-value[e]
Norovirus GII[f]	Drain	43	3.15 (1.15)	0.96, 5.94	-0.3433	-0.7089, 0.0222	-1.89	0.0649
	Canal	4	2.80 (0.09)	2.69, 2.89				
Vibrio cholerae	Drain	59	3.96 (1.34)	0.43, 6.70	-0.3655	-1.4019, 0.6708	-0.70	0.4836
	Canal	7	3.59 (0.69)	2.30, 4.34				
Giardia	Drain	31	2.73 (1.12)	0.62, 4.83	-	-	-	-
	Canal	1	0.54	0.54, 0.54				
Shigella	Drain	61	4.16 (1.19)	0.62, 6.62	-0.4522	-1.3319, 0.4276	-1.03	0.3086
	Canal	8	3.71 (1.05)	2.49, 5.01				
Salmonella Typhi	Drain	17	1.54 (1.12)	0.008, 3.46	0.3255	-0.7153, 1.3663	0.65	0.5225
	Canal	6	1.87 (0.79)	1.00, 2.81				

[a] Pathogen concentrations were calculated excluding samples with concentrations of <1 genomic copies/100 mL
[b] SD denotes standard deviation
[c] Min, Max denotes the minimum and maximum mean values
[d] CI denotes confidence interval
[e] t-values and p-values were estimated using two sample t-tests
[f] Norovirus GII denotes Norovirus Genogroup II

Table 3.5. Comparison Between Pathogen Concentrations ($\log_{10}$ genomic copies/100 mL)[a] Between Grab Samples and Ultrafiltration Samples

Pathogen	Collection Type	N	Mean (SD[a])	Min, Max[b]	Mean Difference	95% CI Difference[c]	t-value[e]	p-value[e]
Norovirus GII[f]	Grab	17	3.93 (1.45)	0.96, 5.94	-1.2778	-2.0325, -0.5231	-3.57	0.0023
	Ultrafiltration	30	2.65 (0.41)	1.90, 3.40				
Vibrio cholerae	Grab	33	4.63 (1.21)	2.30, 6.70	-1.4110	-1.9461, -0.8759	-5.27	<0.0001
	Ultrafiltration	33	3.21 (0.95)	0.43, 4.59				
Giardia	Grab	15	3.70 (0.57)	2.57, 4.83	-1.9399	-2.4046, -1.4751	-8.53	<0.0001
	Ultrafiltration	17	1.76 (0.70)	0.54, 2.80				
Shigella	Grab	34	4.73 (1.05)	2.49, 6.62	-1.2350	-1.7163, -0.7537	-5.12	<0.0001
	Ultrafiltration	35	3.50 (0.95)	0.621, 5.24				
Salmonella Typhi	Grab	10	2.52 (0.67)	1.49, 3.46	-1.5816	-2.1748, -0.9884	-5.54	<0.0001
	Ultrafiltration	13	0.94 (0.69)	0.008, 2.10				

[a] Pathogen concentrations were calculated excluding samples with concentrations of <1 genomic copies/100 mL
[b] SD denotes standard deviation
[c] Min, Max denotes the minimum and maximum mean values
[d] CI denotes confidence interval
[e] t-values and p-values were estimated using two sample t-tests
[f] Norovirus GII denotes Norovirus Genogroup II

Table 3.6. Estimated Monthly Risks of Pathogen Infection for Children from Canal and Drain Exposures

Infection Risk[a]	Norovirus GII[b]		*Vibrio cholerae*		*Giardia*		*Shigella*		*Salmonella* Typhi	
	Drain	Canal	Drain	Canal	Drain	Canal	Drain	Canal	Drain	Canal
Mean	0.99	0.61	0.98	0.76	0.75	9.20e-03	0.92	0.74	0.0024	0.02
2.5%	0.92	0.02	0.88	0.24	0.16	2.92e-06	0.70	0.27	0.0004	0.01
50%	1.00	0.76	0.99	0.82	0.83	3.46e-03	0.94	0.79	0.0014	0.02
97.5%	1.00	0.74	1.00	0.99	1.00	9.00e-02	1.00	0.98	0.0180	0.05

