Diarrheal Disease in Northwestern Ecuador: Prevalence, Pathogenicity, and Transmission
of Enteric Pathogens Across the Region

by

Darlene Bhavnani

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Doctoral Committee:

        Associate Professor Joseph N.S. Eisenberg, Chair
        Professor Mark L. Wilson
        Associate Professor Carl F. Marrs
        Associate Professor Bhramar Mukherjee

Dedicated to Mom, who instilled in me a love for literature.

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Abstract

Over four billion episodes of diarrhea occur worldwide each year. The burden of diarrhea is particularly high for children under five years living in developing countries. One in five child-deaths are attributed to diarrhea making it the second leading cause of child-mortality. Motivated by the large diarrheal disease burden, this body of research seeks to better understand the causes of diarrhea in developing country settings. We focus our research on diarrhea in northwestern Ecuador, a remote and underdeveloped region with limited access to health care and poor sanitation infrastructure. In chapter two, we estimate the prevalence and pathogenicity of three marker pathogens circulating in the region; rotavirus, *Giardia* and *Escherichia coli* (*E. coli*). We take this up further by addressing the effects of coinfections on diarrhea. Our main finding is evidence for synergistic interactions between rotavirus and coinfecting pathogens, such that the pathogenic potential of each organism may be enhanced. In chapter three, we identify and characterize a regional epidemic of one of these pathogens, enteroinvasive *E. coli* (EIEC). We show that EIEC swept through northwestern Ecuador causing a prolonged epidemic that lasted nearly 3 years. The epidemic involved at least six communities and 31 unique genotypes. Based on spatial and temporal data we suggest that a centrally located community, Borbón, may have played a key role in sustaining these infections while they spread to surrounding communities. Changing the focus from transmission

between communities to transmission within a community, we shift our attention to Borbón. In chapter four, we highlight the importance of environmental context such as extreme rainfall events on the effectiveness of diarrhea interventions. We show that the protective effects of improved sanitation facilities decrease after heavy rainfall. In contrast, the protection offered by safe water sources increase after heavy rainfall. These results suggest a "flushing effect", where during heavy rainfall, pathogens in the environment are flushed out of contaminated latrines and flushed into unsafe water sources, such as uncovered wells and rivers. This body of research highlights the importance of both biological and environmental interventions for diarrhea.

Chapter I

Introduction

Diarrheal Disease Burden

Worldwide, over four billion episodes of diarrhea occur each year (1, 2). Since the introduction of Oral Rehydration Therapy in the 1960's, diarrheal disease mortality in children younger than five years has dropped substantially. Estimates of mortality have fallen from 4.6 million in 1982 (data from 1954-1979, (3)), to 3.0 million in 1992 (data from 1980-1990, (4)), to 2.5 million in 2003 (data from 1980-2000, (5)). Analysis of Global Burden of Disease data puts this estimate at 2.2 million in 2004 (2). Despite the decreasing trend in mortality, morbidity of diarrheal illness in children younger than five remains high. Estimates of morbidity have ranged from 2.2 to 3.2 episodes per child-year since the 1950's (5, 6). Diarrheal morbidity is a significant public health concern because of its effects on quality of life, malnutrition, stunted growth and poor physical and cognitive development (5, 7-9). Moreover, it contributes to socio-economic inequality, as developing countries share a disproportionate amount of the global burden of diarrhea (5). In low-income countries diarrheal disease is the 3rd leading cause of mortality and, when measured in Daily Adjusted Life Years, is the 2nd leading cause of burden of disease (2).

Enteric Pathogens

Common causes of diarrhea in children are pathogenic *Escherichia coli* (*E. coli*), rotavirus, *Giardia lamblia*, *Shigella*, *Campylobacter*, *Cryptosporidium parvum*, *Aeromonas*, *Salmonella*, *Entamoeba histolytica* and *Virbriones* (10). Case control data collected from the proposed study site demonstrate high levels of three marker pathogens: pathogenic *E. coli*, rotavirus, and *Giardia* (11). Pathogenic *E. coli* and rotavirus are among the leading causes of infantile diarrhea, while *Giardia* infection is often found at high rates in developing country settings (12, 13). Enteric pathogens may be transmitted directly from person-to-person via the fecal-oral route or indirectly through the environment. Although the three marker pathogens highlighted here may exploit similar transmission pathways, they have distinct patterns of epidemiology, different environmental tolerances, and unique pathologies, which are summarized below.

Pathogenic Escherichia coli

In developing countries, 30-40% of acute diarrhea is attributed to infection with diarrheagenic *E. coli* (12). Diarrheagenic *E. coli* include the following pathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), *E. coli* Shigelloses (*Shigella*), enterohaemorrhagic *E. coli*, enteroaggregative *E. coli*, and diffusely adherent *E. coli* (14). The principle reservoir of these motile gram negative bacteria are the intestines of mammals and birds (14). *E. coli* may also survive in environments such as soil, manure, and water (15). In freshwater, they may survive for up to 12 months (16, 17). However, it is unclear whether the bacteria can also multiply in

the environment (14). There are several important pathogenic mechanisms that characterize and distinguish the clinical presentation and epidemiology of EPEC, ETEC, EIEC and *Shigella*.

Infection with EPEC is characterized by a distinct pattern of lesions formed on the surface of the small intestine epithelium referred to as 'attaching and effacing' lesions. These lesions are the result of: 1) intimate attachment to enterocytes; 2) cytoskeletal rearrangement of the host cell; 3) pedestal formation; and 4) a general disruption to brush border surface (18). Diarrhea results from decreased surface area due to a loss of microvilli in the epithelium (14). The infectious dose of EPEC (ID_{50}) is estimated to be 10^8-10^{10} organisms, the incubation period is between 9-12 hours, and transmission may be mostly via person-to-person (14, 19).

ETEC, like EPEC, may also adhere to the small intestinal epithelium but is further characterized by enterotoxin production and secretion of the heat-stable (ST) and heat-labile (LT) toxins (20). ETEC causes watery diarrhea with no mucus, pus or blood. The infectious dose is 10^8-10^{10} organisms, the incubation period can be up to 24-72 hours, and transmission occurs mostly through food and water (14, 19). Symptomatic infection is common in infants and travelers due to a lack of mucosal immunity to the pathogen (14).

EIEC primarily adheres to the mucosa of the large intestine, produces toxins, and invades the mucosal epithelium (14). EIEC, like *Shigella*, is typically associated with exudative, bloody diarrhea with fever (21). The infectious dose ranges from 10^6-10^{10} organisms, the incubation period is typically 10-18 hours, and transmission includes person-to-person, foodborne and waterborne pathways (14, 19, 22-24). Though EIEC

and *Shigella* share similar molecular mechanisms and transmission pathways, *Shigella* has a much lower infectious dose (10-100 organisms), and a longer incubation period (1-4 days) compared to EIEC (25).

Rotavirus

Rotavirus infection is the most common cause of diarrhea in children younger than two years. In developing areas, infection is more likely to occur in children younger than 12 months while in developed areas it is more common in children aged 12 to 24 months (12). Rotavirus is a double stranded RNA virus that selectively infects and kills differentiated and highly absorptive epithelial cells of the small intestine leading to both secretory and osmotic diarrhea (26, 27). The principle reservoir of the virus is humans and the mean infectious dose is estimated to be 200 viral particles (28). Rotavirus may survive for several hours on hands; for days in air, on surfaces and in food; for weeks in water; and for months in fecal matter (29). However, the virus cannot multiply outside a human host. The incubation period ranges from 24-72 hours and the infectious period averages at 8 days. Rotavirus is mostly transmitted from person-to-person through the fecal-oral route (19) however, there is also evidence for spread through the air (29).

Giardia

Giardia infection is a common cause of diarrhea in all ages regardless of region (12, 30). *Giardia lamblia* is a protozoite that is ingested as a cyst and adheres to the small

intestinal epithelium. Cysts release trophozoites in the small intestine, which then

multiply through binary fission. Trophozoites damage the mucosal surface of the

epithelium and trigger an immune response causing secretion of fluid and often, diarrhea

(31). Both trophozoites and cysts are released through feces yet only cysts can survive in

the environment (up to several months in cold water (30)). The typical infectious dose is

only 10-25 cysts (31). Of those who ingest cysts, 35-70% may be asymptomatic (30). The

principle reservoirs of *Giardia* include humans, beavers and other animals (wild and

domestic (31)). The median incubation period of *Giardiasis* is typically 7-8 days,

although the distribution can range from 1 to 60 days (32, 33), and the infectious period

can last for months (13). *Giardia* transmission occurs most often through consumption of

contaminated water and direct person-to-person contact (19, 31).

Diarrhea Transmission

Enteric pathogens employ multiple and often inter-dependent transmission routes

which can take place in the public or household domain (34). As described above, *E. coli*,

rotavirus and *Giardia* may be transmitted from feces to a new host through food, water or

direct contact. Curtis et al summarize and extend these transmission routes in the F-

diagram (Figure 1.1), highlighting the importance of fluids, fields, flies, fingers and food

(34).
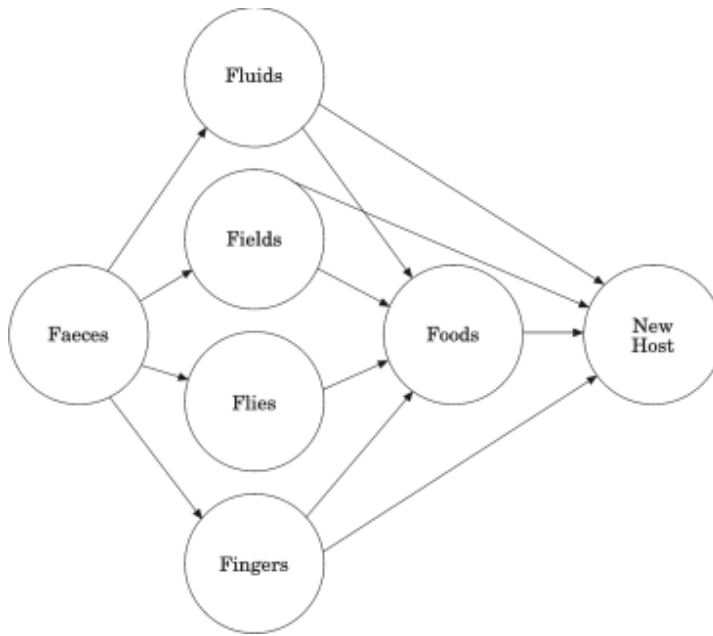
Figure 1.1. The F-diagram describing transmission of enteric pathogens (34).

Food may be contaminated through exposure to enteric pathogens in water (if improperly washed), soil (if unwashed), flies that land or regurgitate on food (if not stored safely), and on fingers (during food preparation). Fluids such as water can be contaminated with infected animal and human feces. Fingers may also harbor pathogens if unwashed after handling feces and contaminated soil (34). These vehicles of transmission may be realized through unsafe drinking water, unimproved sanitation and poor hygiene.

Water, Sanitation, and Hygiene

Water

Various aspects of drinking water are relevant to diarrheal disease. Water source, storage, and treatment can be defined and measured differently, have relevance at the

household-, neighborhood-, and community-level, and have varying associations with diarrhea. For example, Esry et al classified water supply from Demographic Health Surveys (DHS) as unimproved (rivers, ponds, lakes and unprotected springs), intermediate (centrally located hand pump, tap or well) and optimal (household water supply), and found no differences in risk for diarrhea (35). In an more extensive review of the DHS data, Fink et al used similar categorizations and showed significant protective effects of optimal and intermediate water sources compared to unimproved sources (36). While the protective effects of an improved water source are now widely accepted (2), there has been a recent shift in focus to water treatment and storage, owing to the risk of recontamination in the home. Meta-analyses of randomized and quasi-randomized control trials have shown that water quality interventions at point-of-use (chemical treatment, flocculation-disinfection, filtration, and solar disinfection) are protective for diarrhea (37, 38). Adding observational studies to their analyses, Gundry et al found that household water treatment (chlorination or solar disinfection) and/or water storage interventions (improved storage containers) were protective for diarrhea (39). Checkley et al reported that storage in small containers (which tended to be uncovered pots, pans, and buckets) compared to medium and large containers increased risk for diarrhea (40). Despite larger effect sizes reported for point-of-use interventions in the home compared to source-based interventions (41), poor compliance and lack of sustainability may actually decrease the effectiveness of these household-level interventions. This realization has motivated a re-evaluation of community- or neighborhood-wide water source improvements (42).

Sanitation

Sanitation is typically characterized by sewage disposal but may also include disposal of solid waste by the household, neighborhood, or community (43). Like drinking water, sanitation may be defined in different ways and may have varying associations with diarrhea. For example, when Checkley et al compared use of a sewage connection and use of a latrine to no facility, the authors found no associations with diarrheal disease (40). However, both Esry's and Fink's meta-analyses employed similar categories of sanitation: unimproved (holes in the ground, bushes and other open areas); intermediate (pit latrine or equivalent); and improved (flush toilets or water-seal latrines), and found that incremental improvements to sanitation resulted in a lower incidence of diarrhea (35, 36). The former study reported larger effect sizes in urban compared to rural areas (35). In a longitudinal study conducted in an urban area of Brazil, Genser et al classified the presence of a household toilet as in-habitation, outside habitation, and no toilet. Incremental and significant effects on the hazard for diarrhea were noted as the facility moved from inside to outside the house (44). Shifting the focus to those with access to latrines, Galal et al reported that latrine cleanliness (summarized by nine features: tap water connection, soap, flush, door, lid over toilet, flies, cockroaches, dirt and bad smell), was not associated with diarrhea. However, the presence of flies alone was associated with greater incidence (45). In contrast, Moll et al found that use of a hygienic latrine (defined as fewer than three flies present and no feces outside the latrine) was not associated with diarrhea. Interestingly, the authors did report a greater risk associated with more people sharing the latrine (46). Altering the definition of sanitation to one that involves the local environment, Genser et al report a higher hazard for diarrhea in young children associated with the presence of open sewage near the

household (44). Similarly, associations have been made between type of solid waste disposal and risk of diarrheal disease, where households that practice waste disposal in streets, streams and vacant lots are at higher risk for diarrhea than those which have garbage collected (43). Together, these studies suggest that open defecation and open waste disposal, which contaminate the local environment and promote the breeding of flies, are important causes of diarrhea.

Hygiene

The majority of the literature on hygiene has focused on the effects of handwashing on diarrhea. Literature reviews, including observational and intervention designs, have reported a 42-48% reduction in diarrhea risk associated with hand washing with soap (41, 47). Results from a cluster randomized control trial conducted over the span of a year, showed that handwashing promotion reduced diarrhea incidence in children younger than 15 years by 53% compared to children in control groups (48). Handwashing may be a simple practice, yet on average only 20% of mothers in developing countries wash their hands with soap after coming into contact with fecal matter (49). This low prevalence of handwashing may be partially explained by limited access to water for washing and poor hygiene education. Recently, public health agencies have begun teaming up with private industry to promote improved hygiene through marketing of soap (34). The results of these campaigns have yet to be summarized. Fly control, and protection of food from contact with flies may also fall under hygiene (34),

however, this pathway is strongly tied to the presence of fecal matter in the environment, and may be addressed through improved sanitation.

Global Development Targets

At the turn of this century, the United Nations together with the international community pledged to reduce conditions of extreme poverty worldwide. Eight Millennium Development Goals were established based on universally accepted human rights and values. Goal number four aimed to reduce the under-five mortality rate by two-thirds by the year 2015. By 2006, we were one-third of the way there (50). But, child mortality is still unacceptably high, especially in developing regions. The 2nd leading cause of child death in these regions is diarrhea (2) and thus, efforts to meet this goal are largely dependent on our ability to prevent diarrheal disease. Related to diarrheal disease prevention is Millennium Development Goal number seven, which aims to cut in half the proportion of those without sustainable access to safe drinking water and basic sanitation by 2015 (50). Recent reports project that we will meet safe the water target but will fall short on providing basic sanitation (51). This shortfall is unfortunate considering that improved sanitation may prevent more diarrhea than an improved water source (35, 36, 41). Currently, 2.6 billion people lack access to improved sanitation facilities, while 1.1 billion defecate in the open (52). With the 2015 target date nearing, we will likely witness renewed political will and international interest in improving sanitation. Yet, we will still have to work with limited resources to effectively target sanitation interventions within a community.

Direct and Indirect Effects of Diarrhea Interventions

Environmental (water and sanitation), behavioral (handwashing) and biological interventions (rotavirus vaccination) for diarrhea may have direct effects in a population. That is, they may lower the risk of diarrhea in the intervention group. However, just as important, are the indirect effects of an intervention in a neighborhood, community, or region. Indirect effects result from protection offered by the intervention group to the rest of the population, as a result of their reduced risk of disease (53). For example, Root et al reported lower risk of diarrhea in households where neighbors owned a latrine compared to households where neighbors did not, demonstrating the presence of indirect effects (56). Ali et al and Emch et al have shown that higher neighborhood coverage of a cholera vaccine reduced incidence in non-vaccinated persons more rapidly (through indirect effects) than vaccinated persons (through direct and indirect effects) (54, 55). Finally, Huq et al provide evidence for indirect effects of sari cloth water filtration to neighboring households that practice no filtration (57). While herd immunity may be a common consideration during vaccination campaigns, the indirect effects of environmental and behavioral interventions for diarrhea remain largely under addressed. In this dissertation, we add to the evidence for indirect effects by considering the effects of improved water and sanitation in neighboring households.

The Study Region

The study region is located in the cantón Eloy Alfaro in the province of Esmeraldes in northwestern Ecuador. The region sits at the southern end of the Chocó rainforest and is classified as a biodiversity hotspot with high levels of endemic plant and animal species (58). Since the 1980's, the region has seen high rates of deforestation. Around this time, dirt roads developed for commercial logging and land clearing paved the way for export-oriented agriculture, including the farming of cocoa, banana, palm oil and eucalyptus. By the 1990's, the political and economic climate in Ecuador heavily discouraged small-scale farming practices, which were widely practiced in the Andes to the East. Both road development and large-scale agriculture encouraged the in-migration of Mestizos (people of mixed origin) from other parts of Ecuador and Colombia. Before this, the region's population was made up of mostly Chachis (the indigenous) and Afro-Ecuadorians, who have been living here for over 200 years. Today, Mestizos represent a growing segment of the region's population (59).

