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Signature:

Miranda Delahoy

Date

Challenges in reducing the burden of childhood diarrhea in Peru

By

Miranda Delahoy
Doctor of Philosophy

Environmental Health Sciences

Karen Levy, PhD, MPH
Advisor

Thomas Clasen, PhD, JD, MSc
Committee Member

Benjamin Lopman, PhD, MSc
Committee Member

Kyle Steenland, PhD, MS
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

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By

Miranda Delahoy
MSPH, Rollins School of Public Health, Emory University, 2013
BA, Oberlin College, 2007

Advisor: Karen Levy, PhD, MPH

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in
Environmental Health Sciences
2019

Abstract

Challenges in reducing the burden of childhood diarrhea in Peru

By Miranda Delahoy

Diarrhea is an important cause of childhood morbidity. Improving drinking water and sanitation and vaccinating infants for rotavirus can reduce diarrhea cases; however, these interventions face challenges in achieving optimal performance. Associations between higher temperature and diarrhea highlight challenges in reducing childhood diarrhea as climate change progresses. A spatially-detailed national longitudinal dataset was constructed to examine these factors and the rate of clinic visits for diarrhea in children under five from 2005-2015 in Peru. Drinking water and infant stool samples were collected from 96 households in Piura, Peru in 2016 to examine water quality and infant enteropathogen infections and diarrhea.

In Peru, the rate of childhood diarrhea clinic visits decreased by 3% per year from 2005-2015. The rate was 6.7% lower in the rotavirus vaccine era (2010-2015, when most infants were vaccinated; incidence rate ratio (IRR): 0.93, 95% confidence interval (CI): 0.90-0.97); however, there was no impact of ongoing rotavirus immunization in provinces in the lowest quartiles of piped water or sewerage access. A 1 °C temperature increase was associated with a 3.8% higher rate of childhood diarrhea clinic visits (IRR: 1.04, 95% CI: 1.03-1.04). Controlling for temperature, there was higher diarrhea incidence during El Niño periods (IRR: 1.03, 95% CI: 1.01-1.04). In Piura, we found high prevalence of arsenic, pesticides/herbicides, and *E. coli* in drinking water samples mostly originating from piped water connections. Storing drinking water was associated with higher odds of *E. coli* (odds ratio (OR): 4.50, 95% CI: 2.04-9.95), and pesticide/herbicide detection (OR: 6.55, 95% CI: 2.05-20.96). Infants had high prevalence of diarrhea (14%) and enteropathogen infections (68%); enteropathogen detection was higher among infants from households with pesticide/herbicide presence in drinking water (OR: 2.93, 95% CI: 1.13-7.61).

These results underscore the importance of considering multiple strategies to reduce diarrheal disease: water/sanitation improvements may operate synergistically with rotavirus vaccination to reduce childhood diarrhea. Provision of piped drinking water should be accompanied by concurrent microbiological and chemical assessments and messaging on safe storage. Although childhood diarrhea clinic visits are declining in Peru, higher temperatures and intensifying El Niño events potentially resulting from climate change could threaten this progress.

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Acknowledgements

I would like to thank my advisor, Karen Levy, for her constant support and guidance. Karen taught me so much about public health and writing, but also taught me to look beyond the details and see the big picture, and to recognize the strengths of my work. Thank you also to Kyle Steenland, for going above and beyond as a committee member in mentoring me. Kyle made me a stronger epidemiologist and I will carry his teachings into my career. Thank you to Thomas Clasen for teaching me so much throughout my studies and for being a strong advocate for students. I would also like to thank Benjamin Lopman for his thoughtful and challenging questions, which helped me better understand my work. Ciara Sugerman has been a mentor to me throughout my entire public health career, and I will always be grateful for this mentorship.

Thank you to the members of the GeoHealth team. A special thank you to Angela Rozo, who not only provided unparalleled commitment and organization to the team, but who always cheered me on and cheered me up. And to Vanessa Vasquez, who was very supportive throughout our research and travels. I would also like to thank the faculty and staff of the Environmental Health department at Emory, especially Jeremy Sarnat, Stefanie Sarnat, Paige Tolbert, Robin Thompson, and Ariadne Swichtenberg.

Many people contributed to this research, and are mentioned in the acknowledgements of each chapter. Thank you to the families who participated in the Pervuain censuses and to the caretakers and infants who participated in our study in Piura, Peru. Thank you also to the Peruvian census enumerators.

A very warm thank you to my family and friends. They say it takes a village (to raise a dissertation, of course). For me it felt like many villages. I appreciate the colleagues and friends I have gained among the Environmental Health Science PhD students. Tuesday Thomas-Jackson and the rest of my gym

community may not realize just how much they helped me stay mentally fit by keeping me physically fit. Tuesday told me I'd better work, so I did! Katie and Caryn have been constant anchors in my life for decades, especially during my PhD. And Manoah and Celeste showed me how it's all done, while being great friends of mine. Many thanks to Emily, Nicole, and Dahni for getting me out of the house in Atlanta and for being such wonderful friends. My entire family and in-laws have also provided endless support and comforting. Thank you to Patrick and Elizabeth for adventuring with me and keeping my spirits up.

I'd like to dedicate this work to my parents: Mom, my desire to go into a field that would help children comes from your amazing dedication as both a teacher and a mother, and Dad, your example of persistence and hard work was so inspirational during my doctoral research. Most of all, thank you to Kevin for his loving support and care for me. Kevin always listened and pushed me to achieve more. I am forever grateful.

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Chapter 1 Introduction

Diarrhea is a leading cause of global morbidity and mortality for children.¹⁻⁴ There are numerous bacteria, protozoa, and viruses that cause diarrhea, as well as several exposure pathways through which children become infected.⁵⁻⁷ The multitudes of hosts, environmental reservoirs, and exposure pathways for these pathogens pose challenges in reducing the disease burden. Water, sanitation, and hygiene (WASH) interventions can interrupt the transmission of these pathogens across several pathways; yet billions of people still lack access to reliable safe water and basic sanitation facilities.⁸ In low- and middle-income countries (LMICs), the majority of diarrheal deaths are attributed to inadequate WASH.⁹

Health challenges related to inadequate WASH extend beyond acute diarrheal disease. Early childhood diarrhea and enteric pathogen infections have been associated with poor physical growth, decreased cognitive function, lower performance in school, decreased physical fitness, and increased future susceptibility to diarrhea;¹⁰⁻¹³ even asymptomatic infections have been associated with adverse health and development outcomes.^{14,15} Repeated exposure to enteric pathogens can lead to environmental enteric dysfunction, a subclinical condition that may lead to increased vulnerability to future infections, reduced nutrient uptake, and oral vaccine failure.¹⁶⁻¹⁸ In addition to microbiological threats, unsafe drinking water can contain heavy metals and chemicals that can have long-lasting impacts on immune function and cognitive development for children, especially when exposed during critical development periods.¹⁹⁻²¹

While WASH interventions can prevent transmission of diverse diarrheal pathogens across several exposure pathways, public health interventions targeted at specific diarrheal pathogens have also been successful at reducing the disease burden. The introduction of rotavirus vaccination was associated with large declines in gastroenteritis hospitalizations globally during the first decade of

licensure (2006-2016).²² Both WASH and non-WASH interventions that address diarrheal diseases face challenges in optimizing health impact.

New challenges to combatting diarrheal disease are expected in the coming decades as climate change progresses. The World Health Organization (WHO) projects an annual increase of 48,000 diarrheal deaths in children (under the age of 15) worldwide in 2030 attributable to climate change.²³ This estimated increase is largely based on the association between temperature and diarrheal disease; however, climate change may also result in an increase in extreme weather events that can damage WASH infrastructure.²⁴ Countries bordering the eastern Pacific Ocean, such as Peru, may also experience more intense El Niño events;^{25,26} El Niño seasons have previously been associated with increased diarrhea cases in Peru.²⁷⁻³¹

In this introductory chapter, I elaborate on diarrheal disease and enteric pathogen transmission, with a focus on possible WASH improvements/interventions (particularly water and sanitation interventions, as hygiene is not explicitly addressed in this dissertation research). I discuss associated challenges for interrupting transmission, including increases in temperature expected under climate change. I then discuss these aspects more specifically for the country of Peru, the setting of this research. Finally, I present the dissertation chapters, which examine WASH improvements, rotavirus vaccination, and challenges to reducing the diarrheal disease burden in Peru.

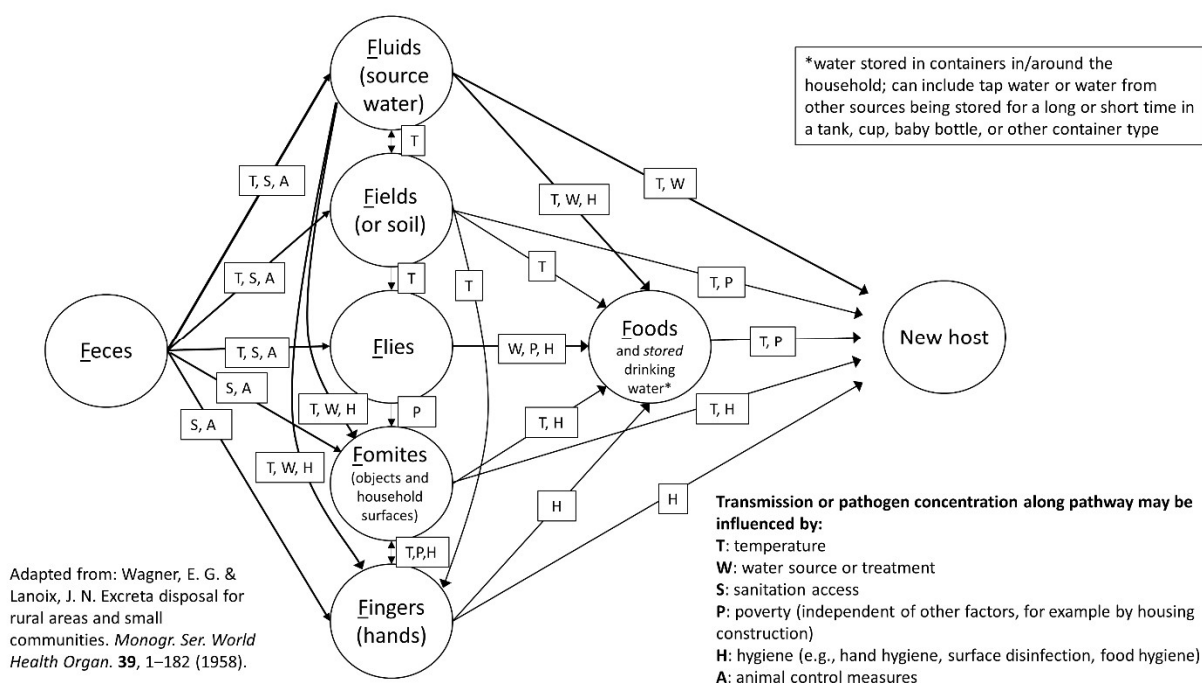
Diarrhea and transmission of enteric pathogens

Diarrheal diseases in children are often caused by enteropathogen infections. Two large multi-center studies conducted in the past decade have shed light on which enteric pathogens are associated with childhood diarrhea cases in LMICs: the Global Enteric Multicenter Study (GEMS) and the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study.^{32,33} GEMS, conducted from 2007-2011 at sites

that had not yet introduced rotavirus vaccination, underscored the importance of rotavirus as the leading cause of moderate-to-severe diarrhea in young children under two years old, with prevalence in infants nearly double that of the next leading pathogen.^{5,32,34} MAL-ED was conducted from 2009-2014 in three sites that had introduced rotavirus vaccination and three sites that had not, and considered less-severe diarrhea in the community that did not necessarily warrant a clinic visit. In MAL-ED sites where rotavirus vaccination had not been introduced, rotavirus was the pathogen to which the highest proportion of cases of diarrhea in children under two years old were attributable.³³ In a re-analysis of MAL-ED stool samples using quantitative molecular diagnostic methods, rotavirus had the highest attributable incidence of diarrhea among infants (0-11 months).³⁵ Other pathogens notable for their contributions to moderate-to-severe and community childhood diarrhea in GEMS and MAL-ED included the bacterial pathogens *Shigella*, enterotoxigenic *E. coli* (ETEC), and *Campylobacter*; viral pathogens sapovirus, adenovirus, norovirus, and astrovirus; and the protozoan parasite *Cryptosporidium*. The relative contributions of each to the diarrheal disease burden varied by age group and by laboratory methods employed, and are described elsewhere.³²⁻³⁵

There are several important pathways of exposure and risk factors for diarrheal disease transmission. Possible risk factors for diarrhea include poor water quality; inadequate sanitation coverage; inadequate hand hygiene; poor health, nutritional, or vaccination status; and other environmental risk factors, such as higher ambient temperature.³⁶⁻³⁹ A classic representation of the exposure pathways of diarrheal pathogens is the “F-diagram”, so named because feces are transferred to fingers, fomites, fields, flies, fluids, and foods, and eventually to a new host.⁴⁰ I present an adapted version of the F-diagram below, in which various environmental factors influence pathways of transmission, as discussed in this chapter.

Figure 1.1 Modified F-diagram of diarrheal disease transmission



Rotavirus and other enteric viruses are primarily transmitted from person-to-person.^{41,42} The low infectious dose and persistence on surfaces and hands may contribute to this transmission route.^{43–45} Rotavirus can survive in water and has been detected in drinking water;⁴³ however, interventions to improve water quality may lead to greater reductions in bacterial diarrhea compared to viral diarrhea.^{41,46} Rotavirus is not considered an important foodborne pathogen⁴⁷ and does not replicate in food.⁴⁸ Transmission from infected individuals to food during preparation via aerosols, hands, and/or fomites may contribute to spread through food, though this is better described as indirect person-to-person transmission.^{43,49}

While person-to-person transmission is perhaps the most important transmission route for rotavirus, other pathways of exposure are more important for other pathogens. Animals carry different strains of rotavirus than those found in humans and zoonotic transmission is not thought to contribute to human rotavirus infections.^{42,48} On the other hand, chickens and other animals may be important sources of pathogens such as *Campylobacter*, *Salmonella*, and *Cryptosporidium*, for which

zoonotic transmission is likely substantial.^{50–58} Exposure to chickens in the household has been shown to be a risk factor for *Campylobacter* infections in Peru.⁵³ Foodborne transmission, of little importance to rotavirus, is considered a dominant transmission route for pathogens such as *Campylobacter* and non-typhoidal *Salmonella*.⁴⁷ Waterborne transmission may be important for pathogens such as *Cryptosporidium* and *Shigella*,⁴⁷ but of relatively less importance for rotavirus transmission.⁴⁶

Compared to research on child diarrhea, there has been less attention on child enteropathogen infections and their association with drinking water quality measurements. GEMS and MAL-ED considered WASH factors associated with enteric pathogen transmission, but mostly considered caretaker-reported water treatment and source types in the absence of household water samples.^{32,33}

Improved drinking water and drinking water treatment

The WHO/United Nations International Children's Emergency Fund (UNICEF) Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (JMP) maintains classifications of whether drinking water sources are “improved” or “unimproved” that are widely used in the WASH research community.⁵⁹ The definition of improved drinking water focuses on the ability to deliver safe water, and includes piped water, boreholes or tubewells, protected wells and springs, rainwater, and purchased water (from a tanker-truck, kiosk, or other delivery method, in bottles, sachets, or small tanks).⁵⁹ The JMP has updated these criteria to suggest a gold standard of safely managed drinking water that is located on the premises, available when needed, and free of fecal and chemical contamination.⁵⁹ This new standard addresses several important aspects of water quality, including intermittent availability, as well as chemical aspects of drinking water, which traditionally have been given less attention in defining improved water sources.⁶⁰

Piped drinking water supplies in LMICs often fall short of meeting these criteria, in part because they fail to provide continuous service. Intermittent piped water service can pose challenges to maintaining high water quality within a system, and in the household, if water is stored for use during service cuts.^{61,62} The modified F-diagram (Figure 1.1) differentiates between source water and drinking water stored in the house, which highlights the potential for contamination of stored water by hands, objects (such as cups dipped into larger containers of water), and flies (or other animals). Previous research has demonstrated post-collection contamination of stored water, with considerable change in quality for water that was relatively uncontaminated at the source.⁶³ There is, however, potential to intervene to improve water quality between the source and consumption, by treating household drinking water. While some household drinking water treatments, such as boiling drinking water, are effective against bacterial, viral, and protozoal enteric pathogens, other treatments are ineffective against certain taxa of enteropathogens, such as chlorination, which does little to inactivate protozoa such as *Cryptosporidium*, or using water filters, which often have inadequate pore sizes to physically remove viruses from water.⁶⁴ High adherence to household water treatment is necessary to achieve health gains through averting waterborne pathogen transmission.^{65,66} Furthermore, household drinking water treatment options in LMICs tend to focus on microbiological water contamination; low-cost point-of-use treatments that address chemical/metal contamination in drinking water are lacking.⁶⁷

Improved sanitation

The JMP classifies sanitation systems as improved if they hygienically separate human excreta from human contact; included in this group are flush (and pour-flush) toilets that flush to a piped sewer system, septic tank, or pit latrine, as well as pit latrines with slabs, and composting toilets.⁵⁹ The JMP defines a gold standard of safely managed sanitation, which refers to the use of improved facilities

that are not shared, where feces are disposed of safely or transported elsewhere for treatment.⁵⁹

Globally, access to improved sanitation facilities lags behind access to improved drinking water sources, with approximately one-third of the world population lacking access to improved sanitation facilities.⁶⁸

Improving sanitation can reduce the spread of diarrheal pathogens across several pathways, by preventing human fecal waste from contaminating food, water, household surfaces, and child play spaces via contact with infected hands or vectors such as flies (Figure 1.1). The benefits of improved sanitation can extend beyond those who own the improved facilities to the surrounding community, which benefits from a type of herd immunity provided by reducing overall environmental contamination.^{69,70}

Global disparities in access to improved sanitation persist between urban and rural areas. Especially poignant are challenges in increasing rural sewer coverage: globally in 2015, 63% of the population of urban areas had a sewer connection compared to only 9% of those in rural areas.⁷¹ Improved sanitation, including toilets connected to sewerage networks, can fail to safely separate humans from fecal contamination in the environment. Not all sewage from piped systems goes to treated wastewater plants.⁷¹ Having improved sanitation does not always equate with use;⁷² improved sanitation facilities are not always utilized by all household members and having an improved facility does not preclude unsafe disposal of infant feces.⁷³ Furthermore, improved sanitation facilities are designed to separate humans from contact with their own excreta, but not from animal excreta. Several pathogens found in animal feces pose a risk to human health (Appendix Chapter 6).

Mixed evidence on water and sanitation improvements

Systematic reviews demonstrate that interventions to improve drinking water, sanitation, and hygiene can result in substantial reductions in childhood diarrheal disease, with provision of treated,

piped drinking water demonstrating some of the largest disease reductions, and sanitation interventions being particularly effective when high coverage is attained.^{74–76} Nevertheless, several large recent trials have failed to find an association between improved WASH conditions and child diarrhea.^{76–80} These trials highlight some of the aforementioned challenges to interrupting enteric pathogen transmission via drinking water and sanitation improvements. In the two trials that addressed drinking water quality, the choice of drinking water improvement was point-of-use (or point-of-collection) chlorination, which is ineffective at inactivating *Cryptosporidium*, an important cause of childhood diarrhea, and performs less well at inactivating viruses compared to bacteria.^{81,82} Increasing sanitation coverage may not achieve reductions in diarrhea in the absence of high adoption; furthermore, sanitation interventions do not consistently reduce fecal exposure along critical pathways.^{78,83}

Rotavirus vaccination

In 1999, an oral rotavirus vaccine (introduced in 1998) was withdrawn because of increased risk of intussusception.⁸⁴ Two oral rotavirus vaccines commonly in use today (Rotarix® (GlaxoSmithKline Biologicals, Wavre, Belgium) and RotaTeq® (Merck & Co., Kenilworth, New Jersey, USA)) were licensed in 2006, which was followed by a global recommendation of use in 2009.^{85–88} Rotavirus vaccination has since provided >80% protection against severe rotavirus illness in several middle- and high-income countries; however, low- and middle-income countries have experienced both reduced efficacy and effectiveness of the vaccine compared to high-income countries.⁸⁹ Hypotheses for attenuated effectiveness include that (1) underlying health conditions (including environmental enteropathy, helminth infection, and/or poor nutritional status) may reduce oral vaccine immune response, or (2) that natural and vaccine-derived immunity may be insufficient to combat the diversity of strains and the very high incidence of rotavirus in endemic lower-income settings.^{90–94}

Improvements to water and sanitation may diminish these barriers to oral rotavirus vaccine efficacy.^{39,95} Recent evidence suggests that improved water, sanitation, and hygiene conditions can improve seroconversion of oral rotavirus vaccination.⁹⁶ A 2017 review of the global impact of rotavirus vaccination identified 26 studies of rotavirus vaccine impact conducted in LMICs; none of these explicitly considered vaccine impact at varying levels of water and sanitation access.²² National analyses of the impact of rotavirus vaccination stand to benefit from including local factors related to water and sanitation infrastructure. Improvements in water and sanitation may affect the secular trend of diarrheal disease, which is important in analyses of diarrhea rates before and after vaccination; furthermore, better access to water and sanitation may operate synergistically with rotavirus vaccination to reduce diarrhea.

Temperature, climate change, and all-cause diarrhea

Average temperatures are projected to rise globally by 2 °C or more by the end of the 21st century,^{97,98} which is expected to impact the incidence of vector-borne diseases and diseases caused by pathogens that can survive or multiply in the environment.^{99–101} A large percentage of the burden of climate change-related morbidity is expected to be borne by children,¹⁰² who have unique vulnerabilities to climate change.¹⁰³ Climate change is expected to impact rates of many conditions that adversely affect child health such as malaria, other vector-borne diseases, malnutrition, and diarrhea.¹⁰³

Globally, warmer temperatures are associated with increased diarrhea incidence. A systematic review and meta-analysis investigating the relationship between temperature and diarrheal illness found a 1 °C increase in temperature to be associated with a pooled estimate of 7% higher incidence of diarrhea.³⁶ Temperature increases associated with climate change are expected to greatly increase the global risk of diarrhea by the end of the century,^{36,104,105} with even a moderate increased risk having

the potential to greatly affect the burden of disease attributable to diarrhea.^{98,105} The WHO projects an annual increase of 48,000 child diarrheal deaths worldwide in 2030 attributable to climate change.²³

Temperature may influence diarrheal disease transmission through a number of pathways, illustrated in Figure 1.1, and previously enumerated elsewhere.¹⁰⁶ The concentration and transport of viable pathogens in soil, water, food, and on surfaces can vary based on air and water temperature.

Temperature may also affect fly abundance, which could be of importance in considering associations between climate and diarrheal disease transmission.¹⁰⁷ Temperature can influence human behavior and exposure pathways, including time spent outdoors and in contact with surface water, as well as changes in water consumption.