[a] Infection Risk percentages denote the median risk as well as the 95% credible interval
[b] Norovirus GII denotes Norovirus Genogroup II

Table 3.7. Estimated Monthly Risks of Pathogen Infection for Adults from Canal and Drain Exposures

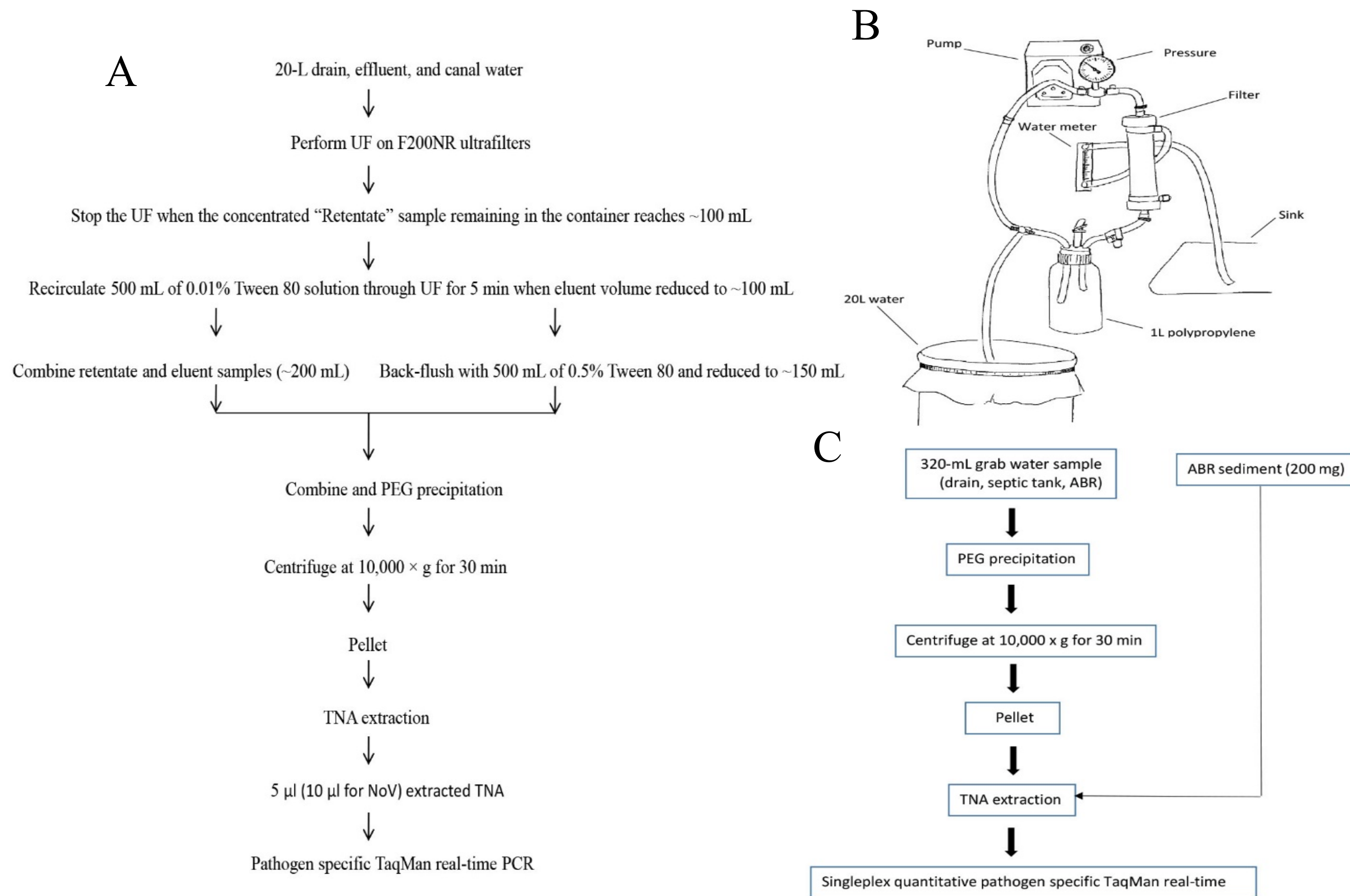
Infection Risk[a]	Norovirus GII[b]		*Vibrio cholerae*		*Giardia*		*Shigella*		*Salmonella* Typhi	
	Drain	Canal	Drain	Canal	Drain	Canal	Drain	Canal	Drain	Canal
Mean	0.94	0.59	0.81	0.61	0.29	7.73e-04	0.55	0.49	0.00003	0.0019
2.5%	0.72	0.04	0.47	0.16	0.01	2.93e-08	0.20	0.09	0.00001	0.0008
50%	0.97	0.65	0.84	0.64	0.20	2.86e-04	0.56	0.49	0.00003	0.0017
97.5%	1.00	0.96	0.98	0.93	0.89	9.09e-03	0.85	0.87	0.00011	0.0046