The region has approximately 150 communities located along one of three river systems (Río Cayapas, Río Santiago and Río Onzole), which all drain towards Borbón, the region's population center (Figure 1.2). Borbón and the surrounding region have recently undergone dramatic change due to the construction of a new highway, linking the town to the coast on the west and the Andes to the east. The highway has encouraged the movement of people, commercial goods and information in and out of the region. In Borbón, residential development is occurring at a rapid pace yet, the development of water and sanitation infrastructure lags behind. For example, in July 2004, a water treatment plant opened up in Borbón. However, it does not extend to all neighborhoods, and the supply is unreliable, forcing some to continue using river or rainwater for

consumption and hygiene. Residents of the city use either flush toilets, pit latrines, open spaces or the river as sites for defecation and currently there is no working public sewage system in place, although one was being constructed during the course of our study (2009). In the communities upstream of Borbón, there is heavier dependence on the river as a water source and sanitation facilities tend to be more rudimentary. Consistent with tropical culture, domestic activities such as washing, bathing, and socializing usually take place outside and around the house. The social culture and mixed water and sanitation practices make this an excellent population in which to study transmission of diarrhea through environmental pathways.

Dissertation Overview

Motivated by the large diarrheal disease burden in developing countries, we identified three major enteric pathogens that cause diarrhea in northwestern Ecuador. In chapter one, we estimate the prevalence and pathogenicity of these three pathogens across all age groups in the region. Our specific focus however, is on the pathogenicity of coinfecting pathogens, which, considering the large diarrheal disease burden, is under-addressed in the literature. In chapter two, we identify and characterize a regional outbreak of one of these pathogens, enteroinvasive *E. coli*. We estimate the spatial and temporal extent of the outbreak in the study region, and consider Borbón as a central source of the pathogen. Few, if any studies, have addressed regional outbreaks of this *E. coli* pathotype in the developing world. Our focus in this chapter is on *between-*community transmission whereas in the next, it is on *within*-community transmission. In

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chapter three, we present evidence for pathogen transmission through the environment in Borbón. We highlight the importance of environmental context, such as neighboring household practices and extreme rainfall events, on the effectiveness of water and sanitation improvements. Our hope is that these three chapters will provide insight into the causes of diarrhea in developing areas as well as effective intervention strategies to prevent them.

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Figure 1.2. Map of study communities in northwestern Ecuador. Communities were studied from 2003-2008 (triangles), 2008-2010 (squares) and 2003-2010 (circles).

Study Region in Northwestern Ecuador (2003-2010)
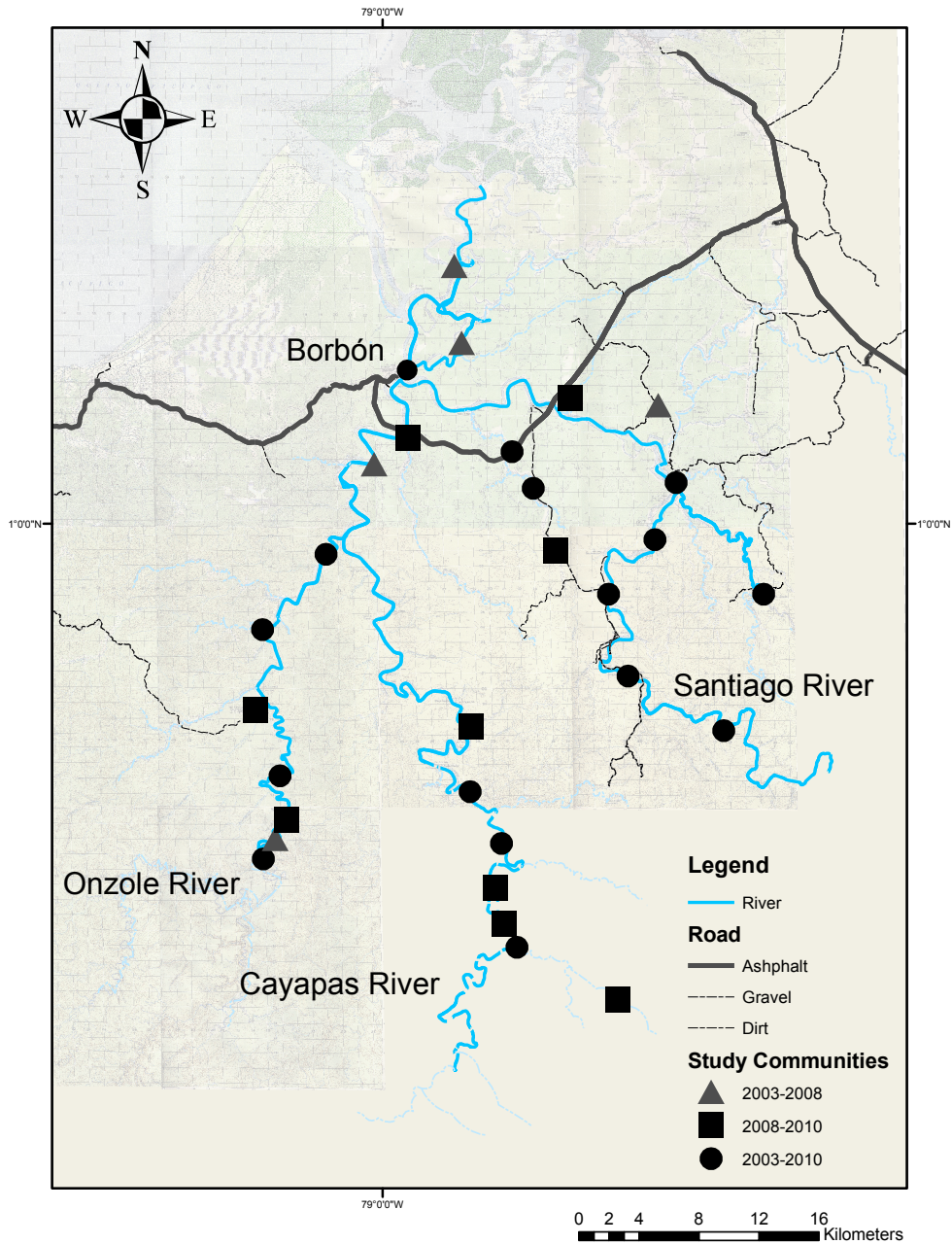
Chapter II

Synergistic Effects Between Rotavirus and Coinfecting Pathogens on Diarrheal
Disease

Abstract

In developing countries where diarrheal disease is a leading cause of morbidity
and mortality in children under five, enteric coinfection is common. There is little
understanding, however, of the biological interaction between coinfecting pathogens. We
investigated the potential for synergistic interaction between coinfecting pathogens on
diarrhea pathogenesis using an epidemiological framework. We conducted community-
based case control studies in 22 communities in northwestern Ecuador between 2003 and
2008. Risk ratios of diarrhea associated with single infections and coinfections were
estimated. Biological interaction between coinfecting pathogens was assessed through
departure from risk ratio additivity and multiplicativity after adjusting for age. On the
additive scale, we found departure from the null value of zero for rotavirus-*Giardia*
coinfections (8.0 (95% CI: 3.1, 18.9)) and for rotavirus-*E. coli* coinfections (9.9 (95% CI:
2.6, 28.4)). Departure from multiplicativity (i.e. departure from a value of one) for
rotavirus-*Giardia* coinfections was 3.6 (95% CI: 1.3, 8.7). This research provides
epidemiological evidence for synergism between rotavirus and other enteric pathogens.
During coinfection, the pathogenic potential of each organism appears to be enhanced.

The potential for pathogenesis to be more severe in the presence of a rotavirus coinfection amplifies the need for rotavirus vaccination.

Introduction

Diarrheal disease is the fifth leading cause of death in low- and middle-income countries (1). Although mortality rates have declined in the past several decades (2), diarrhea still causes up to 1.9 million childhood deaths each year (3). Many enteric viruses, bacterial pathogens and parasites likely contribute to this disease burden both individually and together (2). Together, coinfecting pathogens may cause more severe diarrhea than infection with either pathogen alone (4). Specific coinfecting pathogens may also act synergistically, resulting in even greater pathogenesis and a larger contribution to the overall diarrheal disease burden.

Mixed infections are commonly detected in case control studies making it difficult to establish causal links between a pathogen and diarrhea. Coinfecting pathogens may confound these relationships and if ignored, potentially lead to false inferences. Previous case control studies (5-12) have found mixed infections in 10% to 40% of cases and in 0% to 15% of controls, and as many as five coinfecting pathogens (7, 12). Though mixed infections were discussed in all eight studies, only three reported associations between single infections and diarrhea (6, 10, 11). By consistently distinguishing between single and mixed infections, we may be able to improve our understanding of the pathogenic potential of enteric infections.

Mixed infections have been shown to exacerbate diarrheal illness. Earlier animal studies support increased morbidity and mortality from rotavirus-*Escherichia coli* (*E. coli*) coinfections (13-17). Clinic-based studies of diarrhea cases have also suggested greater severity of diarrhea in the presence of a rotavirus-*E. coli* coinfection (4, 6, 10, 18, 19). In contrast, Unicomb et al. have shown no greater severity than that of single infections with rotavirus or *E. coli* (20). Unhoo et al. have also demonstrated no increase in severity from mixed viral-bacterial infections (21). Yet, the authors did report prolonged diarrhea associated with coinfections compared to single infections with rotavirus. The pathogenicity of enteric coinfections has been the focus of very few community-based studies, (see for example (22)). However, much of the clinical research to-date supports enhanced pathogenesis from the combined independent actions of two pathogens.

Little is known about the potential for pathogens to act synergistically to cause diarrhea. In vitro models have provided us with some insight on the biological mechanisms behind synergism. These studies (23-25) have shown that intestinal cell lines incubated with rotavirus may predispose cells to increased adhesion, invasion and multiplication by invasive bacteria. Superti et al also reported increased levels of viral replication in coinfected cells, demonstrating a synergistic interaction (23). Relationships were time-dependent; higher levels of adhesion, invasion and multiplication were noted as the rotavirus-incubation period lengthened. Furthermore, the inability of non-invasive bacterial strains to traverse the host-cell membrane, points to a specific transport mechanism and not just a general increase in permeability. While these in vitro models

support a synergistic interaction between rotavirus and coinfecting pathogens, evidence from community-based studies will be important to confirm this action in vivo.

In this study, we use community-based case control data to estimate the prevalence and pathogenicity of *Giardia*, rotavirus, *E. coli* (including *Shigella*), and *Plesiomonas shigelloides* (*P. shigelloides*) across all ages in northern coastal Ecuador between 2003 and 2008. In our assessment of pathogenicity we distinguish between single and coinfections. We also examine the evidence for biological synergism between coinfecting pathogens by estimating their interaction on both additive and multiplicative scales.

Methods

Study Region

The study region is located in the northwestern coastal area of Ecuador in the Cantón Eloy Alfaro. This area has experienced rapid development since the introduction of a paved road in 2001. The new road links the region to the coast on the west and the Andes to the east, facilitating the movement of products, people and pathogens in and out of the region. We sampled 21 villages in the study area, each of which is located along one of three rivers that drain into Borbón, the urban center of the region also included in the study. These 22 communities generally rely on river water, although some have access to well or piped water. Sanitation facilities range from pit latrines to flush toilets.

Study Design

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Between August 2003 and February 2008 we conducted up to seven 15-day case control studies in each of the 22 communities. Before the case control period began, we conducted a census of the community. During the case control period we visited each house daily to identify all cases of diarrhea in the 21 villages (ranging in size from 5 to 200 households) or in a random sample of 200 households in Borbón, which has a population of approximately 1000 households. For every case, we randomly selected one household control and two community controls at the time of case identification. Cases were defined as anyone with three or more loose stools in a 24-hour period. Controls were eligible if they were free of diarrhea in the previous six days (no other inclusion or exclusion criteria were applied). Stool specimens were collected from all cases and controls. We obtained oral consent from each village and household in the study. Approximately 99% of houses consented to participate in the study and 93% of cases submitted stool specimens. IRB committees at the University of Michigan, Trinity College, and Universidad San Francisco de Quito approved all protocols.

Pathogen Detection

Stool samples were tested for rotavirus, *P. shigelloides*, pathogenic *E. coli*, *Shigella* spp., and *Giardia*. Rotavirus was detected in the field using a commercial immunochromatographic test (RIDA Quick Rotavirus, R-Biopharm, Darmstadt, Germany). Fecal samples were plated directly onto XLD or MacConkey agar. All lactose negative colonies were selected and analyzed with the API 20E assay (bioMerièux Marey l'Etoile), which was used to detect *P. Shigelloides*. Lactose-negative isolates, identified as either *E. coli* or *Shigella* by API 20E, and a random sample of five lactose-positive

isolates, were analyzed using PCR. Pathotype-specific primers were used to identify

ETEC (*toxA* and *ST 1b*), EPEC (*bfpA*), and EIEC or *Shigella* (*ipahH*). Additionally, an

aliquot of fecal material was frozen in liquid nitrogen and transported to a laboratory in

Quito where *Giardia* was detected using an ELISA kit (RIDASCREEN *Giardia*; R-

Biopharm). Further details can be found in (26).

Statistical Analysis

  We pooled 152 case control studies and analyzed these together using R v.

2.11.1. Estimates of diarrhea prevalence were based on the number of cases identified,

the number of household residents, and the community population during each case

control visit. To estimate prevalence of enteric infection, we assigned inverse probability

sampling weights to all cases and controls. Probability sampling weights were adjusted to

the age distribution of the community population for age-specific estimates only. Using

these weights and the standard Horvitz-Thompson theory (27), unbiased estimation of the

15-day prevalence was achieved. The pathogenicity of each organism was quantified by

the risk ratio between presence of the pathogen and diarrheal symptoms. These risk ratios

were calculated directly from the 2x2 table whose entries were filled in with the weighted

proportions. To investigate pathogenic effects both within and across age groups, we

estimated the strata-specific, crude, and Mantel-Haenszel (MH) pooled risk ratio (RR) for

diarrhea associated with single and multiple infections (28). Biological interaction

between two coinfecting pathogens was assessed on the additive scale using the

interaction contrast ratio (ICR, (28)) and age-standardized RR's:

$$\mathrm{ICR} = \mathrm{RR}_{\text{coinfection}} - \mathrm{RR}_{\text{single infection 1}} - \mathrm{RR}_{\text{single infection 2}} + 1$$

And on the multiplicative scale by estimating departure from multiplicativity of the age-standardized RR's (28), which we refer to as multiplicative interaction (MI):

$$\mathrm{MI} = \mathrm{RR}_{\text{coinfection}} / (\mathrm{RR}_{\text{single infection 1}} \ \mathrm{x} \ \mathrm{RR}_{\text{single infection 2}})$$

Use of age-standardized RR's allowed us to account for potential confounding by age. To make statistical inferences, we characterized the sampling distribution of the ICR, MI and all RR by bootstrapping the data. We sampled with replacement from the original dataset a number of observations equal to the original sample size. Using this new dataset, estimates of the ICR, MI, and all RR were calculated. This process was repeated 1000 times to produce estimates of the sample distributions associated with each statistic. The lower 0.025 and upper 0.975 percentiles of the bootstrap distribution of these statistics are presented as 95% confidence intervals. Since EIEC and *Shigella* share similar molecular mechanisms and phylogeny, these organisms were grouped together and are noted as EIEC/*Shigella* in the subsequent text.

Results

Case Control Sample

Between August 2003 and February 2008, 3314 stool samples were collected from 883 cases and 2431 controls in the study region. We isolated pathogens in 499 cases (56.5%) and in 780 controls (32.1%, Table 2.1). Mixed infections were detected in 21.2%

of cases and in 4.2% of controls. The most common infections were either single or

mixed infections with *Giardia*, found in 31.5% of cases and 20.4% of controls. Rotavirus

was detected in 22.2% of cases and 2.6% of controls, *P. shigelloides* in 10.4% of cases

and 6.6% of controls, and *E. coli* or *Shigella* in 17.1% of cases and 6.8% of controls.

Multiple *E. coli* pathotypes were isolated in 21 individuals (Table 2.2). Our sample size,

which was originally reduced from 3326 to 3314 observations due to missing information

on case control status, was further reduced to 3107 due to missing individual census data.

For all subsequent analyses we use a sample size of 3107. The median age of cases was 3

years (range: 0-81) and controls 16 years (range: 0-99).

Community Prevalence

The 15-day period prevalence of diarrhea was 2.3% (95% CI: 2.1, 2.5, Table 2.3).

Although there were cases of diarrhea in older children and adults, diarrhea was most

prevalent in children younger than five years (Figure 2.1). The prevalence of *Giardia* and

rotavirus was 20.3% (95% CI: 18.1, 22.5) and 3.2% (95% CI: 2.2, 4.2) respectively.

Giardia and rotavirus infections were prevalent across all age categories, though the

latter were most prevalent in children under one. Of the *E. coli* pathotypes, EIEC/*Shigella*

was the most prevalent (4.5%, 95% CI: 3.3, 5.7) and was evenly distributed across the

age groups with the exception of infants; only one EIEC/*Shigella* infection was identified

in infants younger than one year.

Pathogenicity

A single infection with rotavirus was significantly associated with diarrhea in one to four year olds (RR = 2.4, (95% CI: 1.1, 6.2)) and in those older than 13 years (RR = 6.6, (95% CI: 3.2, 15.1), Table 2.4). In comparison, a single infection with ETEC increased the risk of diarrhea in children aged one through 12 years, but not in adults. Single infections with rotavirus were significantly associated with diarrhea across all age groups (MH-RR = 2.3, (95% CI: 1.3, 4.8). In contrast, single infections with *Giardia*, EIEC, and *P. shigelloides* were not pathogenic (RR for P. *shigelloides* = 1.5, 95% CI: 0.9, 2.2). Age appeared to confound the associations between diarrhea and each of *Giardia*, rotavirus, ETEC and EPEC but not EIEC (Table 2.4). Age did not appear to confound the association between *P. shigelloides* and diarrhea (data not shown). In all instances, crude estimates of the risk ratios were higher than MH risk ratios pooled across age groups. And, where we had enough data to report estimates across infection categories, MH risk ratios associated with a coinfection and any infection were greater than risk ratios associated with single infections by the same pathogen.

Coinfections

We found evidence for greater than additive and greater than multiplicative effects of rotavirus coinfections on the risk of having diarrhea. Under the null hypothesis of no biological interaction on the additive and multiplicative scales, we would expect the ICR to equal zero and the MI to equal one, respectively. The ICR specific to coinfection with rotavirus and *Giardia* was 7.96 (95% CI: 3.13, 18.92) and the MI was 3.61 (95% CI: 1.33, 8.71, Table 2.5). Coinfections with rotavirus and *Giardia* were found in all age categories. Excluding two coinfected individuals with missing birthdates, 31 (63%) of

rotavirus-*Giardia* coinfections occurred in children under five. The ICR related to coinfection with rotavirus and *E. coli* was 9.93 (95% CI: 2.61, 28.41) while the MI was 3.06 (95% CI: 0.75, 7.27). Of these 24 coinfections, one was missing a birth date, 10 (43%) were found in children under five, and 16 were specific to EIEC/*Shigella*. We found no interaction effects associated with a *Giardia-E. coli* coinfection on diarrhea.