The forecasted increased temperatures in the 21st century may give rise to a range of adverse health outcomes, with likely variation in the magnitude of these effects by geography and socioeconomic status.⁹⁸ Effective adaptation strategies are needed to mitigate excess morbidity that will be brought on by rising temperatures; however, investment in plans to adapt to climate change with a focus on health has been lacking,¹⁰¹ especially outside of high-income countries.⁹⁸ The concept of climate vulnerability focuses on the potential for health hazards to affect a given population, and that population's ability to react to hazards in a way that would mitigate adverse outcomes.¹⁰⁸

Adaptations for reducing the adverse health and well-being burden due to climate change after often focused on direct responses to extreme weather events (*e.g.*, housing modifications or early-warning systems);¹⁰³ however, the United Nations Intergovernmental Panel on Climate Change (UN IPCC) and WHO recommend strengthening current disease control measures to build long-term resilience to infectious diseases as climate change progresses.^{101,109} Interventions to increase access to improved

water and sanitation have been implemented worldwide to reduce diarrhea morbidity, but can also serve as climate adaptations.¹⁰⁰

While there is substantial literature examining the relationship between temperature and diarrhea, little attention has been given to how this relationship may be modified or affected by other environmental variables such as access to improved water and sanitation.^{36,110} More information on how water, sanitation, and hygiene interventions could be effective adaptation methods under future climate scenarios is needed.^{98,111} None of the models in the global systematic review of diarrhea and temperature explicitly considered access to water and sanitation.³⁶ The lack of consideration of non-climate variables in models related to climate and health makes informed decision-making from these models challenging.^{111,112} When examining associations between temperature and diarrhea, understanding the historic role of social and environmental factors is critical for making meaningful future projections.¹¹¹ Considering factors such as water and sanitation infrastructure in historic analyses of diarrhea and meteorological conditions is important to informing future projections of the diarrheal disease burden and prioritizing mitigation strategies; yet this remains an under-studied topic when considering the potential impacts of climate on diarrheal diseases.¹¹¹

Temperature and rotavirus

When all etiologies of diarrhea are considered together, there is a positive association between temperature and diarrhea. However, certain diarrheal pathogens, notably viral enteric pathogens, exhibit a different association with temperature.³⁶ Rotavirus is negatively associated with temperature.^{36,113} It may thus be hypothesized that rotavirus vaccination may reduce cooler-temperature diarrhea cases and strengthen the association between temperature and diarrhea. Indeed, other studies have demonstrated rotavirus vaccination shifting or diminishing the seasonality of rotavirus infections.^{114–116} As rotavirus vaccination becomes more widespread, the leading

etiologies of diarrheal disease may shift more toward bacterial diarrhea, which exhibit a stronger temperature-diarrhea relationship; thus the residual burden of diarrhea may be more prone to increase under climate change warming scenarios. It is therefore important to consider changes in the temperature-diarrhea relationship that may occur after rotavirus vaccination when considering the potential effects of climate change on diarrheal disease.

Precipitation and diarrhea

Precipitation, including both heavy rainfall and drought, also has the potential to affect diarrheal disease through many of the pathways displayed on the F-diagram, described elsewhere.¹⁰⁶ Heavy rainfall and flooding have been associated with increased diarrhea; data on the association between droughts and diarrhea is lacking, but there is some evidence that drought conditions may be associated with higher diarrhea rates.¹⁰⁶ Rainfall patterns tend to be highly localized and more spatially variable than temperature estimates; use of typical rainfall datasets can lead to large bias in analyses of waterborne disease.¹¹⁷ The association between rainfall and diarrhea is complex: although heavy rainfall can be associated with increased diarrhea, this relationship may depend on antecedent conditions.¹¹⁸

Peru

The research in this dissertation considers multiple factors associated with childhood diarrhea in Peru. Peru is located on the Pacific Ocean just south of the equator. It is characterized by three main geographic regions: the coast, which includes the capital city of Lima and is the most populous region of the country; the Andes Mountains, a high-elevation region that runs roughly north-to-south and divides the coast from the third region, the Amazon basin, comprised of high and low jungle climatic zones. The decade between 2005 and 2015 was characterized by strong economic development in Peru; Peru was one of the fastest-growing economies in Latin America during this

period.¹¹⁹ In this decade, there were appreciable improvements in the proportion of the population with access to improved drinking water and sanitation, driven primarily by increased access to piped drinking water and sewer connections.¹²⁰ While the JMP estimates that 91% of all Peruvians had an improved drinking water supply in 2015, only 73% of improved supplies were available when needed, and it was estimated that only 50% were free of contamination. Drinking water metrics were worse in rural areas, where an estimated 74% of the population had access to improved water source, of which 51% were available when needed and only an estimated 20% were free of contamination. Differences between urban and rural areas were more pronounced when considering improved sanitation: in 2015, 75% of households in urban areas had a sewer connection, compared to only 13% in rural areas.¹²⁰ There was a large national handwashing promotion campaign in Peru from 2008-2010, which increased reported handwashing before eating and preparing food, but did not translate into reductions in contamination of drinking water or child diarrhea.¹²¹

Childhood deaths from diarrhea in Peru decreased from 1,224 deaths in children under five years old in 2005 to 447 deaths in 2015;¹²² however, there is still a high burden of disease, explored further in Chapter 2. Prior to the introduction of the rotavirus vaccine, rotavirus was identified as the most common cause of severe diarrhea in Peruvian children (<5 years old), responsible for an estimated 384,000 cases, with 63% of Peruvian children experiencing at least one episode of rotavirus diarrhea by age five.¹²³ A model of introducing a rotavirus vaccine program in Peru estimated that more than 200,000 rotavirus cases and 90,000 outpatient visits for rotavirus could be prevented in the year 2015.¹²⁴ Peru added the rotavirus vaccine--specifically Rotarix® (GlaxoSmithKline Biologicals, Wavre, Belgium), a two-dose vaccine given to infants--to its national vaccine schedule in 2009. There was a significant decrease in rotavirus diarrhea in a peri-urban community in Lima in the two years after vaccine introduction;¹²⁵ a national analysis of the impact on rotavirus vaccination on childhood diarrhea has not previously been conducted.

One of the MAL-ED sites was located in a rural area of Loreto, Peru, a province in the Amazon region that has worse access to water/sanitation and generally worse child health indicators than the rest of the country.¹²⁶ In that cohort, the pathogens to which the highest proportion of community diarrheal cases in children under two years old were attributable were *Campylobacter*, norovirus, and astrovirus. Despite the rotavirus vaccine having been introduced before the start of MAL-ED, rotavirus still remained the fourth leading pathogen associated with diarrhea in children aged 12-24 months.³³ Results from MAL-ED suggest that in Loreto, Peru, the rotavirus vaccine was successful at preventing rotavirus diarrhea in children under one year old, but that protection was not sustained beyond infancy, *i.e.*, when children were 1-2 years old.¹²⁷ While not directly comparable to the study in peri-urban Lima, it is possible that heterogeneity in vaccine impact may exist across settings within Peru, although this has yet to be rigorously evaluated across multiple geographic areas.

El Niño in Peru

The El Niño Southern Oscillation (ENSO, or simply “El Niño”) is a global pattern of climate variability associated with unusual warming of the Pacific Ocean near the equator occurring approximately every 2-7 years.¹²⁸ El Niño events are associated with increased temperature and changes in precipitation patterns in Peru. During the 1997-1998 El Niño, temperatures rose up to 5 °C above normal in Lima, Peru.¹²⁹ El Niño events have been associated with extreme flooding in areas of northern coastal Peru that are usually arid; conversely, on the other side of the Andes Mountains, El Niño events are associated with lower rainfall and have triggered droughts in the Amazon basin.^{130,131} Similarly, drought conditions have been associated with El Niño in water-scare southern Peru, at the base of the Andean Mountains.¹³²

While prediction of global El Niño events has greatly improved, local weather phenomena associated with El Niño remain challenging to forecast.¹³³ The 2015-2016 El Niño in Peru was

heavily anticipated: Peru declared a state of emergency and spent >\$20 million on flood and drought prevention upon the forecast of a strong El Niño for 2015.^{128,134} The early forecasting of this event differed from the 2017 El Niño that developed rapidly and was associated with extreme flooding, death, and infrastructure damage in northern Peru.¹³³

Several studies have demonstrated a strong association between El Niño events and increased diarrhea incidence in Peru.^{27–31,129,135} Notably, all of these analyses consider El Niño events in the 1990s: the two El Niño events in that decade (1991-1992 and 1997-1998) coincided with cholera epidemics in Peru.²⁹ These analyses were limited to coastal Peru (Lima and Piura); no research linking El Niño events to diarrhea cases in regions other than coastal Peru or in eras that were not characterized by cholera epidemics was identified.

Climate change is expected to increase the frequency and intensity of El Niño periods;²⁶ in recent decades, El Niño events have been intensifying in the Eastern Pacific, near the coast of Peru.²⁵ The strong historical association between El Niño and diarrhea in Peru, combined with predictions of future intensifying of El Niño events, underscores the importance of analyzing the impact of El Niño on diarrhea in Peru. It is important to assess whether El Niño events are associated with increases of childhood diarrhea in non-coastal regions of Peru, and whether the association exists during years not characterized by cholera epidemics.

Previous temperature and diarrhea research in Peru

Two studies have explicitly examined the association between temperature measurements and diarrhea in Peru. Checkley *et al.* (2000) assessed the relationship between temperature and clinic admissions for diarrhea in children under five years old in Lima, Peru. This study consisted of a time series analysis before and during the 1997-1998 El Niño event and found an 8% increase in diarrhea for a 1 °C increase in temperature.¹³⁶ Lama *et al.* (2004) examined emergency department visits for

diarrhea in Lima between 1991 and 1998, a period beginning and ending with El Niño events and corresponding cholera epidemics. They found an 11% increase in emergency department visits for acute diarrhea in adults for a 1 °C increase in mean monthly temperature.²⁸ While several other studies have examined seasonality of diarrhea in Peru, to our knowledge, an association between air temperature measurements and diarrhea incidence has not been estimated outside of the 1990s, a decade characterized by two El Niño events with co-occurring cholera epidemics.

Dissertation chapters

In summary, there are many pathways through which enteric pathogens can be transmitted, and many possible interventions to reduce the burden of disease by interrupting transmission across these pathways. However, these interventions—including improving water, improving sanitation, and rotavirus vaccination—face many challenges in achieving optimal performance. Furthermore, future challenges may be presented in lowering diarrhea morbidity as climate change progresses. In Peru, access to improved water and sanitation has improved since 2005, and the rotavirus vaccine was introduced nationally in 2009; however, there has not been an evaluation of whether these improvements have resulted in a lower rate of childhood diarrhea. Furthermore, although provision of improved drinking water (especially piped water) has increased, data on water quality (including both microbiological and chemical quality) and the relationship between household drinking water quality and child enteropathogen infection are sparse in Peru. Finally, associations between meteorological conditions such as temperature and childhood diarrhea have not been estimated in the 21st century, although such information could contribute to climate vulnerability assessments for childhood diarrhea in Peru. These research gaps are addressed in the chapters of this dissertation, as summarized below.

In Chapter 2, I present a national analysis of the association between rotavirus immunization and childhood clinic visits for diarrhea in Peru utilizing data from 194 provinces. I estimate an overall effect of the introduction of the rotavirus vaccine, and also examine whether changes in the rate of childhood clinic visits for diarrhea from the pre- to post-vaccine era varied based on provincial-level access to piped drinking water, sewerage connections, and poverty levels. This is the first national analysis of the impact of the rotavirus vaccine in Peru, and addresses the lack of accounting for differential WASH access when analyzing associations between the rotavirus vaccine and diarrhea incidence. This is one of few national analyses of the rotavirus vaccination that accounts for different underlying conditions across the country.

In Chapter 3, I utilize time series regression to estimate the association between ambient temperature and weekly clinic visits for diarrhea in Peru, controlling for other climatic variables, including El Niño events and the wet/dry season. I assess whether the association between temperature and diarrhea cases changed after the introduction of rotavirus vaccine, and whether this association differed based on level of access to piped drinking water and sewer connections. To my knowledge, this is the first analysis of how incremental temperature changes are associated with diarrhea cases in Peru outside of two El Niño periods in the 1990s that were characterized by cholera epidemics. Estimating this association can inform future climate vulnerability research.¹¹¹ This analysis also addresses the lack of data on the association between El Niño events and diarrhea outside of major coastal cities in Peru.

In Chapter 4, I characterize microbiological, chemical, and heavy metal contamination of drinking water in 96 households with infants in Piura, Peru and examine how drinking water contamination is associated with infant diarrhea and enteropathogen infection. This study considers three aspects of drinking water quality and health that have been under-studied. Firstly, it characterizes both

microbiological and chemical/heavy metal contamination in drinking water in a low-income setting, which are usually studied in isolation. Secondly, this study examines the relationship between drinking water quality measurements and infant enteropathogen infections. Finally, the study compares prospective vs. cross-sectional analyses between microbiological drinking water contamination and child health using a study design that could plausibly establish causality between drinking water consumption and health outcomes. While others have found high prevalence of arsenic in groundwater and surface water in Peru,^{137,138} this is one of the first characterizations of arsenic and pesticides/herbicides in household drinking water samples.

I also include an Appendix Chapter in this dissertation of a published review titled “Pathogens transmitted in animal feces in low- and middle-income countries”. In this manuscript, I led a review compiling evidence on which pathogens may contribute to the burden of disease in LMICs through transmission in animal feces. This built off of another review of the impact of animal feces on child health that I was involved in¹³⁹ that critically examined how animal feces can contribute to the childhood disease burden through pathways that are often overlooked when designing interventions to interrupt enteric disease transmission.

Chapter 1 References

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Chapter 2 Impact of rotavirus vaccination varies with differential access to piped water and sewerage: an analysis of childhood clinic visits for diarrhea in Peru, 2005-2015

Miranda J. Delahoy¹, Cesar Carcamo², Luís Ordoñez³, Vanessa Vasquez², Benjamin Lopman¹,

Thomas Clasen¹, Gustavo F. Gonzales², Kyle Steenland¹, Karen Levy¹

Author Affiliations: (1) Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA; (2) Universidad Peruana Cayetano Heredia, Lima, Peru; (3) Ministerio de Salud (Ministry of Health), Lima, Peru

ABSTRACT

Background: Peru added rotavirus vaccination to its national immunization schedule during an era of poverty declines and increases in access to improved water supply and sanitation. A national evaluation of the impact of the rotavirus vaccine on childhood diarrhea has not previously been conducted; concurrently evaluating poverty and water/sanitation conditions is important to understanding vaccine performance.

Methods: A national dataset was compiled from Peruvian governmental data sources including weekly diarrhea surveillance records, annual administered doses of rotavirus vaccination, annual piped water and sewerage access estimates, and poverty rates. We fit negative binomial models investigating the impact of rotavirus vaccination, piped water access, sewerage access, and poverty on the rate of clinic visits for diarrhea in children (< 5 years) in 194 of 195 Peruvian provinces from 2005-2015, considering the interaction between these factors, and controlling for long-term and seasonal (El Niño) trends. We compared the “pre-(rotavirus) vaccine” (2005-2009) and “post-vaccine” (2010-

2015) eras. We considered poverty and access to piped water and sewerage in separate models, and considered them in quartiles calculated from the province-level estimates for 2005-2015.

Results: The 2005 childhood diarrhea rate was 29 annual clinic visits per 100 children and decreased by 3% per year. We found no significant impact of rotavirus vaccination in the lowest quartile of piped water access, *i.e.*, when <40% of households in a province had access to piped water, or in the lowest quartile of sewerage access (<17% of households had a toilet connected to the sewerage system). Controlling for long-term trend, compared to the pre-vaccine era, the diarrhea rate was lower in the post-vaccine era by 7% (95% CI: 2-12%), 13% (95% CI: 7-19%), and 15% (95% CI: 10-20%) in the 2nd, 3rd, and 4th quartiles of piped water access, respectively. In the post-vaccine era, the diarrhea rate was 9% lower in the 2nd and 3rd quartiles of sewerage access, and 13% (95% CI: 6-19%) lower in the highest quartile. Diarrhea rates were significantly higher (6%, 95% CI: 4-8%) during moderate and strong El Niño events.

Conclusions: There was a significantly lower rate of childhood diarrhea clinic visits in the post-rotavirus vaccine era; however, the benefits of vaccination were not fully realized among those provinces with the lowest levels of access to piped water and sewerage connections, perhaps due to poor vaccine coverage and/or poor water/sanitation conditions negatively impacting vaccine performance.

INTRODUCTION

Diarrhea is a leading cause of global childhood morbidity and mortality.¹⁻⁴ Diarrhea deaths in children under five years old have declined by approximately one third since 2005; however, in 2015 there were still half a million annual child diarrhea deaths.⁵ Rotavirus, a pathogen associated with severe gastroenteritis, remained the leading etiologic agent implicated in these deaths in 2015, despite the licensure and increased use of rotavirus vaccines since 2006.^{5,6}

Two oral rotavirus vaccines commonly in use today [Rotarix® (GlaxoSmithKline Biologicals, Wavre, Belgium) and RotaTeq® (Merck & Co., Kenilworth, New Jersey, USA)] were licensed in 2006, which was followed by a global recommendation of use in 2009.^{5,7,8} Rotarix® is used in dozens of countries on six continents; RotaTeq® has also been introduced in several countries primarily in North America, Australia, Europe, and Northern Africa.⁹ Rotavirus vaccination has provided >80% protection against severe rotavirus illness in several middle- and high-income countries; however, both reduced efficacy and effectiveness of the vaccine have been observed in low- and middle-income countries compared to high-income countries.¹⁰ Hypotheses for attenuated effectiveness include that (1) underlying health conditions (including environmental enteropathy, helminth infection, and/or poor nutritional status) may reduce oral vaccine immune response, or (2) that natural and vaccine-derived immunity may be insufficient to combat the diversity of strains and the very high incidence of rotavirus in endemic lower-income settings.^{11–15} Improvements to water and sanitation may diminish these barriers to oral rotavirus vaccine efficacy;^{16,17} indeed, recent evidence suggests that improved water, sanitation, and hygiene conditions can improve seroconversion of oral rotavirus vaccination.¹⁸

In Latin America, rotavirus was responsible for approximately one third of inpatient and outpatient diarrhea visits prior to the introduction of the rotavirus vaccine.¹⁹ Prior to vaccine introduction, rotavirus was identified as the most common cause of severe diarrhea in Peruvian children (<5 years old), responsible for an estimated 384,000 cases, resulting in 64,000 outpatient visits with 63% of Peruvian children experiencing at least one episode of rotavirus diarrhea by age five.²⁰ A model of introducing a rotavirus vaccine program in Peru estimated that more than 200,000 rotavirus cases and 90,000 outpatient visits for rotavirus could be prevented in the year 2015.²¹

Peru added the oral rotavirus vaccine Rotarix® to its national vaccine schedule in 2009.²² Rotarix® is intended to be administered to infants in two doses at two and four months of age; immunization is not recommended before age six weeks and doses should be at least four weeks apart.^{23,24} Sub-par adherence to rotavirus vaccine dosage timing was reported for Peru using data from 2012.²⁴ The vaccine introduction occurred during a decade characterized by rapid economic development and corresponding poverty declines, during which Peruvians experienced increases in access to improved water and sanitation, although these changes were not realized equally across geographic regions.^{25,26} There was a significant decrease in rotavirus diarrhea in a peri-urban community in Lima in the two years after vaccine introduction.²⁷ At a study site in Loreto, Peru with demonstrated high enteropathogen exposure, the rotavirus vaccine was successful at preventing rotavirus diarrhea in children under one year old, but that protection was not sustained beyond infancy, *i.e.*, when children were 1-2 years old.²³ While the study outcomes in Lima and Loreto differed, it is possible that heterogeneity in vaccine impact may exist across settings within Peru, although this has yet to be rigorously evaluated across multiple geographic areas.

While health and infrastructure improvements would be expected to contribute to lower diarrhea rates, Peru faces the added challenge of being vulnerable to increased temperatures and altered precipitation patterns brought on by El Niño events, which have been associated with increased diarrhea cases in Peru.^{28–30}

In this study, we take advantage of a highly spatially and temporally resolved dataset of clinic visits for diarrhea in Peru from 2005–2015, as well as extensive data on water access, sewerage, poverty, and population. We examine long-term trends to understand the impacts of the introduction of routine rotavirus vaccination in Peru, and to explore how sociodemographic factors modify this impact.

METHODS

Background and geographic scope

Data on clinic visits for diarrhea and rotavirus vaccination were collected by the Peruvian Ministry of Health (MINSA: Ministerio de Salud) and data on poverty and access to piped water and sewerage were collected by the National Institute of Statistics and Informatics (INEI: Instituto Nacional de Estadística e Informática). Peru is comprised of 25 departments, which encompass 195 provinces. Provinces are sub-divided into districts, with each district belonging to a single province, allowing for aggregation of district data to the provincial level. Analyses were conducted across the 195 provinces of Peru for the years 2005-2015. Province populations range from <4,000 residents (Purus province, Ucayali department) to >7 million residents (Lima province); areas range from ~150 km² (province of Callao) to >67,000 km² (province of Loreto).³¹ A 196th province was created in 2014 (Putumayo, Loreto department); data from the districts forming this province were included as part of their original province (Maynas) in analyses.

Data sources and definitions

Diarrhea cases

MINSA collects obligatory weekly surveillance data on diarrhea visits from all public inpatient and outpatient clinics in Peru. Private clinics also send regular weekly reports if they opt into the reporting system. Diarrhea cases refer to patients presenting to a clinic with an increase in frequency of bowel movements (three or more bowel movements in 24 hours), or in fluidity or volume of stool compared to usual, with onset within the past two weeks. Cases are aggregated by age group (<1 year old, 1-4 years old, ≥5 years old), and assigned to the patient's district of residence. We use the term “childhood diarrhea rate” to describe the rate of clinic visits for diarrhea in children under five years old.

Rotavirus vaccination

MINSA provided counts of the first and second doses of rotavirus vaccine given to infants in each district of Peru from 2008-2015, as well as census-derived estimates of the annual district-level infant population. Coverage increased quickly after the national vaccine introduction in 2009, with most infants receiving both doses of vaccine starting in 2010. Based on vaccine administration data in Peru, we classified 2005-2009 as the “pre-(rotavirus) vaccine era” and 2010-2015 as the “post-vaccine era”, or simply the “rotavirus vaccine era”. We considered vaccination in eras rather than utilizing provincial-level estimates of the percentage of infants vaccinated, due to difficulties in obtaining stable coverage estimates at this geographic level.

National census: water, sanitation, and population

INEI conducted national decadal censuses in 2007 and 2017. Participation is obligatory, with residents legally required to be at home during enumeration hours on the national census day. An interim census, the household and population register (Empadronamiento Distrital de Población y Vivienda, or SISFOH), was conducted from February 2012-September 2013. SISFOH participation was not obligatory, and in contrast to the 2007 and 2017 censuses, enumerated respondents were limited to those residing in their households for at least 6 months; temporary residents were excluded. SISFOH enumerated approximately 24 million residents (compared to ~27 million in the 2007 census).³²

Population by age was enumerated for each district during the 2007 and 2017 censuses. The population of children under five years old for non-census years was imputed assuming a linear change in population in this age group between censuses. These estimates were used for the child population when determining the diarrhea rate.

SISFOH and the national census collect data on the main source of drinking water used in each household, and on household sanitation. Households were categorized as having access to piped water if they identified the primary source of water as tap water, either piped into the interior of the house, or piped to the outside of the house but within the building area. This includes piped water in the yard area of a building, or an interior passageway, alley, or parking area, but does not include public taps located on the street or in another public space. Households were considered to have access to sewerage if they had a toilet with a connection to a treated sewage network; the connection could be either within the house or within the building area. Households with toilets emptying to septic tanks, households with pit latrines, households emptying human waste to a river or ditch, and households without any latrine/toilet were not considered to have access to the sewerage network.

The percentages of households that had access to piped water and access to sewerage were calculated for each province in the years that census data were available. Data from SISFOH were used as annual estimates for 2012, although enumeration continued into 2013. Data for interim years were imputed for each province from available data, assuming a linear change in percentage access between data points. The continuous percentage of province households with access to piped water was split into four quartiles, based on the annual estimates for 2005-2015. Using this categorization allowed us to maintain adequate data in each group in the pre- and post-vaccine eras, and, in contrast to a continuous variable, does not impose a linear relationship between coverage and the childhood diarrhea rate. Access to sewerage was likewise categorized into quartiles.

Poverty

INEI calculated the percentage of each district falling below the poverty line for the years 2007, 2009, and 2013 based on data from the national census, the national household survey ENAHO (Encuesta Nacional de Hogares/National Survey of Households), and other annual surveys.

Residents of households were considered below the poverty line if monthly per capita expenditure was below the amount needed to acquire goods and services adequate to satisfy basic needs.^{32–34} For years with available data, provincial level estimates of the percentage of households in poverty were created using population-weighted district-level data. Data for interim years were imputed, assuming a linear change in the poverty rate between reporting years. The continuous percentage of province households that were in poverty was split into four quartiles, based on the annual estimates for 2005–2015.