[a] Infection Risk percentages denote the median risk as well as the 95% credible interval
[b] Norovirus GII denotes Norovirus Genogroup II

H. FIGURES

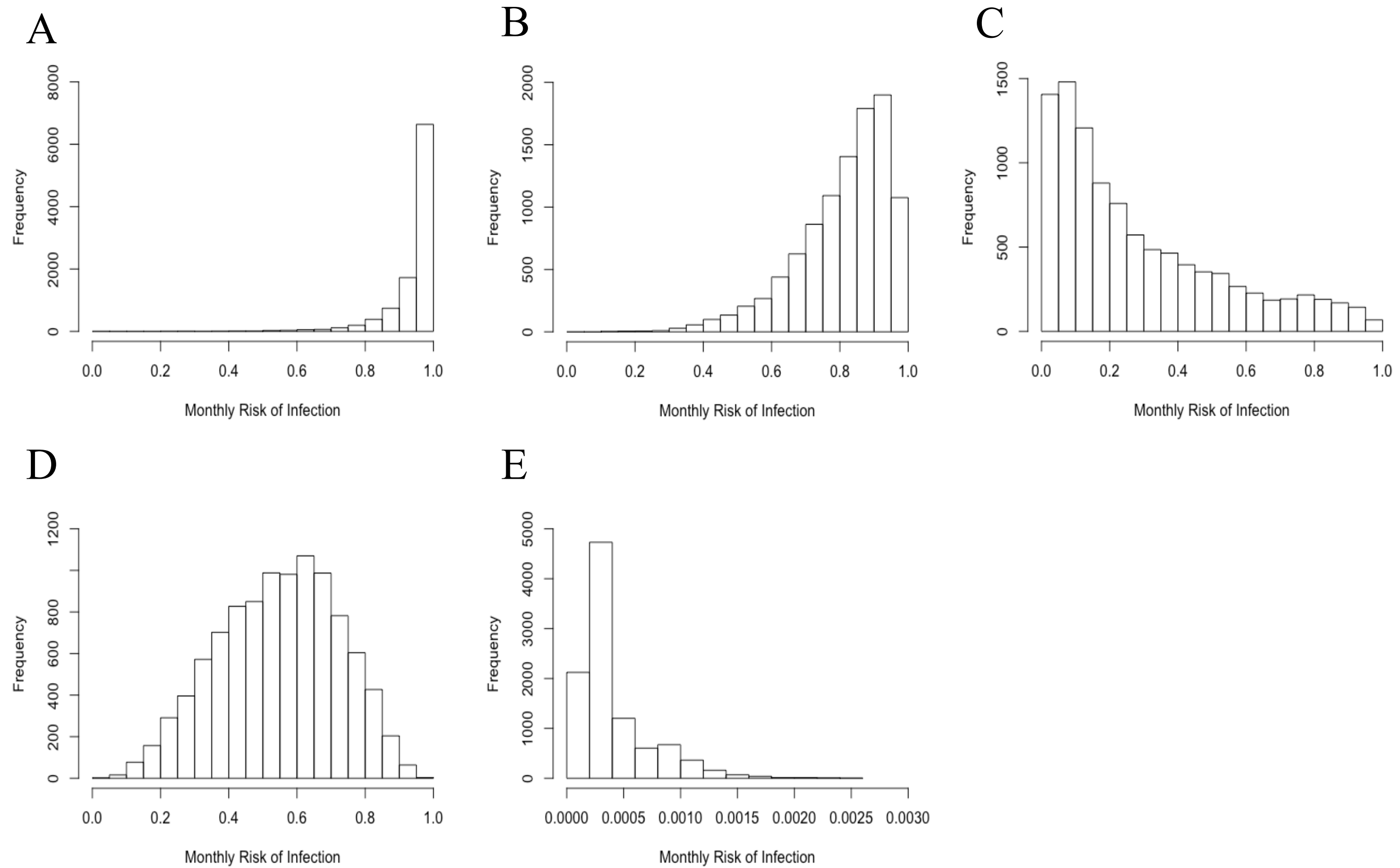
Figure 2.1. Sampling Locations in Urban Neighborhoods