Discussion

Using community-level data, we provide evidence that coinfecting pathogens act synergistically with rotavirus to cause diarrhea. Possible mechanisms for these synergistic effects may be specific, involving attachment and invasion of the intestinal epithelia by pathogens, or non-specific resulting from inflammation. We also found evidence that a single infection with rotavirus is pathogenic in young children and adults. The potential for rotavirus to cause disease in these age groups, and the potential for pathogenesis to be more severe in the presence of a rotavirus coinfection, may warrant targeting rotavirus prevention efforts to both young children and adults.

Synergism of coinfecting pathogens

Simultaneous infection with rotavirus and *Giardia* or rotavirus and *E. coli* (including *Shigella*) resulted in a greater risk of having diarrhea than would be expected if the coinfecting organisms acted independently of one another. The idea that rotavirus and *Giardia* act synergistically contradicts findings by Bilenko et al (22), who compared severity scores of single and mixed infections in Bedouin infants. However, their inferences were based on a small sample size with only 12 single rotavirus infections and

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three rotavirus-*Giardia* coinfections. Regarding coinfection with rotavirus and *E. coli,* our findings disagree with Unhoo et al (21), which could be the result of differences between developed and developing country settings. Unicomb et al also reported dissimilar findings to ours, but the *E. coli* pathotypes detected were different (20). Approximately two-thirds of the rotavirus-*E. coli* coinfections found in their study were specific to diffuse-adherent *E. coli* and enteroaggregative *E. coli* while in our study a similar proportion involved EIEC/*Shigella*. On the other hand, the ability of these coinfecting pathogens to have at least additive effects is supported by other studies in children (4, 6, 10, 18, 19). Our community-based study provides evidence for superadditive effects of coinfecting pathogens. Unlike these studies, ours includes older age groups and uses a different outcome measure. Rather than severity of diarrhea, we have considered the ability of coinfecting pathogens to cause diarrhea, defined as three or more loose stools passed in a 24-hour period. Since our definition likely includes diarrhea ranging in severity, our findings that coinfecting pathogens act synergistically to cause either mild or severe diarrhea compliments these previous works.

The evidence for synergistic interaction between rotavirus and other coinfecting pathogens is important to the global burden of diarrhea given that developing and rural regions may experience a high prevalence of enteric infections. *Giardia*, for example, was estimated to affect one-fifth of the population in our study region creating potential for high levels of coinfection. Furthermore, the lack of improved water and sanitation in developing regions may facilitate simultaneous transmission of enteric pathogens. Synergistic interaction between rotavirus and coinfecting pathogens calls for targeted

rotavirus prevention as well as more general water, sanitation, and hygiene improvements to curb transmission of coinfecting pathogens.

Mechanisms for Synergistic Interaction

In vitro models of pathogenesis indicate that synergism between rotavirus and invasive bacteria involve specific biological pathways (23-25). These pathways may involve the attachment of, or the invasion by, coinfecting pathogens through an up-regulation of specific receptors. It is interesting that we found no evidence for pathogenic effects of EIEC/*Shigella* alone, yet when infection occurred in the presence of rotavirus the risk of diarrhea was enhanced. It should be noted that rotavirus, ETEC, EPEC, and *Giardia* predominately affect the small bowel while EIEC and *Shigella* colonize the large bowel (29). Although pathogenesis studies in the Rhesus monkey (30) and rabbits (31), have suggested that *Shigella* passage through the jejunum of the small intestine may alter secretion of sodium and water, potentially contributing to watery diarrhea. The heightened pathogenicity of *Giardia* in the presence of rotavirus may be related to a more successful attachment of the trophozoite ventral disk to the infected epithelium (32).

Alternatively, the biological mechanisms behind synergistic interaction may be less specific than in-vitro models predict. The inflammatory response induced by rotavirus likely damages the epithelium and alters the mucosal structure facilitating the attachment and invasion of coinfecting pathogens. Inflammation is also characterized by the release of fluid, mucin, and cellular debris, which potentially contain high-energy nutrients for pathogens (33). Furthermore, the secretion of antimicrobials during inflammation could alter the composition of the gut microbiota, allowing pathogens to

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occupy the commensal niche (34). More research is needed to elucidate the pathogenesis of diarrhea during rotavirus coinfection.

Prevalence and Pathogenicity

Regarding pathogenicity of single infections, our findings that rotavirus is associated with diarrhea when pooled across age groups is consistent with other community- and clinic-based studies (7, 35, 36). However, the lack of an association between rotavirus and diarrhea in infants younger than one year is inconsistent with the literature (6, 7, 37) and may correspond to the presence of maternal antibodies (38), our small sample size in this age group, or misclassification of diarrhea owing to looser stools in infants at baseline. Despite the probable exposure to early infection with rotavirus, we observed the ability of rotavirus to cause diarrhea in adolescents and adults. The presence of rotavirus-induced diarrhea in older age groups may be accounted for by frequent transmission between infants and their caregivers, potential inclusion of immuno-compromised and malnourished individuals in our sample, inclusion of mild diarrhea events, waning of rotavirus immunity over time, and exposure to a variety of circulating genotypes (39). A previous study from this region found high rates of the emerging G9 genotype (40), while another reported subsequent replacement of the G9 genotype by the G1 and G2 genotypes (41). In developing countries, rotavirus-specific interventions may also need to be targeted to older children and adults.

Overall, we estimated that 2.3% of the population, and 9.3% of children under five, had diarrhea during any given 15-day period. While diarrhea prevalence in the total population may have been low, we found high prevalence of infection. High prevalence

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of asymptomatic infection may reflect early exposure to enteric pathogens and naturally acquired immunity. High prevalence of *Giardia* in our region may be further explained by frequent exposure, rapid re-colonization rates, and long-term shedding (42). The overall isolation rates of *Giardia*, rotavirus, EIEC/*Shigella*, ETEC and EPEC from cases and controls were comparable to those found in other studies of children under five (5, 8, 9, 35). Our detection of pathogens in 56.5% of cases was similar to isolation rates from other community-based studies (5, 43, 44) and as expected, was lower than those from hospital-based studies in children, which capture more severe diarrhea (7, 10, 45).

Although cases and controls were not age-matched while sampling, all age categories had a sufficient number of cases and controls for analyses. Our age-specific estimates of pathogenicity were solely limited by the prevalence of pathogen infections within each age category. Confounding by age was addressed in our analyses. Additional confounders of the association between enteric infection and diarrhea, such as environmental or social variables, potentially act through other enteric pathogens to cause diarrhea. Therefore, adjusting for other enteric pathogens addresses confounding, which in our analyses was done through the exclusion of other measured pathogens from the risk ratio estimates. There are unmeasured pathogens that may have resulted in confounding. However, unpublished data from our region indicate that the majority of the helminthes in circulation are non-pathogenic and there are low rates of other potentially pathogenic organisms such as *Vibrio cholerae*, *Cryptosporidium*, *Salmonella* spp., and *Aeromonas* spp. Published data from other regions suggest that the predominant pathogens are those captured in our study (11, 35, 36).

Conclusions

Given the high prevalence of enteric pathogen coinfection found in our study and others (7, 9, 10, 12, 45), a true understanding of the pathogenesis of diarrheal disease is incomplete without a thorough understanding of the biological interaction of these pathogens. Furthermore, reducing the diarrheal disease burden is dependent upon our knowledge of the pathogenesis of diarrhea. This study is one of the few to consider pathogenicity of both single and mixed infections in all age groups, with a particular focus on the synergistic interaction between coinfecting pathogens. Our literature search suggests that this is the first community-based study of diarrhea to examine synergistic effects using theory rooted in foundational epidemiology. Further research is needed to address the specific biological mechanisms of enteric pathogens resulting in their synergistic interaction.

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Table 2.1. Infection patterns identified in cases and controls from 22 communities in northwestern Ecuador, 2003-2008

Mutually exclusive infection categories	Cases (%) N=883	Controls (%) N=2431
Giardia	137 (15.5)	408 (16.8)
Rotavirus	85 (9.6)	47 (1.9)
Rotavirus + *Giardia*	52 (5.9)	9 (0.4)
E. coli[a]	52 (5.9)	107 (4.4)
Giardia + E. coli[a]	46 (5.2)	42 (1.7)
P. Shigelloides	38 (4.3)	116 (4.8)
Rotavirus + *E. coli*[a]	23 (2.6)	4 (0.2)
Giardia + P. Shigelloides	17 (1.9)	32 (1.3)
Rotavirus + *P. Shigelloides*	12 (1.4)	2 (0.1)
E. coli[a] + *P. Shigelloides*	9 (1.0)	8 (0.3)
Rotavirus + *Giardia + E. coli*[a]	12 (1.4)	0 (0.0)
Rotavirus + *Giardia + P. Shigelloides*	7 (0.8)	1 (0.0)
Giardia + E. coli[a] + *P. Shigelloides*	4 (0.5)	3 (0.1)
Rotavirus + *Giardia + E. coli*[a] + *P. Shigelloides*	3 (0.3)	0 (0.0)
Rotavirus + *E. coli*[a] + *P. Shigelloides*	2 (0.2)	1 (0.0)
Total	499 (56.5)	780 (32.1)

[a]Includes *Shigella*.

Table 2.2. *Escherichia coli* (*E. coli*) and *Shigella* infection patterns identified in cases and controls exclusively infected with *E. coli* and/or *Shigella* in 22 communities in northwestern Ecuador, 2003-2008

Mutually exclusive infection categories	Cases (%) N=883	Controls (%) N=2431
EIEC	11 (1.2)	48 (2.0)
Shigella	6 (0.7)	16 (0.7)
ETEC	22 (2.5)	23 (0.9)
EPEC	4 (0.5)	8 (0.3)
EIEC + *Shigella*	5 (0.6)	9 (0.4)
EIEC + ETEC	2 (0.2)	1 (0.0)
Shigella + ETEC	1 (0.1)	0 (0.0)
Shigella + EPEC	1 (0.1)	1 (0.0)
EIEC + Shigella + ETEC	0 (0.0)	1 (0.0)
Total	52 (5.9)	107 (4.4)

Enterotoxigenic *E. coli* (ETEC); Enteroinvasive *E. coli* (EIEC); Enteropathogenic *E. coli* (EPEC)

Table 2.3. Weighted community prevalence of diarrhea and enteric infections and 95% confidence intervals estimated using the Horvitz-Thompson theory, 2003-2008

	Prevalence[a] (%)	95% Confidence Interval
All-Cause Diarrhea	2.3	2.1, 2.5
Giardia	20.3	18.1, 22.5
Plesiomonas shigelloides	6.9	5.4, 8.4
Rotavirus	3.2	2.2, 4.2
EIEC/*Shigella*	4.5	3.3, 5.7
ETEC	2.0	1.2, 2.7
EPEC	0.6	0.1, 1.0

Enteroinvasive *E. coli* (EIEC); Enterotoxigenic *E. coli* (ETEC); Enteropathogenic *E. coli* (EPEC); [a]Weighted prevalence estimates were based on analyses of case and control stool samples.

Table 2.4. Age-specific, Mantel-Haenszel (MH) pooled and crude risk ratios (RR) for diarrhea and bootstrap 95% confidence intervals (95% CI)

Infection Category	<1 Year [a-d] RR	95% CI	1-4 Years [a-d] RR	95% CI	5-12 Years [a-d] RR	95% CI	13+ Years [a-d] RR	95% CI	MH-Pooled [a-d] RR	95% CI	Crude [a-c] RR	95% CI
Giardia (any infection)	2.3	1.2, 4.5	1.8	1.2, 2.7	1.1	0.6, 1.8	1.6	0.9, 2.7	1.7	1.3, 2.3	2.6	2.1, 3.2
Giardia (single infection)	1.4	0.5, 3.8	1.2	0.8, 1.9	0.6	0.3, 1.2	0.6	0.1, 1.3	1.1	0.8, 1.5	1.5	1.2, 2.0
Giardia (any coinfection)			3.9	2.4, 7.4	3.6	1.7, 7.2	7.8	3.3, 16.7	4.1	2.9, 6.0	7.6	5.6, 10.4
Rotavirus (any infection)	2.0	0.7, 5.1	4.2	2.3, 8.0	7.0	3.2, 20.6	14.1	7.8, 27.2	4.3	2.8, 7.1	10.7	7.9, 15.1
Rotavirus (single infection)	1.4	0.4, 5.2	2.4	1.1, 6.2	2.5	0.6, 10.9	6.6	3.2, 15.1	2.3	1.3, 4.8	5.8	3.8, 9.4
Rotavirus (any coinfection)			8.7	6.2, 12.1	20.2	8.5, 58.3	59.0	30.6, 122.1	9.4	6.9, 12.7	25.5	17.3, 39.6
EIEC (any infection)			2.4	1.2, 5.0	3.9	1.8, 7.5	4.1	2.0, 7.9	2.9	1.8, 4.8	3.6	2.4, 5.0
EIEC (single infection)			1.2	0.4, 5.7			2.6	0.8, 5.6	1.5	0.7, 3.2	1.6	0.9, 2.7
EIEC (any coinfection)			3.4	1.7, 7.9	7.4	3.5, 15.7	10.3	3.4, 33.1	4.6	2.7, 8.4	6.7	4.1, 10.5
ETEC (any infection)	1.5	0.4, 4.9	5.4	3.1, 9.8	8.8	3.4, 21.4	3.3	0.9, 12.2	3.7	2.2, 6.5	7.1	4.6, 12.1
ETEC (single infection)			4.1	1.5, 10.7	8.2	1.8, 24.5	1.8	0, 9.8	2.2	1.0, 6.3	3.8	2.0, 8.2
ETEC (any coinfection)			6.5	3.4, 11.1	9.4	2.0, 41.0	16.2	0, 158.5	6.0	3.8, 8.8	13.9	7.5, 28.7
EPEC (any infection)			2.7	0.9, 8.1					1.7	0.7, 4.8	5.7	2.0, 14.3
EPEC (single infection)												
EPEC (any coinfection)			3.5	1.1, 10.8					3.4	1.2, 8.7	11.6	4.2, 44.9

Enteroinvasive *E. coli* and *Shigella* (EIEC); Enterotoxigenic *E. coli* (ETEC); Enteropathogenic *E. coli* (EPEC)

[a]Risk ratios compare risk of diarrhea in those exposed to risk in those unexposed to *Giardia*, rotavirus, pathogenic *E. coli*, *Shigella* and *P. shigelloides*.

[b] Risk ratios based on cell counts less than five were excluded.

[c]All risk ratios were weighted by inverse sampling probabilities.

[d]Strata-specific and pooled estimates are weighted by inverse age-specific sampling probabilities.

Table 2.5. Assessment of the interaction between coinfecting pathogens associated with diarrhea on additive and multiplicative scales using age-standardized risk ratios (RR) and bootstrap 95% confidence intervals (95% CI)

Infection Category	RR	Age-Standardized Estimates				
		Additive Model			Multiplicative Model	
		ICR[a]	95% CI		MI[b]	95% CI
Rotavirus (single infection)	2.63					
Giardia (single infection)	1.13					
Rotavirus and *Giardia* (coinfection)	10.72					
		7.96	3.13, 18.92		3.61	1.33, 8.71
Rotavirus (single infection)	2.63					
E. *coli*/*Shigella* (single infection)	1.64					
Rotavirus and E. *coli*/*Shigella* (coinfection)	13.20					
		9.93	2.61, 28.41		3.06	0.75, 7.27
Giardia (single infection)	1.13					
E.*coli*/*Shigella* (single infection)	1.64					
Giardia and E. *coli*/*Shigella* (coinfection)	3.02					
		1.25	-1.48, 3.13		1.63	0.47, 3.06

[a]Interaction contrast ratio (ICR) = $RR_{coinfection}$ - $RR_{single\ infection\ 1}$ - $RR_{single\ infection\ 2}$ + 1;
[b]Multiplicative interaction (MI) = $RR_{coinfection}$ / ($RR_{single\ infection\ 1}$ x $RR_{single\ infection\ 2}$).

Figure 2.1. Weighted prevalence (%) and upper confidence limit of all-cause diarrhea, *Giardia*, rotavirus, enteroinvasive *E. coli* or *Shigella* (EIEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC) by age category in 22 communities in northwestern Ecuador, 2003-2008.
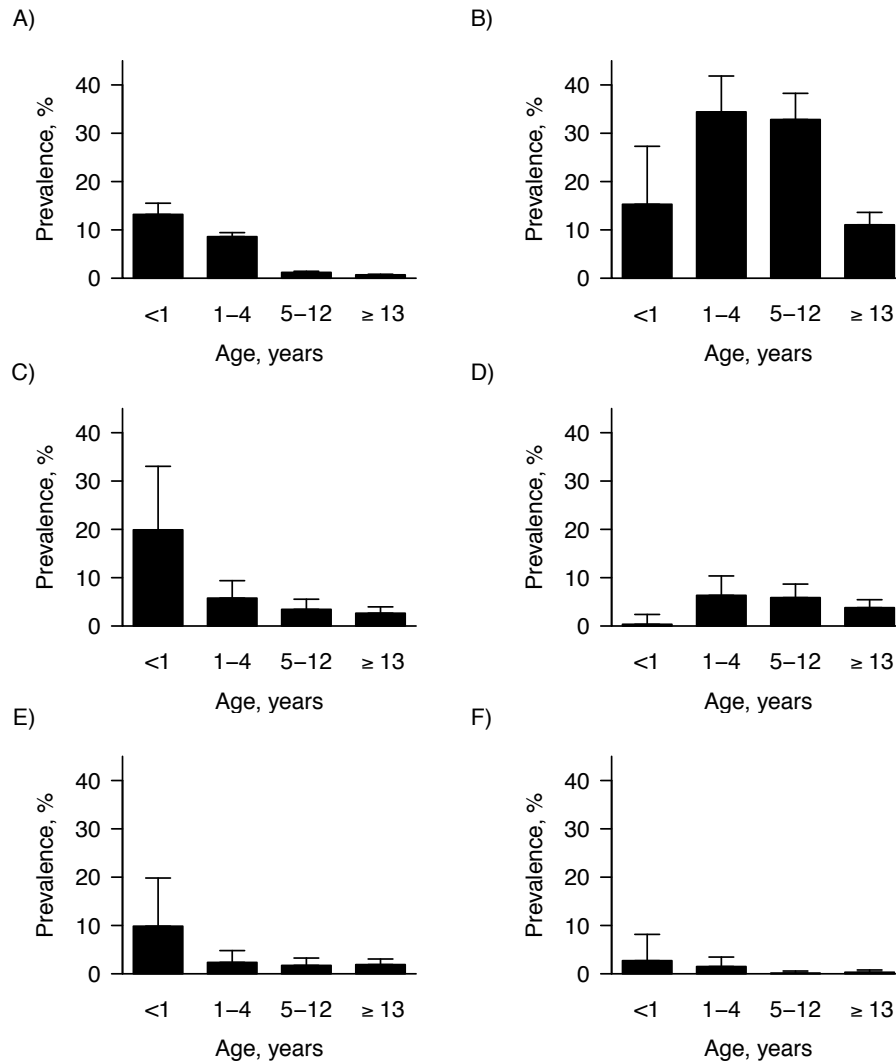
Chapter III

Prevalence Patterns of Four Diarrheagenic *E. coli* Pathotypes Across Space and
Time in Northwestern Ecuador

Abstract

In developing areas, enteroinvasive *Escherichia coli* (EIEC) is generally found in
small numbers. Yet, high prevalence of EIEC was reported in northwestern Ecuador prior
to 2005. We used case control data from this region to characterize the prevalence of
EIEC and three other *E. coli* pathotypes in 16 communities between 2004 and 2010. Our
analysis indicates that EIEC swept through the region causing a prolonged epidemic
between 2004 and 2007, involving at least 6 communities and 31 unique genotypes. At its
peak, the regional prevalence of EIEC was 8.3 cases/100 persons (95% CI = 5.0-11.5).
As the EIEC epidemic waned, enterotoxigenic *E. coli* became more prevalent in the
region. Prevalence and genotype patterns suggest that a centrally located community
played a key role in sustaining these infections while they spread to surrounding
communities. The use of spatially explicit longitudinal data has allowed us to refine our
understanding of EIEC transmission in the region.