El Niño

The U.S. National Oceanic and Atmospheric Administration reports data on the Oceanic Niño Index (ONI), calculated using a standard three-month mean of sea surface temperature anomalies in the Niño 3.4 region of the Pacific Ocean.³⁵ El Niño periods were defined using the ONI, with values in the ranges 0.5–0.9, 1.0–1.4, and ≥ 1.5 corresponding to weak, moderate, and strong El Niño events, respectively.³⁶ We compared three-month periods with a weak El Niño or no El Niño to the three-month periods with moderate/strong El Niño events: October–December 2009 (moderate), January–March 2010 (moderate), April–June 2015 (moderate), July–September 2015 (strong), and October–December 2015 (strong).

Statistical Analysis

Data cleaning and analyses were performed using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) and SAS 9.4 (SAS Institute, Inc., Cary, NC). We analyzed the provincial-level quarterly rate of childhood clinic visits for diarrhea using negative binomial generalized estimating equations (GEEs), after finding over-dispersion of the outcome in a Poisson model. We considered the number of clinic visits for childhood diarrhea by three-month intervals (January–March, April–June, July–September, and October–December) and modeled these provincial-level case counts

against the risk factors and control variables of interest. We chose three-month intervals to assess longer-term trends, to align our data with El Niño seasons, and to limit the influence of potential autocorrelation between weekly cases. We ran three models to separately consider quartiles of access to piped water, access to sewerage, and poverty, as these risk factors were highly correlated with one another. Each model included: (1) a binary term for the rotavirus vaccine era, (2) one of the risk factors of interest (categorical quartile of access to piped water, access to sewerage, or poverty), (3) the interaction between the first two variables, to assess whether the risk factors modified the rotavirus vaccine/diarrhea relationship, (4) a continuous variable for year, to control for secular trend, and (5) an indicator for whether there was a moderate or strong El Niño event in the three-month period. We accounted for clustering at the province level with an exchangeable correlation structure. The models include a population offset for the natural log of person-weeks (some three-month periods had 13 epidemiologic weeks, whereas some had 14), based on the provincial child (<5 years) population.

A small subset of districts did not participate in diarrhea surveillance for the entire study period. If a district did not start contributing diarrhea surveillance data until after January 2005, or if the district stopped sending reports prior to the conclusion of the study period (December 2015), the district's child population was not counted toward the provincial population during the weeks the district was not reporting. In other words, we did not consider children from the non-reporting districts to be at risk in the weeks that they were not reporting data.

Sensitivity analysis

Models were also run using weekly diarrhea cases, in order to assess whether the choice of analyzing data aggregated in three-month periods was influential. An autoregressive correlation structure was used for these weekly models. For these sensitivity analysis models, a slightly different definition of

El Niño periods was used, namely the same definition as used in Chapter 3. Specifically, for each month in our study, we assigned the corresponding ONI value of the three-month running average in which the study month was the midpoint. For example, the ONI for February 2005 was assigned the ONI running average for January-March 2005. Thus, in sensitivity analyses, the El Niño variable was resolved to the month, rather than three-month season.

Ethical review

Emory University's Institutional Review Board reviewed the study protocol and determined that the use of aggregated unidentified data in these analyses does not constitute human subjects research.

RESULTS

The analysis ultimately included data from 194 of the 195 Peruvian provinces and had 8,536 observations (data from 194 provinces in 11 years, with four seasons per year). Ocos province in Ancash was not included, due to inconsistent diarrhea surveillance reporting, with little data reported in the pre-vaccine era. Data were analyzed from 1,838 districts in the remaining 194 provinces, with less than 1% of districts (18 districts) having any missing diarrhea reports. Most missing reports were from the years 2005 and 2006.

Rate of childhood clinic visits for diarrhea

Nationally, there were 28.9 annual clinic visits for diarrhea per 100 children < 5 years old in 2005, and the rate generally decreased throughout the study period (Figure 2.1). In 2009, the year during which the rotavirus vaccine was added to the national immunization schedule, there was an annual rate of 25.6 clinic visits for diarrhea per 100 children. The lowest annual rate was in 2014 (18.6 diarrhea clinic visits per 100 children); in the last year of the study (2015), there was an annual rate of 20.2 diarrhea clinic visits per 100 children. There was substantial variability in the rate of clinic visits for childhood diarrhea between provinces (Figure 2.1 and Figure 2.2).

Rotavirus vaccine coverage

Rotavirus vaccination began in approximately 40% of provinces in 2008. By 2009, the year the vaccine was added to the national immunization program, all but three provinces were administering rotavirus vaccines. As of 2010, the majority of Peruvian infants (estimated 74.8%) were receiving both doses of rotavirus vaccine (Figure 2.3). Coverage increased until 2012, then remained relatively stable through 2015.

Piped water and sanitation access

Within provinces, access to piped water and piped sewerage generally increased over time. There was large variability in access between provinces. In 2005, the percentage of households in each province with access to a piped water connection ranged from 0% to 94.8% (median 42.8%); by 2015, the median percentage of households in a province with piped water access was 69.7% (Appendix Table 2.1). Access to a toilet connected to the sewerage system varied from 0-86.0% of households across provinces in 2005, with a median of 20.0% of households in a province having sewerage access in 2005 and 40.2% in 2015.

Poverty rates

The percentage of households in each province living below the poverty line was high at the beginning of the study period (2005), when the median provincial poverty rate was 66.2% (Appendix Table 2.1). Poverty generally declined throughout the study period; the median provincial poverty rate was 48.6% in 2015. Poverty varied substantially between provinces: the range of poverty rates in 2005 was 9%-100%.

Model results

Secular trends

Full model results are presented in Tables 2.1-2.2 and Appendix Table 2.2. Across models, the continuous variable for year was consistently a strong predictor of diarrhea rates. Controlling for the other factors in each model, there was a reduction of 3% in the childhood diarrhea rate each year (Tables 2.1-2.2 & Appendix Table 2.2).

Effect of rotavirus vaccination on the rate of childhood clinic visits for diarrhea

Controlling for secular trends and El Niño events, the rate of childhood clinic visits for diarrhea was 6.7% lower in the post-vaccine era compared to the pre-vaccine era (incidence rate ratio (IRR): 0.93, 95% confidence interval (CI): 0.90-0.97); in the models considering piped water or sewerage access, the association varied by quartile of access. There was no significant difference in the childhood diarrhea rate between the pre- and post-vaccine eras in the first quartile of piped water access (*i.e.*, for provinces in which less than 40% of households had piped water access). Compared to the pre-vaccine era, there were lower diarrhea rates in the post-vaccine era from the second to fourth quartiles of access to piped water (2nd quartile IRR: 0.93 (95% CI: 0.88-0.98), 3rd quartile IRR: 0.87 (95% CI: 0.81-0.93), 4th quartile IRR 0.85 (95% CI: 0.80-0.90); Table 2.1 & Figure 2.4). A similar trend was observed in the model considering access to piped sewerage, in which there was no significant difference in the childhood diarrhea rate in the pre- and post-vaccine eras in the lowest quartile of access, but there was a significantly lower childhood diarrhea rate in the post-vaccine era in the second, third, and fourth quartiles (2nd quartile IRR: 0.91 (95% CI: 0.86-0.95), 3rd quartile IRR: 0.91 (95% CI: 0.84-0.98), 4th quartile IRR: 0.87 (95% CI: 0.81-0.94); Table 2.2 & Figure 2.4).

Piped water, sewerage access, and poverty as risk factors for childhood diarrhea

Higher access to piped water was associated with a lower rate of clinic visits for childhood diarrhea in the rotavirus vaccine era only. In that era, the incidence rate of childhood diarrhea cases ranged from 9-18% lower in the 2nd-4th quartiles of access to piped water, compared to the quartile of lowest access to piped water. There was no statistically significant association between the diarrhea rate and access to the sewerage (Table 2.2) or poverty (Appendix Table 2.2), in either the pre- or post-vaccine eras, although higher access to piped sewerage tended to be associated with a lower diarrhea rate in the rotavirus vaccine era (Table 2.2).

El Niño seasons

The effect of El Niño events was consistent across the three models. Childhood diarrhea rates were 6% higher (IRR: 1.06, 95% CI: 1.04-1.09) during three-month periods characterized by a moderate or strong El Niño event (compared with seasons with a weak El Niño, or no El Niño; Tables 2.1-2.2 & Appendix Table 2.2).

Sensitivity analysis

Results of the interaction models for piped water and sewerage from the sensitivity analysis are displayed alongside original results in Appendix Table 2.3 and Appendix Table 2.4. The overall interpretation of the results was similar: namely, the rate of clinic visits for childhood diarrhea was lower in the post-vaccine era compared to the pre-vaccine era only in higher quartiles of access to piped water and sewerage. A notable difference between the results from the two methods, however, was that in the analysis conducted in three-month aggregated seasons, the rate of clinic visits for childhood diarrhea was significantly lower in the post-vaccine era in the second, third, and fourth quartiles of piped water coverage, whereas in the model run on weekly data this was only true

in the third and fourth quartiles. Considering results from both methods, the secular trend and association between childhood clinic visits for diarrhea and El Niño events were similar.

DISCUSSION

In this study, we utilized a spatially and temporally detailed dataset to examine if and how access to piped water, sewerage, and poverty modify the impact of rotavirus vaccination on childhood clinic visits for diarrhea in Peru, and to examine the impact of El Niño events on trends in childhood diarrhea rates. The rotavirus vaccine era was associated with a significantly lower rate of childhood clinic visits for diarrhea, controlling for long-term trend (IRR: 0.93, 95% CI: 0.90-0.97); however, the benefits of vaccination were not fully realized among those provinces with the lowest levels of access to piped water and sewerage connections, perhaps due to poor vaccine coverage and/or performance. There was a significantly higher rate of clinic visits for diarrhea during moderate/strong El Niño events.

Secular trend and impact of the rotavirus vaccine era

There was a strong secular trend in the diarrhea rate, even controlling for other factors of interest. This trend may encompass a composite of health and demographic changes that have occurred in Peru in the last decade beyond the ones we have measured, such as parental education, malnutrition, and hygiene education. There was a large national handwashing promotion campaign in Peru from 2008-2010, which increased reported handwashing before eating and preparing food, although evidence suggests it did not translate into reductions in child diarrhea.³⁷

In studies conducted in other middle-income countries in Latin America, rotavirus vaccination reduced annual acute gastroenteritis cases by 17-55% in children under two years old.¹⁰ While we saw lower overall reductions in childhood diarrhea (6.7% lower in the post-vaccine era compared to the pre-vaccine era), we analyzed a broader age group of children under five years old, as our data

were aggregated by age group in a way that did not allow us to consider 0-2 year olds separately. The rotavirus vaccine is given to infants and national vaccination did not begin until 2009, thus vaccination was unlikely to reach high levels among the entire group of children under five years old until the last years of our study, although older children may have benefited from indirect effects of vaccination. Rotavirus infections are more common in children aged 0-2 years (compared to 3-4 years),²² thus we may expect a higher proportion of diarrhea cases to be averted in younger age groups.

Associations between water and sanitation access and rotavirus vaccine impact

There are several potential explanations for the findings of greater reductions in the rate of clinic visits for childhood diarrhea from the pre- to post-vaccine era in provinces with higher access to piped water and sewerage. First, children without access to piped water may have worse drinking water quality and reduced water quantity for hygiene. Children living in these conditions, and without access to sewerage, could be predisposed to environmental enteric dysfunction, which may diminish oral vaccine impact.^{12,38} Second, the leading etiologies of diarrhea cases may differ between areas with varying piped water and sewerage coverage. Compared to bacterial diarrheagenic pathogens, person-to-person transmission may be a more important transmission route for rotavirus, with food and water having a lesser role.^{39,40} There could be more bacterial diarrheal infections in areas with low piped drinking water and sewerage access, where people rely on surface water and other potentially unsafe drinking water sources; thus reducing viral diarrhea may not have had an appreciable impact on the rate of clinic visits for childhood diarrhea. Third, areas with higher access to piped water and sewerage could also have higher vaccine coverage. Despite unstable provincial-level estimates of the percentage of infants that received rotavirus vaccines, we assessed whether province-level access to piped water or sewerage was associated with the percentage of

infants receiving a second dose of rotavirus vaccine, and found that provincial-level piped water access and rotavirus vaccine coverage were only weakly correlated (Appendix Figure 2.1) and that provincial-level sewerage access and rotavirus vaccine coverage were moderately positively correlated (Appendix Figure 2.2).

Association between water and sanitation access and childhood clinic visits for diarrhea

In other research, access to improved drinking water, especially piped water, as well as access to improved sanitation are generally associated with lower diarrhea rates.⁴¹ Curiously, we did not see a strong association between piped water access and sewerage access and the childhood diarrhea rate in the pre-vaccine era. It may be the case that our interpolated province-level estimates did not adequately capture effects of piped water and sewerage beyond the secular childhood diarrhea trend, or the improvements may not have reduced diarrhea cases. Piped water is not necessarily microbiologically safe; furthermore, service may be intermittent, potentially leading to unsafe storage methods and/or supplementation with unimproved water sources.⁴² Likewise, access to piped sewerage may be insufficient to limit children's exposure to human/animal feces. In the post-vaccine era higher access to piped drinking water was associated with a significantly lower diarrhea rate, and the childhood diarrhea rate tended to be lower in areas with higher access to sewerage. Again, this difference in the impact of piped water and sewerage between the pre- and post-vaccine eras may be explained by shifting dominant diarrhea etiologies. Water and sewer interventions may have been insufficient to interrupt transmission of diarrheal disease pathogens in the pre-vaccine era, but may have had a greater impact on the residual diarrhea burden after the introduction of the rotavirus vaccine.

El Niño events and childhood clinic visits for diarrhea

We found that childhood diarrhea rates were significantly elevated during seasons with a moderate or strong El Niño event. This result is in agreement with other assessments of El Niño and diarrhea in Peru,^{29,30,43–47} although the effect of El Niño on diarrhea has not been shown before for the entire country of Peru.

Limitations

We were unable to evaluate the percentage of infants fully vaccinated for rotavirus (*i.e.*, who received both doses of rotavirus vaccine) at the provincial level. The number of second dose vaccines given to infants exceeded the total estimated infant population in many provinces (Figure 2.3). This could result from an underestimated infant population, or an overcount in the number of second dose vaccines given. Accurately capturing the annual number of infants residing in each province is challenging with a decadal census, especially in a country with a rapidly-changing population pyramid and high rates of migration (both internationally, and internally from rural to urban areas).⁴⁸

We did not have access to data on the total number of all-cause clinic visits throughout the study period, which likely varied across provinces, as well as over time. It is possible that fewer cases of childhood diarrhea, even if equally severe, result in clinic visits in high poverty and/or remote areas. High levels of clinic avoidance have been observed in the Peruvian Amazon, where respondents cite distance and wait times as barriers to visiting a clinic.⁴⁹ Children presenting to clinics with diarrhea are likely those with the most severe cases, so our analysis may better apply to more severe cases of childhood diarrhea. We did not have data on the etiology of the diarrhea cases and were unable to estimate the impact of the rotavirus vaccine on diarrhea caused specifically by rotavirus.

Provincial-level estimates of access to piped water, sewerage access, and poverty were estimated for the 11-year study period using a linear interpolation of the available data, which were collected in

three years (2007, 2012, and 2017 for water and sewerage; 2007, 2009, and 2013 for poverty). Abrupt coverage changes at the province level may not be captured by a linear interpolation, although we did not observe gross violations of linearity among the data used for the interpolations, and the linear changes were consistent with annual Demographic and Health Survey (DHS) data for the 25 departments of Peru. The high correlation between piped water access, sewerage access, and poverty led us to examine these factors in isolation and therefore limited our ability to differentiate between their possible effects.

Conclusions

This analysis utilized an ecologic study design, which is well-suited to study large-scale impacts of population-level interventions.⁵⁰ It is one of few studies to consider other risk factors for diarrheal disease in a national evaluation of the rotavirus vaccine, and the results underscore the importance of considering modifying factors in such national analyses. Water and sanitation conditions may be operating synergistically with rotavirus vaccination to reduce childhood clinic visits for diarrhea in Peru. Our results suggest that implementation of rotavirus vaccination with lower provision of piped water access (<40%) or sewerage access (<17%) may reduce the health impact of vaccination efforts, although vaccine coverage may have been worse in these areas. Additionally, improving national levels of access to piped water and sewerage may be more important to address the residual burden of diarrheal diseases in the rotavirus vaccination era.

FUNDING/DISCLAIMER

Research reported in this publication was supported by the NIH Fogarty International Center, National Institutes of Environmental Health Sciences, National Cancer Institute, Centers for Disease Control and the NIH under Award Number [U01 TW0101 07]. Karen Levy was supported by the National Institute for Allergy and Infectious Diseases [grant number 1K01AI103544]. The

content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ACKNOWLEDGEMENTS

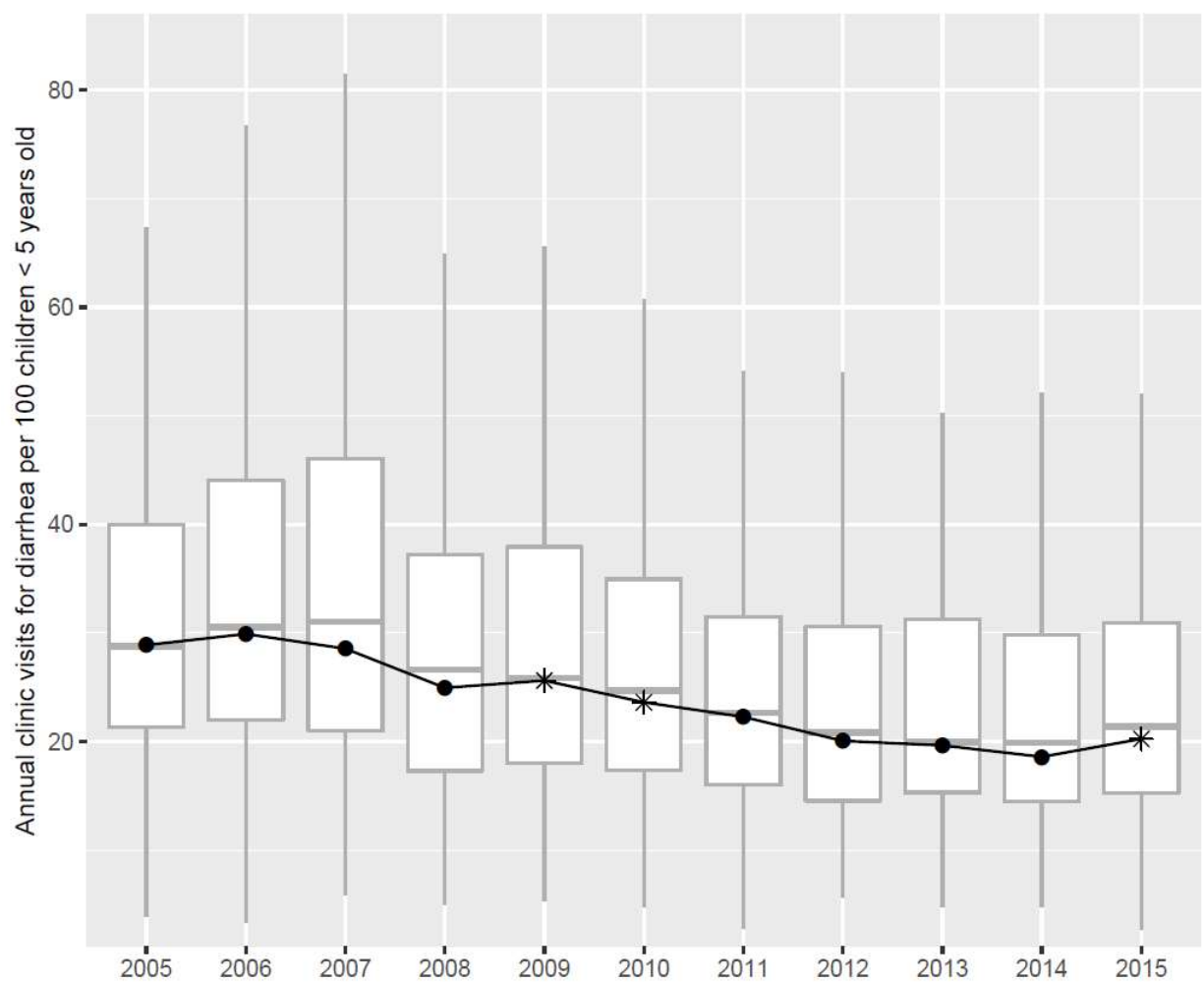
This study utilized data collected by the Peruvian Ministry of Health and the Peruvian National Institute of Statistics and informatics (INEI). We acknowledge their contributions and thank their staff, as well as the national census enumerators, for their data collection efforts. We thank Gaspar Moran, Nancy Hidalgo, and Hector Benavides Rullier of INEI for their guidance in data collection and interpretation. We thank Angela Rozo at Emory University, and Diego Fano and Vilma Tapia from Universidad Peruana Cayetano Heredia for assistance in project management and translation, and Mitchel Klein and Howard Chang (Emory University) for epidemiologic and statistical guidance.