Figure 2.2. Flow Diagrams for Environmental Sample Processing Procedures for Large Volume (20 L) and Small Volume (400 mL) Samples



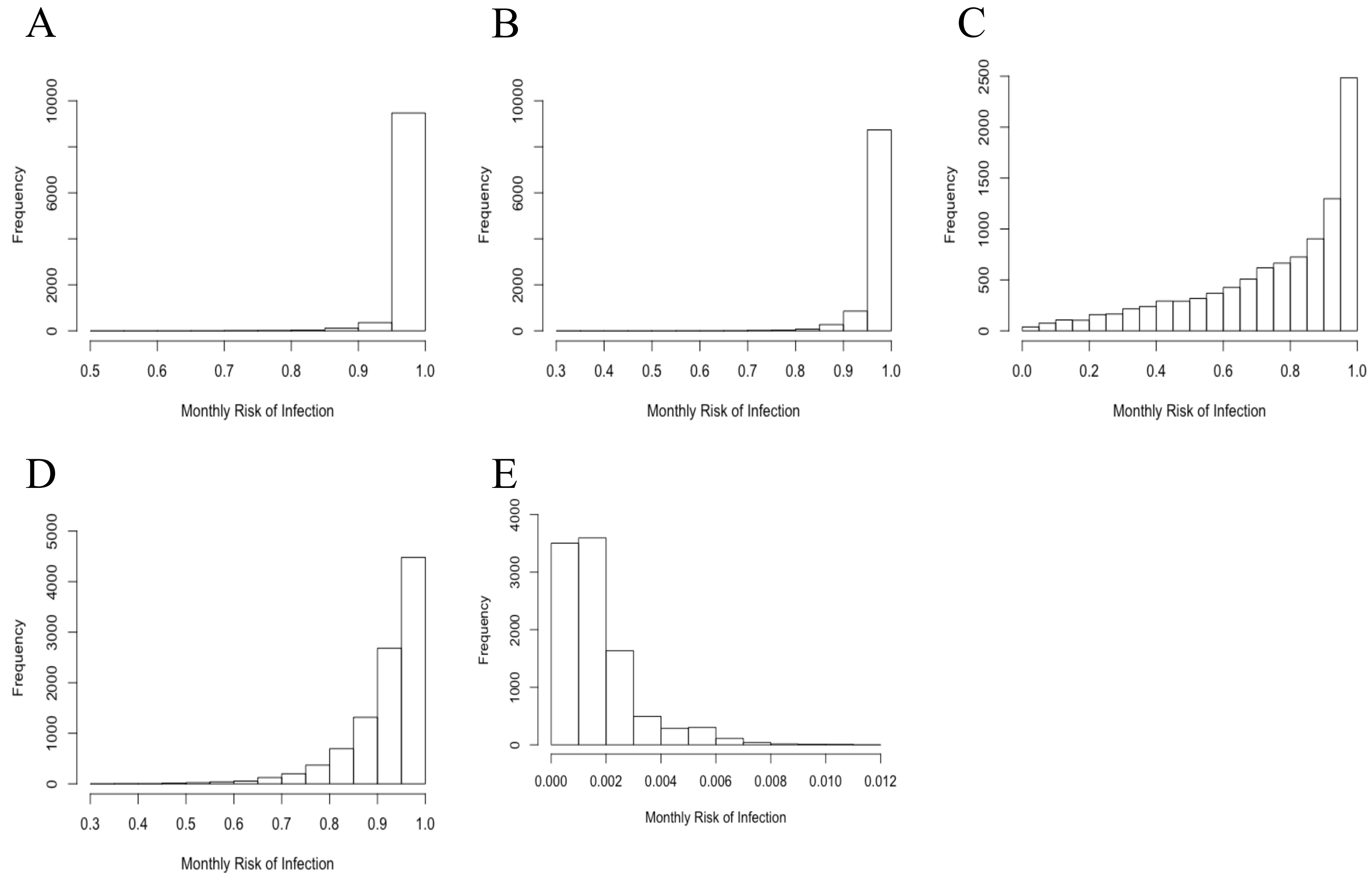
(A) Procedures for processing 20 L ultrafiltration samples. (B) Diagram of ultrafiltration set up (C) Procedures for processing 400 mL grab samples