Introduction

In developing regions, diarrheagenic *Escherichia coli* (DEC) causes up to 40% of diarrhea in children under five (1). The diarrheagenic group of *Escherichia coli* (*E. coli*) includes enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), *E. coli* Shigelloses (*Shigella*), and enteropathogenic *E. coli* (EPEC) and may employ multiple transmission pathways involving either fecal-oral contact or ingestion of contaminated food and water (2). Given that *E. coli* can be transmitted from person-to-person directly or indirectly, though a contaminated environment (3), pathogens may move between communities that are socially or geographically connected. Capturing *E. coli* prevalence trends in communities that are connected may help us better understand their transmission within a geographical region.

The transmission of ETEC has been associated with consumption of contaminated food (4-7), and water (8). Rarely has ETEC been associated with person-to-person transmission (4, 5, 8, 9). In an experiment where volunteers were infected with ETEC, investigators found no evidence of direct transmission to their close contacts (10). In contrast, EIEC may be transmitted more frequently through person-to-person contact (11). Food manipulation (12, 13) and imported food products may also be important vehicles for EIEC (14, 15). EPEC has been implicated in person-to-person transmission (8) while *Shigella* has been associated with all of the aforementioned transmission pathways, potentially owing to a lower infectious dose than the other pathotypes (10-100 organisms, (16)). Given these differences in transmission pathways, we would expect pathotypes to move between communities differently, resulting in different prevalence trends across geographical regions and over time.

The literature on spatial and temporal trends in *E. coli* prevalence is limited to a few studies, most of which focus on one pathotype (see for example (17-21)). Yet, there are many studies that have estimated prevalence of *E. coli* pathotypes at one point in time and in one location. Most have suggested that ETEC is the predominant pathotype in circulation (5, 22-26). Others have shown that when enteroaggregative *E. coli* (EAEC) and atypical EPEC are included in the study, these pathotypes are often more prevalent than ETEC (27-30). The typical form of EPEC, EIEC, and *Shigella* are often less prevalent than ETEC (22, 23, 25, 31). Longitudinal data would be important to confirm that these patterns hold over time. In this study, we estimate the prevalence of ETEC, EPEC, EIEC, and *Shigella* in 16 communities along three different river basins in northwestern Ecuador at seven sampling points between 2004 and 2010. Using prevalence trends and genotype patterns in space and time, we aim to better understand *E. coli* transmission between communities in the region.

Methods

Study Area

We conducted seven 15-day case control studies in 16 communities (described in Table 3.1) in the Cantón Eloy Alfaro between November 2004 and December 2010. Fifteen of these communities are located on one of three river systems: the Cayapas, the Onzole, and the Santiago. These river systems drain into Borbón, the 16th community in the study, which is also the main population and commercial center in the region. Borbón has a water distribution system that supplies piped water to the majority of its households. Sanitation practices are widely mixed and range from flush toilets to open

defecation. Communities upstream of Borbón tend to use the river as their primary water source and are largely reliant on unimproved sanitation facilities. Before the study began, all roads, rivers and communities in the region were mapped using GPS. Oral consent was obtained from all households in our study. The University of Michigan institutional review board and Universidad San Francisco de Quito bioethics committees approved all protocols.

Sample Collection

During each 15-day case control period, fecal samples were collected from both cases and controls in the community. Cases were defined as having three or more loose stools in a 24-hour period while controls were defined those without diarrhea in the previous six days. No other inclusion or exclusion criteria were applied to controls. Between 2005 and 2008, one household- and two community-controls were randomly sampled per case. From 2009 to 2010, at least 10% of all non-cases in the community were randomly sampled as controls. Each community was visited approximately every nine months between November 2004 and December 2010. We refer to this nine-month period as a sampling period in the subsequent text.

Microbiological Analysis and Genotyping

Samples were cultured on the following media: xilose lisine desoxicholate agar, *Salmonella* and *Shigella* agar, and MacConkey agar. *E. coli* were identified by selecting lactose fermenting colonies and testing β-glucoronidase activity using ChromoCult ® Coliforms Agar (Merck, Darmstadt, Germany). Lactose negative colonies were analyzed using API ® 20 E (BioMérieux, Marcy l'Etoile, France). A random sample of five lactose

positive *E. coli* and any lactose negative *E. coli* or *Shigella* isolates were analyzed with

PCR for the presence of pathotype-specific virulence genes (*toxA* and *ST 1b* for ETEC,

bfpA for EPEC, and *ipaH* for EIEC and *Shigella* (32)). Isolates identified as *Shigella* with

no corresponding *ipaH* gene were also included in the study. Pathogenic *E. coli* isolates

were genotyped using our validated Probe Hybridization Array Typing (PHAT) method

with 28 gene probes (33, 34) on the Library on a Slide platform (35). Each typing probe

generated a binary outcome of presence or absence of the probed gene. Isolates that

matched on all 28 probing outcomes were considered to have the same genotype.

Statistical Analysis

To estimate the prevalence of each pathotype, we assigned inverse probability

sampling weights (w_i) to all cases and controls. We assumed that all cases were identified

during the 15-day visit to a community and thus, cases were assigned a weight of one.

Control weights reflected a random sampling of houses and communities during the case

control visit. Using these weights and the standard Horvitz Thompson theory (36),

unbiased estimation of the 15-day prevalence was achieved.

$$\text{Weighted Prevalence} = \frac{\sum_{i}^{i=1} w_i X_i}{\sum_{i}^{i=1} w_i}$$

Equation 3.1. Weighted Prevalence; *I* = the total number of cases and controls sampled
in the community or region and tested for pathogenic *E. coli*; w_i = weight of individual *i;*
X_i = 1 if pathogen is detected in individual *i* , X_i = 0 otherwise.

To obtain 95% confidence intervals (95% CI) for these estimates, we bootstrapped our sample with replacement 1000 times and took the 2.5[th] and 97.5[th] percentiles of the weighted prevalence distribution as our lower and upper limits, respectively. Based on preliminary data collected from the region in 2003, we expected pathogenic *E. coli* infection to be rare in most communities, such that our confidence interval would contain the value zero. Thus, community outbreaks were identified when the lower prevalence limit was greater than zero. All analyses were carried out using R. v 2.11.1.

Results

A total of 4,196 fecal samples (from 916 cases and 3280 controls) were collected from 16 communities in northern coastal Ecuador during seven sampling periods between November 2004 and December 2010. Pathogenic *E. coli* (including *Shigella*) were found in 327 samples (144 cases and 183 controls). The regional prevalence of EIEC ranged from 0.1% to 8.3% and peaked during sampling period 2 (August 2005 – March 2006, Figure 3.1). Between sampling periods 3 and 7 (May 2006 – December 2010), ETEC was more prevalent than EIEC, though infection with either pathotype declined. The regional prevalence of ETEC was less variable over time than that of EIEC, ranging from 0.8% to 3.7%. *Shigella* prevalence varied widely (0% - 4.9%), while that of EPEC was consistently low (0% - 1.3%).

In Borbón, the temporal trend of EIEC prevalence resembled the regional pattern shown in Figure 3.1 (Figure 3.2). Large EIEC outbreaks were found at sampling points 1

and 2 (prevalence = 15.0% and 18.6%, respectively). By our definition (a lower prevalence limit greater than 0), there was also an outbreak of EIEC at sample point 3, however the lower prevalence limit just barely made our cutoff (prevalence = 2.6% (95% CI = 0.1% - 7.2%). Small ETEC outbreaks were found at 6 of the 7 sampling points in Borbón (prevalence range during outbreaks = 1.6% - 3.8%). In the 15 communities upstream of Borbón, both EIEC and ETEC infections were less common.

In addition to Borbón, EIEC outbreaks were found in communities 1, 2 and 3 (located on the road), community 6 (Santiago river) and community 15 (Onzole river, Figure 3.3). Given our observation of each community approximately every 9 months, our data are interval censored. Thus, we know the interval during which the EIEC outbreak arose, but not the exact date. The impact of interval censoring is most apparent in communities 1 and 2 where the time between finding a prevalence of zero in these communities and the outbreak in Borbón spans eight months (November 2004 – July 2005, Figure 3.4). The impact is less apparent in community 15, where this period is only three months (April 2005 – July 2005). Interval censoring did not however, preclude us from inferring that an outbreak began in Borbón before community 3. Comparing community 6 with Borbón, we note that the data in Borbón are left censored such that there is uncertainty about which community experienced an outbreak first. ETEC outbreaks were found in 6 communities, located on the road, the Santiago and Cayapas river systems (Figure 3.5). Here, interval censoring was not an issue. We observed an ETEC outbreak in Borbón prior to each interval in which an outbreak in communities 2, 3, 6, 11, and 12 arose (Figure 3.6).

We identified 100 EIEC isolates in outbreak communities (1, 2, 3, 6, and 15) between sampling periods 1 and 4. Of these isolates, 57 were genotyped according to the presence or absence of 28 genes (Table 3.2). From 57 isolates, we identified 31 unique genotypes. Seven of these unique genotypes were detected in Borbón and at least one other outbreak community (Figure 3.7).

Discussion

We found evidence for a regional outbreak of EIEC in six communities of northwestern Ecuador between November 2004 and July 2007. We hypothesize that the source of this outbreak was Borbón, the main commercial and population center of the region. Borbón geographically connects communities on three different river systems with those on the main road. High prevalence of EIEC in Borbón preceded outbreaks in at least one of the other communities. Genotype patterns revealed similar strains circulating in Borbón and the other outbreak communities. During non-outbreak periods, ETEC was the dominant pathogen in the region, and may be endemic in Borbón. Our estimates of prevalence across the region and in time suggest that like EIEC, ETEC may be transmitted from Borbón to other communities.

An earlier report from our region describes high prevalence of EIEC in 2005 and suggests that EIEC is the predominant *E. coli* pathotype in our region (37). The longitudinal nature of the current study has allowed us to characterize this high prevalence as a regional epidemic. To date, there are several studies from the United States that have also described the spatial extent and duration of EIEC epidemics. Gordillo et al, provide evidence for the movement of EIEC from Mexico into Houston

two months prior to a large food-related outbreak (12). Harris et al posture that a 2-3 month outbreak at a Missouri school was related to a staff member's acquisition of traveler's diarrhea in the Bahamas (11). Finally, Marrier et al describe a domestic outbreak related to the consumption of imported French cheese across 14 states lasting 40 days (15). Our study demonstrates that EIEC epidemics may persist for a much longer time period in a developing region compared to one that is more developed.

The 2-3 year epidemic of EIEC in our study region may have been sustained by a prolonged multistrain outbreak in Borbón. Previous works have shown that densely populated geographical sites can be central to transmission of other infectious diseases (38-41). Broutin et al demonstrate that pertussis epidemics occurring over a 15-year period began in two urban centers of Senegal before spreading to 28 surrounding villages (42). Wallace et al describe tuberculosis spread from Manhattan, where incidence was high in the 1980's, to districts in the Bronx and in Brooklyn (39). And, Chevallier et al point to two specific epicenters (one of which includes the current study region) of the cholera pandemic that swept through Ecuador in the 1990's (41).

The introduction of EIEC infections into Borbón may have been related to its connectivity to Colombia (40km north) and to the rest of Ecuador (to the east), via a recently constructed primary road. This hypothesis is consistent with observations by Bharti et al, who show that regional persistence of measles and meningococcal meningitis in Niger are related to high connectivity with Nigeria and human movement via primary roads (43, 44). The association between sexually transmitted diseases and human migration along national highways has also been shown (45-49). Persistence of EIEC

infections in Borbón between July 2005 and July 2007 may be attributed to its higher population density compared to surrounding communities.

The spread of infection outward from Borbón may have resulted from human movement between this urban center and surrounding communities. Borbón is the only site on the three river basins with market stalls, restaurants, hotels, and a hospital and is therefore, the most likely destination for services and provisions in the region. For anyone traveling into and out of the study region, passage through Borbón's river depot and bus station is often necessary. As expected, outbreaks were observed in all road communities in our sample, these being the least remote (measured using cost and travel time) from Borbón. Alternatively, one could argue that EIEC infections may arise independently in surrounding communities and be transmitted to Borbón via human movement or the downstream current of the river. Future research with finer temporal sampling may better address this hypothesis.

In contrast to the large epidemics caused by EIEC, smaller epidemics of ETEC were observed. These small but frequent epidemics may be due to repeated introduction of ETEC into Borbón by infected persons and contaminated foods or persistence in the local environment. The confluence of these small epidemics may drive ETEC to be endemic in Borbón. ETEC appears to be endemic in other urban and developing sites of Latin America (18, 22, 23, 27, 28). Differences in epidemic behavior between EIEC and ETEC may be explained by infectious dose and environmental tolerance. EIEC has a lower range of its infectious dose than ETEC (10^6-10^{10} organisms compared to 10^8-10^{10}, (8)) and thus, amplification of an outbreak through person-to-person transmission may be more likely. ETEC, on the other hand, may be able to survive longer in the environment

(50-53). Through widespread consumption of untreated potable water, uncooked seafood products, and survival in the soil, environmental sources may provide a constant and low dose of ETEC to residents of Borbón. As with EIEC, we hypothesize that ETEC may have spread from Borbón to surrounding communities.

To further address the hypothesis that Borbón was a source of EIEC and ETEC in the region, we suggest the application of two additional analytic approaches: regression modeling and phenetic analysis (i.e. grouping based on overall similarity, independent of their phylogeny and evolutionary relationships). These approaches would be applied separately to EIEC and ETEC infections. The regression approach includes a series of 16 different binomial models each fit to the same dataset. The predicted outcome would always be the same, the presence or absence of an outbreak of EIEC (or ETEC). The only exposure for each model would be distance from the source community. Using 16 different models, we may assume 16 different source communities. This would result in 16 different odds ratios associated with distance from each of the communities in our sample. Comparison of these odds ratios would indicate proximity to which of the 16 communities corresponds most strongly with outbreaks of EIEC (or ETEC) in our sample. Our hypothesis that Borbón is a source of infection would be supported by a large odds ratio associated with distance to Borbón relative to the other 15 odds ratios. Though this approach excludes the use of temporal data, we assume that Borbón is a constant source of pathogens for other communities. Potential drawbacks of this approach include: 1) an inability to test our assumption that Borbón is a constant source, owing to the course temporal resolution of our data; 2) the course spatial resolution of our data, which may reduce our power to obtain accurate odds ratio

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estimates; And 3) the assumption of a monotonic trend between distance and odds of an outbreak. More complex models like point-source regression models (e.g. (54)), that assume an exponential decay in risk, may also be employed. However, inferences from these models may also suffer from a lack of power and an inability to establish causality.

The second analytic approach involves simple phenetic methods, such as hierarchical clustering, that group bacterial strains based on similarity in presence or absence of a set of genes. We recommend clustering on similarity in binary outcomes associated with the 28 gene probes described in the current paper. Clustering algorithms based on distance matrices, such as minimizing Euclidean distance between matched pairs, and the neighbor-joining algorithm are two widely accepted methods that can be used to construct a dendrogram (55). To estimate the similarity between different clusters, measures such as the DICE coefficient would be used (56). Given our hypothesis that Borbón is a source of pathogens in the region, we would expect a high degree of similarity between strains from Borbón and other outbreak communities. Inclusion of an outgroup (e.g. strains from another region of Ecuador) would allow for a comparison of similarity in strains within and between geographic regions. The major limitation of this method is that it does not provide insight into the order of transmission, such that causality cannot be established. Phylogenetic analysis, used to infer evolutionary relationships, may better address the origin of the outbreak and order of transmission by estimating the time at which two strains diverged. However, complete nucleotide sequences of at least one locus or gene are required for this approach (57).

In the current study, weighted prevalence estimates were based on a series of 15-day sampling periods in each community carried out approximately every nine months.

While we may not have captured small fluctuations in prevalence estimates, our spatial and temporal resolution did allow us to identify and characterize a regional epidemic of EIEC, which we hypothesize began in Borbón before spreading to other communities in the region. Future research using regression models and phenetics may further address this hypothesis. We also found that ETEC is the predominant pathogen in the region during non-epidemic periods. While our analysis did not include other *E. coli* pathotypes such as enterohemorrhagic *E. coli* (EHEC), atypical EPEC, and EAEC, unpublished genotype data from our final sampling period suggest that EHEC is not circulating in our study region, and though EPEC and EAEC are circulating, they may be less pathogenic than the pathotypes considered here (L. Zhang, unpublished data). Other researchers have found year-to-year variation in the prevalence of one particular *E. coli* pathotype, (17, 19, 20, 58) or have focused on various pathotypes isolated from diarrhea samples (21). To our knowledge this may be one of the few studies to consider spatial and temporal trends of four *E. coli* pathotypes isolated from symptomatic and asymptomatic individuals.

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Table 3.1. Characteristics of 16 sampled communities in northwestern Ecuador (2005-2010)

Community	Average Sample Population	Road/River Basin	Remoteness **
1	283	Road	0.01
2	816	Road	0.01
3	517	Road	0.03
4	131	Santiago	0.12
5	242	Santiago	0.16
6	306	Santiago	0.15
7	75	Santiago	0.05
8	145	Santiago	0.04
9	90	Cayapas	0.06
10	96	Cayapas	0.16
11	336	Cayapas	0.16
12	138	Cayapas	0.20
13	76	Onzole	0.08
14	99	Onzole	0.14
15	427	Onzole	0.19
Borbón	800-2000*	Road	0

*Between sample points 1 and 5, we followed 800 persons in Borbón. At sample point 6 this population was increased to approximately 2000. **Metric is based on time and cost of travel to Borbón; higher values indicate more remote communities.