AUTHOR CONTRIBUTIONS

Conceptualization: MJD, KS, KL; **Data collection:** LO; **Data curation:** MJD, LO, VV; **Data analysis:** MJD, KS; **Methodology:** MJD, BL, KS, KL; **Project administration:** MJD, CC, LO, VV, GFG, KS, KL; **Writing (original draft preparation):** MJD; **Writing (review and editing):** MJD, CC, LO, VV, BL, TC, GFG, KS, KL

TABLES AND FIGURES

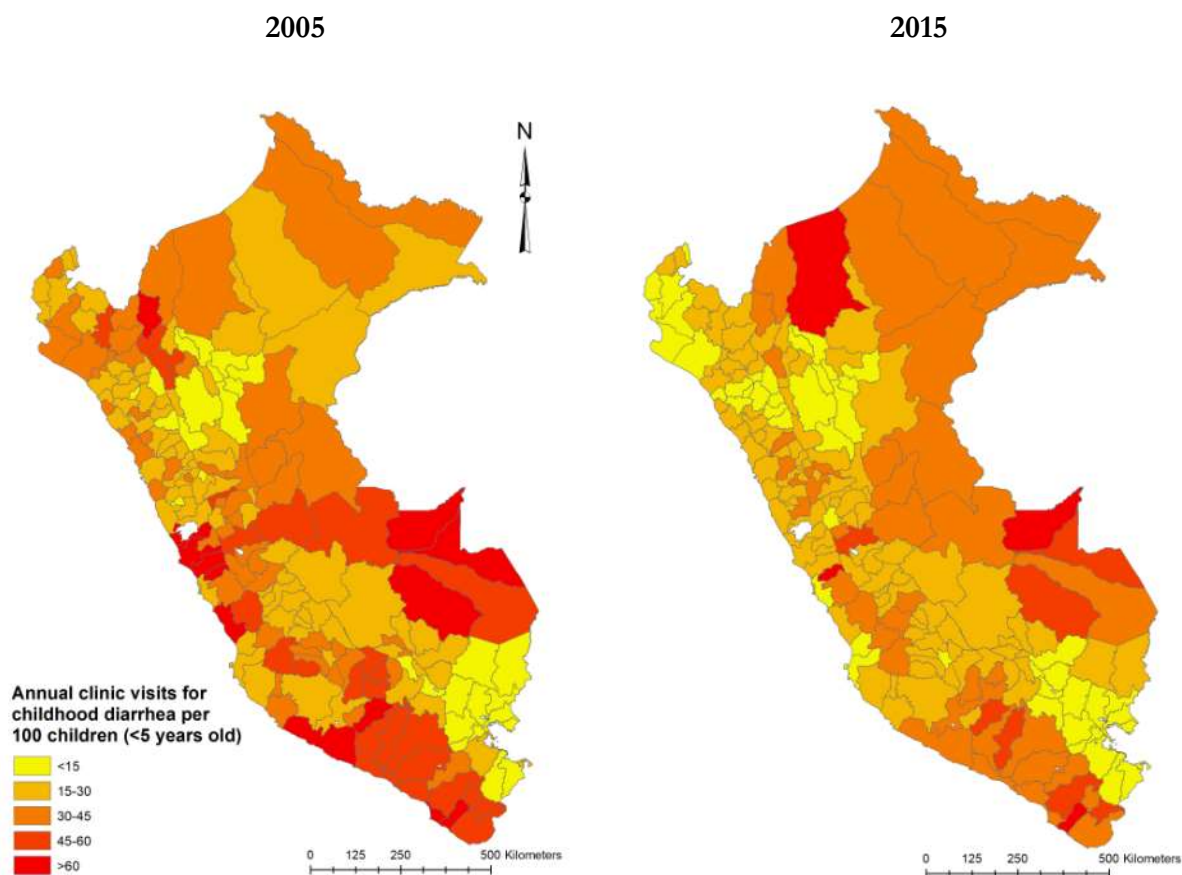
Figure 2.1 Child (<5 years) clinic visits for diarrhea, province-level and national estimates, Peru, 2005-2015



National rate of child (<5 years) clinic visits for diarrhea * El Niño year ● non-El Niño year

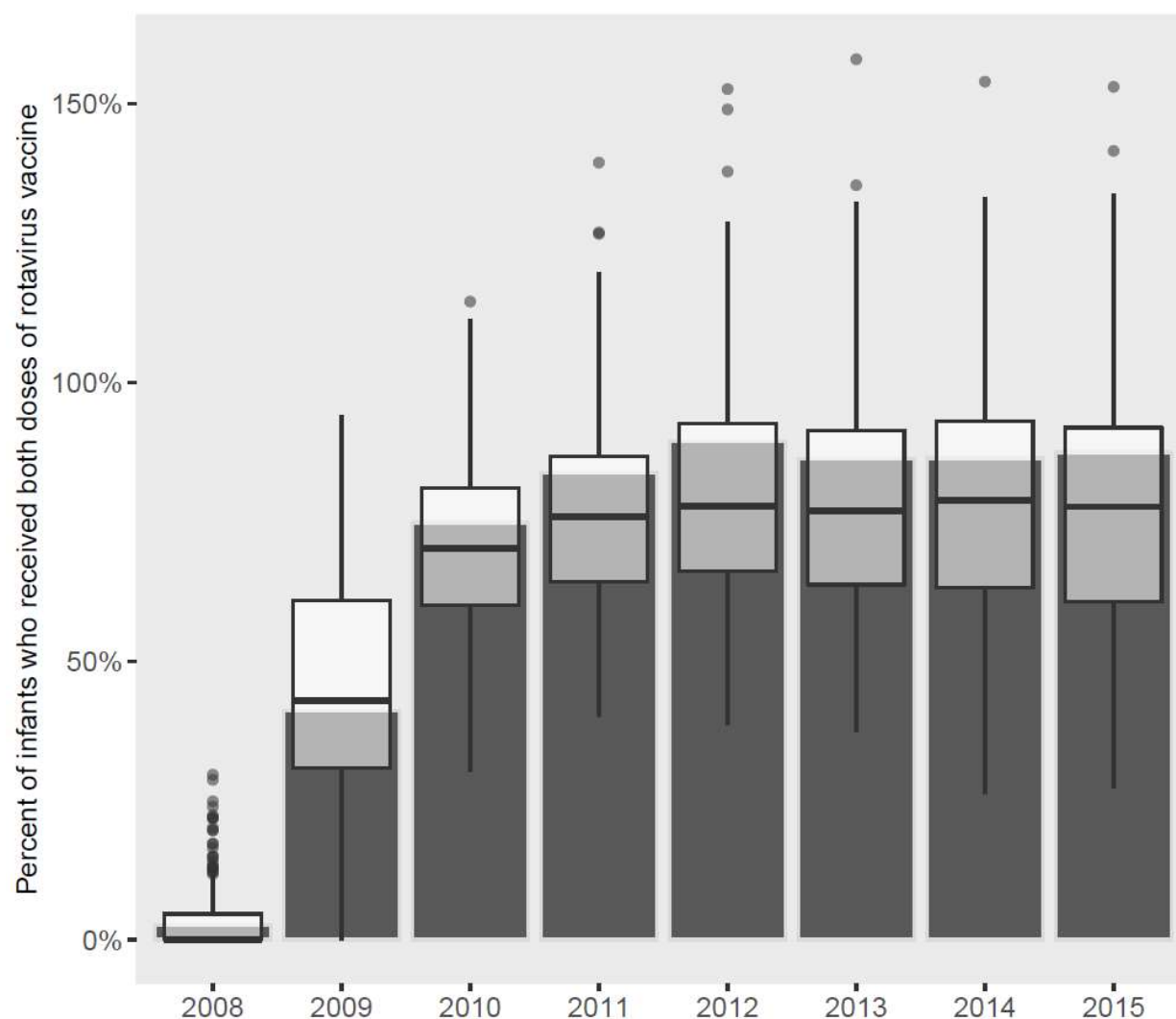
El Niño years: years with moderate/strong El Niño events. Boxplots display provincial statistics.

Figure 2.2 Annual rate of clinic visits for diarrhea in children < 5 years old, provinces of Peru



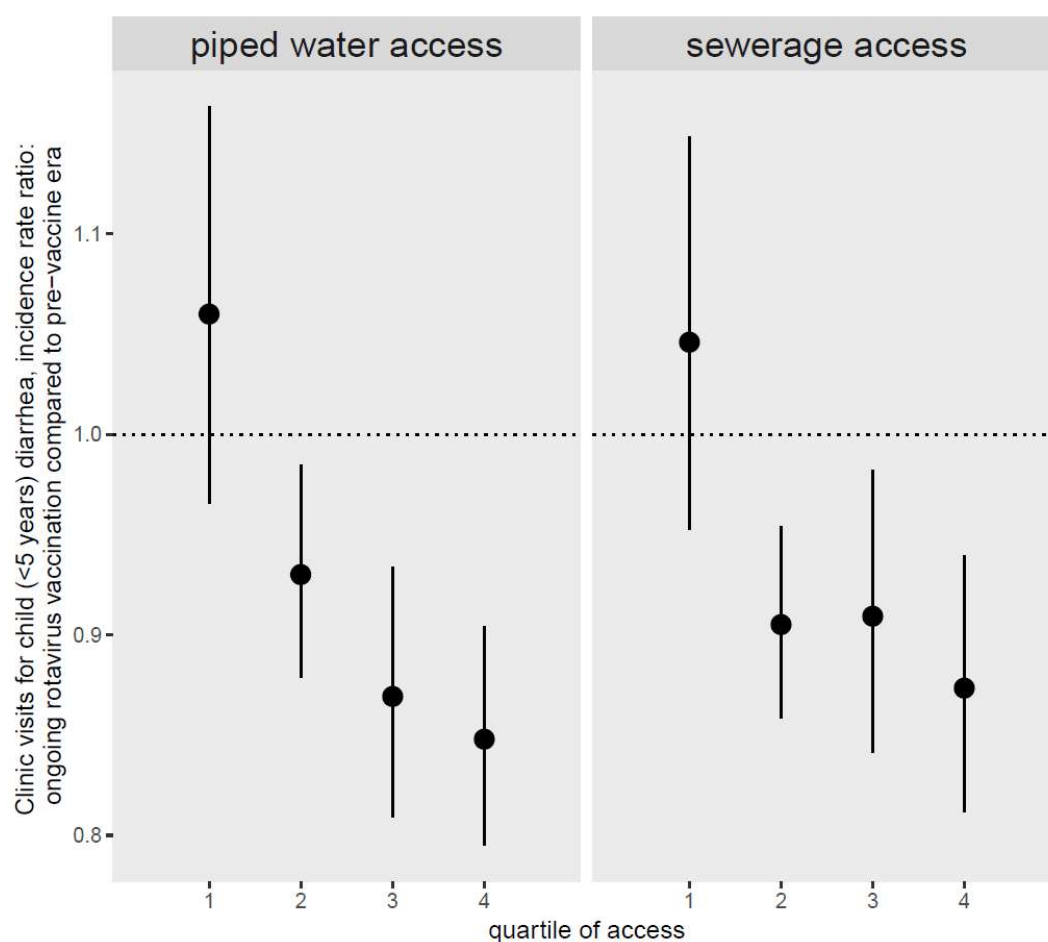
Provincial-level rates of clinic visits for childhood diarrhea displayed for first and last years of the study. There was a strong El Niño event in the second half of 2015. Moderate/strong El Niño events were associated with a higher rate of clinic visits for childhood diarrhea. In the study period (2005-2015), the annual rate of clinic visits for childhood diarrhea was lowest in the year 2014.

Figure 2.3 Percentage of infants who received both doses of rotavirus vaccine, Peru, 2008-2015



Shaded bars reflect national estimates; boxplots (with outlier points) are province-level estimates. The percentage of infants vaccinated is based on the total number of second doses of rotavirus vaccine administered, divided by the estimated infant (<12 months) population. Estimates exceed 100% if the count of second vaccine doses exceeded the estimated infant population, which may have resulted from an overcount of vaccine doses administered, or an underestimated infant population. Infant population estimates are Ministry of Health-derived estimates based on the 2007 Peruvian national census and other administrative data.

Figure 2.4 Incidence rate ratios for childhood clinic visits for diarrhea in the rotavirus vaccine era (2010-2015) compared to the pre-rotavirus vaccine era (2005-2009), by level of access to piped water and sewerage



Piped water access is defined by the percentage of households in a province that use piped water as their drinking water source. Sewerage access is defined by the percentage of households in a province for which the method of excreta disposal is a toilet connected to a piped sewerage system. Quartiles are based on the data collected and defined as follows. Piped water access: quartile 1: 0-40%, quartile 2: 40-58%, quartile 3: 58-72%, quartile 4: 72-95%. Sewerage access: quartile 1: 0-17%, quartile 2: 17-29%, quartile 3: 29-47%, quartile 4: 47-92%.

Table 2.1 Effect of rotavirus vaccination on child (<5 years) clinic visits for diarrhea, by level of piped water access, provinces of Peru (N=194), 2005-2015

Main effect model	Diarrhea IRR (95% CI)
ongoing rotavirus vaccination (2010-2015; ref: 2005-2009)	0.93 (0.90, 0.98)
piped water access^a quartile 2 (ref: quartile 1) piped water access quartile 3 (ref: quartile 1) piped water access quartile 4 (ref: quartile 1)	1.02 (0.95, 1.09) 0.95 (0.86, 1.05) 0.90 (0.79, 1.03)
moderate/strong El Niño period^b year	1.06 (1.04, 1.08) 0.97 (0.96, 0.98)
model QIC	-81380596.48
Interaction model*	Diarrhea IRR (95% CI)
Effect of rotavirus vaccination, by piped water quartile ^c	
at 1 st quartile of piped water access	1.06 (0.97, 1.16)
at 2 nd quartile of piped water access	0.93 (0.88, 0.98)
at 3 rd quartile of piped water access	0.87 (0.81, 0.93)
at 4 th quartile of piped water access	0.85 (0.80, 0.90)
Effect of piped water (pre-vaccine era, 2005-2009):	
piped water quartile 2 (ref: quartile 1)	1.04 (0.96, 1.11)
piped water quartile 3 (ref: quartile 1)	1.02 (0.92, 1.13)
piped water quartile 4 (ref: quartile 1)	1.03 (0.91, 1.15)
Effect of piped water (vaccine era, 2010-2015):	
piped water quartile 2 (ref: quartile 1)	0.91 (0.82, 1.01)
piped water quartile 3 (ref: quartile 1)	0.84 (0.74, 0.95)
piped water quartile 4 (ref: quartile 1)	0.82 (0.71, 0.96)
moderate/strong El Niño period year	1.06 (1.04, 1.09) 0.97 (0.96, 0.98)
model QIC	-83143414.88

IRR = incidence rate ratio; CI = confidence interval; ref. = referent group

*effect modification term significance: $p=0.02$

a. Defined by the percentage of households in a province that use piped water as their drinking water source. Quartile 1: 0-40%, quartile 2: 40-58%, quartile 3: 58-72%, quartile 4: 72-95%. Referent is the first (lowest) quartile of access.

b. Refers to a three-month period classified as having a moderate or strong El Niño event. (Referent: no El Niño or weak El Niño event).

c. Post-vaccine era (2010-2015), compared to pre-vaccine era (2005-2009)

Table 2.2 Effect of rotavirus vaccination on child (<5 years) clinic visits for diarrhea, by level of sewerage access, provinces of Peru (N=194), 2005-2015

Main effect model	Diarrhea IRR (95% CI)
ongoing rotavirus vaccination (2010-2015; ref: 2005-2009)	0.93 (0.90, 0.97)
sewerage access ^a quartile 2 (ref: quartile 1)	1.00 (0.93, 1.07)
sewerage access quartile 3 (ref: quartile 1)	0.99 (0.89, 1.11)
sewerage access quartile 4 (ref: quartile 1)	0.94 (0.79, 1.12)
moderate/strong El Niño period ^b	1.06 (1.04, 1.08)
year	0.97 (0.96, 0.98)
model QIC	-79985163.67
Interaction model*	Diarrhea IRR (95% CI)
Effect of rotavirus vaccination, by sewerage quartile ^c	
at 1 st quartile of sewerage access	1.05 (0.95, 1.15)
at 2 nd quartile of sewerage access	0.91 (0.86, 0.95)
at 3 rd quartile of sewerage access	0.91 (0.84, 0.98)
at 4 th quartile of sewerage access	0.87 (0.81, 0.94)
Effect of sewerage (pre-vaccine era, 2005-2009):	
sewerage quartile 2 (ref: quartile 1)	1.04 (0.96, 1.11)
sewerage quartile 3 (ref: quartile 1)	1.04 (0.92, 1.16)
sewerage quartile 4 (ref: quartile 1)	1.03 (0.88, 1.21)
Effect of sewerage (vaccine era, 2010-2015):	
sewerage quartile 2 (ref: quartile 1)	0.90 (0.80, 1.01)
sewerage quartile 3 (ref: quartile 1)	0.90 (0.78, 1.04)
sewerage quartile 4 (ref: quartile 1)	0.86 (0.71, 1.05)
moderate/strong El Niño period	1.06 (1.04, 1.08)
year	0.97 (0.96, 0.98)
model QIC	-81935159.59

IRR = incidence rate ratio; CI = confidence interval; ref. = referent group

* effect modification terms not significant in a chunk test at $p < 0.05$ ($p = 0.17$)

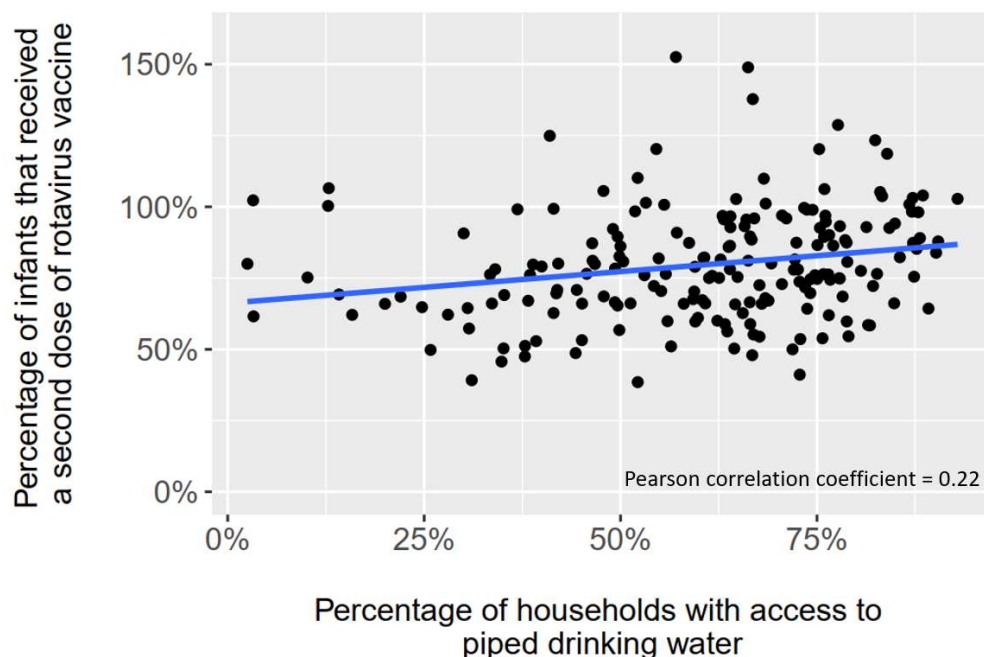
a. Defined by the percentage of households in a province for which the method of excreta disposal is a toilet connected to the sewerage system. Quartile 1: 0-17%, quartile 2: 17-29%, quartile 3: 29-47%, quartile 4: 47-92%. Referent is the first (lowest) quartile of coverage.

b. Refers to a three-month period classified as having a moderate or strong El Niño event. (Referent: no El Niño or weak El Niño event).

c. Post-vaccine era (2010-2015), compared to pre-vaccine era (2005-2009)

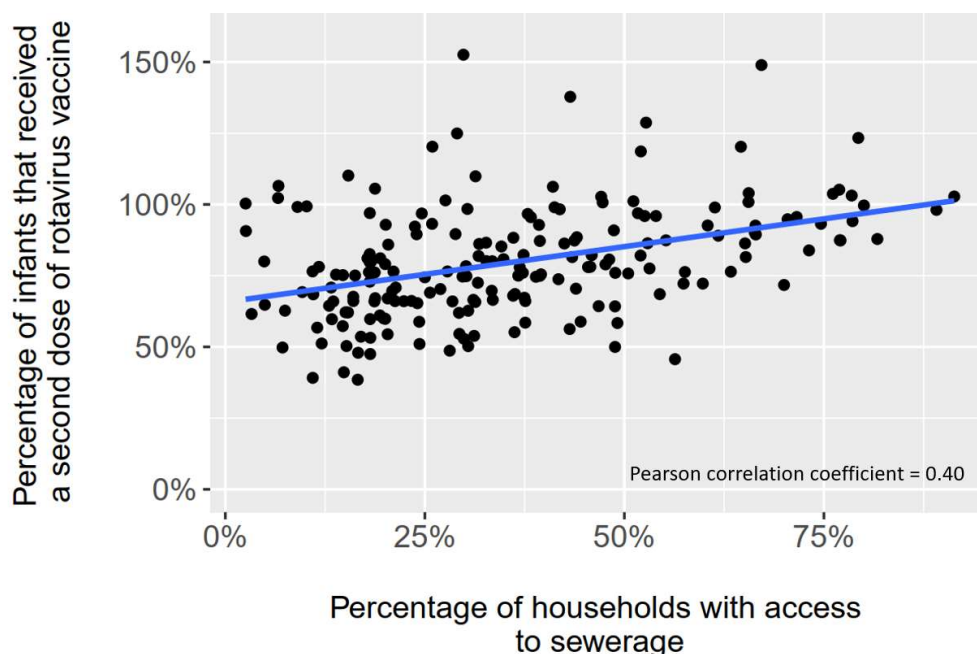
APPENDIX

Appendix Figure 2.1 Association between province-level piped water access and percentage of infants receiving a second dose of the rotavirus vaccine, provinces of Peru (N=194), 2012



A single year of data was assessed because both variables considered increased over time (*i.e.*, both were correlated with year). The year 2012 was chosen arbitrarily as a data point in the middle of the rotavirus vaccine era. The percentage of households with access to piped drinking water is defined as the percentage of households in a province using piped water (piped either inside of, or outside of the home, but within the building area) as the primary drinking water source. The percentage of infants vaccinated is based on the total number of second doses of rotavirus vaccine administered in each province, divided by the estimated infant (<12 months) population. Estimates exceed 100% if the count of second vaccine doses exceeded the estimated infant population, which may have resulted from an overcount of vaccine doses administered, or an underestimated infant population. Infant population estimates are Ministry of Health-derived estimates based on the 2007 Peruvian national census and other administrative data.

Appendix Figure 2.2 Association between province-level sewerage access and percentage of infants receiving a second dose of the rotavirus vaccine, provinces of Peru (N=194), 2012



A single year of data was assessed because both variables considered increased over time (*i.e.*, both were correlated with year). The year 2012 was chosen arbitrarily as a data point in the middle of the rotavirus vaccine era. The percentage of households with access to sewerage is defined as the percentage of households in a province for which the method of excreta disposal is a toilet connected to the sewerage system. The percentage of infants vaccinated is based on the total number of second doses of rotavirus vaccine administered in each province, divided by the estimated infant (<12 months) population. Estimates exceed 100% if the count of second vaccine doses exceeded the estimated infant population, which may have resulted from an overcount of vaccine doses administered, or an underestimated infant population. Infant population estimates are Ministry of Health-derived estimates based on the 2007 Peruvian national census and other administrative data.

Appendix Table 2.1 Access to piped water, access to toilets connected to sewerage, and poverty; provinces of Peru (N=194), 2005-2015

	2005	2015	2005-2015
Access to piped water^a			
1st quartile	0.0-21.0%	3.6-58.5%	0.0-40.1%
2nd quartile	21.0-42.8%	58.5-69.7%	40.1-58.2%
3rd quartile	42.8-61.7%	69.7-78.5%	58.2-71.6%
4th quartile	61.7-94.8%	78.5-92.6%	71.6-94.8%
Toilet connected to the sewerage network^b			
1st quartile	0.0-10.9%	4.3-26.0%	0.0-17.1%
2nd quartile	10.9-20.0%	26.0-40.2%	17.1-29.4%
3rd quartile	20.0-40.0%	40.2-54.2%	29.4-47.0%
4th quartile	40.0-86.0%	54.2-91.7%	47.0-91.7%
Poverty^c			
1st quartile	8.8-42.9%	0.0-18.0%	0.0-30.2%
2nd quartile	42.9-66.2%	18.0-35.8%	30.2-48.6%
3rd quartile	66.2-80.9%	35.8-50.3%	48.6-65.6%
4th quartile	80.9-100.0%	50.3-85.5%	65.6-100.0%

a. Defined as the percentage of households in a province using piped water (piped either inside of, or outside of the home, but within the building area) as the primary drinking water source.

b. Defined as the percentage of households in a province for which the method of excreta disposal is a toilet connected to the sewerage system.

c. Defined by the percentage of households in the province living below the poverty line.

Appendix Table 2.2 Effect of rotavirus vaccination on child (< 5 years) clinic visits for diarrhea, by poverty level, provinces of Peru (N=194), 2005-2015

Main effect model	Diarrhea IRR (95% CI)
ongoing rotavirus vaccination (2010-2015; ref: 2005-2009)	0.93 (0.90, 0.96)
poverty ^a quartile 2 (ref: quartile 1)	1.04 (0.95, 1.14)
poverty quartile 3 (ref: quartile 1)	0.96 (0.86, 1.07)
poverty quartile 4 (ref: quartile 1)	0.91 (0.81, 1.02)
moderate/strong El Niño period ^b	1.06 (1.04, 1.08)
year	0.96 (0.96, 0.97)
model QIC	-80275772.17

IRR = incidence rate ratio; CI = confidence interval; ref. = referent group

In an interaction model (not presented), the effect modification term was not significant at $p < 0.05$ ($p = 0.73$).

a. Defined by the percentage of households in a province below the poverty line. Referent is the first (lowest) quartile.

b. Refers to a three-month period classified as having a moderate or strong El Niño event. (Referent: no El Niño or weak El Niño event).