Figure 3.1. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Adults from Open Drain Exposure



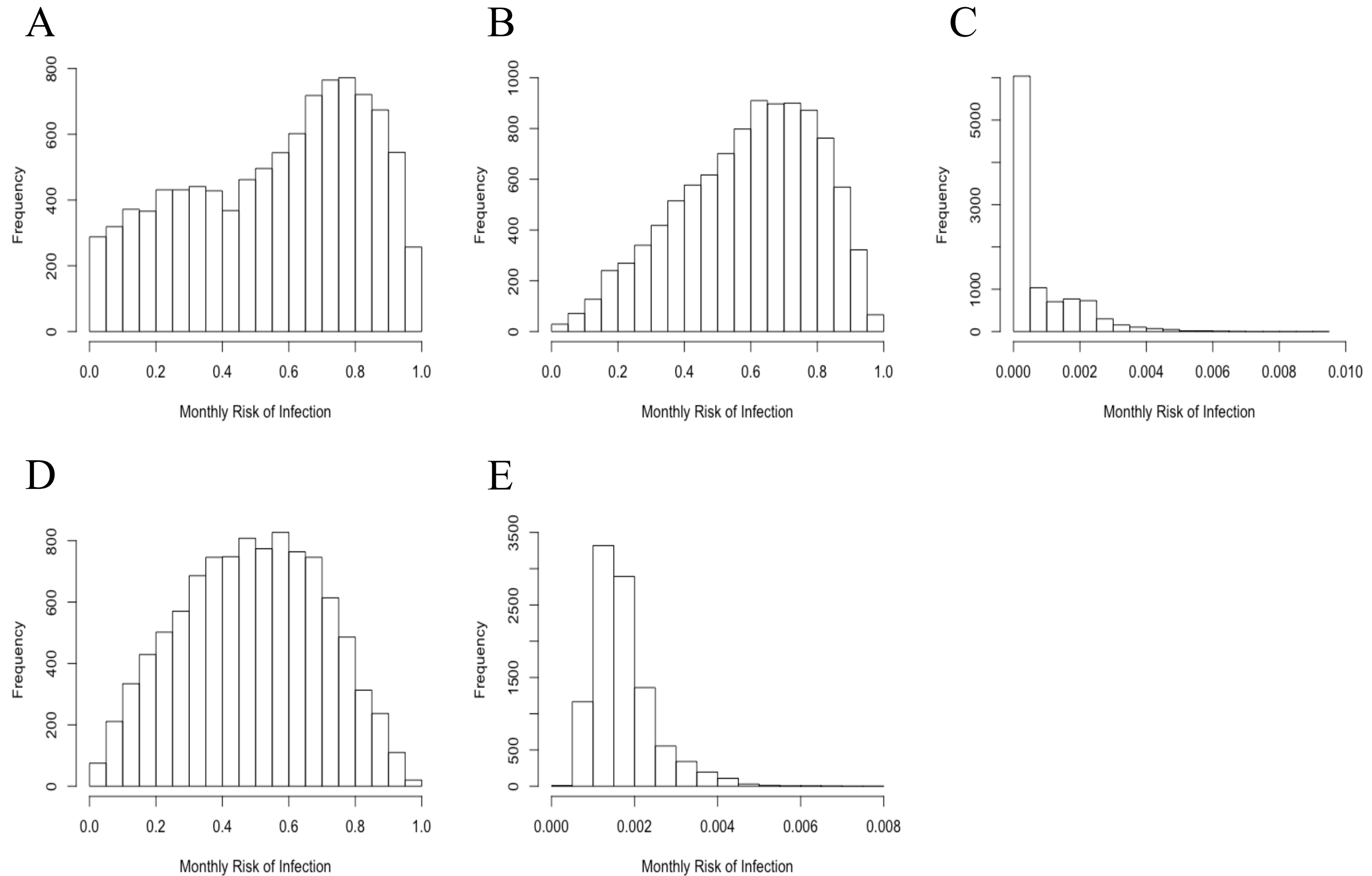
(A) Distribution of Norovirus GII infection risk. (B) Distribution of *Vibrio cholerae* infection risk. (C) Distribution of *Giardia* infection risk. (D) Distribution of *Shigella* infection risk. (E) Distribution of *Salmonella* Typhi infection risk. .