Table 3.2. Enteroinvasive *Escherichia coli* genotypes found in outbreak communities in northwestern Ecuador (2004-2007)

	Sampling Point			
	1	2	3	4
Community	November 2004-July 2005	August 2005-March 2006	May 2006-December 2006	January 2007-July 2007
Borbón	**T7, T11,** T12, T20, T23, T24, T25, T43, T47, **T49, T49, T49, T49, T49, T50, T50,** T52, T54, T94, X, X, X, X, X, X, X	Missing 19 Genotypes.	**T7, T45,** X, X	**T26,** T54
1	T78	T3, T19, T28, **T45, T45**	**T43,** X, X, X	**T7,** X, X, X, X
2	-	T2, T15, T45, **T50**	**T7, T26,** X, X, X	**T7, T7,** T30
3	**T49,** X	-	T3, T10, T27, T27, T36, T39, X, X	-
6	T2, **T7,** T34, T79, X	X	-	-
15	-	T3, T3, **T11,** T58, T78	T5	-

Genotypes in bold indicate identification in Borbón and in at least one other outbreak community, T = genotype, X = an individual with missing genotype data.

Figure 3.1. Weighted prevalence of four *Escherichia coli* pathotypes in 16 communities across northwestern Ecuador during sampling periods: 1 (November 2004-July 2005); 2 (August 2005-March 2006); 3 (May 2006-December 2006); 4 (January 2007-July 2007); 5 (September 2007- March 2008); 6 (December 2008-November 2009); and 7 (January 2010-December 2010).
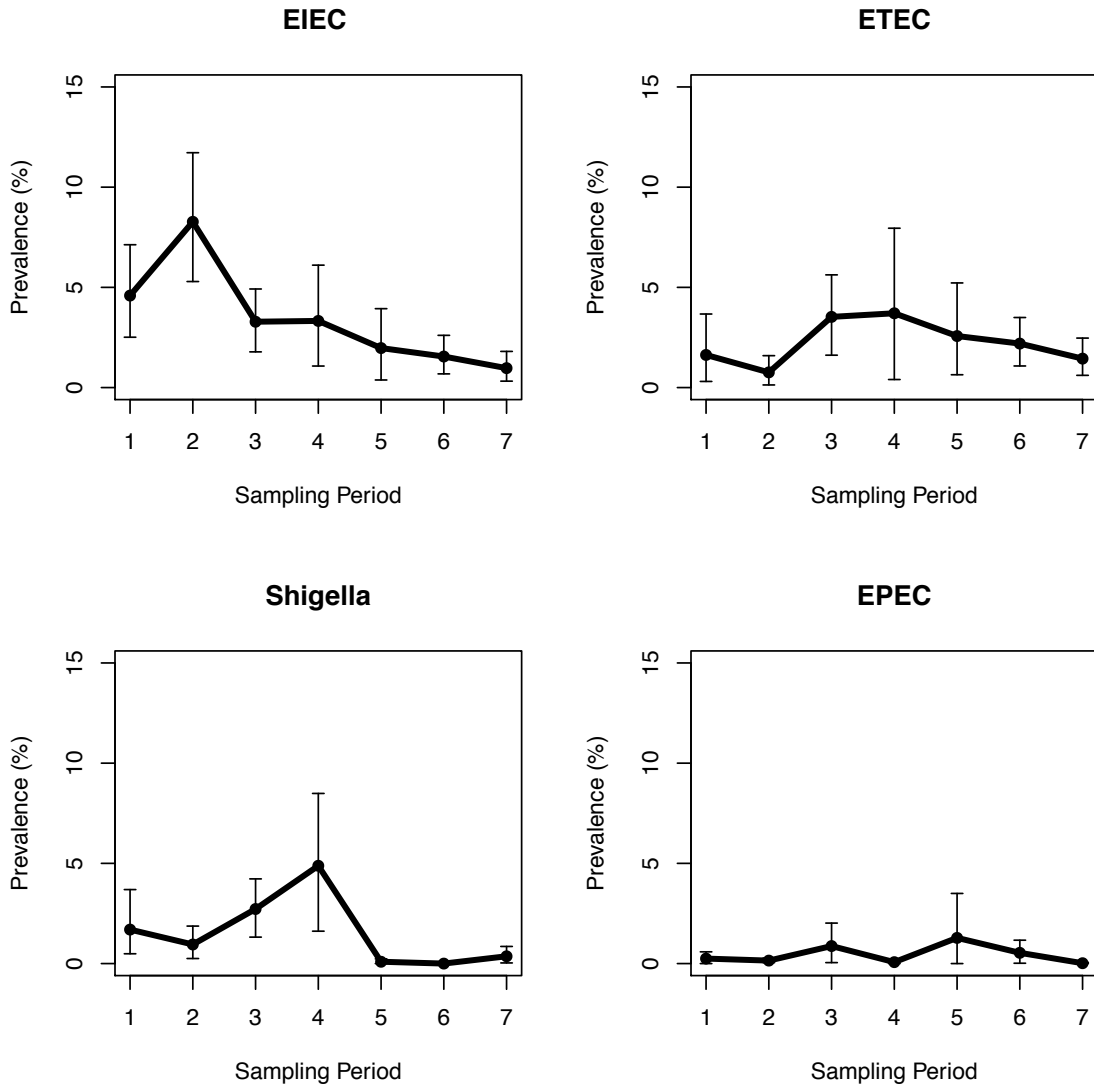
Figure 3.2. Weighted prevalence of four *Escherichia coli* pathotypes in Borbón, Ecuador at sampling points: 1 (July 2005); 2 (March 2006); 3 (December 2006); 4 (July 2007); 5 (March 2008); 6 (December 2008-January 2009); and 7(January 2010-February 2010).
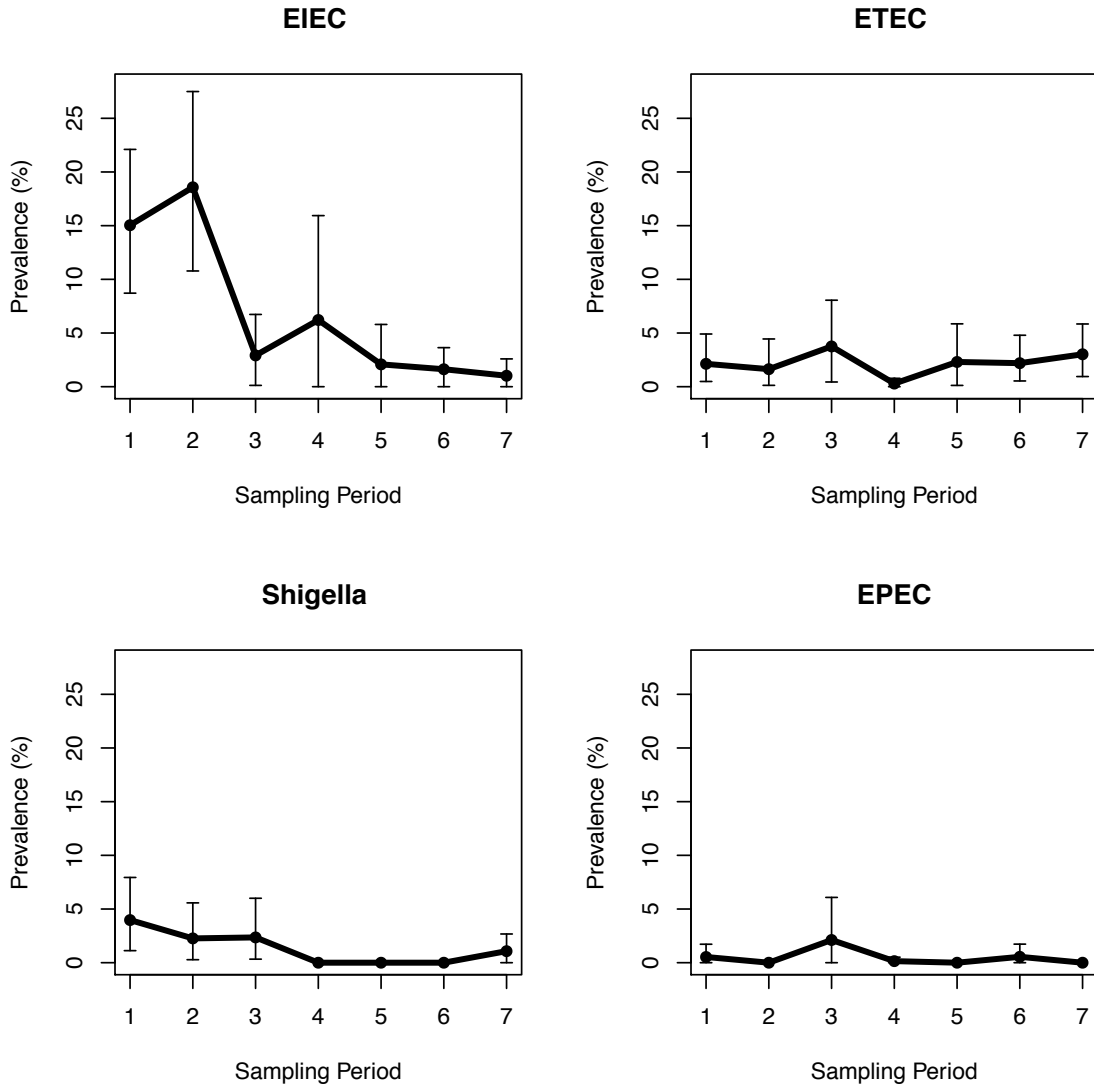
Figure 3.3. Map of the study region in northwestern Ecuador; enteroinvasive *Escherichia coli* (EIEC) outbreak communities (large red dots); non-EIEC outbreak communities (small black dots).
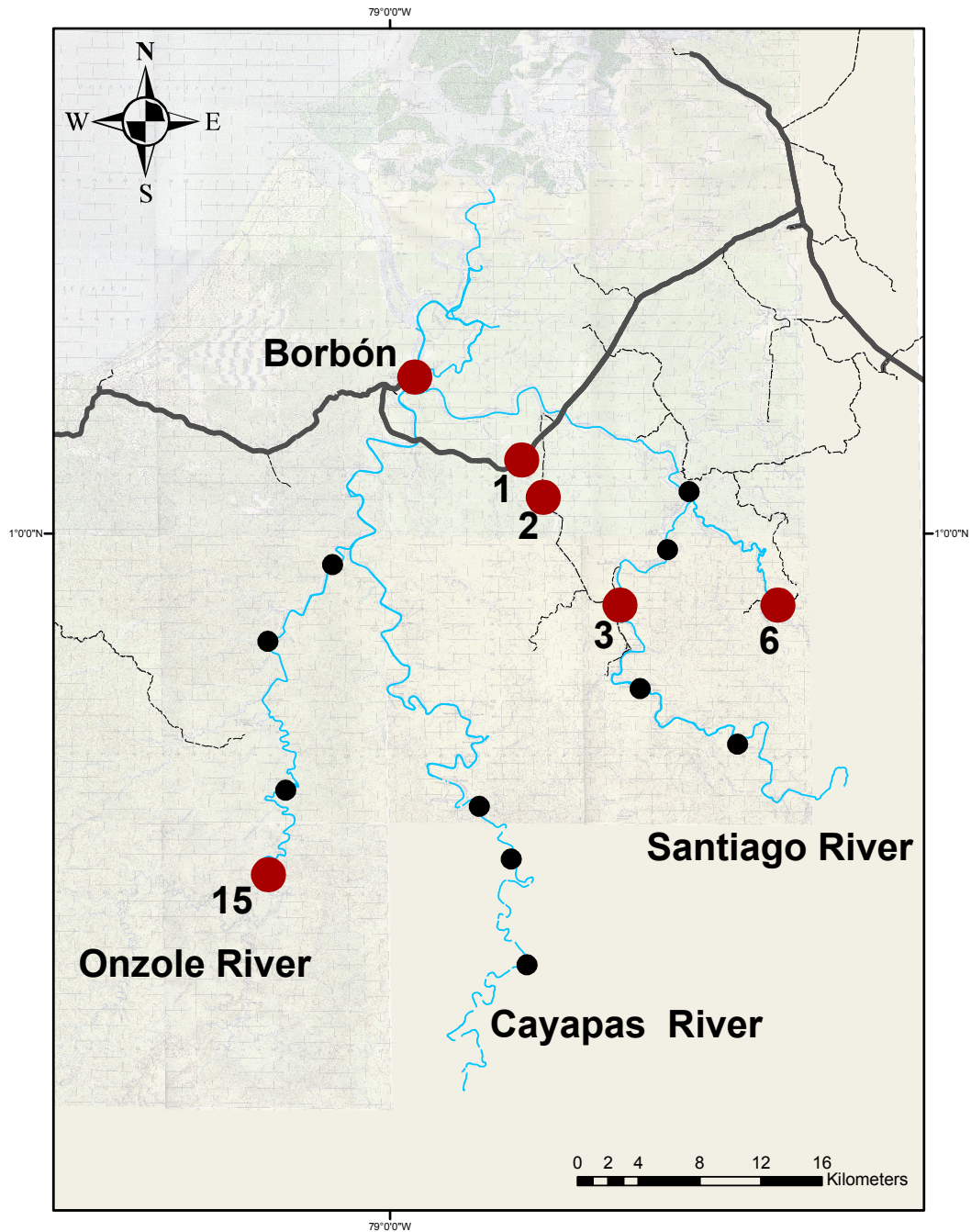
Figure 3.4. Weighted prevalence of enteroinvasive *Escherichia coli* in outbreak communities in northwestern Ecuador at seven sampling points (2005-2010). Black circles represent 15-day sampling points; those larger in size indicate the presence of an outbreak.
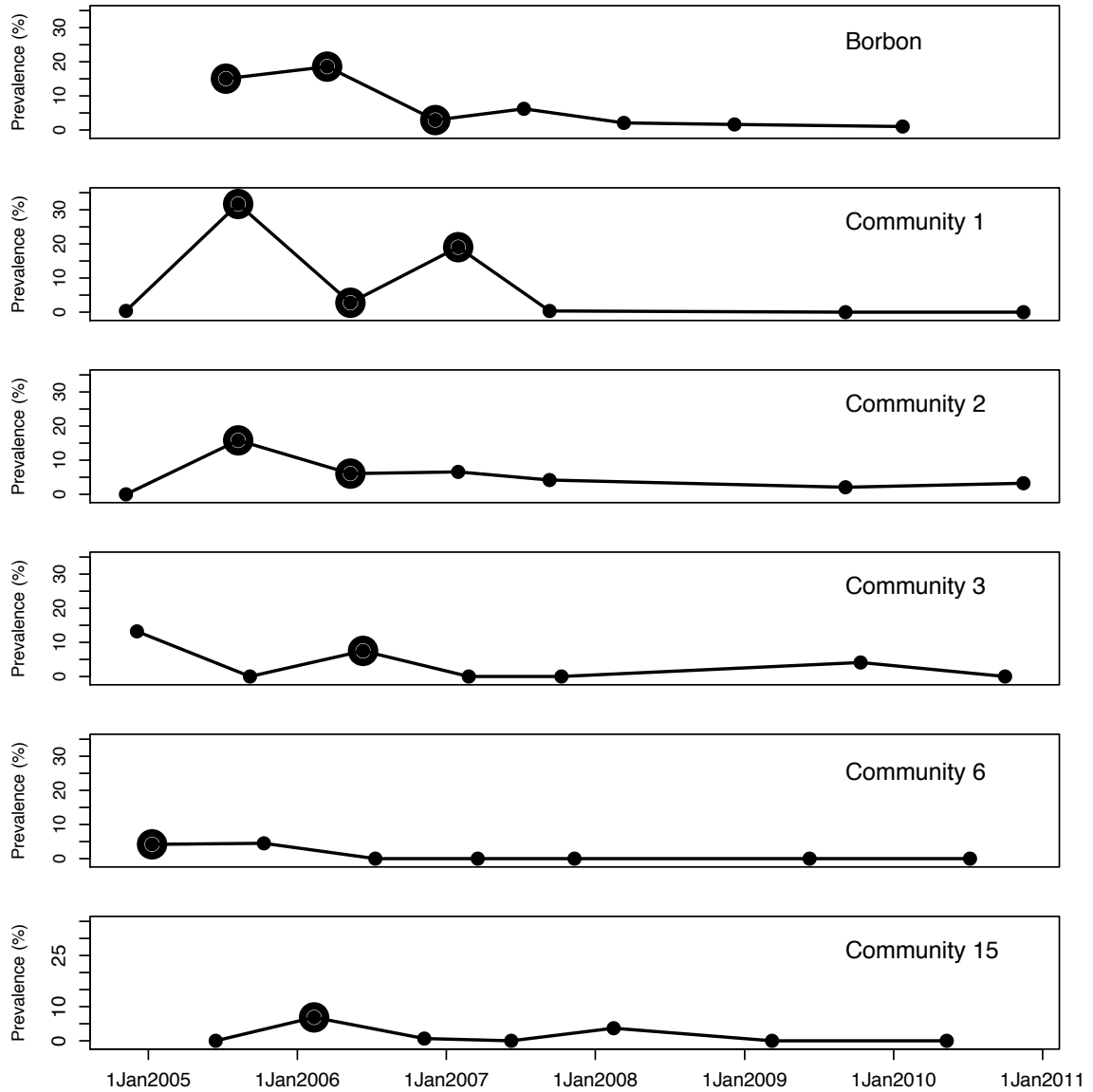
Figure 3.5. Map of the study region in northwestern Ecuador; enterotoxigenic *Escherichia coli* (ETEC) outbreak communities (large red dots); non-ETEC outbreak communities (small black dots).
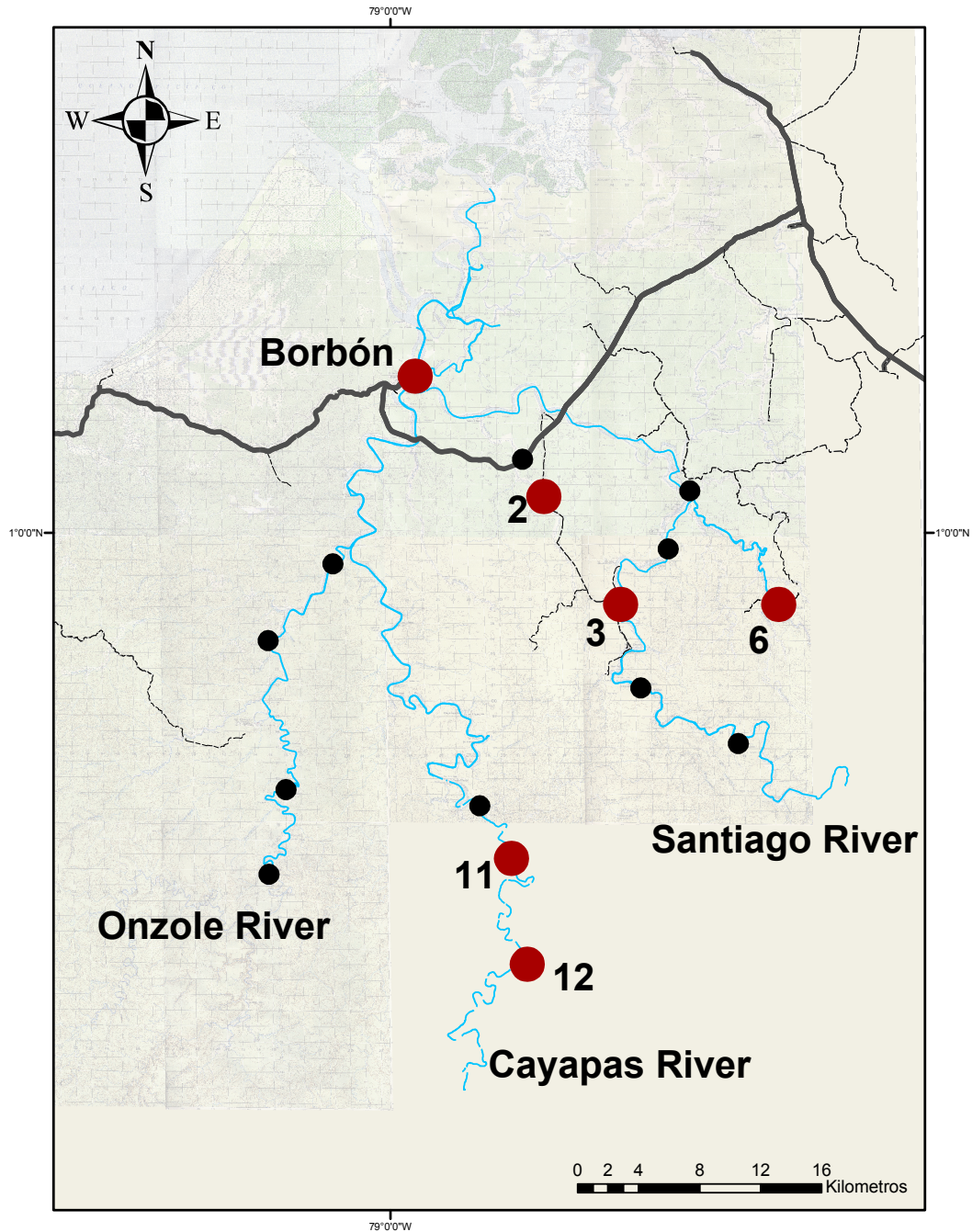
Figure 3.6. Weighted prevalence of enterotoxigenic *Escherichia coli* in outbreak communities in northwestern Ecuador at seven sampling points (2005-2010). Black circles represent 15-day sampling points; those larger in size indicate the presence of an outbreak.
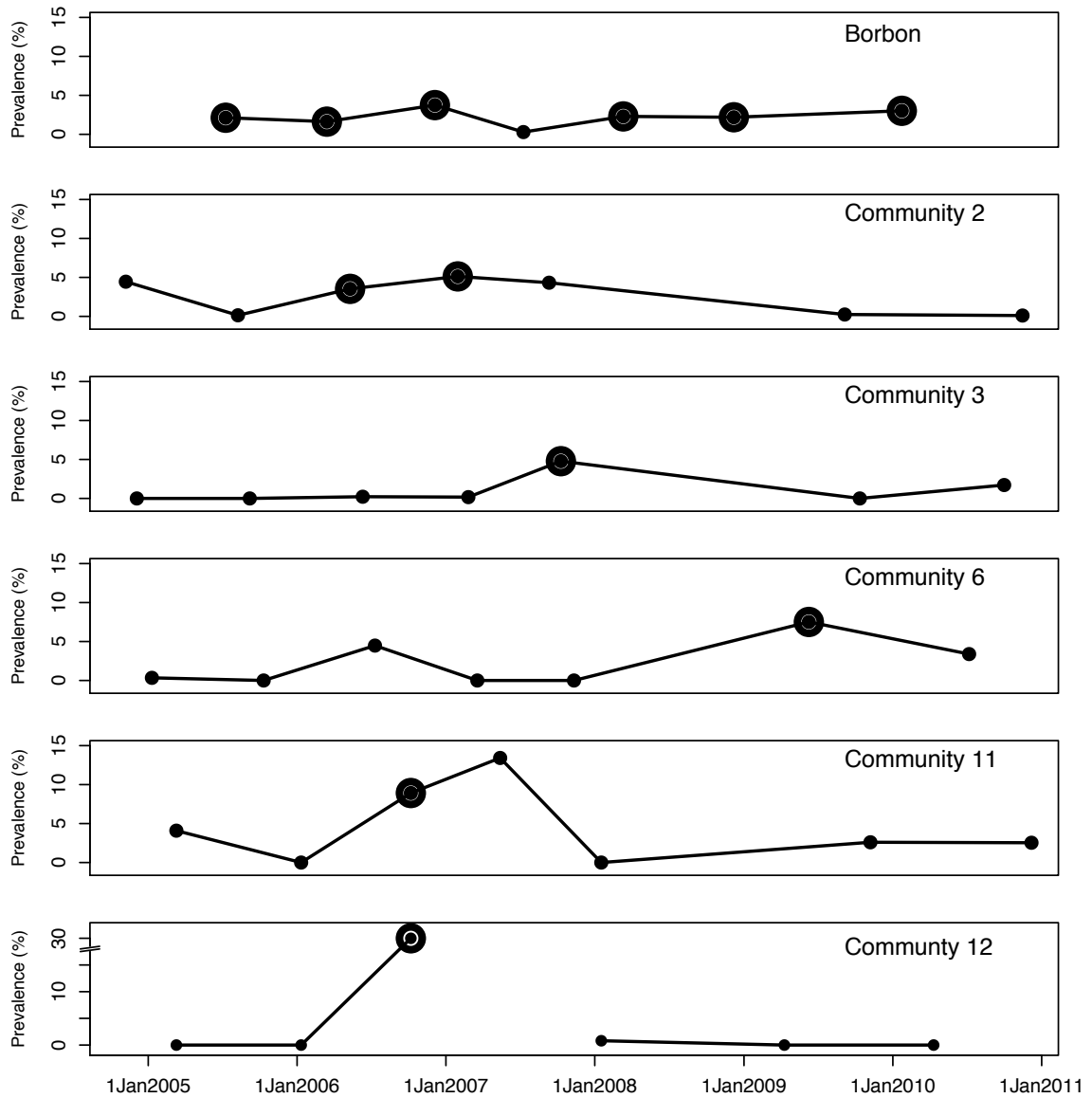
Figure 3.7. Frequency (y-axis) of seven common enteroinvasive *Escherichia coli* genotypes shared between Borbón and at least one other outbreak community at sampling points 1- 4 (x-axis): T7 (blue), T11 (cyan), T43 (yellow), T49 (purple), T50 (lime green), T45 (red), and T26 (pink).
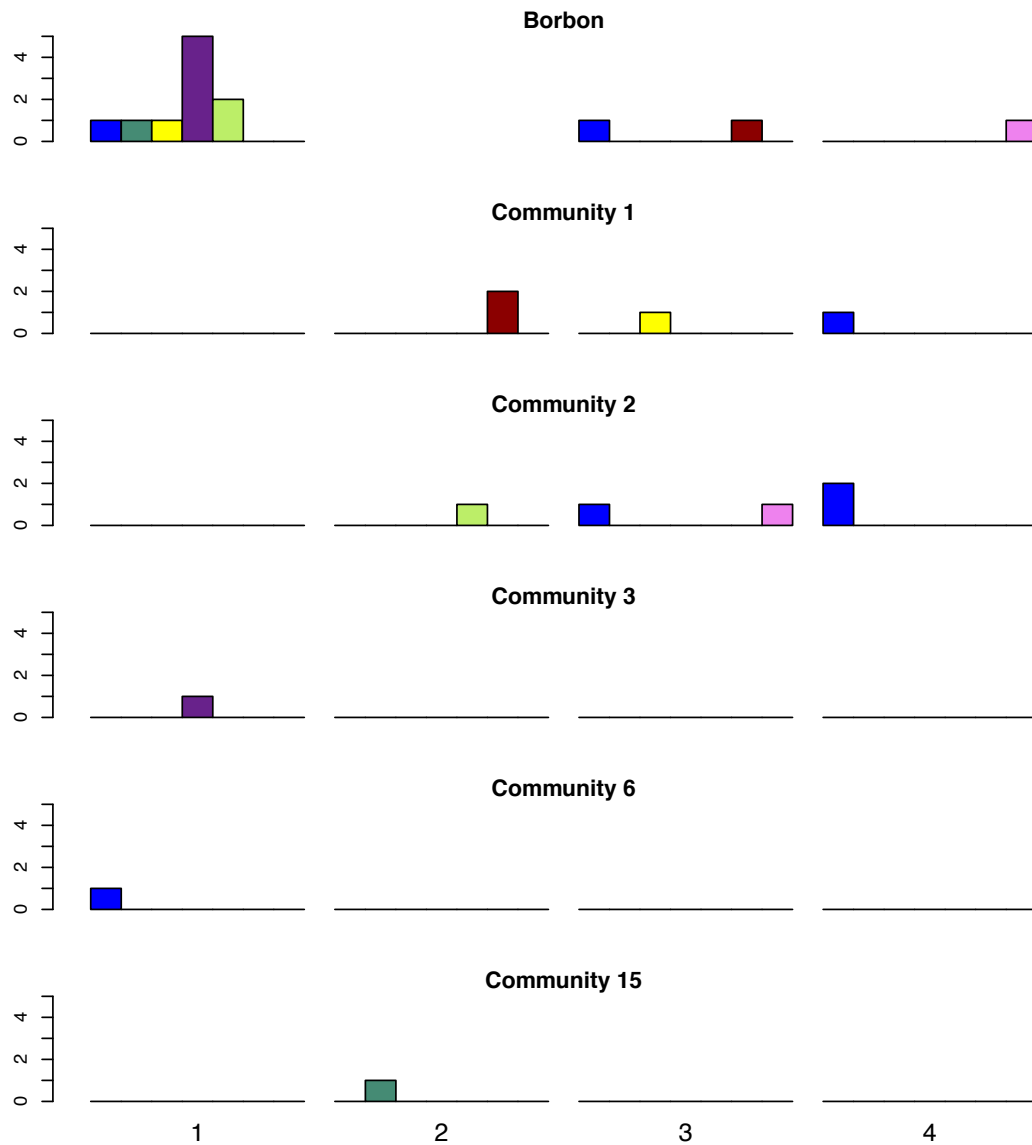
Chapter IV