Appendix Table 2.3 Effect of rotavirus vaccination on child (<5 years) clinic visits for diarrhea, by level of piped water access, provinces of Peru (N=194), 2005-2015; sensitivity analysis comparison

Interaction model	Diarrhea IRR (95% CI)	
	3-month season analysis ^a	weekly case analysis ^a
Effect of rotavirus vaccination ^b , by piped water quartile ^c		
at 1 st quartile of piped water access	1.06 (0.97, 1.16)	1.03 (0.89, 1.19)
at 2 nd quartile of piped water access	0.93 (0.88, 0.98)	1.06 (0.96, 1.18)
at 3 rd quartile of piped water access	0.87 (0.81, 0.93)	0.79 (0.69, 0.90)
at 4 th quartile of piped water access	0.85 (0.80, 0.90)	0.88 (0.79, 0.98)
Effect of piped water (pre-vaccine era, 2005-2009):		
piped water quartile 2 (ref: quartile 1)	1.04 (0.96, 1.11)	0.91 (0.78, 1.05)
piped water quartile 3 (ref: quartile 1)	1.02 (0.92, 1.13)	1.02 (0.86, 1.21)
piped water quartile 4 (ref: quartile 1)	1.03 (0.91, 1.15)	0.97 (0.81, 1.17)
Effect of piped water (vaccine era, 2010-2015):		
piped water quartile 2 (ref: quartile 1)	0.91 (0.82, 1.01)	0.94 (0.74, 1.19)
piped water quartile 3 (ref: quartile 1)	0.84 (0.74, 0.95)	0.78 (0.61, 1.00)
piped water quartile 4 (ref: quartile 1)	0.82 (0.71, 0.96)	0.83 (0.64, 1.06)
moderate/strong El Niño period ^d		
year	1.06 (1.04, 1.09)	1.04 (1.03, 1.06)
	0.97 (0.96, 0.98)	0.97 (0.96, 0.98)

IRR = incidence rate ratio; CI = confidence interval; ref. = referent group

a. The three-month seasonal analysis is the main analysis presented in the text, in which diarrhea cases were aggregated in three-month seasons. The weekly case analysis is the sensitivity analysis, in which weekly diarrhea cases were used with an autoregressive correlation structure and more temporally-resolved El Niño term.

b. Post-vaccine era (2010-2015), compared to pre-vaccine era (2005-2009)

c. Defined by the percentage of households in a province that use piped water as their drinking water source. Quartile 1: 0-40%, quartile 2: 40-58%, quartile 3: 58-72%, quartile 4: 72-95%. Referent is the first (lowest) quartile of access.

d. Refers to a three-month period classified as having a moderate or strong El Niño event. (Referent: no El Niño or weak El Niño event).

Appendix Table 2.4 Effect of rotavirus vaccination on child (<5 years) clinic visits for diarrhea, by level of sewerage access, provinces of Peru (N=194), 2005-2015; sensitivity analysis comparison

Interaction model	Diarrhea IRR (95% CI)	
	3-month season analysis ^a	weekly case analysis ^a
Effect of rotavirus vaccination ^b , by sewerage quartile ^c		
at 1 st quartile of sewerage access	1.05 (0.95, 1.15)	1.11 (0.96, 1.27)
at 2 nd quartile of sewerage access	0.91 (0.86, 0.95)	0.86 (0.78, 0.96)
at 3 rd quartile of sewerage access	0.91 (0.84, 0.98)	0.92 (0.83, 1.03)
at 4 th quartile of sewerage access	0.87 (0.81, 0.94)	0.91 (0.83, 1.00)
Effect of sewerage (pre-vaccine era, 2005-2009):		
sewerage quartile 2 (ref: quartile 1)	1.04 (0.96, 1.11)	1.06 (0.92, 1.23)
sewerage quartile 3 (ref: quartile 1)	1.04 (0.92, 1.16)	1.17 (0.98, 1.40)
sewerage quartile 4 (ref: quartile 1)	1.03 (0.88, 1.21)	1.15 (0.96, 1.39)
Effect of sewerage (vaccine era, 2010-2015):		
sewerage quartile 2 (ref: quartile 1)	0.90 (0.80, 1.01)	0.83 (0.65, 1.06)
sewerage quartile 3 (ref: quartile 1)	0.90 (0.78, 1.04)	0.98 (0.76, 1.27)
sewerage quartile 4 (ref: quartile 1)	0.86 (0.71, 1.05)	0.95 (0.73, 1.24)
moderate/strong El Niño period ^d		
year	1.06 (1.04, 1.08)	1.04 (1.02, 1.06)
	0.97 (0.96, 0.98)	0.96 (0.95, 0.98)

IRR = incidence rate ratio; CI = confidence interval; ref. = referent group

a. The three-month seasonal analysis is the main analysis presented in the text, in which diarrhea cases were aggregated in three-month seasons. The weekly case analysis is the sensitivity analysis, in which weekly diarrhea cases were used with an autoregressive correlation structure and more temporally-resolved El Niño term.

b. Post-vaccine era (2010-2015), compared to pre-vaccine era (2005-2009)

c. Defined by the percentage of households in a province for which the method of excreta disposal is a toilet connected to a piped sewerage system. Quartile 1: 0-17%, quartile 2: 17-29%, quartile 3: 29-47%, quartile 4: 47-92%. Referent is the first (lowest) quartile of coverage.

d. Refers to a three-month period classified as having a moderate or strong El Niño event. (Referent: no El Niño or weak El Niño event).

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Chapter 3 Historic associations between meteorological factors and childhood diarrhea cases in Peru (2005-2015): implications for climate change

Miranda J. Delahoy¹, Cesar Carcamo², Adrian Huerta³, Waldo Lavado³, Yury Escajadillo³,
 Luís Ordoñez⁴, Vanessa Vasquez², Benjamin Lopman¹, Thomas Clasen¹, Gustavo F. Gonzales,
 Kyle Steenland¹, Karen Levy¹

Author Affiliations: (1) Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA; (2) Universidad Peruana Cayetano Heredia, Lima, Peru; (3) Ministerio del Ambiente: Servicio Nacional de Meteorología e Hidrología del Perú (SENAMHI; National Meteorology and Hydrology Service of Peru, Lima, Peru (4) Ministerio de Salud (MINSA; Ministry of Health), Lima, Peru

ABSTRACT

Background: Global temperatures are projected to rise by ≥ 2 °C by the end of the century, with expected impacts on infectious disease incidence, including diarrheal disease. Establishing the historic relationship between temperature and childhood diarrhea for Peru is important to inform future vulnerability under projected climate change scenarios.

Methods: A national dataset was compiled for Peru from government data sources including weekly diarrhea surveillance records, annual administered doses of rotavirus vaccination, annual piped water and sewerage access estimates, and interpolated daily temperature estimates. We used generalized estimating equations to quantify the association between ambient temperature and government-reported childhood (<5 years) weekly clinic visits for diarrhea from January 1, 2005-December 16, 2015 in 194 of 195 Peruvian provinces. We estimated the combined effect of the

mean daily high temperature lagged one, two, and three weeks, in the era before (2005-2009) and after (2010-2015) widespread rotavirus vaccination in Peru, in provinces with varying levels of piped water and sewerage access.

Results: Nationally, the 2005 childhood diarrhea rate was 29 annual clinic visits per 100 children and decreased by 3% per year. An increase of 1 °C in the temperature across the three prior weeks was associated with a 3.8% higher rate of childhood clinic visits for diarrhea [incidence rate ratio (IRR): 1.04, 95% confidence interval (CI): 1.03-1.04]. Controlling for temperature, there was a significantly higher incidence rate of diarrhea during moderate/strong El Niño events (IRR: 1.03, 95% CI: 1.01-1.04) and during the dry season (IRR: 1.01, 95% CI: 1.00-1.03); these effects tended to be stronger in areas with low access to piped water and low access to sewerage connections. Nationally, there was no evidence that the association between ambient temperature and the rate of childhood clinic visits for diarrhea changed between the pre- and post-rotavirus vaccine eras, or that higher levels of access to piped water or sewerage mitigated the effects of temperature on the childhood diarrhea rate.

Conclusions: Higher temperatures and intensifying El Niño events that may result from climate change could increase clinic visits for childhood diarrhea in Peru. This finding can be applied to vulnerability assessments and contribute to planning mitigation efforts.

INTRODUCTION

Global temperatures are projected to rise globally by 2 °C or more by the end of the 21st century,^{1,2} which is expected to impact the incidence of diseases caused by pathogens that can survive or multiply in the environment, including diarrhea.^{3,4} A large percentage of the burden of climate change-related morbidity is expected to be borne by children,⁵ who have unique vulnerabilities to climate change.⁶

The World Health Organization (WHO) projects an annual increase of 48,000 diarrheal deaths (in children under 15 years old) worldwide in 2030 attributable to climate change.⁷ In Peru, the rate of childhood clinic visits for diarrhea has been declining since 2005 (Chapter 2); however, countries that have made substantial gains toward lowering diarrhea morbidity may have these trends slowed or reversed as climate change and increasing temperatures bring additional challenges to reducing diarrheal disease morbidity.² The El Niño phenomenon may also contribute to such challenges: research in Peru has shown significant increases in diarrhea cases during El Niño events.^{8–14} Climate change is expected to increase the frequency and intensity of El Niño periods;¹⁵ in recent decades, El Niño events have been intensifying in the Eastern Pacific, near the coast of Peru.¹⁶

Estimations of increased diarrhea mortality as a result of climate change are based on systematic reviews that have demonstrated an overall positive association between temperature and diarrhea cases; however not all diarrheagenic pathogens display this relationship.^{7,17,18} Diarrhea caused by rotavirus, historically a leading cause of childhood diarrhea, is more common at lower temperatures.^{17,19,20} In 2009, WHO announced a recommendation for rotavirus vaccine inclusion in national immunization programs.²¹ Global rotavirus vaccination has since increased, with early studies showing large reductions in severe rotavirus cases.²² Reducing rotavirus cases through vaccination could be expected to strengthen the overall positive association between temperature and diarrhea, as more cases of diarrhea may be averted during cooler periods. Other studies have demonstrated rotavirus vaccination shifting or diminishing the seasonality of rotavirus infections.^{23–25}

While there have been many published works examining the relationship between temperature and diarrhea,¹⁷ little attention has been given to how this relationship may be modified by other environmental variables such as access to improved water and sanitation; establishing these historic relationships is important to climate change vulnerability and mitigation assessments.^{17,26}

Using an extensive national surveillance dataset, we estimated the association between temperature and clinic visits for childhood diarrhea in Peru, accounting for El Niño, wet/dry seasons, and the introduction of rotavirus vaccination. We also examined whether the temperature-diarrhea association varied by level of water and sewerage access, hypothesizing that water and sanitation improvements might interrupt some of the pathways through which higher temperatures may increase diarrhea cases.¹⁸

METHODS

Geographic scope

Peru is comprised of 25 departments, which encompass 195 provinces. Provinces are sub-divided into 1,876 districts, with each district belonging to a single province, allowing for aggregation of district data to the provincial level. The study included data from 194 of the 195 provinces of Peru: Ocos province in Ancash was not included, due to inconsistent diarrhea surveillance reporting, with little data prior to rotavirus vaccine introduction. Province populations range from <4,000 residents (Purus province, Ucayali department) to >7 million residents (Lima province); areas range from ~150 km² (province of Callao) to >67,000 km² (province of Loreto).²⁷ A 196th province was created in 2014 (Putumayo, Loreto department); data from the districts forming this province were included as part of their original province (Maynas) in analyses.

Data sources and definitions

Diarrhea cases and rotavirus vaccine data

The Peruvian Ministry of Health (MINSA) collects obligatory weekly surveillance data on diarrhea visits from all public inpatient and outpatient clinics in Peru. Private clinics also send regular weekly reports if they opt into the reporting system. Diarrhea cases refer to patients presenting to a clinic with an increase in frequency of bowel movements (three or more bowel movements in 24 hours),

or in fluidity or volume of stool compared to usual, with onset within the past two weeks. Cases are aggregated by age group (<1 year old, 1-4 years old, ≥ 5 years old), and assigned to the patient's district of residence. We use the term "childhood diarrhea rate" to describe the rate of clinic visits for diarrhea in children under five years old. We analyzed cases reporting to clinics between January 5, 2005 and December 16, 2015. A total of 111,162 observations were analyzed (weekly clinic visits in 194 provinces over a total of 573 weeks).

MINSA also collects rotavirus immunization data. Rotarix® is intended to be administered to infants in two doses between approximately six weeks and six months of age. The vaccine was added to the national immunization schedule in 2009, although some provinces started administering the vaccine in 2008. MINSA provided counts of the first and second doses of rotavirus vaccine given to infants in each district of Peru from 2008-2015. By 2010, most Peruvian infants were receiving both doses of the rotavirus vaccine (Chapter 2). Data were analyzed for the "pre-(rotavirus) vaccine era" (2005-2009) and "post-vaccine era" or "rotavirus vaccine era" (2010-2015). We considered vaccination in eras rather than provincial-level estimates of the percent of infants vaccinated, due to difficulties in obtaining stable coverage estimates at this geographic level (see Chapter 2).

Piped water, sewerage, and population

Data from the Peruvian national censuses (2007 and 2017) and the household and population register (Empadronamiento Distrital de Población y Vivienda, or SISFOH, 2012-2013) were used to estimate the percentages of households in each province with access to piped water and with access to a toilet connected to the sewerage system. The national censuses in 2007 and 2017 were used to estimate the population of children under five years old. A small subset of districts did not participate in diarrhea surveillance for the entire study period. If a district did not start contributing

diarrhea surveillance data until after the start of the study, or if the district stopped sending reports prior to the conclusion of the study period, the district's child population was not counted toward the provincial population during the weeks the district was not reporting. In other words, we did not consider children from the non-reporting districts to be at risk in the weeks that they were not reporting data. Detailed methods of obtaining annual piped water, sewerage, and population estimates, and definitions of piped water and sewerage have been described previously (Chapter 2).

Provinces were divided into three groups of water and sewerage access: (1) provinces that had consistently lower access than the rest of the country throughout the study period, (2) provinces that had consistently higher access, and (3) provinces that fit into neither category, namely that transitioned from lower to higher access throughout the study period. Low piped drinking water access provinces were defined as those in which $<60\%$ of households had access to a piped water connection in all study years (2005-2015), or all but one year. High piped drinking water access provinces were those in which $\geq 60\%$ of households had access to a piped water connection in all study years, or all but one year. Transitional provinces were those in which $<60\%$ had access to piped water for at least two study years, but transitioned to higher access ($\geq 60\%$ for at least two years). Low sewerage access provinces were defined as those in which $<30\%$ of households had access to a toilet connected to the sewer system in all study years (2005-2015), or all but one year. High sewerage access provinces were those in which $\geq 30\%$ of households had access to a toilet connected to the sewer system in all study years, or all but one year. Transitional provinces were those in which $<30\%$ had access to a toilet connected to the sewer system for at least two study years, but transitioned to higher access ($\geq 30\%$ for at least two years). The choice of cut-off for the categories was based on the median access level across all years and all provinces, to maximize the number of provinces in the high and low categories.

Temperature data source

The Peruvian National Meteorology and Hydrology Service (SENAMHI) provided daily minimum temperatures (tmin) and maximum temperatures (tmax) at 0.1° gridded spatial resolution (each grid approximately 10 km x 10 km) for the country of Peru. Estimates were constructed from interpolated data from 684 air temperature monitors, and from remote-sensed data.²⁸ The tmin and tmax values were averaged to created gridded daily mean temperature (tmean) values.

Construction of meteorological variables

Control for dry/rainy season

Peru consists of three major geographic regions: the coast, mountains, and the Amazon jungle. Provinces were classified into one of these three groups.²⁹ June-August was considered the dry season in the mountain provinces and the Amazon jungle provinces. Coastal provinces receive very little rainfall, nevertheless have a drier season from June to November, which was classified as the dry season in analyses.³⁰

El Niño

The U.S. National Oceanic and Atmospheric Administration (NOAA) reports data on the Oceanic Niño Index (ONI), calculated using a standard three-month mean of sea surface temperature anomalies in the Niño 3.4 region of the Pacific Ocean.³¹ For each month in our study, we assigned the corresponding ONI value of the three-month running average in which the study month was the midpoint. For example, the ONI for February 2005 was assigned the ONI running average for January-March 2005. El Niño periods were defined using the ONI, with values in the ranges 0.5-0.9, 1.0-1.4, and ≥ 1.5 corresponding to weak, moderate, and strong El Niño events, respectively.³² We compared months with a moderate or strong El Niño to months with a weak El Niño or no El

Niño. Moderate/strong El Niño periods in the study were from October 2009 to February 2010 and May-December 2015.

Province-level temperature estimates

The gridded tmax temperature values were averaged within each district boundary, giving a daily high temperature for each district. Province-level daily temperature estimates were estimated using population-weighted averages of the district-level temperatures, giving more weight to temperatures in more populous districts of the province. The same process was used to estimate province-level daily tmean values.

Three weekly province-level temperature variables were compared for model fit: (1) the weekly maximum of the daily high temperatures, (2) the weekly average of the daily high temperatures, and (3) the weekly average of the daily mean temperatures. We ran three negative binomial generalized estimating equation (GEE) models using each of the three temperature variants above for the week before the diarrhea data were recorded (“1 week lag”), as well as the 2- and 3- week lagged temperatures. Other variables were specified in the model as described in the next paragraph. We found the model with the weekly mean of the daily high temperatures to have the best fit, based on lowest QIC value (Appendix Table 3.1). In this model, the association between temperature and diarrhea was highest for the 2-week lagged value, but was of similar magnitude for the 1-week lag. After a 2-week lag, the association began to drop off, but was still significant at a 3-week lag.

Statistical analysis

Data were compiled and cleaned in R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) and analyzed using SAS 9.4 (SAS Institute, Inc., Cary, NC). We analyzed province-level weekly counts of clinic visits for diarrhea in children <5 years using negative binomial GEEs with autoregressive correlation structures, after finding over-dispersion of the outcome in a Poisson

model. We first examined whether temperature was associated with childhood clinic visits for diarrhea in a model that included the following variables: the weekly mean of the daily high temperatures at 1-week, 2-week, and 3-week lags; an indicator for the rotavirus vaccine era; an indicator for dry/wet season (which varied by region of Peru); an indicator for moderate/strong El Niño events; a continuous variable for the study year (to account for secular trend); and a variable to control for province, to focus on week-to-week temperature changes within each province, while controlling for other unmeasured province-level factors. We also included an offset for population (child population <5 years; see Chapter 2 for more detail).

To examine whether the association between temperature and childhood clinic visits for diarrhea differed between the pre- and post-vaccine eras, we ran the model above with additional terms for the interaction between each lagged temperature value and the rotavirus vaccine era term. All analyses were performed on all provinces combined, and also stratified by the three groups of access to piped water and the three groups of access to sewerage.

As noted above, for our exposure variable we used a distributed lag across three different weekly lags. To obtain a single exposure metric, we summed the coefficients across the lags and then exponentiated them, *i.e.*, we calculated the incidence rate ratio for a one degree increase in temperature in each of the three lag periods and multiplied them together for a single measure. We deem this term the “temperature-diarrhea association” for short. The incidence rate ratio for each lag period is also displayed in model results.

Sensitivity analyses

We conducted two sensitivity analyses. Many provinces of Peru have low temperature variability, *i.e.*, temperature is relatively constant throughout the year. Hypothesizing that these provinces might contribute little to the temperature-diarrhea association and bias the effect toward the null, for our

first sensitivity analysis we ran models only in provinces with higher temperature variability (Appendix Figure 3.3).

To consider the variability in temperature throughout the year in each province, we constructed monthly average temperatures, which were the mean of the daily high temperatures in each month of the year, excluding data from El Niño periods. As an example, the January mean daily high for a province was an average of all daily high temperatures from any January in the study period (2005-2015), excluding any January temperatures when an El Niño was ongoing. Of the 194 provinces, 133 (68.6%) had less than a 3 °C difference between the mean temperature in the warmest month and coolest month. These provinces were classified as having low temperature variability, and were excluded in the sensitivity analysis models.

For our second sensitivity analysis, we defined high and low sewerage access using the same cut-off for sewerage as we did for piped drinking water (60%). While there were a very small number of provinces in the highest access group (N=11), this analysis allowed us to examine the hypothesis that a high threshold of coverage may be needed for sewerage improvements to yield health benefits.^{33,34}

Ethical review

Emory University's Institutional Review Board reviewed the study protocol and determined that the use of aggregated unidentified data in these analyses does not constitute human subjects research.

RESULTS

Diarrhea trend and piped water/sewerage access

There was a strong, secular, downward trend in the rate of clinic visits for childhood diarrhea throughout the study period. The main time series showed a decline of 3.2% per year in the

incidence of childhood clinic visits for diarrhea, controlling for other variables in the model (the daily high temperatures at 1-week, 2-week, and 3-week lags, the rotavirus vaccine era, dry/wet season, moderate/strong El Niño events, and province, Table 3.1). Controlling for this secular trend, the childhood diarrhea rates were 8.7% lower in the rotavirus vaccine era.

Generally, coastal provinces had higher levels of access to piped drinking water and sewerage, with lowest coverage in the Amazon jungle provinces (Figure 3.1 and Figure 3.2). Provinces in southern Peru near Lake Titicaca also tended to have lower piped drinking water and sewerage coverage. In the first year of the study (2005), provinces with low and high piped drinking water access had similar annual rates of clinic visits for childhood diarrhea (27.8 and 28.3 visits per 100 children under five years old, respectively), and provinces with lower sewerage access reported lower rates of clinic visits for childhood diarrhea compared to provinces with higher access (24.8 compared to 29.9 visits per 100 children under five years old, respectively, Appendix Figure 3.1 and Appendix Figure 3.2). Beginning in 2007, the childhood diarrhea rate was consistently lower in provinces with high piped drinking water access and high sewerage access, compared to provinces with low access.

Association between air temperature, El Niño events, season, and childhood clinic visits for diarrhea

Nationally, an increase of 1 °C in temperature across the three weeks prior to diarrhea cases was associated with a 3.8% higher rate of childhood clinic visits for diarrhea [incidence rate ratio (IRR): 1.038, 95% confidence interval (CI): 1.032-1.044; Table 3.1]. Controlling for temperature, there was still a significantly higher rate of clinic visits for diarrhea during moderate/strong El Niño events (IRR: 1.026, 95% CI: 1.009-1.044). There was also a significantly higher diarrhea rate in the dry season (IRR: 1.014, 95% CI: 1.002-1.027). In the pre-vaccine era, an increase of 1 °C in the temperature across the three weeks prior to diarrhea cases was associated with a 3.6% higher

childhood diarrhea; in the post-vaccine era it was 4.0%. There was no statistically significant difference in the temperature-diarrhea relationship from the pre- to post-vaccine era ($p=0.37$).

Temperature-diarrhea association at varying levels of piped water access

When analyses were stratified based on access to piped water, the increase in the incidence of childhood clinic visits for childhood diarrhea per 1° C temperature increase was smaller in the 64 provinces with consistently low access to piped water. In these provinces, an increase of 1 °C in the temperature across the three weeks prior to diarrhea cases was associated with a 1.7% higher rate of childhood clinic visits for diarrhea (IRR: 1.017, 95% CI: 1.007-1.027), compared to a 4.3% higher incidence in high piped water access provinces (IRR: 1.043, 95% CI: 1.034-1.051; Table 3.2).

Transitional piped water access provinces displayed similar patterns to high piped water access provinces.

In all piped water access groups, there was a higher incidence of childhood clinic visits for diarrhea during moderate/strong El Niño events, controlling for temperature and other variables in the model. This effect was stronger and statistically significant in provinces with consistently low piped water access. In these provinces, moderate/strong El Niño events were associated with a 4.2% higher childhood diarrhea incidence (IRR: 1.042, 95% CI: 1.008-1.077). In high piped water access provinces and transitional piped water provinces, the association was somewhat lower (2.3% and 2.6%, respectively), and not statistically significant. There was a higher incidence of childhood clinic visits for diarrhea in the dry season in provinces with low piped water access (IRR: 1.036, 95% CI: 1.014-1.059); there was no significant difference in diarrhea rates between wet and dry seasons in high and transitional piped water access provinces. The rate of childhood clinic visits for diarrhea was lower in the post-rotavirus vaccine era, though not significantly so in provinces with low piped water access.