Figure 3.2. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Children from Open Drain Exposure



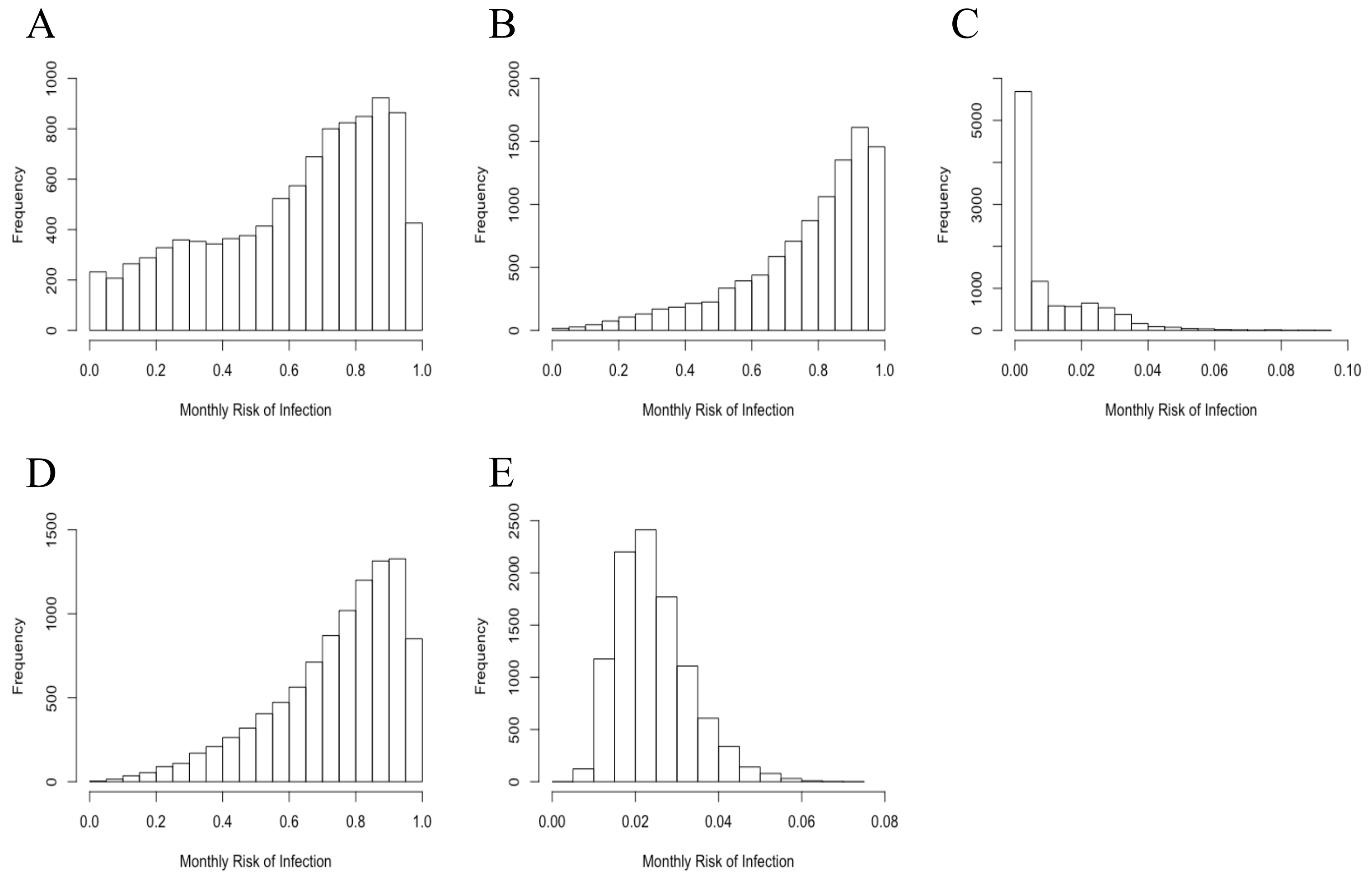
(A) Distribution of Norovirus GII infection risk. (B) Distribution of *Vibrio cholerae* infection risk. (C) Distribution of *Giardia* infection risk. (D) Distribution of *Shigella* infection risk. (E) Distribution of *Salmonella* Typhi infection risk. .

Figure 3.3. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Adults from Canal Exposure



(A) Distribution of Norovirus GII infection risk. (B) Distribution of *Vibrio cholerae* infection risk. (C) Distribution of *Giardia* infection risk. (D) Distribution of *Shigella* infection risk. (E) Distribution of *Salmonella* Typhi infection risk. .

Figure 3.4. Distribution of Estimated Monthly Risks of Pathogen-Specific Infections in Children from Canal Exposure



(A) Distribution of Norovirus GII infection risk. (B) Distribution of *Vibrio cholerae* infection risk. (C) Distribution of *Giardia* infection risk. (D) Distribution of *Shigella* infection risk. (E) Distribution of *Salmonella* Typhi infection risk.

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CHAPTER III: SUMMARY, PUBLIC HEALTH IMPLICATIONS, POSSIBLE FUTURE DIRECTIONS

A. Summary and Public Health Implications

Bangladesh experiences a high prevalence of enteric diseases each year, however, there is limited data on fecal contamination in the environment (56). In Dhaka, Bangladesh, there is a clear need to understand and quantify the health risks associated with exposure to fecal contamination in the environment to guide advocacy and investments in water and sanitation infrastructure. This study found that ultrafiltration methods followed by quantitative PCR can be useful in concentrating and detecting pathogens, Norovirus GII, *Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi, from large volume samples and increase the likelihood of pathogen detection in low-income countries, such as Bangladesh. Ultrafiltration methods allowed us to successfully detect a wide range of pathogens from a variety of environmental samples in a low-income setting. However, ultrafiltration methods are time consuming and may not be efficient or practical for widespread use.