Rainfall Alters the Protective Effects of Improved Water and Sanitation on Diarrheal Disease in Northwestern Ecuador

Abstract

Background. With increasing concern about the relationship between climate change and health, many studies have examined the association between climate and disease. Less attention has been paid to how climate might impact the association between exposure and disease. Here, we examine the impact of extreme rainfall on the association between two exposures, safe water sources and improved sanitation, and diarrheal disease.

Methods. We conducted a series of six nested case control studies in northwestern Ecuador between December 2008 and May 2009. Using multiple logistic regression models we assessed the protective effects of safe water sources and improved sanitation facilities on household-level diarrhea and whether extreme rainfall events modified these protective effects.

Results. Protective factors for diarrhea included a safe water source (OR=0.28, 95% CI=(0.13, 0.60)), improved sanitation (OR=0.59, (95% CI=0.40, 0.86)) and the number of extreme rainfall days in the previous 5 weeks (OR=0.83, 95% CI=(0.74,

0.93)). The protective effect of an improved sanitation facility decreased with increasing rain. In contrast, the protective effect of safe water sources increased with increasing rain.

Conclusions. More attention should be paid to how risk factors, such as water and sanitation, are modified by climate. Given the increasing frequency of extreme weather events, our analysis suggests the need to promote improved sanitation during periods of drought and the consumption of safe water after extreme rainfall events.

Introduction

Diarrheal disease continues to be a leading cause of mortality in children younger than five years. Nearly 1.9 million children die annually from diarrheal disease, accounting for 19% of all deaths in this age group (1). This global burden of diarrheal disease disproportionately affects developing regions, largely be due to the lack of safe water and basic sanitation (2-4). United Nations Millennium Development Goal number seven aims to cut in half the proportion of those living without access to safe water and basic sanitation by 2015. Recent reports project that we will meet safe the water target but will fall short on providing basic sanitation (5). Currently, 2.6 billion people lack access to improved sanitation facilities, and 1.1 billion defecate in the open (5). As we strive to improve coverage of basic sanitation it will be important to have a broader understanding of the environmental context (defined here as the built and ecological environment) in which improved sanitation can prevent exposure to enteric pathogens.

Enteric pathogens are shed by infected individuals into the environment (6). These pathogens can flow throughout the community through various environmental

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pathways that are facilitated by both built and ecological environmental factors. For example, inadequate sanitation in the community (the built environment) can cause the release of pathogens into the soil or promote the transport of pathogens via flies (7). Water runoff (governed by ecological factors such as rain and soil moisture content) may facilitate the spread of these pathogens throughout a neighborhood or community, resulting in contamination of primary water sources (8, 9). Thus, inadequate sanitation and piped water (components of the built environment) and rainfall (an ecological factor) may have interdependent effects on diarrhea.

The literature on rainfall and diarrhea pays little attention to the use of improved water and sanitation. It may be for this reason that the relationship between rainfall and diarrhea remains unclear. Recent studies have shown positive, (10-12) negative, (10) and threshold-type associations between rainfall and diarrheal disease (13, 14) (Table 4.1). Rainfall indicators include total, average, and presence or absence of rainfall, as well as extreme rainfall events defined by some threshold over a period of time, referred to as a lag period (10-14). Lag periods in which rainfall is summarized have ranged from zero days to four months prior to diarrhea reports (10-14). In one study, changing the lag period led to a significant change in the direction of rainfall effects (10). Research to-date indicates a lack of consensus on the most relevant indicator of rainfall, the direction of rainfall effects on diarrhea, and the appropriate lag period in which to summarize rainfall. Developing a better understanding of how rainfall events direct pathogen transport and potentially modify the effects of improved water and sanitation is important to the design of effective interventions for diarrhea.

In the present study, we use a community-based case control design to capture diarrhea events in Borbón, Ecuador over a six-month period. We estimate the associations between diarrhea and household water and sanitation practices. We also examine the correlation between rainfall and diarrhea, using two different indicators, total rainfall and extreme rainfall events, and three different lag periods, ranging from three to five weeks. Finally, we address the potential for effect modification of improved water and sanitation by extreme rainfall events on diarrhea. We hypothesize that rainfall alters the ecology of the environment, directing pathogen flow throughout the community, and thus, modifies the effectiveness of water and sanitation interventions.

Methods

The Study Site

Borbón is located on the northwestern coast of Ecuador in the province of Esmeraldas. The city of Borbón has a population of approximately 5000 people, 1175 houses, and spans a geographic area of $1.3km^2$. Though once considered remote, Borbón is now connected to the coast on the west and the Andes to the east via a paved road. The new road has encouraged in-migration, creating potential for new settlements on the outer edges of the town where water and sanitation infrastructure may not reach. A water treatment plant was established in 2004 but it does not supply water to all households and the supply is unreliable, forcing some to use unimproved sources for consumption and hygiene. Sanitation options in the town are mixed and include flush toilets, latrines and

open pits. At the time of the study there was no functioning sewage system in place, although one was being constructed.

Cohort and Census

In November 2008, all houses in Borbón were mapped and enumerated using GPS. Two distinct cohorts of approximately 200 households were randomly selected and recruited into the study. The only requirement for study inclusion was that the selected house was occupied during the two-week recruitment period. During recruitment we collected census data and obtained oral consent from a household representative. IRB committees at the University of Michigan and Universidad San Francisco de Quito approved all protocols.

Serial Case Control

A 15-day case control study was conducted each month between December 2008 and May 2009. The six case control studies were nested within one of the two distinct cohorts described above. We alternated between the two cohorts, allowing us to follow a larger sample of households over time. Two days before the case control period, we updated our census and conducted cross-sectional surveys and observations on household water, sanitation and hygiene practices. During the case control period, we made daily visits to each of the 200 houses in the cohort to identify cases of diarrhea. Cases were defined as having three or more loose stools in a 24-hour period.

Rainfall and Diarrhea

Daily precipitation was collected using a Hoboware Data Logging Rain Gauge (RG3, Onset Computer Corporation) situated in Borbón, from November 2008 through May 2009. Rainfall was summarized as: 1) total rainfall and 2) the number of days of extreme rainfall events. Extreme rainfall days were those exceeding the 90th percentile of the empirical distribution of daily rainfall, a threshold selected based on previous literature (8, 12). Both total rainfall and extreme rainfall days were summarized over lag periods of three weeks, four weeks, and five weeks prior to the end of the 15-day case control period. These lag periods are comparable to those found in the literature (10, 13, 14).

Risk and Protective Factors

Some household factors were assumed to be static over the 7-month study period. These included an ownership score ranging from zero to one, (based on ownership of the house, vehicles, electronics and other household items), and a construction score ranging from 0-5 (based on the quality of materials used for house walls, floors and roof). We collected monthly data on other factors, assuming these would vary by month. These factors were: 1) household demographics (i.e. residents of the household, age distribution, and education level); 2) a hygiene score ranging from 0-1 (based on a set of observations made by our field assistants); 3) drinking water related factors such as source, storage and treatment; and 4) sanitation facility and garbage disposal. We considered piped, commercial, rain, and covered-well water to be improved water sources. Improved sanitation included flush toilets and latrines with raised platforms (15). Garbage disposal was categorized as improved (collected or burned) and unimproved (disposed of in a field, ditch, or river). Neighborhood-level factors, such as the percent of

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sampled houses with an improved water source and an improved sanitation facility within 50 meters of the house, were also considered. These neighborhood-level variables were calculated using a distance matrix created in ArcGIS (v.9.3). The complete list of risk and protective factors are presented in Table 4.2.

Logistic Regression Models

Given the high probability of secondary infections within a household and our interest in household-level risk factors, we assigned the household as the unit of analysis for diarrhea. We defined a case-household as any household with at least one case of diarrhea in the 15-day case control period. Control households were defined as all non-case households in the cohort. In order to characterize the marginal effects of each variable, simple logistic regression models were used to estimate the association between household-level diarrhea and potential risk factors during each month of study. To account for multiple statistical tests, we used Bonferonni-adjusted critical values. However, we also report *p*-values less than 0.05 for the interested reader. Next, we analyzed all six months of data in one logistic regression model. Robust z-statistics (16) were used to allow unbiased inference in the presence of repeated measurements on households. The best fitting multiple regression model was identified using backward stepwise regression. Age, a well-documented risk factor, was not considered as a candidate for deletion and was kept in the model. Additionally, we expected that as the number of household residents increased, the probability of finding a case of diarrhea in the household would also increase, and so, we kept household population size in the model as well. We estimated interactions between extreme rainfall and any water,

sanitation, or hygiene factors in the final model. All statistical analyses were done in R v.2.11.1.