In provinces with consistently low access to piped drinking water, there was a significantly different temperature-diarrhea relationship in the pre- and post-rotavirus vaccine eras ($p=0.02$). In the pre-vaccine era, an increase of 1 °C in temperature across the three weeks prior to diarrhea cases was not significantly associated the diarrhea rate (IRR 1.009, 95% CI: 0.998-1.020); an increase of 1 °C in the temperature across the three weeks prior to diarrhea cases was associated with a 2.4% increase in the diarrhea rate in the post-vaccine era (IRR: 1.024, 95% CI: 1.012, 1.037). There was no significant difference in the temperature-diarrhea relationship from the pre- to post-vaccine era in the transitional water access provinces ($p=0.75$) or high water access provinces ($p=0.32$).

Temperature-diarrhea association at varying levels of sewerage connection access

When analyses were stratified based on sewerage connection access, the increase in the incidence of childhood clinic visits for childhood diarrhea per 1° C temperature increase tended to be smaller in the 72 provinces with consistently low access to a sewerage connection. In provinces with low sewerage access, an increase of 1 °C in the temperature across the three weeks prior to diarrhea cases was associated with a 2.2% higher rate of childhood clinic visits for diarrhea, compared to a 3.9% higher incidence in high sewerage access provinces, and 4.7% in transitional sewer access provinces (Table 3.3).

In all sewerage access groups, there was a higher incidence of childhood clinic visits for diarrhea during moderate/strong El Niño events, controlling for temperature and other variables in the model. This effect was stronger and statistically significant in provinces with low sewerage access. In these provinces, moderate/strong El Niño events were associated with a 4.9% higher childhood diarrhea incidence (IRR: 1.049, 95% CI: 1.016-1.084). In high sewerage access provinces and transitional sewerage access provinces, the association was lower (1.6% in both), and not statistically significant. There was a significantly higher incidence of childhood clinic visits for diarrhea in the dry

season in provinces with low sewerage access (IRR: 1.031, 95% CI: 1.011-1.051); there was no significant difference in diarrhea rates between wet and dry seasons in high and transitional piped water access provinces. The rate of childhood clinic visits for diarrhea was lower in the post-rotavirus vaccine era across all three groups of sewerage access.

In provinces with low access to sewer connections, there was a significantly different temperature-diarrhea relationship in the pre- and post-rotavirus vaccine eras ($p=0.01$). In the pre-vaccine era, an increase of 1 °C in the temperature in the three weeks prior to diarrhea cases tended to be associated with a higher diarrhea rate, but not significantly so (IRR: 1.012, 95% CI: 1.000-1.025); an increase of 1 °C in the temperature in the three weeks prior to diarrhea cases was associated with a 3.1% increase in the diarrhea rate in the post-vaccine era (IRR: 1.031, 95% CI: 1.019-1.043). There was no significant difference in the temperature-diarrhea relationship from the pre- to post-vaccine era in the transitional sewer access provinces ($p=0.84$) or high sewer access provinces ($p=0.55$).

Sensitivity analyses

Temperature variability

Results of the sensitivity analysis modeling only provinces with higher temperature variability are shown in Appendix Table 3.2 and Appendix Table 3.3. The temperature-diarrhea association was somewhat higher when considering only these provinces (IRR: 1.045, 95% CI: 1.038-1.052); there was still no significant difference in the temperature-diarrhea relationship from the pre- to post-vaccine era ($p=0.12$). When subset to these provinces, there was less of a difference in the temperature-diarrhea association between low piped water access provinces (IRR: 1.034, 95% CI: 1.018-1.050) and high piped water access provinces (IRR: 1.044, 95% CI: 1.034-1.054), and there

was no difference in the temperature-diarrhea association between provinces with low and high sewerage access.

Highest sewerage vs. lower sewerage

Comparisons between the temperature-diarrhea association in 11 provinces with the highest sewerage access using the 60% cut-off value are displayed in Appendix Table 3.4. Generally, provinces with the highest sewerage access displayed similar associations between meteorological variables and the childhood diarrhea rate as did provinces with just “high access” to sewerage (Appendix Table 3.4, compared to Table 3.3). Furthermore, the association between meteorological factors and childhood diarrhea was similar between the 11 provinces with highest sewerage access and the provinces that had lower sewerage access. There was no significant difference in the temperature-diarrhea association from the pre- to post-vaccine era in the 11 provinces with highest access to sewerage ($p=0.49$).

DISCUSSION

We found a positive association between ambient temperature and childhood diarrhea even when controlling for El Niño events, and also that El Niño events are associated with more childhood clinic visits for diarrhea even when controlling for temperature. These results are consistent with global research,¹⁷ as well as research in Peru.^{8,12} The overall increase in the incidence of childhood clinic visits for diarrhea associated with a 1 °C increase in temperature (3.8%) is in line with previous global estimates, which range from 3-11% (pooled estimate: 7%) but is lower than other findings specific to Peru (8-11%). Other research on the association between temperature and diarrhea in Peru was conducted in Lima in the 1990s, a decade characterized by two El Niño events that were associated with cholera epidemics.^{8,12,14} The temperature-diarrhea association may be less pronounced in the absence of epidemic cholera, and outside of Lima.

The positive associations between childhood clinic visits for diarrhea and El Niño events, as well as the dry season, were significant and strongest in areas with low piped water and sewerage access. Other research in Peru has found increased risk of diarrhea in children >5 years old during El Niño in households lacking a sewerage connection, but not in households that do have a sewerage connection.⁹ The authors suggest that these children may be more susceptible to the effects of El Niño when they leave their homes to defecate. It is also possible that in the absence of piped water, worse hand hygiene is practiced, leaving children more vulnerable to become infected with pathogens circulating during dry and El Niño seasons.

In the Amazon jungle region of Peru, El Niño events are associated with lower rainfall and have triggered droughts.^{35,36} Similarly, drought conditions have been associated with El Niño in water-scarce southern Peru, at the base of the Andean Mountains.³⁷ Notably, these are the areas in which many of the provinces with low access to piped water and sewerage are located (Figure 3.1 and Figure 3.2), and that had the strongest association with El Niño events and the dry season. Dry conditions may therefore be a risk factor for childhood diarrhea in Peru, although we did not assess precipitation or drought specifically. Mechanisms through which drought or low rainfall conditions can pose a risk of diarrheal disease have been previously enumerated.¹⁸ In brief, dry conditions may lead to accumulation/increased concentration of fecal pathogens in water and on household surfaces.¹⁸ It is also possible that people travel further distances to obtain drinking water in the dry season, leaving drinking water susceptible to contamination between the source and consumption.^{18,38} The main source of drinking water may also change between seasons, especially if certain communities rely on rainwater, an improved drinking water source, during the rainy season and switch to an unimproved source in the dry season. Lack of water may also change handwashing behaviors during the dry season.³⁹ Compared to research on temperature and heavy

rainfall/flooding, there has been less attention on the effects of drought on diarrhea,¹⁸ though further consideration of this may be relevant in this setting.

We hypothesized that improved water and sanitation conditions might interrupt some of the pathways through which temperature influences diarrhea, thereby dampening the temperature-diarrhea association. Contrary to this hypothesis, the association between temperature and clinic visits for childhood diarrhea was weaker in areas with lower levels of access to piped water and sewerage; although these differences were not apparent when limiting analyses to provinces with higher temperature variability. Many of the provinces with low piped water and sewerage access are geographically large provinces in the Amazon region of the country. There may be more exposure misclassification in temperature estimates in these provinces, given their large surface areas. This may bias the association between temperature and clinic visits for child diarrhea toward the null. The lower population densities in the jungle region may also limit the potential of pathogens to circulate through a population, interrupting some of the pathways through which temperature may influence sustained transmission of diarrheal disease.

Also contrary to what we hypothesized, there was no overall difference in the temperature-diarrhea association from the pre- to post-rotavirus vaccine era. Curiously, the one context in which the temperature-diarrhea relationship did significantly change from the pre- to post-rotavirus vaccine era was in provinces with lower access to piped drinking water and sewerage. In the current study, as well as in our previous research (Chapter 2), reductions in overall childhood diarrhea from the pre- to post-rotavirus vaccine era were lowest in areas with low access to piped water. In other words, it's possible that vaccine performance was worse in those areas; however, there are several alternative explanations for this difference (Chapter 2). The significant change in the temperature-diarrhea

association in these provinces from the pre- to post-vaccine era, however, is in line with what might be expected if viral diarrhea (usually associated with cooler temperatures) was reduced.

Limitations

This study utilized an ecologic study design, which limits causal inference. Interpretation of analyses that compare provinces with low and high access to piped drinking water and sewerage are limited due to spatial confounding. Provinces with low access to piped drinking water and sewerage tended to be geographically larger, have lower population densities, and be located in the Amazon region. It was also difficult to compare factors associated with lower piped water access to factors associated with lower sewerage access. There were larger increases in access to piped water than to access to sewerage in the study period (Chapter 2), meaning there were more “transitional” piped water provinces; however, places with lower water access tended to also have lower sewerage access, limiting our ability to distinguish between associations related to water versus sewerage access.

We assigned a single weekly temperature value to each province, which involved averaging temperature estimates over both space and time. Some provinces were geographically large, especially in the Amazon region. The choice of conducting the analysis at a province level, rather than at a district level, was based on very small child populations in many districts. This meant there were a high proportion of weeks with no diarrhea cases, as well as difficulties in obtaining estimates of the child population and piped water/sewerage access at a district level. Furthermore, similarly to provinces, districts also tend to be geographically much larger in the Amazon region. Notably, many of the provinces that were geographically large had rather homogenous temperature estimates within them, so the choice of averaging the temperature in these provinces may have led to less exposure misclassification than in smaller provinces on the border of the Andean Mountains that have high within-province temperature differences due to differences in elevation (Appendix Figure 3.4).

Imprecise temperature estimates may have biased our temperature-diarrhea association toward the null.

We did not consider precipitation measurements in this analysis. Rainfall patterns tend to be highly localized and more spatially variable than temperature estimates; use of typical rainfall datasets can lead to large bias in analyses of waterborne disease.⁴⁰ The association between rainfall and diarrhea is complex: though heavy rainfall can be associated with increased diarrhea, this relationship may depend on antecedent conditions.⁴¹ Because we did not use precipitation data, we used more general classifications of the wet and dry season. Wet/dry season was defined based on whether a province was in the coast, mountain, or jungle region of Peru; however, we used classifications of these three regions that were previously established at the department level, thus we assigned all provinces within each of the 25 departments of Peru to the same geographic region. This may be problematic in departments such as Ancash, which contain provinces along the coast as well as in the mountains. However, this may have been of limited importance since the dry season was similar across the three regions.

Conclusions

We utilized extensive spatially-detailed weekly data from the country of Peru and established that--despite low temperature variability in several provinces--increases in ambient temperature are associated with significantly higher rates of childhood clinic visits for diarrhea. Moderate/strong El Niño events and the dry season were significantly associated with higher diarrhea rates, controlling for temperature, especially in provinces with low access to piped water and piped sewerage. Unlike earlier research on these topics, these associations are demonstrated in a decade that did not encompass major cholera epidemics concurrently with its El Niño events, and in eras both with and without rotavirus vaccination ongoing. Thus, with data that reflect more recent conditions in Peru,

such as ongoing rotavirus vaccination and high access to piped drinking water, we demonstrate that rising temperatures and intensifying El Niño events may pose a risk to child health. Such analyses can be useful in informing vulnerability assessments and mitigation strategies for the effects of climate change on childhood diarrhea.

FUNDING/DISCLAIMER

Research reported in this publication was supported by the NIH Fogarty International Center, National Institutes of Environmental Health Sciences, National Cancer Institute, Centers for Disease Control and the NIH under Award Number [U01 TW0101 07] and by the National Institute for Allergy and Infectious Diseases under Award Number [1K01AI103544]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ACKNOWLEDGEMENTS

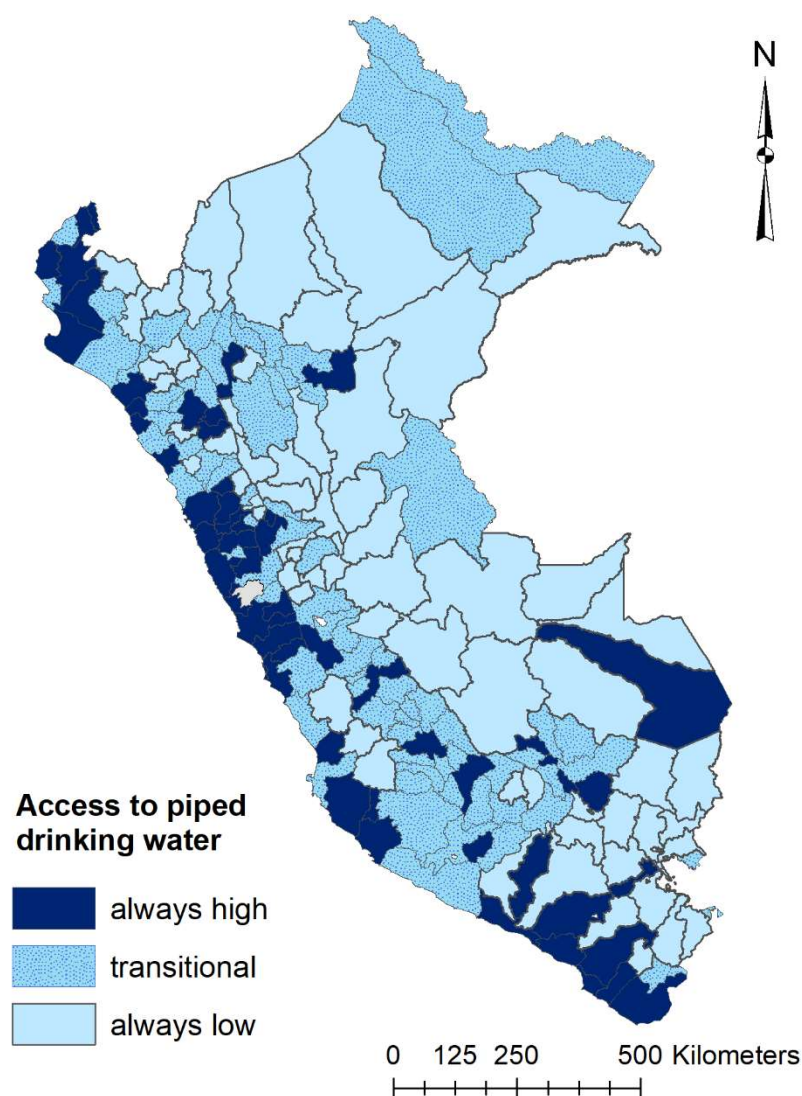
This study utilized data collected by the Peruvian Ministry of Health and the Peruvian National Institute of Statistics and informatics (INEI). We acknowledge their contributions and thank their staff, as well as the national census enumerators, for their data collection efforts. We thank Gaspar Moran, Nancy Hidalgo, and Hector Benavides Rullier of INEI for their guidance in data collection and interpretation. We thank Angela Roza at Emory University, and Diego Fano and Vilma Tapia from Universidad Peruana Cayetano Heredia for assistance in project management and translation, and Mitchel Klein and Howard Chang (Emory University) for epidemiologic and statistical guidance. We thank Ken Takahashi of the Peruvian National Meteorological and Hydrological Service for early guidance on data sources and study design.

AUTHOR CONTRIBUTIONS

Conceptualization: MJD, CC, KS, KL; **Data collection:** LO; **Data curation:** MJD, AH, WL, YE, LO, VV; **Data analysis:** MJD, KS; **Methodology:** MJD, AH, WL, YE, KS, KL; **Project administration:** MJD, CC, LO, VV, GFG, KS, KL; **Writing (original draft preparation):** MJD; **Writing (review and editing):** MJD, AH, LO, VV, BL, TC, GFG, KS, KL

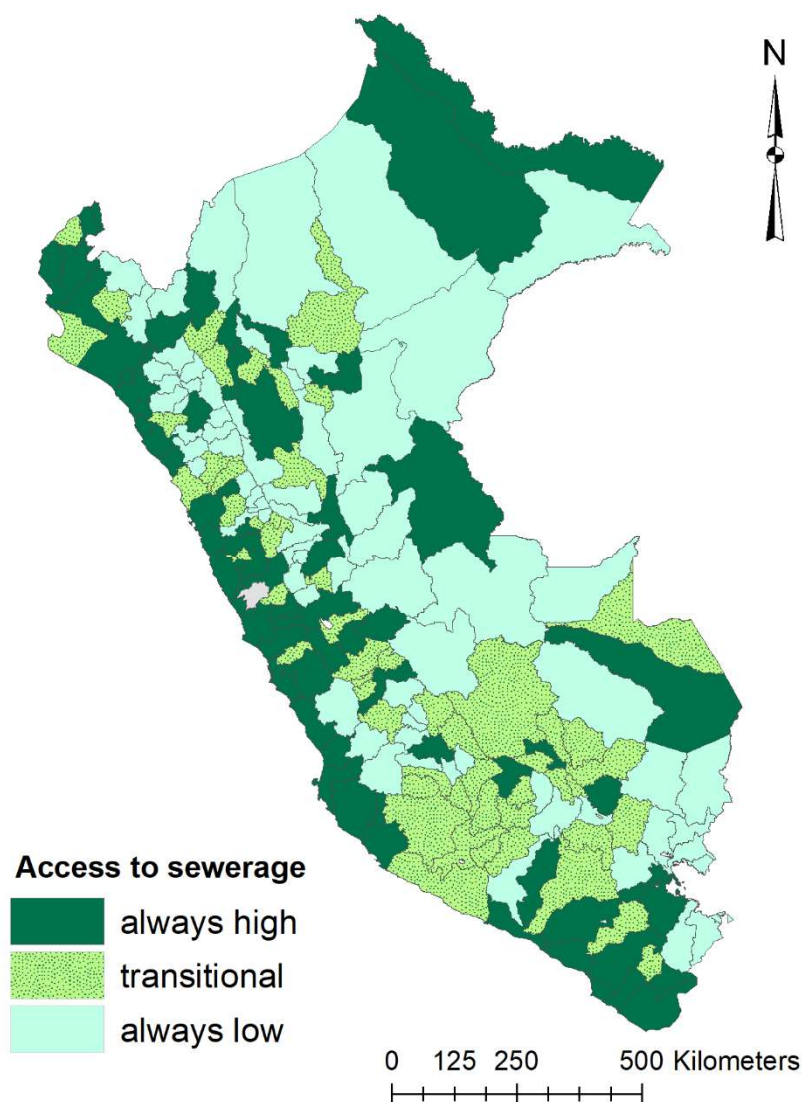
TABLES AND FIGURES

Figure 3.1 Piped drinking water access, provinces of Peru, 2005-2015



“Always high” water access refers to provinces in which $\geq 60\%$ of households had access to piped drinking water for every year (or all but one year) from 2005-2015. “Always low” water access refers to provinces in which $< 60\%$ of households had access to piped drinking water for every year (or all but one year) from 2005-2015. “Transitional” provinces were those that did not fall into either category, *i.e.*, those that transitioned from lower piped water access ($< 60\%$ of households with a piped connection) to higher water access ($\geq 60\%$ of households with a piped water connection) between 2005 and 2015. Statistics on piped drinking water access from the Peruvian National Institute of Statistics and informatics (Instituto Nacional de Estadística e Informática). Province boundaries obtained from the Permanent Coordinating Committee of the Spatial Data Infrastructure of Peru (Comité Coordinador Permanente de la Infraestructura de Datos Espaciales del Perú).

Figure 3.2 Sewerage access, provinces of Peru, 2005-2015



“Always high” sewerage access refers to provinces in which $\geq 30\%$ of households had a toilet connected to the sewerage network for every year (or all but one year) from 2005-2015. “Always low” sewerage access refers to provinces in which $< 30\%$ of households had a toilet connected to the sewerage network for every year (or all but one year) from 2005-2015. “Transitional” provinces were those that did not fall into either category, *i.e.*, those that transitioned from lower sewerage access ($< 30\%$ of households with a toilet connected to the sewerage network) to higher sewerage access ($\geq 30\%$ of households with a toilet connected to the sewerage network) between 2005 and 2015. Statistics on sewerage access from the Peruvian National Institute of Statistics and informatics (Instituto Nacional de Estadística e Informática). Province boundaries obtained from the Permanent Coordinating Committee of the Spatial Data Infrastructure of Peru (Comité Coordinador Permanente de la Infraestructura de Datos Espaciales del Perú).

Table 3.1 Association between meteorological factors and incidence rate of childhood clinic visits for diarrhea, controlling for rotavirus vaccination and secular trend, 194 provinces of Peru, 2005-2015

	IRR (95% CI)
Temperature across three weeks prior to diarrhea cases ^a	1.038 (1.032, 1.044)
1-week temperature lag ^b	1.014 (1.011, 1.017)
2-week temperature lag ^b	1.016 (1.013, 1.019)
3-week temperature lag ^b	1.008 (1.005, 1.010)
Moderate/strong El Niño period	1.026 (1.009, 1.044)
Dry season	1.014 (1.002, 1.027)
Rotavirus vaccine era (2010-2015) ^c	0.913 (0.886, 0.941)
Year (secular trend) ^d	0.968 (0.961, 0.974)

IRR = incidence rate ratio; CI = confidence interval

a.) Combined effect of temperature across three weeks prior to weekly diarrhea report.

b.) The 1-week temperature lag is the effect of temperature in the week before the diarrhea cases, the 2-week lag refers to the week before that, etc.

c.) Compared to the pre-rotavirus vaccine era (2005-2009).

d.) Continuous term for year.

Table 3.2 Association between meteorological factors and incidence rate of childhood clinic visits for diarrhea, controlling for rotavirus vaccination and secular trend, 194 provinces of Peru, by piped water access, 2005-2015

	Piped water access ^a		
	Low provinces (N=64) IRR (95% CI)	High provinces (N=56) IRR (95% CI)	Transitional provinces (N=74) IRR (95% CI)
Temperature across three weeks prior to diarrhea cases ^b	1.017 (1.007, 1.027)	1.043 (1.034, 1.051)	1.042 (1.033, 1.051)
1-week temperature lag ^c	1.004 (1.000, 1.009)	1.021 (1.016, 1.026)	1.013 (1.008, 1.019)
2-week temperature lag ^c	1.008 (1.003, 1.014)	1.016 (1.011, 1.021)	1.019 (1.014, 1.024)
3-week temperature lag ^c	1.004 (0.999, 1.009)	1.005 (1.000, 1.010)	1.009 (1.004, 1.013)
Moderate/strong El Niño period	1.042 (1.008, 1.077)	1.023 (1.000, 1.046)	1.026 (0.996, 1.057)
Dry season	1.036 (1.014, 1.059)	1.007 (0.989, 1.026)	1.012 (0.992, 1.032)
Rotavirus vaccine era (2010-2015) ^d	0.955 (0.895, 1.019)	0.892 (0.851, 0.934)	0.901 (0.862, 0.942)
Year (secular trend) ^e	0.974 (0.962, 0.985)	0.957 (0.946, 0.968)	0.970 (0.960, 0.981)

IRR = incidence rate ratio; CI = confidence interval

a.) “Low piped water access” provinces were defined as those in which <60% of households had access to a piped water connection in all study years (2005-2015), or all but one year. “High piped water access” provinces were those in which ≥60% of households had access to a piped water connection in all study years, or all but one year. “Transitional” provinces were those that did not fall into either category, *i.e.*, those that transitioned from lower piped water access (<60% of households with a piped connection) to higher water access (≥60% of households with a piped water connection) between 2005 and 2015.

b.) Combined effect of temperature across three weeks prior to weekly diarrhea report.

c.) The 1-week temperature lag is the effect of temperature in the week before the diarrhea cases, the 2-week lag refers to the week before that, etc.

d.) Compared to the pre-rotavirus vaccine era (2005-2009).

e.) Continuous term for year.