Open drains and canals represent two distinct fecal exposures in Dhaka, however there was no evidence of statistically significant differences between pathogen concentrations in these samples. Our results indicate that both open drain and canal waters in the study neighborhoods were highly contaminated with enteric pathogens. This suggests that any contact with open drains or canals will likely result in a high level of exposure to fecal contamination and therefore an increased risk for infection by enteric pathogens.

Infection risks from two fecal exposure pathways by five enteric pathogens were estimated in children and adults. The highest average monthly risks of infection from exposure to open drains in adults and children were for Norovirus GII and the lowest risks were for *Salmonella* Typhi. The highest average monthly risks of infection associated with canal exposure for adults and children was for *Vibrio cholera* and the lowest was for *Giardia*. The infection risks by pathogen vary based on the different dose-response relationships as well as the duration of contact adults and children have with the fecal exposure sources. Estimating risks of infection by Norovirus GII*, Vibrio cholerae, Giardia, Shigella,* and *Salmonella* Typhi from open drains and canals adds to the body of knowledge around risk assessment research, especially in Dhaka, Bangladesh.

B. Recommendations

1. Sample collection and sample processing dates were found to be inconsistent in the Microsoft Excel Sheet (Microsoft, Redmond, WA) obtained for data analysis. I recommend drain and canal characterization forms to take place at the times of water sampling and sample processing. This would help maintain quality control and ensure that samples are being processed at the appropriate times.
2. Since the pathogen concentration values from open drain and canal samples are incorporated into our exposure models, I recommend calculating an adequate sample size for both fecal exposure pathways so that better estimates of infection risk could be obtained.
3. I recommend a sensitivity analysis of selected exposure parameters to determine which are most influential in estimating health risks. Specifically, identifying

behaviors associated with ingesting water in open drains and canals and the duration of exposure to open drains and canals should be assessed.

4. The estimated infection risks for the majority of the target pathogens were high for both children and adults and for both fecal exposure routes. I recommend local government administrators or local NGOs develop targeted interventions to reduce exposure to fecal contamination from open drains and canals.
5. Open drains and canals are highly contaminated with enteric pathogens and pose a threat to the community. To help eliminate, if not minimize the exposure to these fecal exposure pathways, I recommend drains to be covered and barriers to be placed around canals. These measures would help prevent community members from having contact with these fecal exposure pathways.
6. To help reduce the magnitude of fecal contamination in open drains and canals so that the environment is less contaminated, I recommend Dhaka city develop better sanitation infrastructure and public health communications to prevent discharge from septic tanks and other untreated sanitation effluent into the environment and prevent open defecation into the drains and canals.

C. Future Directions

1. Structured observations could be conducted to supplement household surveys to better understand the frequency and types of behaviors when adults and children have contact with open drain and canal waters. They could be useful for identifying locations for environmental sampling (53). While household survey

data were incorporated in this thesis, structured observations could help assess the validity of the data reported in the household surveys (125).

2. For future studies that cannot conduct household surveys and/or structured observations due to time constraints and/or the lack of resources, community mapping methods could be adopted. Community mapping could provide information about locations of human contact with fecal exposure pathways and behaviors commonly exhibited when in contact. This information could identify locations for environmental sampling.
3. Future QMRA studies could assess pathogen-specific infection risks from open drain and canal exposures in children and adults during wet and dry seasons. This could identify how pathogen-specific infection risks vary by season.
4. Future QMRA studies could assess infection risks from other fecal exposure pathways that adults and children have frequent contact with in the study location. This would allow for a more comprehensive understanding of the risk of enteric infections associated with other fecal exposure pathways.
5. While this thesis only incorporated water sample data into our exposure models, sediment samples were collected from open drains. Sediment samples were found to have high pathogen concentrations for Norovirus GII, *Vibrio cholerae*, and *Shigella.* Future QMRA studies could include drain sediment into their exposure models to assess how contact with drain sediment and drain water would affect infection risks.