Results

Cohort Characteristics

We followed 2295 individuals in 433 different households in Borbón between December 2008 and May 2009. The age distribution of the total cohort had a median of 17 years, and ranged between 0 and 93 years. The average individual had up to a third grade education. During the six 15-day case control periods we made 1035 household observations, identifying 204 case households and 831 control households (Table 4.2). Approximately 48% of the 1035 households had one or more children younger than 5 years. The median household population size was five persons (range = 1-25) and the median household ownership score was 0.33 (range = 0-1), reflecting widespread ownership of homes, television sets and DVD players but limited ownership of vehicles, businesses, and farms. There was little to no difference between the two distinct cohorts in the factors shown in Table 4.2.

Diarrhea Prevalence and Rainfall

The 15-day period prevalence of diarrhea was highest in December 2008 (7.2 cases per 100 persons) and lowest in January 2009 (3.3 cases per 100 persons, Figure 4.1). Both total rainfall and the number of extreme rainfall days were negatively correlated with diarrhea. Total rainfall was most correlated with diarrhea when

summarized over 4 weeks compared to 3 and 5 weeks. In contrast, extreme rainfall days (>18 mm of rainfall) summarized over 5 weeks had a higher correlation with diarrhea prevalence than that summarized over 3 and 4 weeks (Figure 4.2). *P*-values are not reported here since the strength, and not statistical significance, of the correlation was used to select the most appropriate lag period for the multiple regression model.

Simple Regression Models

We observed positive effects of age and population size on household diarrhea each month from December through May (Table 4.3). Having at least one child younger than five years and a higher household population size were positively associated with case-household status, with odds ratios (OR) ranging from 2.6 to 7.6, and from 1.1 to 1.4, respectively). Use of an improved sanitation facility was significantly protective for diarrhea in January (OR = 0.21 (95% CI = 0.09, 0.49), *p*<0.0006), while small container storage was significantly protective for diarrhea in May (OR=0.20 (95% CI = 0.08, 0.50), p<0.0006). Other protective factors included the use of improved sanitation in December, use of an improved water source in March and April, and having neighbors within 50 meters of the house who used improved sanitation facilities in April. These associations however, were not statistically significant according to the Bonferonni-adjusted critical value.

Multiple Regression Models

The final model included household ownership, water source, sanitation facility and the number of extreme rainfall days in the previous five weeks (Table 4.4, Model 1). A higher number of extreme rainfall days was protective for diarrhea (adjusted OR = 0.83

(95% CI = 0.74-0.93). Total rainfall was also protective for diarrhea in the final model

(adjusted OR = 0.995, (95% CI = (0.992, 0.998) but was excluded because of co-linearity

with extreme rainfall. Extreme rainfall modified the effect of improved sanitation on

diarrhea ($p = 0.12$, Table 4.4, Model 2). After one day of extreme rainfall, households

with improved sanitation had a 26% lower probability of diarrhea than households with

unimproved sanitation. This probability difference changed to 4% after 5 days of extreme

rainfall (Figure 4.3a). Similarly, extreme rainfall modified the effects of improved water

source on diarrhea ($p = 0.09$), Table 4.4, Model 3). However, unlike improved sanitation,

use of an improved water source was protective after five days of extreme rainfall. The

difference in probability of diarrhea between improved water source users and

unimproved users changed from 7% after one day of extreme rainfall to 42% after five

days of extreme rainfall (Figure 4.3b).

Discussion

We observed four main protective factors for diarrhea: 1) Extreme rainfall events;

2) Improved household sanitation after periods of low rainfall; 3) Use of improved water

sources after heavy rainfall; and 4) Having neighbors with improved sanitation after

heavy rainfall. Extreme rainfall events may reduce the risk of diarrhea by potentially

flushing enteric pathogens from the local environment. Our finding that improved

household sanitation was protective for diarrhea in dry conditions but not in wet

conditions supports this flushing hypothesis. After five days of extreme rainfall, many

enteric pathogens may already be flushed out of unimproved sanitation facilities,

reducing the level of risk they pose to a household. In contrast, household use of an improved water source was most protective after five days of extreme rainfall. Here, enteric pathogens may be flushed into unimproved sources of water increasing risk for diarrhea. Having neighbors who used improved sanitation was protective for diarrhea in April. The limitation of this result to a month associated with five days of extreme rainfall lends evidence to the movement of enteric pathogens through a flooded neighborhood environment.

The observed negative association between rainfall and diarrhea agrees with findings by Chou et al during the month of case identification (10). Our results however, are inconsistent with those from these studies (11, 13, 14, 17), which report either a threshold effect or a positive association between rainfall (total or extreme events) and diarrhea. This inconsistency may be explained by a difference in rainfall patterns between sites. For example, both Borbón and Dhaka, Bangladesh are characterized by a tropical monsoon climate. Yet, total weekly rainfall in Dhaka peaked to over 200 mm 11 times between 1996 and 2003 (13) while in Borbón, the same was observed only three times between 2003 and 2011 (unpublished data). Furthermore, monsoon rains and coastal storm surges cause intense flooding in Bangladesh (18), but not in coastal Ecuador. Therefore, compared to this site, Borbón experiences less severe flooding. Rainfall patterns in Borbón may be enough to flush pathogens from the local environment but insufficient to cause the flood-associated stagnant water shown to increase risk of diarrhea (19).

Differences in the effects of rainfall on diarrhea found in previous studies and ours may also be attributed to varying etiology of diarrhea between sites. The major

pathogens in our region are rotavirus, pathogenic *E. coli*, and *Shigella*, and in the context

of coinfections, *Giardia* and *Plesiomonas shigelloides* (20, 21). Others have reported in

addition to these, a high prevalence of *Vibrio cholerae*, *Aeromonas* spp., and

Cryptosporidium spp., (22-24) which are rarely found in our study region. Given

differences in pathogen prevalence, and that pathogens may respond differently to

rainfall, the combined effects of rainfall on pathogen-associated diarrhea is likely to

differ between sites. The variability in pathogen prevalence between regions may also

explain the wide range of lag periods associated with diarrhea across studies (10-14). The

five-week lag period used in our study reflects the environmental persistence of rotavirus

and *Giardia* cysts in water, sewage and soil for up to several weeks (25, 26). This time

period is also several times longer than the incubation periods of rotavirus, *E. coli,* and

often *Giardia*, allowing enough time for secondary transmission events to noticeably

affect diarrhea prevalence (27, 28).

A third and likely explanation for the differences between rainfall effects at our

site and at others may have to do with the prevalence of households using unsafe water

sources. We estimate that 4% of households in Borbón use unsafe water sources, which

compared to other study sites such as Dhaka (19) and rural areas of the Pacific Islands

(14), is very low. Given our finding that use of unsafe water sources increases risk for

diarrhea in wet conditions, we would expect that in populations where reliance on unsafe

water is prevalent, extreme rainfall events would promote diarrheal disease.

Our finding that unimproved sanitation facilities were no longer protective in wet

conditions is consistent with Hashizume et al, who reported no effect of unimproved

sanitation during a flood period but found a positive effect up to six months post-flooding

(19). The most common unimproved sanitation facilities in Borbón are pit latrines. Pit

latrines, unlike flush toilets, are located outdoors and, unlike latrines with raised

platforms, are often poorly constructed, facilitating the movement of pathogens into the

surrounding environment. As described by Cronin et al, pathogen movement can be

subsurface from dug latrines or through surface runoff from flooded latrines (29). Surface

runoff was recently demonstrated by Knappet et al who found evidence for the movement

of *E. coli* from unsanitary latrines (defined as open pits or visible effluent) to nearby

ponds at distances ranging from 15 to 80 meters (30).

Unlike sanitation, we found that use of a safe water source was most protective

for diarrhea after five days of extreme rainfall. These protective effects may arise because

of higher microbial contamination of unimproved sources, such as the river and

uncovered wells, after heavy rainfall. A previous study by Levy et al, conducted in a

village 15 km southeast of Borbón, showed significantly higher *E. coli* counts in surface

and stored water during the rainy season compared to the dry season (31). Surface and

shallow groundwater may be more contaminated after heavy rainfall due to both the

flushing of human and animal fecal material and the transport of soil-resident bacteria

from the land environment into the water. Furthermore, the authors reported higher

microbial contamination of river water at sites located downstream of the village

compared to those located upstream of, and along the village, linking human settlement

to fecal contamination of river water (31). Other water quality studies have linked higher

concentrations of fecal indicator bacteria in shallow groundwater with rainfall, solid

waste, and pit latrines in the environment (29, 32). During our study, illegal use of the

sewage pipes still under construction may have resulted in sewage overflow after heavy

rainfall, sending fecal matter up into the streets. And, given that sanitation practices in Borbón included the use of pit latrines, the river, open fields and disposal of fecal matter along with household solid waste, there may have been a variety of fecal sources in the environment leading to high levels of source water contamination after heavy rainfall.

Having neighbors with an improved sanitation facility was protective for diarrhea in the month of April, following five extreme rainfall events. This result suggests a greater potential for pathogen flow between neighboring household environments under wet, but not dry conditions. Previous studies have also reported protective effects of neighboring practices on diarrhea. Root et al found that having a nearest neighbor with an improved latrine lowered the risk of diarrhea in those without a latrine (33). Ali et al. reported protective effects of higher cholera-vaccine coverage in a neighborhood in those who were unvaccinated. These findings were robust to two alternate definitions of the neighborhood, one based on Euclidean distance (34) and another based on shared water bodies (35), supporting our hypothesis of enteric pathogen movement between neighbors through a shared environment.

This study focuses on rainfall effects over a six-month period. While the time frame is not long enough to capture seasonal effects on diarrhea, results from four-years of active surveillance in the region indicate minimal seasonality of diarrhea (36). Nevertheless, longer studies are needed to confirm the association between rainfall and diarrhea. It is possible that the overall decline in diarrhea over the six-month period was the result of a reporting bias. However, we believe reporting fatigue to be minimal as our staff visited households on a daily basis to limit the effort required by study participants. We observed the ability of extreme rainfall to modify the effects of improved sanitation

and water source on diarrhea. Though these associations were not statistically significant, we believe that this may have been a power issue due to small sample size. Effect modifications were consistent in direction and strength when a lag of 4 weeks instead of 5 weeks was used, and when extreme rainfall was defined according to the 95th percentile instead of the 90th percentile of the daily rainfall distribution. Our study of diarrhea and its protective factors has several advantages over others including a local measurement of rainfall, a community-based design that followed participants of all ages, and sampling of both mild and severe cases of diarrhea.

Reducing the diarrheal disease burden in low- and middle-income countries is dependent on implementing effective household interventions such as the use of safe water sources, household water treatment and improved sanitation facilities. Furthermore, understanding the effectiveness of these interventions during extreme weather events is critical given current climate change. Our finding that use of safe water and improved sanitation may be protective under different ecological conditions highlights the need for integrated intervention strategies. This study is one of the few to consider the impact of extreme rainfall events on diarrhea interventions.

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Table 4.1. Reported associations between rainfall and diarrhea or acute gastrointestinal illness

Study	Location	Case Detection (Temporal Scale)	Rainfall Indicator	Lag Periods	Direction of Association with Diarrhea
Hashizume et al 2007 (13)	Dhaka, Bangladesh	Hospital-based (weekly)	Average of total weekly rainfall > 52 mm	0-16 weeks, 0-8 weeks	Positive
			Average of total weekly rainfall < 52 mm	0-16 weeks	Positive
Singh et al 2001 (14)	Pacific Islands	National case reports (monthly)	Rate of rainfall > 5 x 10^{-5} kg/m^2/min	No lag	Positive
			Rate of rainfall > 5 x 10^{-5} kg/m^2/min	1 month	Negative*
			Rate of rainfall < 5 x 10^{-5} kg/m^2/min	No lag, 1 month	Positive
Drayna et al 2010 (11)	Wauwatosa, Wisconsin	Hospital-based (daily)	Any rainfall	4 days	Positive
			Extreme rainfall events (2.54 cm/24 hours or 3.76 cm/48 hours)	1-7 days	Not reported*
Chou et al 2010 (10)	Taiwan	National reports of hospital admission (monthly)	Total Rainfall	No lag, 1 month, 2 months	Negative*
			Extreme rainfall days (> 40mm)	No lag	Negative
			Extreme rainfall days (> 40mm)	1 month	Positive*
			Extreme rainfall days (> 40mm)	2 months	Positive

*Not statistically significant

Table 4.2. Characteristics of case and control households from a series of six community-based case control studies in Borbón, Ecuador (December 2008 – May 2009)

	Number of Case Households (%), N=204	Number of Control Households (%), N=831
Age		
One or More Children < 5 Years of Age	157 (77.0)	335 (40.3)
Household Population Size		
≥5	157 (77.0)	400 (48.1)
Ownership Score (0-1)		
<0.3	94 (46.1)	319 (38.4)
≥0.3 - <0.6	107 (52.5)	470 (56.6)
≥0.6	3 (1.5)	42 (5.1)
Education		
Completed High School	64 (31.4)	339 (40.8)
Housing Construction Score (0-5)		
<4	107 (52.7)	333 (40.1)
Drinking Water Source		
Improved		
Piped Water	160 (78.4)	593 (71.4)
Bottled Water	16 (7.8)	149 (17.9)
Rain	8 (3.9)	36 (4.3)
Covered Well	2 (1.0)	9 (1.1)
Unimproved		
Uncovered Well	9 (4.4)	11 (1.3)
River	7 (3.4)	9 (1.1)
Point-of-use Water Treatment		
Yes	56 (27.5)	250 (30.1)
Water Storage Vessel Mouth Size		
Small (<8 cm)	128 (62.7)	556 (66.9)
Large (≥8 cm)	74 (36.3)	251 (30.2)
Type of Sanitation Facility		
Improved		
Flush Toilet	112 (54.9)	558 (67.1)
Latrine with Raised Platform	16 (7.8)	69 (8.3)
Unimproved		
Pit Latrine	50 (24.5)	116 (14.0)
Open Field	12 (5.9)	21 (2.5)
River	4 (2.0)	12 (1.4)
In a Bag, Newspaper or Basin	8 (3.9)	30 (3.6)
No. of Families Sharing Sanitation Facility		
1	112 (54.9)	527 (63.4)
≥2	63 (30.9)	174 (20.9)
Household Hygiene Score (0-1)		
<0.6	41 (20.1)	105 (12.6)
≥0.6 – <0.8	79 (38.7)	115 (32.9)
≥0.8	75 (36.8)	416 (50.1)
Waste Disposal		
Improved		
Collected by the City	134 (65.7)	601 (72.3)
Burned at Home	13 (6.4)	47 (5.7)
Unimproved		
Field, Ditch, or River	55 (27.0)	154 (18.5)

Table 4.3. Simple logistic regression model odds ratios (and 95% confidence intervals) for household-level diarrhea from a series of six community-based case control studies in Borbón, Ecuador (December 2008-May 2009)

	December	January	February	March	April	May
Household Population Size	**1.36 (1.16, 1.59)****	**1.14 (1.01, 1.28)***	**1.34 (1.16, 1.56)****	**1.21 (1.07, 1.36)***	**1.22 (1.05, 1.42)***	**1.44 (1.22, 1.71)****
Children Under Five Years	**7.64 (3.59, 16.26)****	**3.37 (1.41, 8.04)***	**7.51 (2.90, 19.46)****	**2.62 (1.18, 5.82)***	**5.69 (2.18, 14.86)****	**5.70 (2.01, 16.15)***
Ownership Score	0.23 (0.02, 2.28)	0.19 (0.01, 5.45)	0.16 (0.01, 2.39)	**0.03 (0.00, 0.71)***	0.11 (0.01, 2.15)	0.20 (0.01, 6.65)
High School Education	**0.44 (0.21, 0.91)***	0.53 (0.22, 1.25)	0.50 (0.22, 1.16)	0.74 (0.33, 1.64)	1.52 (0.69, 3.35)	0.85 (0.35, 2.07)
Construction Score	**0.69 (0.48, 1.00)***	0.77 (0.49, 1.20)	1.00 (0.67, 1.51)	0.67 (0.43, 1.05)	0.93 (0.60, 1.43)	0.64 (0.39, 1.07)
Improved Water Source	0.69 (0.16, 2.98)	0.27 (0.06, 1.21)	0.59 (0.05, 6.72)	**0.14 (0.03, 0.68)***	**0.08 (0.01, 0.77)***	0.33 (0.06, 1.93)
Water Treatment	1.48 (0.75, 2.89)	0.44 (0.17, 1.14)	0.80 (0.34, 1.88)	1.16 (0.50, 2.66)	0.34 (0.11, 1.04)	0.93 (0.34, 2.52)
Small Container Storage	0.99 (0.50, 1.94)	0.93 (0.41, 2.14)	0.96 (0.43, 2.13)	1.12 (0.49, 2.56)	0.91 (0.38, 2.17)	**0.20 (0.08, 0.50)****
Improved Sanitation	**0.40 (0.19, 0.84)***	**0.21 (0.09, 0.49)****	0.72 (0.32, 1.62)	0.53 (0.24, 1.19)	0.65 (0.28, 1.50)	0.62 (0.24, 1.57)
Number Sharing Sanitation	0.98 (0.68, 1.41)	0.91 (0.58, 1.42)	1.45 (0.76, 2.78)	0.92 (0.54, 1.56)	1.05 (0.80, 1.39)	0.99 (0.74, 1.32)
Improved Waste Disposal	**0.34 (0.16, 0.69)***	0.68 (0.26, 1.73)	0.87 (0.35, 2.16)	1.42 (0.46, 4.41)	0.68 (0.29, 1.62)	0.99 (0.34, 2.87)
Higher Hygiene Score	0.24 (0.03, 2.00)	**0.08 (0.01, 0.56)***	0.10 (0.01, 1.15)	0.23 (0.02, 3.16)	0.09 (0.01, 1.53)	1.28 (0.04, 41.76)
Neighboring Water Source[#]	0.50 (0.03, 8.72)	0.49 (0.04, 5.48)	7.03 (0.01, 7221.69)	0.30 (0.03, 2.73)	0.02 (0.00, 1.00)	1.55 (0.05, 53.48)
Neighboring Sanitation[#]	0.29 (0.07, 1.24)	0.38 (0.08, 1.68)	0.24 (0.04, 1.39)	0.51 (0.11, 2.33)	**0.15 (0.02, 0.97)***	1.95 (0.22, 17.07)
Neighboring Density[#]	0.96 (0.93, 1.00)	0.99 (0.95, 1.03)	1.01 (0.97, 1.05)	0.97 (0.93, 1.01)	0.99 (0.95, 1.04)	1.02 (0.98, 1.07)

*$p<0.05$, **$p<0.0006$, the Bonferonni-adjusted value, [#]Neighborhood is defined using a 50 meter radius around the house

Table 4.4. Generalized estimating equation model odds ratios and robust 95% confidence intervals (95% CI) for household-level diarrhea from a series of six community-based case control studies in Borbón, Ecuador (December 2008-May 2009).