Table 3.3 Association between meteorological factors and incidence rate of childhood clinic visits for diarrhea, controlling for rotavirus vaccination and secular trend, 194 provinces of Peru, by sewerage access, 2005-2015

	Sewerage access ^a		
	Low provinces (N=72)	High provinces (N=68)	Transitional provinces (N=54)
	IRR (95% CI)	IRR (95% CI)	IRR (95% CI)
Temperature across three weeks prior to diarrhea cases ^b	1.022 (1.010, 1.033)	1.039 (1.032, 1.047)	1.047 (1.037, 1.058)
1-week temperature lag ^c	1.005 (1.000, 1.010)	1.019 (1.014, 1.024)	1.015 (1.010, 1.020)
2-week temperature lag ^c	1.010 (1.004, 1.015)	1.015 (1.011, 1.019)	1.022 (1.016, 1.028)
3-week temperature lag ^c	1.007 (1.001, 1.012)	1.005 (1.001, 1.009)	1.010 (1.004, 1.015)
Moderate/strong El Niño period	1.049 (1.016, 1.084)	1.016 (0.992, 1.042)	1.016 (0.986, 1.046)
Dry season	1.031 (1.011, 1.051)	1.008 (0.992, 1.025)	1.014 (0.989, 1.040)
Rotavirus vaccine era (2010-2015) ^d	0.909 (0.855, 0.967)	0.918 (0.881, 0.956)	0.918 (0.869, 0.969)
Year (secular trend) ^e	0.979 (0.968, 0.991)	0.957 (0.946, 0.967)	0.968 (0.958, 0.979)

IRR = incidence rate ratio; CI = confidence interval

a.) “Low sewerage access” provinces were defined as those in which <30% of households had access to a toilet connected to the sewer system in all study years (2005-2015), or all but one year. “High sewerage access” provinces were those in which ≥30% of households had access to a toilet connected to the sewer system in all study years, or all but one year. “Transitional” provinces were those that did not fall into either category, *i.e.*, those that transitioned from lower sewerage access (<30% of households with access) to higher sewerage access (≥30% of households with a piped water connection) between 2005 and 2015.

b.) Combined effect of temperature across three weeks prior to weekly diarrhea report.

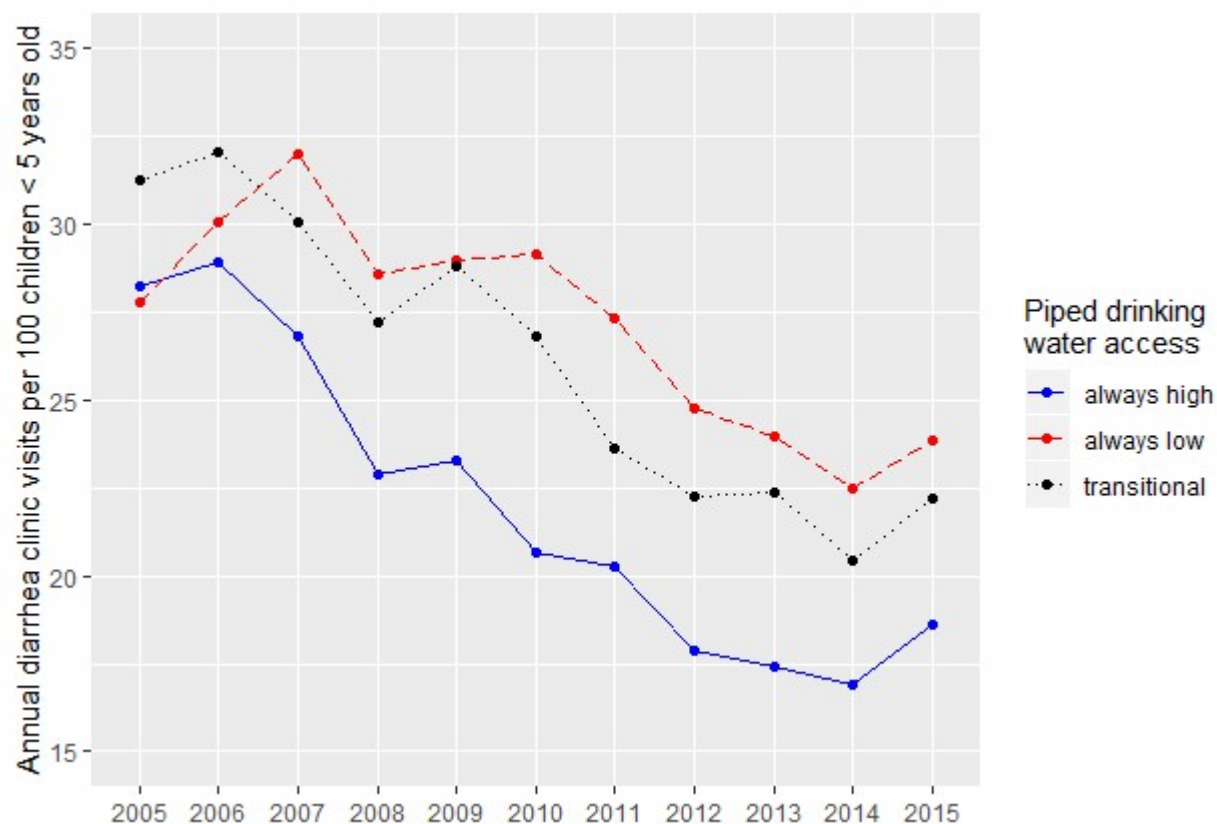
c.) The 1-week temperature lag is the effect of temperature in the week before the diarrhea cases, the 2-week lag refers to the week before that, etc.

d.) Compared to the pre-rotavirus vaccine era (2005-2009).

e.) Continuous term for year.

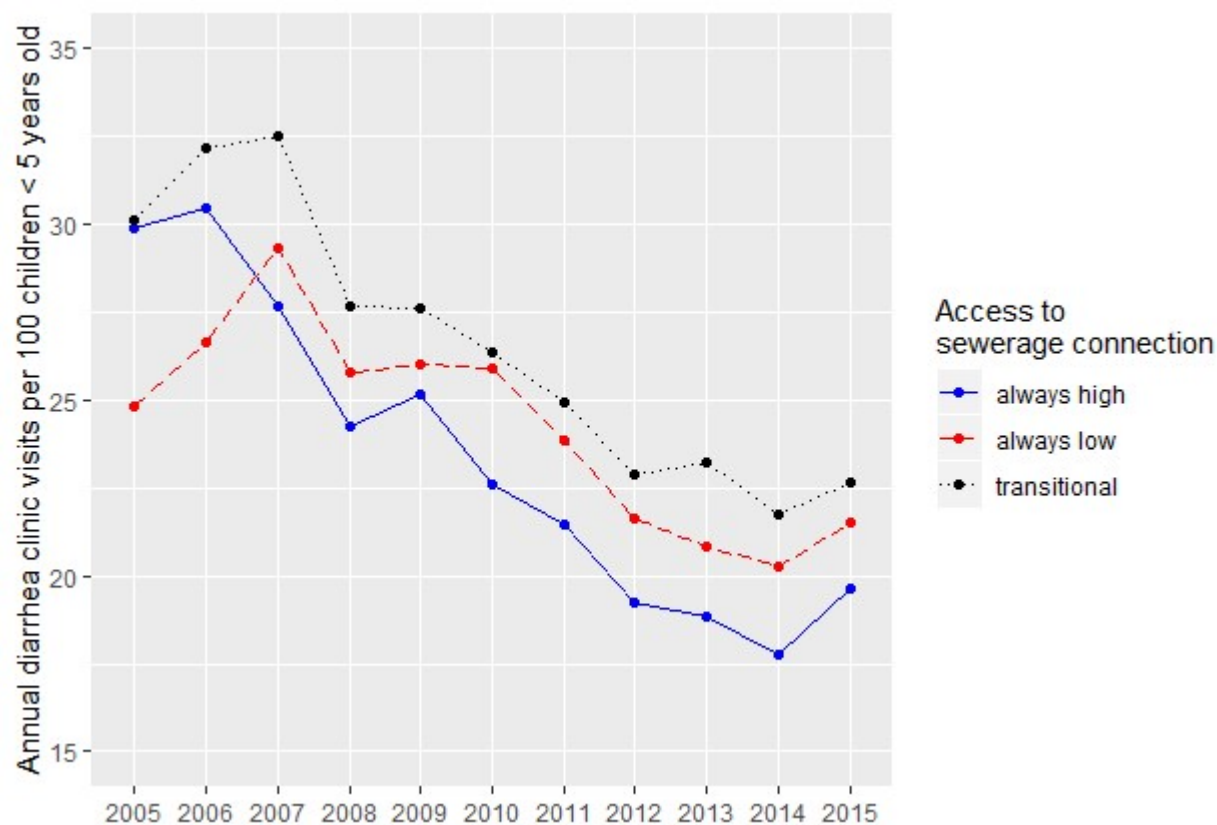
APPENDIX

Appendix Figure 3.1 Annual rate of clinic visits for childhood diarrhea in Peru, by level of access to piped water, 2005-2015



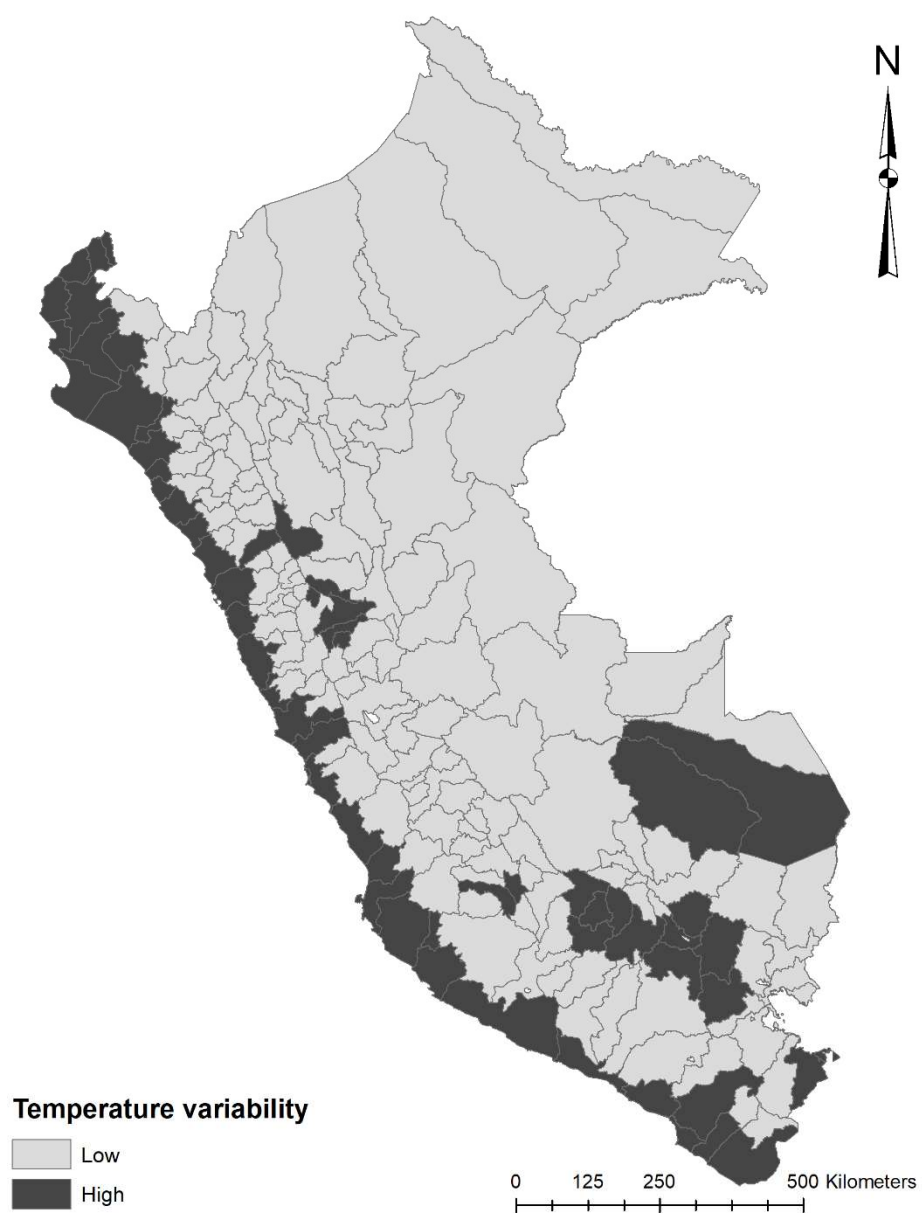
“Always high” water access refers to provinces in which $\geq 60\%$ of households had access to piped drinking water for every year (or all but one year) from 2005-2015. “Always low” water access refers to provinces in which $< 60\%$ of households had access to piped drinking water for every year (or all but one year) from 2005-2015. “Transitional” provinces were those that did not fall into either category, *i.e.*, those that transitioned from lower piped water access ($< 60\%$ of households with a piped connection) to higher water access ($\geq 60\%$ of households with a piped water connection) between 2005 and 2015.

Appendix Figure 3.2 Annual rate of clinic visits for childhood diarrhea in Peru, by level of access to sewerage, 2005-2015



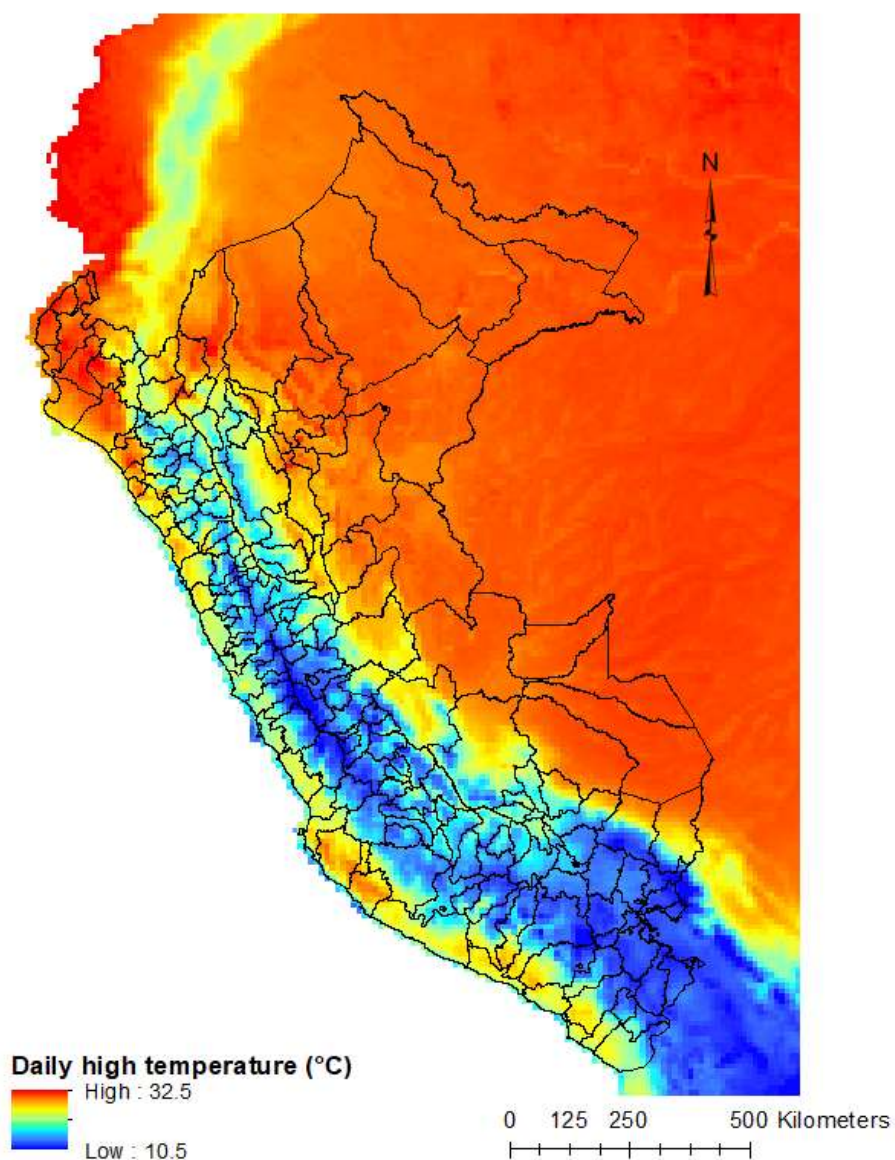
“Always high” sewerage access refers to provinces in which $\geq 30\%$ of households had a toilet connected to the sewerage network for every year (or all but one year) from 2005-2015. “Always low” sewerage access refers to provinces in which $< 30\%$ of households had a toilet connected to the sewerage network for every year (or all but one year) from 2005-2015. “Transitional” provinces were those that did not fall into either category, i.e., those that transitioned from lower sewerage access ($< 30\%$ of households with a toilet connected to the sewerage network) to higher sewerage access ($\geq 30\%$ of households with a toilet connected to the sewerage network) between 2005 and 2015.

Appendix Figure 3.3 Provinces of Peru with high and low annual temperature variability



Provinces with high temperature variability are defined as those in which the average daily high temperature in the warmest month of the year was at least 3 °C higher than it was in the coolest month of the year; other provinces are considered to have low temperature variability.

Appendix Figure 3.4 Sample gridded map of the estimated daily high temperature in provinces of Peru



Source: PISCO product (Peruvian Interpolation data of SENAMHI's Climatological and hydrological Observations); National Meteorology and Hydrology Service of Peru (SENAMHI)²⁸. Sample data for May 15, 2011.

Appendix Table 3.1 Comparison of model fit for different temperature variables

Description	QIC
Weekly average of daily mean temperatures	-55,233,362.89
Weekly average of daily high temperatures	-55,262,848.92
Weekly maximum of daily high temperatures	-55,245,730.09

Appendix Table 3.2 Association between meteorological factors and incidence rate of childhood clinic visits for diarrhea, controlling for rotavirus vaccination and secular trend, 61 provinces of Peru with higher temperature variability, by piped water access, 2005-2015

	Piped water access ^a		
	Low provinces (N=11)	High provinces (N=31)	Transitional provinces (N=19)
	IRR (95% CI)	IRR (95% CI)	IRR (95% CI)
Temperature across three weeks prior to diarrhea cases ^b	1.034 (1.018, 1.050)	1.044 (1.034, 1.054)	1.047 (1.037, 1.058)
1-week temperature lag ^c	1.010 (1.001, 1.019)	1.025 (1.016, 1.033)	1.026 (1.019, 1.033)
2-week temperature lag ^c	1.015 (1.007, 1.024)	1.012 (1.006, 1.018)	1.015 (1.007, 1.024)
3-week temperature lag ^c	1.008 (0.998, 1.019)	1.007 (1.000, 1.014)	1.006 (0.995, 1.016)
Moderate/strong El Niño period	1.047 (0.985, 1.113)	0.994 (0.965, 1.024)	1.005 (0.957, 1.056)
Dry season	1.087 (1.061, 1.113)	1.017 (0.995, 1.039)	0.986 (0.944, 1.030)
Rotavirus vaccine era (2010-2015) ^d	0.948 (0.842, 1.067)	0.924 (0.871, 0.980)	0.919 (0.861, 0.981)
Year (secular trend) ^e	0.951 (0.926, 0.976)	0.947 (0.932, 0.963)	0.950 (0.928, 0.974)

IRR = incidence rate ratio; CI = confidence interval

a.) “Low piped water access” provinces were defined as those in which <60% of households had access to a piped water connection in all study years (2005-2015), or all but one year. “High piped water access” provinces were those in which ≥60% of households had access to a piped water connection in all study years, or all but one year. “Transitional” provinces were those that did not fall into either category, *i.e.*, those that transitioned from lower piped water access (<60% of households with a piped connection) to higher water access (≥60% of households with a piped water connection) between 2005 and 2015.

b.) Combined effect of temperature across three weeks prior to weekly diarrhea report.

c.) The 1-week temperature lag is the effect of temperature in the week before the diarrhea cases, the 2-week lag refers to the week before that, etc.

d.) Compared to the pre-rotavirus vaccine era (2005-2009).

e.) Continuous term for year.

Appendix Table 3.3 Association between meteorological factors and incidence rate of childhood clinic visits for diarrhea, controlling for rotavirus vaccination and secular trend, 61 provinces of Peru with higher temperature variability, by sewerage access, 2005-2015

	Sewerage access ^a		
	Low provinces (N=13) IRR (95% CI)	High provinces (N=36) IRR (95% CI)	Transitional provinces (N=12) IRR (95% CI)
Temperature across three weeks prior to diarrhea cases ^b	1.044 (1.023, 1.065)	1.043 (1.034, 1.053)	1.049 (1.035, 1.063)
1-week temperature lag ^c	1.014 (1.004, 1.024)	1.025 (1.017, 1.033)	1.024 (1.017, 1.030)
2-week temperature lag ^c	1.016 (1.006, 1.026)	1.013 (1.008, 1.018)	1.018 (1.008, 1.027)
3-week temperature lag ^c	1.013 (1.000, 1.027)	1.005 (0.999, 1.012)	1.006 (0.997, 1.016)
Moderate/strong El Niño period	1.023 (0.951, 1.101)	0.996 (0.967, 1.026)	1.016 (0.966, 1.069)
Dry season	1.068 (1.028, 1.109)	1.011 (0.992, 1.031)	0.985 (0.927, 1.047)
Rotavirus vaccine era (2010-2015) ^d	0.963 (0.865, 1.072)	0.946 (0.902, 0.993)	0.815 (0.748, 0.887)
Year (secular trend) ^e	0.964 (0.939, 0.989)	0.942 (0.927, 0.957)	0.959 (0.934, 0.985)

IRR = incidence rate ratio; CI = confidence interval

a.) “Low sewerage access” provinces were defined as those in which <30% of households had access to a toilet connected to the sewer system in all study years (2005-2015), or all but one year. “High sewerage access” provinces were those in which ≥30% of households had access to a toilet connected to the sewer system in all study years, or all but one year. “Transitional” provinces were those that did not fall into either category, *i.e.*, those that transitioned from lower sewerage access (<30% of households with access) to higher sewerage access (≥30% of households with a piped water connection) between 2005 and 2015.

b.) Combined effect of temperature across three weeks prior to weekly diarrhea report.

c.) The 1-week temperature lag is the effect of temperature in the week before the diarrhea cases, the 2-week lag refers to the week before that, etc.

d.) Compared to the pre-rotavirus vaccine era (2005-2009).

e.) Continuous term for year.

Appendix Table 3.4 Association between meteorological factors and incidence rate of childhood clinic visits for diarrhea, controlling for rotavirus vaccination and secular trend, 172 provinces of Peru with highest or lower sewerage access, 2005-2015

	Sewerage access ^a	
	Lower provinces (N=161)	Highest provinces (N=11)
	IRR (95% CI)	IRR (95% CI)
Temperature across three weeks prior to diarrhea cases ^b	1.033 (1.027, 1.040)	1.043 (1.032, 1.055)
1-week temperature lag ^c	1.011 (1.007, 1.014)	1.027 (1.020, 1.034)
2-week temperature lag ^c	1.015 (1.012, 1.019)	1.010 (1.003, 1.017)
3-week temperature lag ^c	1.007 (1.004, 1.011)	1.006 (1.000, 1.011)
Moderate/strong El Niño period	1.030 (1.009, 1.051)	1.025 (0.989, 1.063)
Dry season	1.020 (1.006, 1.034)	1.030 (1.006, 1.055)
Rotavirus vaccine era (2010-2015) ^d	0.911 (0.878, 0.944)	0.918 (0.858, 0.983)
Year (secular trend) ^e	0.971 (0.963, 0.978)	0.960 (0.944, 0.976)

IRR = incidence rate ratio; CI = confidence interval

a.) “Highest sewerage access” provinces were those in which $\geq 60\%$ of households had access to a toilet connected to the sewer system in all study years, or all but one year. “Lower sewerage access” provinces were defined as those in which $<60\%$ of households had access to a toilet connected to the sewer system in all study years (2005-2015), or all but one year.

b.) Combined effect of temperature across three weeks prior to weekly diarrhea report.

c.) The 1-week temperature lag is the effect of temperature in the week before the diarrhea cases, the 2-week lag refers to the week before that, etc.

d.) Compared to the pre-rotavirus vaccine era (2005-2009).

e.) Continuous term for year.