Predictor	Model 1			Model 2			Model 3		
	Beta Estimate	Odds Ratio	95% CI	Beta Estimate	Odds Ratio	95% CI	Beta Estimate	Odds Ratio	95% CI
Household Population Size	0.15	1.16	(1.08, 1.25)	0.15	1.17	(1.09, 1.25)	0.15	1.16	(1.09, 1.25)
Children Under Five Years	1.32	3.75	(2.40, 5.86)	1.33	3.78	(2.41, 5.92)	1.32	3.73	(2.39, 5.82)
Ownership Score	-2.06	0.13	(0.03, 0.51)	-2.15	0.12	(0.03, 0.46)	-2.08	0.13	(0.03, 0.50)
Improved Water Source	-1.28	0.28	(0.13, 0.60)	-1.25	0.29	(0.13, 0.63)	0.11	1.11	(0.17, 7.23)
Improved Sanitation	-0.54	0.59	(0.40, 0.86)	-1.28	0.28	(0.10, 0.78)	-0.55	0.58	(0.39, 0.85)
Extreme Rainfall Days*	-0.19	0.83	(0.74, 0.93)	-0.34	0.72	(0.58, 0.89)	0.19	1.21	(0.77, 1.91)
Sanitation x Extreme Rainfall Days*	-	-	-	0.21	1.23	(0.95, 1.60)	-	-	-
Water Source x Extreme Rainfall Days*	-	-	-	-	-	-	-0.41	0.67	(0.42, 1.07)

*Extreme rainfall (>18 mm) is defined using a threshold equivalent to the 90th percentile of the daily rainfall distribution with a lag of 5 weeks prior to the end of the 15-day case control period.

Figure 4.1. Daily rainfall (black lines) and the 15-day period prevalence of diarrhea from a series of six 15-day community-based case control studies (blue horizontal bars) in Borbón, Ecuador (November 2008 to May 2009).
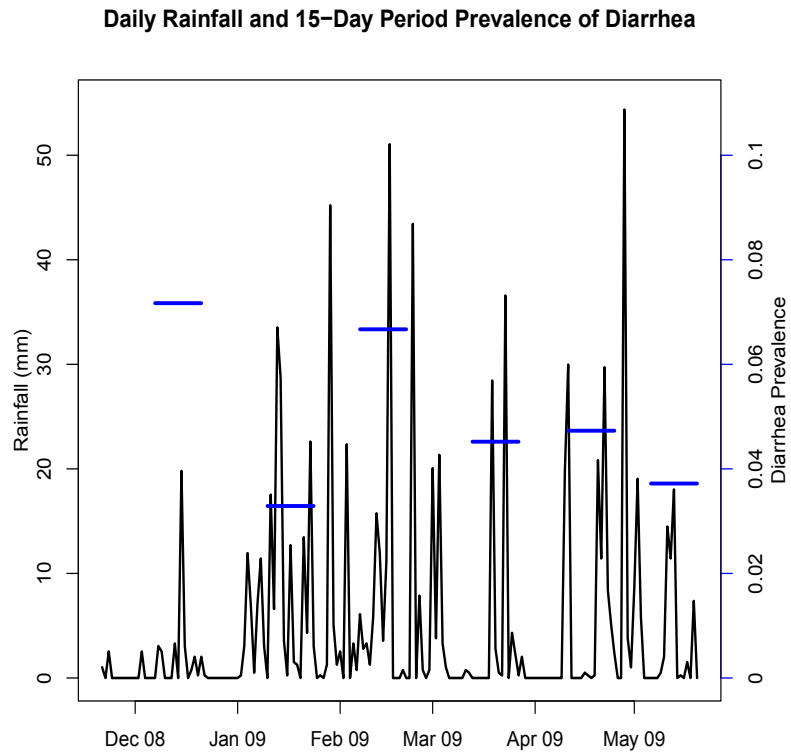

Daily Rainfall and 15−Day Period Prevalence of Diarrhea

Figure 4.2. Scatter plots of diarrhea prevalence versus total rainfall (mm, top) and number of extreme rainfall days (> 18 mm, bottom) summarized across lags 3, 4, and 5 weeks prior to the end of the six 15-day case controls in Borbón, Ecuador (December 2008-May 2009).
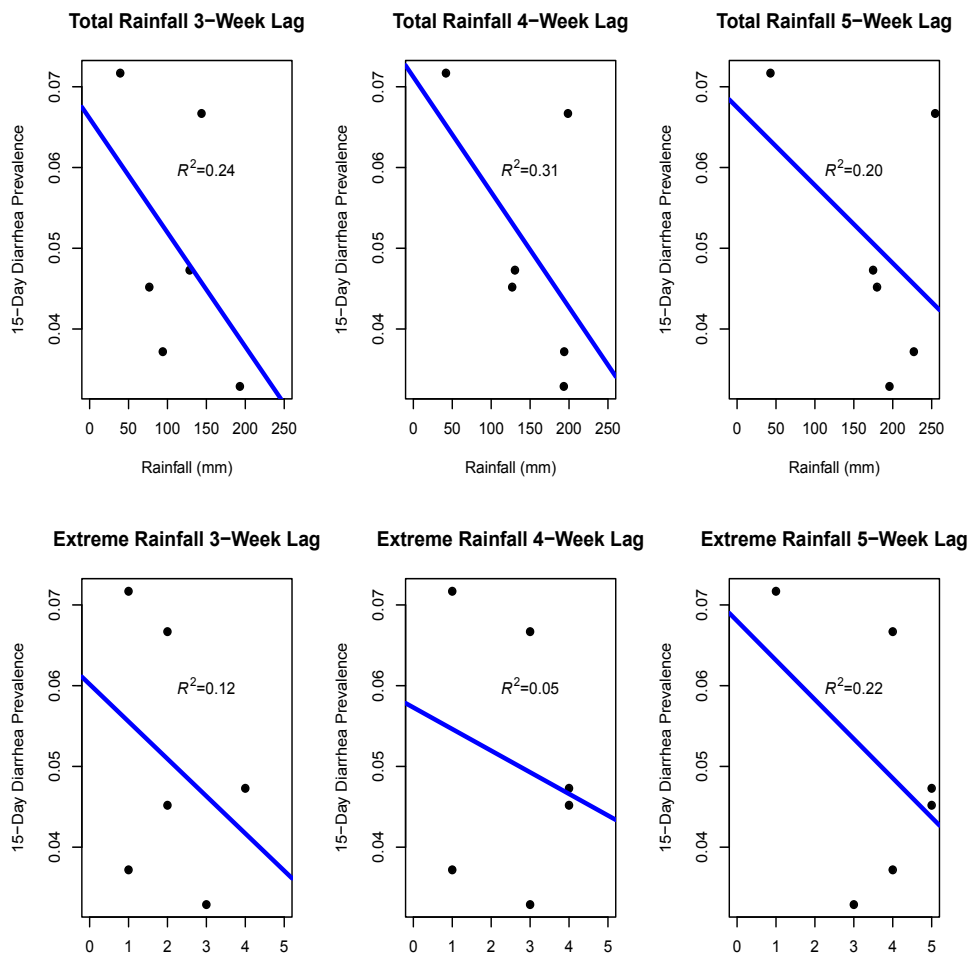
Figure 4.3a. The predicted probability of household-level diarrhea (on the log odds scale, y-axis) for a household with median household size and ownership, at least one child under the age of five, an improved water source, and either improved (dashed line) or unimproved (solid line) sanitation, at each value of extreme rainfall days (x-axis) in the 5 weeks prior to the end of the 15-day case control period in Borbón, Ecuador (December 2008-May 2009).
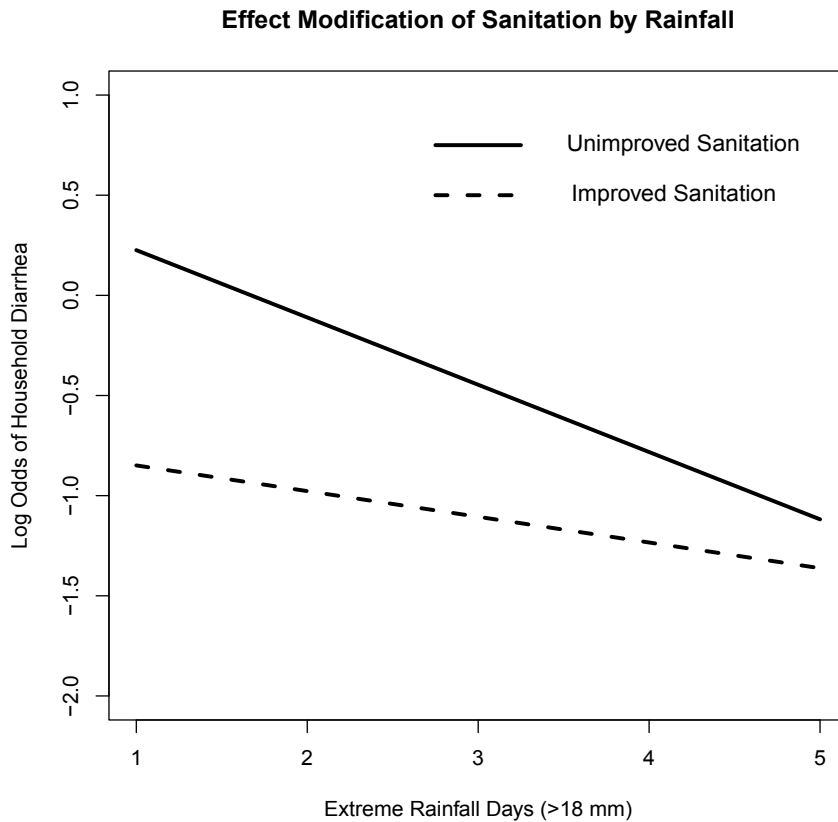
Figure 4.3b. The predicted probability of household-level diarrhea (on the log odds scale, y-axis) for a household with median household size and ownership, at least one child under the age of five, improved sanitation and either an improved (dashed line) or unimproved (solid line) water source, at each value of extreme rainfall days in the 5 weeks prior to the end of the 15-day case control period in Borbón, Ecuador (December 2008-May 2009).

Effect Modification of Water Source by Rainfall
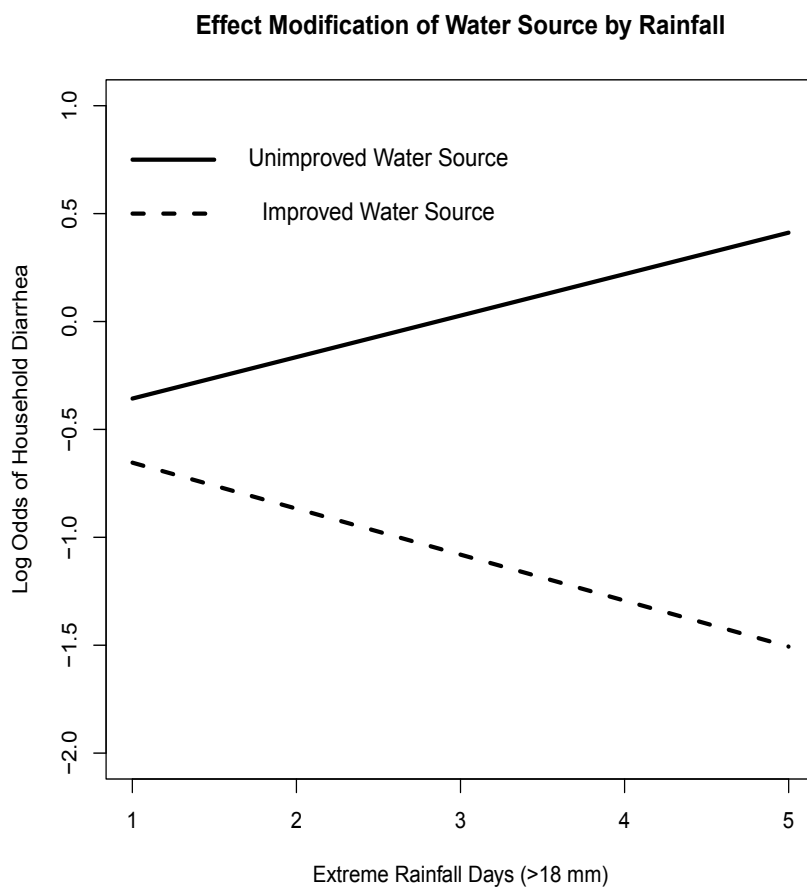
Chapter V

Discussion

Vaccination Against Enteric Pathogens

In this dissertation research, we show that rotavirus coinfections are more likely to result in diarrhea than single infections, and even more, we provide evidence that confecting pathogens interact synergistically to cause diarrhea. These results imply additional and unrealized benefits of rotavirus vaccination in areas where coinfecting pathogens circulate widely. Our findings underscore the need for further research on the mechanisms behind these synergistic interactions, as well as the efficacy of rotavirus vaccination in preventing these mechanisms from playing out.

In developing areas like our study region, the World Health Organization recommends the administration of two doses of the monovalent vaccine Rotarix, or three doses of the pentavalent vaccine, Rotateq, by the age of 32 weeks. In Ecuador, rotavirus vaccination was added to the national vaccination program in 2007 and by 2010, coverage was estimated to be 97% (1). What remains unclear are the coverage rates in the northern coastal region. Additionally, there is uncertainty about the protection offered to those who did complete the schedule in this region. While there is urgent need for surveillance studies to evaluate rotavirus vaccination in developing country settings, our case control results suggest little to no reduction in rotavirus prevalence between 2003

and 2008 in the region. This may be due the central government's neglect of the region, poor access to communities upstream of Borbón, issues maintaining a cold-chain, lack of follow up with children leading to missing doses, and low levels of vaccine efficacy due to the circulation of novel serotypes in the region (2). With such a simple and inexpensive solution in hand, we need to better understand access to and efficacy of the rotavirus vaccine in developing areas such as this one.

Our research also shows that children between the ages of one and five years, and adults are at risk for rotavirus-associated diarrhea. Symptomatic rotavirus infections in adults appear to be common in other endemic settings (3). Previous research has demonstrated that immunity to rotavirus wanes over time (Reviewed in (3)). This, and the potential for frequent re-exposure to the virus in endemic settings, warrants an evaluation of vaccination in older children and adults. High-risk individuals, to whom the vaccine should be targeted, might include those with frequent contact with infants.

Like rotavirus, enterotoxigenic *E. coli* (ETEC) is a leading cause of diarrhea in children in developing countries. ETEC is also a common cause of traveler's diarrhea in adults (4). In our study region, we found that ETEC is pathogenic in children aged one through 12 years, but not in adults. It is likely that the adults in our region have developed immunity to this pathogen through frequent exposure. The potential immunity to ETEC shows promise for a vaccine. While there is no vaccine for ETEC yet on the market, there are oral-inactivated vaccines under development and in clinical trials that demonstrate protection against diarrhea (5). As with rotavirus, it will be important to ensure that these vaccines are made available to those living in developing regions.

Water, Sanitation, and Integrated Interventions

While vaccines reduce the risk of diarrhea in the presence of a pathogen infection, environmental interventions limit *exposure to* these pathogens. Our research illustrates the importance of an improved water source (i.e. piped water), and an improved sanitation facility (flush toilets and pit latrines) in preventing these exposures. Furthermore, water and sanitation interventions may be most effective under different ecological conditions. Where resources are extremely limited, and compliance less than perfect, global health campaigns could do well to emphasize treatment of drinking water after heavy rainfall, and use of pit latrines or flush toilets during periods of drought.

Our results also demonstrate that interventions with an integrated approach (i.e. improving water, sanitation, and hygiene simultaneously) would protect from pathogen exposure over a range of ecological conditions. These findings add to the current debate on whether integrated interventions have any additional benefit over single interventions (6-11). Critics of the integrated approach argue that this would spread resources too thin, and that individual components would suffer from a lack of focus. To address this, Fewtrell et al suggest the phasing in of interventions to ensure that each component is introduced with enough attention (7). Based on our results, we suggest that this could be done seasonally; during the dry season, sanitation could be introduced and before the rainy season hits, water interventions could be phased in.

Diarrhea is a Community Issue

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The evidence that we provide for pathogen movement from household to household through a community environment exemplifies the dependency of diarrhea outcomes. Therefore, we have reason to be invested in the water, sanitation and hygiene practices of those in our community, especially those of our neighbors. The recent focus on in-home water treatment interventions holds much promise yet, these solutions may be too individualistic and focus only on the proximal factors causing diarrhea. We should be careful not to let these household-based interventions distract from community-level solutions that reduce risk equally and address more distal causes of diarrhea. Investment in community infrastructure such as piped and treated water, flush toilets, and sewage systems would reduce pathogen concentration in the environment, alleviate the issues related to poor compliance, and perhaps most important, reduce inequality between community members.

In chapter three, we discuss the potential for the existence of a core population in which high prevalence of enteric pathogens are sustained. Social or geographic contact with this core population carries with it higher risk of enteric infection than contact with other populations. The concept of core populations is widely used in the STD and HIV literature, potentially owing to the dynamic analyses that have been applied to these infectious diseases. Core populations have largely been ignored in the diarrhea literature. This is surprising given the vast number of urban slum dwellers living in crowded conditions without access to safe water, sanitation and health care. The estimated number of slum residents has grown from 767 million in 2000 to 828 million in recent years (12). Conditions in these informal settlements are ripe for high levels of diarrhea transmission. Given the potential for movement of pathogens through the environment, I argue that the

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local community should be more concerned about slum conditions. Furthermore, global health efforts would do well to target vaccination campaigns, support environmental interventions and provide basic health care to these high-risk and underserved populations.

Reducing Poverty and Improving Literacy

The most vulnerable populations in our society seem to be at greatest risk for diarrhea and the most underprepared to prevent mortality. Yet, the relationships between poverty, literacy, and diarrhea are under appreciated. Consistent with the literature we found that higher levels of ownership (used as a proxy for income) are protective for diarrhea. While poverty may be an underlying cause of diarrhea, acting through factors such as malnourishment leading to low immunity, crowded living conditions, poor access to water and sanitation, and illiteracy, diarrheal disease can also deepen poverty. Chronic diarrhea can lead to malnutrition, poor physical and cognitive development of children, economic burden related to treatment and a loss of economic productivity. This feedback loop between diarrhea and poverty underscores the need to integrate poverty reduction measures, such as literacy and educational opportunities, along with more traditional interventions for diarrhea.

Conclusion

By 2030 diarrheal disease is expected to drop from the 2nd to the 23rd leading cause of death (13). But, why wait another 18 years for this to happen? Once we reach the Millennium Development Goal target date of 2015, it will be important to maintain the momentum towards poverty reduction that these goals have generated. While more resources are always needed, targeting our research and public health campaigns towards effective and sustainable intervention strategies for diarrhea, building public, private, and non-profit partnerships, and strengthening relationships across boarders will be important to the reduction of global morbidity and mortality from diarrheal disease.

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