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Chapter 4 Chemical and microbiological drinking water risks for infants in coastal Peru

Miranda J. Delahoy¹, Sydney Hubbard¹, Mia Mattioli¹, Carlos Culquichicón², Rebecca Hodge¹,
Forest Altherr¹, Lilia Cabrera³, Dana Boyd Barr¹, P. Barry Ryan¹, Andres G. Lescano²,
Robert H. Gilman⁴, Karen Levy¹

Author Affiliations: (1) Department of Environmental Health, Emory University, Atlanta, GA, United States, (2) Universidad Peruana Cayetano Heredia, Lima, Peru, (3) Asociación Benéfica PRISMA, Lima, Peru, (4) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

ABSTRACT

Background: Chemical and microbiological drinking water contamination pose numerous short- and long-term risks to child health but are not often evaluated concurrently. We conducted a study in Piura, Peru to assess heavy metals, pesticides/herbicides, and microbiological contamination of drinking water, in relation to diarrhea and enteric pathogen infections for 96 infants enrolled in a birth cohort study.

Methods: At two study visits 4-27 days apart, when study infants were approximately six months old, we collected drinking water samples, administered health and exposure surveys, and collected infant stool samples (at second visit only). Standard methods were used to quantify heavy metals, pesticides/herbicides, and the most probable number of *Escherichia coli* in water samples. Stool samples were assayed for bacterial, viral, and parasitic enteropathogens using the Luminex Multiplex Gastrointestinal Pathogen Panel.

Results: We found high chemical and microbiological exposures in drinking water samples, with arsenic detected in 50.0% of samples (N=96), at least one herbicide or pesticide detected in 65.2% (N=92), and *E. coli* detected in 37.0% (N=319). Storing drinking water was associated with substantially higher odds of both *E. coli* detection (adjusted odds ratio (aOR): 4.50, 95% confidence interval (CI): 2.04-9.95), and pesticide/herbicide detection (OR: 6.55, 95% CI: 2.05-20.96). Water samples from households with animals (aOR: 2.37, 95% CI: 1.09-5.13) and without piped sewerage (aOR: 2.23, 95% CI: 1.05-4.76) also had higher odds of *E. coli* detection. The infants in our study had high prevalence of diarrhea (13.8% of infants) and enteropathogen infections (68.1% with at least one infection) at the second visit. Higher odds of enteropathogen infection were observed in infants from households where pesticides/herbicides detected in drinking water (aOR: 2.93, 95% CI: 1.13-7.61). *E. coli* detection in drinking water was associated with concurrent infant diarrhea at the second visit only (OR: 14.77, 95% CI: 1.69-129.25).

Conclusions: These results show concurrent risks of microbiological and chemical exposures in a low-income setting and suggest that even improved drinking water sources contain contaminants that present health risks for infants. Safer drinking water storage may reduce risk of both chemical and microbiological drinking water contamination.

INTRODUCTION

Drinking water contamination poses several acute and long-term risks to child health. In low- and middle-income settings, extensive research has been conducted on microbial contamination of drinking water but less attention has been given to chemical contamination,¹ and few studies examine chemical and microbiological exposures concurrently. This is in part due to the large burden of disease posed by microbiological exposures in these settings. Nearly half a million global diarrheal deaths can be attributed to inadequate water annually.² Drinking water contaminated by

human or animal feces can cause acute or persistent diarrhea and/or enteropathogen infections, and can also lead to long-term shortfalls in physical growth and cognitive development, and inhibited oral vaccine response.³⁻⁵ Yet exposure to heavy metals and pesticides that may be present in drinking water can also have long-lasting impacts on immune function and cognitive development for children, especially when exposed during critical development periods, and chemical exposures have also been associated with diarrhea.⁶⁻⁸ Still, toxicological drinking water exposures have largely been studied in isolation from infectious contaminants, despite the potential of both types of agents to alter the probability and/or severity of pathogen infections.⁹

Provision of improved drinking water supplies may limit children's exposure to pathogens transmitted in water; however, improved drinking water sources are not always free of microbiological contaminants.¹⁰ Furthermore, traditional definitions of improved drinking water sources give little consideration to the physico-chemical properties of water sources.¹ The World Health Organization/United Nations International Children's Emergency Fund Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (WHO/UNICEF JMP) now suggests a gold standard of safely managed drinking water that is located on the premises, available when needed, and free of fecal and chemical contamination.¹¹ Piped drinking water supplies in low- and middle-income settings often fall short of meeting these criteria, in part because they fail to provide continuous service. Intermittent piped water service can pose challenges to maintaining high water quality within a system, and if water is stored in the household for use during service cuts.^{12,13}

In this study, we examine heavy metal, chemical, and microbiological quality of drinking water samples predominantly from improved water sources, collected from households with infants enrolled in a birth cohort in Piura, Peru. We examine water source characteristics and household-level factors associated with detection of *Escherichia coli*, arsenic, and pesticides/herbicides in drinking

water samples, and examine how the presence of these agents is associated with infant diarrhea and enteropathogen infection.

METHODS

Study site and enrollment

Study participants were randomly selected from a previously-established birth cohort. Midwives enrolled 327 mothers in November and December 2015 when they presented at the José Cayetano Heredia Hospital or the Santa Rosa Hospital in Piura, Peru to give birth. Inclusion criteria for the original birth cohort were that mothers had to reside in the department of Piura or Tumbes without plans to move residence, and had to deliver the infant vaginally, or by Cesarean section for reason of cephalopelvic disproportion or prolonged labor. Infants had to be viable at birth to be included. Exclusion criteria for mothers were lack of informed consent or plans to move residence outside of Piura and Tumbes. The birth cohort study involved a hemoglobin heel sample on infants, thus the following were exclusion criteria for infants: heel injury, skin infection of foot or heel, foot malformation, or edema of the foot. There was no incentive for participation offered for enrollment in the birth cohort.

The current study recruited a randomly selected eligible subset of these participants available for follow-up in June-July 2016. Inclusion criteria for the sub-study were that the infant and their caretaker had to reside in the department of Piura, and the guardian had to provide consent, which included consenting to provide approximately 25 L of drinking water for sampling and a stool sample from the study infant. Participants no longer residing in Piura or refusing consent were excluded; there were no additional exclusion criteria for the sub-study.

Participants in the sub-study resided in the provinces of Piura, Morropon, Paita, Sechura, and Sullana in the department of Piura (Figure 4.1). Piura is situated on the Pacific Coast of Peru and is

generally arid, although the department has experienced major flooding during El Niño events.¹⁴

The main industry in Piura in 2016 was agriculture/fishing. The two hospitals where mothers gave birth are characterized by different patient populations. The José Cayetano Heredia Hospital provides services to those with social security insurance. This health insurance is managed by the Peruvian Department of Labor and is available to those with stable employment. The Santa Rosa Hospital accepts the Peruvian Ministry of Health's universal insurance coverage; patients are generally of lower socio-economic status with unstable employment.

In Piura, piped water comes from two sources: aquifers with high mineral concentration, and surface water from the dam of the Daniel Escobar channel, which is thought to be of good chemical and physical quality. Water is treated at the Curumy water plant, which has been in operation since 2007, and treats water through a process of flocculation, sedimentation, filtration, and disinfection. The main distribution lines in the city are between 21 and 50 years old.¹⁵

Study visits and sample collection

Two study visits were planned for each household when infants were approximately six months old, with the second visit ("Visit 2" or "follow-up visit") occurring approximately one week (target range 4-10 days) after the first visit ("Visit 1") (Figure 4.2). At Visit 1, after obtaining written consent from each caretaker, trained enumerators administered a health and exposure questionnaire, including questions on household characteristics/demographics, water source and treatment, and diarrhea symptoms for the study infant. Diarrhea was defined as having three or more loose stools in a 24-hour period, or presence of blood in the stool. At this visit, we obtained multiple drinking water samples from the household's primary drinking water source: 100 mL for *Escherichia coli*/coliform testing, 15mL for heavy metal testing, and 1 L for pesticide/herbicide testing. Enumerators then asked if there was another source or container of household drinking water that the study infant

drinks, either alone or mixed in formula. If so, 100 mL of water were collected from that tap or container. If not, we asked if there was another source or container of water in the household that any household member drinks, and, where applicable, collected 100 mL from that source. This process was repeated until a total of two secondary/tertiary 100 mL drinking water samples were collected, or until there were no additional drinking water taps or containers to sample. Enumerators placed a numbered sticker on the water tap or container and recorded a written description, so that they could be re-identified and re-sampled at Visit 2.

At Visit 2, a short health questionnaire was administered, in which caretakers were asked whether infants had experienced diarrhea since the first study visit. The drinking water taps and containers sampled at Visit 1 were re-identified, and 100 mL of drinking water was collected from each for microbiological testing if water was available. If an infant defecated during the visit, a stool sample was collected at that time. Otherwise, caretakers were given a plastic container, gloves, and a diaper for collecting an infant stool sample, and the sample was collected later that day.

Participants were provided 25 L of bottled water at the conclusion of Visit 2. While we do not report results in this manuscript, at Visit 1 we collected a 20 L water sample using dead-end ultrafiltration that removes viruses, protozoa, and bacteria from the water;¹⁶ participants could opt to have the filtered water returned to a container in their household at the conclusion of Visit 1. Twenty-three (23) caretakers chose to have some portion of the 20 L returned to a household container.

Laboratory methods

Microbiological water testing

Field staff collected water samples in 100 mL Whirl-Pak sterile bags pre-packed with sodium thiosulfate to neutralize chlorine (Nasco, Fort Atkinson, WI, USA). Samples were transported on ice

from households to the laboratory in Piura and processed the same day with the IDEXX Colilert Quanti-Tray/2000 (IDEXX Laboratories, Westbrook, ME, USA). Samples were incubated at 37 °C for 24 hours, after which the most probable number (MPN) of *E. coli* and total coliforms were quantified, with a detection range of 1-2,419.6 MPN/100 mL. Field staff processed sterilized water samples approximately every other day in the laboratory in Piura (N=30, four of which were poured into a Whirl-Pak bag in the field) to serve as negative controls.

Quantification of heavy metals in water

Field staff collected 15 mL of water in a conical tube from the primary household drinking water source. Samples were stored in a refrigerator and shipped to Atlanta, Georgia for processing. Water samples were diluted with 2% nitric and 1% hydrochloric acid and analyzed using inductively-coupled plasma mass spectrometry (ICP-MS). Water samples (2 mL) were prepared concurrently with three blank samples, calibration samples, NIST reference material SRM 1643f, and two levels of quality control samples per analytic run. In order to dissolve the target elements into solution and to digest organic molecules, sample were digested with nitric acid before dilution with a mixture of internal standards (indium, iridium, lutetium, and rhodium). The digests were then analyzed via ICP-MS, removing spectral interferences with a collision reaction cell. Concentrations of the target elements were determined from the ratio of the instrument response to the native analyte to the response to the internal standards in the sample, by comparison to the standard curve. The limit of detection (LOD) for all heavy metals was 0.1 µg/L.

Quantification of pesticides and herbicides in water

One liter of water was collected from the primary household drinking water source in sterilized glass bottles for pesticide/herbicide analysis. Samples were transported to a laboratory in Piura, where the water was passed through solid phase extraction (SPE) cartridges (Phenomenex 8B-S043-HCH,

Torrance, CA, USA). Dried cartridges were eluted with ethyl acetate and methanol then the eluate was concentrated to dryness. Cartridges were stored in a sealed container with silica gel packets until transferred to the laboratory in Atlanta. Each dried sample was reconstituted with acetonitrile and spiked with isotopically labeled analogues of the target pesticides. Calibration samples, blanks and quality control samples were prepared similarly but were extracted in the lab rather than in the field. Extracts were analyzed using gas chromatography-tandem mass spectrometry with isotope dilution quantification.¹⁷

Target pesticides/herbicides were atrazine (LOD: 0.05 ng/L), diazinon (LOD: 0.125 ng/L), chlorpyrifos (LOD: 1.25 ng/L), p,p'-dichlorodiphenyldichloroethylene (pp-DDE; LOD: 0.05 ng/L), permethrin (LOD: 0.125 ng/L), and cypermethrin (LOD: 0.125 ng/L), chosen because their use patterns give them high potential to get into groundwater.

Enteropathogen detection in stool

Field staff collected stool samples using the OMNIgene-Gut stool collection and stabilization kit (OMR-200) (Genotek, Ottawa, Canada) and stored these at room temperature until processing in Atlanta. Samples were extracted using the QIAamp PowerFecal DNA Kit (Qiagen, Hilden, Germany). Stool samples were assayed for a panel 15 of bacterial, viral, and parasitic enteropathogens using the Luminex multiplex Gastrointestinal Pathogen Panel (Thermo Fisher Scientific, Waltham, MA). Bacterial targets on this panel include *Campylobacter* spp., *Clostridium difficile* toxin A/B, *E. coli* O157, enterotoxigenic *E. coli* (heat-stable toxin (ST) or heat-labile toxin (LT)), *Salmonella* spp., shiga toxin-producing *E. coli* (shiga toxin 1 (stx1) or shiga toxin 2 (stx2)), *Shigella* spp., *Vibrio cholerae*, and *Yersinia enterocolitica*; viral targets were adenovirus 40/41, rotavirus A, and norovirus GI/GII; protozoal targets were *Cryptosporidium* spp., *Giardia* spp., and *Entamoeba histolytica*.

Ethics

Study protocols and procedures were approved by the Emory (#IRB00088348) and A.B. PRISMA (CE1157.16) Institutional Review Boards.

Model descriptions, selection, and statistical analysis

Model descriptions

Appendix Table 4.1 contains a description of all multivariable models considered. Variables were selected for multivariable models associated with the following outcomes: detectable *E. coli* contamination in primary/secondary/tertiary drinking water samples collected at Visit 1 or Visit 2 (Model 1), arsenic concentration exceeding the WHO standard¹⁸ of 10 µg/L in primary drinking water samples collected at Visit 1 (Model 2), detection of any pesticide or herbicide in primary drinking water samples collected at Visit 1 (Model 3), infant diarrhea at Visit 2 (Models 4), and infant infection with any enteropathogen at Visit 2 (Model 5). All outcomes were binary (positive/negative).

In addition to the models described above, we ran additional sub-analyses to explore hypotheses about drinking water contamination and infant diarrhea using prospective vs. cross-sectional measurements. Such comparisons can be useful in generating hypotheses about associations between drinking water contamination and infant diarrhea (*e.g.*, whether drinking water contamination was a risk factor for infant diarrhea, or whether household factors might explain the co-occurrence of contaminated drinking water and diarrhea).

Models 1A-1C consider *E. coli* contamination of primary, secondary, and tertiary drinking water samples as an outcome and considered all the variables selected for Model 1 (factors associated with *E. coli* contamination of drinking water), plus an indicator of infant diarrhea, selected *a priori* for each

model. Model 1A considered drinking water samples collected at Visit 2 and included an indicator of whether the study infant had diarrhea at Visit 1, to examine the prospective association between infant diarrhea and subsequent household drinking water contamination. Model 1B also considered water samples collected at Visit 2 and included an indicator of whether the study infant had diarrhea at Visit 2, to examine the cross-sectional association between infant diarrhea and *E. coli* contamination of household drinking water. An analogous cross-sectional model was also conducted for Visit 1 water samples and infant diarrhea at Visit 1 (Model 1C).

Model 1D considered factors associated with *E. coli* contamination of drinking water samples collected at Visit 1 and Visit 2, but, unlike Model 1, was subset to drinking water samples that were stored in containers in the household. This allowed us to consider treatment and storage variables that were only applicable for stored samples. For this model we screened all the variables screened for Model 1, as well as indicator variables for whether the sample was stored on the ground or floor (versus on a countertop, table, or other household surface), whether the storage container was covered, and whether the caretaker reported the drinking water sample had been treated in the household by boiling, filtering, or chlorination.

Models that considered infant diarrhea and enteropathogen infection as an outcome also compared prospective vs. cross-sectional associations with *E. coli* detection in drinking water. Models 4A-4B consider all the variables selected for Model 4 (factors associated with infant diarrhea at Visit 2), and each also includes a variable selected *a priori* to consider *E. coli* contamination of drinking water. Model 4A includes an indicator of whether *E. coli* was detected in the primary household drinking water sample at Visit 1, to examine whether there was a prospective association between *E. coli* detection in drinking water at Visit 1 and subsequent infant diarrhea at Visit 2. Model 4B includes an indicator for whether *E. coli* was detected in the primary drinking water sample at Visit 2, to compare

the cross-sectional association between *E. coli* in drinking water samples and infant diarrhea. The cross-sectional association between *E. coli* in the primary household drinking water sample at Visit 1 and infant diarrhea at Visit 1 was also examined (Model 4C). The same process was used to assess a prospective vs. cross-sectional association between *E. coli* in the primary drinking water sample and infants having any enteropathogen infection at Visit 2 (Models 5A and 5B; Appendix Table 4.1). We also report separately whether specific enteropathogens were associated with infants having diarrhea at Visit 2; parameter estimates and confidence intervals for these univariable analyses were derived by standard logistic regression maximum likelihood methods.

Statistical analysis

Data were cleaned and analyzed using SAS 9.4 (SAS Institute, Inc., Cary, NC). For all models, variables of interest were screened using univariable logistic regression and included in a multivariable model if the univariable analysis *p*-value was <0.10 . If cell counts were <5 for any pair of variables, a Fisher's exact test was used for screening. We performed backward selection on multivariable models until all variables were statistically significant at $p<0.05$. All variables screened for each model are included in that model's results table with univariable analysis results. The enrollment hospital of each study participant, as well as whether the household had a refrigerator were considered in each analysis as indicators of socio-economic status. Model 1 and Models 1A-1D accounted for household clustering (by accounting for the number of households in the degrees of freedom choice for the Taylor series variance estimation), as multiple drinking water samples were collected in each household. Multi-collinearity was assessed in all multivariable models using conditional indices.¹⁹ No conditional indices in any model were >30 , thus no multi-collinearity problems are reported.

RESULTS

Enrollment, study visits, and infant characteristics

We enrolled 96 infants between June 14 and July 21, 2016. Two caretakers refused the second follow-up visit; 94 infants were available for follow-up and provided a stool sample at Visit 2. Follow-up visits were conducted a median of 6 days after the first visit (range: 4-27 days). Most follow-up visits (94%) occurred 4-10 days after the first visit, but 6% occurred >10 days after Visit 1.

The median age of the enrolled infants was 7.0 months (range 5.7-8.0 months old); 43% of enrolled infants were girls (Table 4.1). We enrolled a balance of infants born at the Santa Rosa hospital (47%) and José Cayetano Heredia hospital (53%). Other demographic and household characteristics are described in Table 4.1.

Caretakers reported at Visit 1 that all infants had ever been breastfed; 94% were still being breastfed and 97% were already receiving complementary solid foods. Not all infants included in analyses were regularly given drinking water: caretakers reported at Visit 1 that 12% of infants (all of whom were still breastfed) were not given any drinking water in the last week (either alone or mixed in formula).

Diarrhea and enteropathogen infections

Caretakers reported that 20% of infants had diarrhea in the past week at Visit 1; of the infants with a follow-up visit, caretakers reported that 14% had had diarrhea since the first visit. Enteropathogens were detected in 68% of the stool samples collected at Visit 2 (Table 4.1). The median number of enteropathogens detected per stool sample was one (range 0-5). The most commonly detected pathogens were *Salmonella* spp. (detected in 25.5% of stool samples), *Campylobacter* spp. (23.4%), and *Clostridium difficile* toxin A or B (23.4%) (Table 4.2). Infants with at least one enteropathogen detected in their stool at Visit 2 had higher odds of having diarrhea symptoms reported at the time of sample

collection [odds ratio (OR) 2.91, 95% confidence interval (CI): 0.60-14.03]. *Campylobacter* spp. and enterotoxigenic *E. coli* (ETEC) were associated with significantly elevated odds of caretaker-reported diarrhea symptoms at the time of sample collection (OR: 3.48, 95% CI: 1.03-11.79 and OR: 6.76, 95% CI: 1.53-29.83, respectively).

***Escherichia coli* detection in drinking water**

We collected and tested 347 household drinking water samples for *E. coli*, and additionally tested 30 negative controls for *E. coli* and total coliforms. None of the negative controls had detectable *E. coli*; however, two of these samples had low levels of total coliforms detected. The 28 household drinking water samples processed for *E. coli* on the two days when coliforms were detected in the negative controls were excluded from analyses. Ultimately, microbiological analyses were conducted on 319 household drinking water samples: 91 primary drinking water samples, 102 additional drinking water samples from secondary and tertiary sources at Visit 1, and 126 drinking water samples from Visit 2 (Figure 4.2).

The source of most of the drinking water samples was a piped water connection (72.1%), usually within the study household, although four samples came from an outdoor tap on the premises, and 17 samples came from a neighbor's piped water connection supply. Most samples (69.3%) were stored in containers in the household (Table 4.3).

Overall, 37% of samples tested positive for *E. coli* (concentration range: 1-1,299.7 MPN/100 mL; median concentration: 10.6 MPN/100 mL). In univariable analyses, the factors associated with significantly increased odds of *E. coli* detection in drinking water included: mother had not completed secondary school, presence of animals, and storage of water (Table 4.3). Factors significantly protective against *E. coli* detection included: piped drinking water source and the household having a toilet connected to the sewerage system.

The variables that remained in the final adjusted multivariable analysis of factors associated with *E. coli* in household drinking water samples (Model 1) included animals in the household (adjusted odds ratio (aOR): 2.37, 95% CI: 1.09-5.13), storage of water (aOR: 4.50, 95% CI: 2.04-9.95), and having a toilet connected to the sewerage system (aOR: 0.45, 95% CI: 0.21-0.95). Per a pre-specified analysis (Model 1A), we used the same final multivariable model to examine samples collected at Visit 2 only, and included an indicator for whether the infant previously had diarrhea at Visit 1. This model found prior infant diarrhea (aOR: 3.45, 95% CI: 1.20-9.96) and water storage (aOR: 13.53, 95% CI: 2.95, 62.09) to be significantly associated with *E. coli* contamination of water samples collected at Visit 2 (Appendix Table 4.2). There was also a strong cross-sectional relationship between infant diarrhea and *E. coli* contamination of drinking water at Visit 2 (Model 1B, Appendix Table 4.3), namely, Visit 2 samples from households in which the infant had diarrhea at that time had significantly elevated odds of *E. coli* contamination (aOR: 73.03, 95% CI: 4.75->999.99). Of the 13 water samples collected at Visit 2 from households where the study infant had diarrhea reported at that visit, only one was negative for *E. coli*. There was no significant cross-sectional association between infant diarrhea and *E. coli* contamination of drinking water samples at Visit 1 (Model 1C, Appendix Table 4.4).

E. coli detection in stored drinking water samples

We also evaluated the subset of 221 stored water samples (collected from 80 households). The associations between demographic/household characteristics and *E. coli* detection in this subset were similar to the associations when considering all drinking water samples (both stored and not stored). In an unadjusted analysis of stored water samples, piped water had marginally lower odds of being positive for *E. coli* (OR: 0.60, 95% CI: 0.29, 1.27). Water samples that caretakers had treated (boiled, filtered, or chlorinated) in the household had lower odds of *E. coli* contamination a univariable

analysis (OR: 0.37, 95% CI: 0.21, 0.65); however, this did not remain significant when controlling for other factors associated with *E. coli* contamination in stored water samples. In the final multivariable model of factors associated with *E. coli* detection in stored water samples (Appendix Table 4.5), stored water samples from households with animals had triple the odds of *E. coli* contamination (aOR: 3.02, 95% CI: 1.24-7.33) and water stored in containers on the ground had quadruple the odds of *E. coli* contamination (aOR: 4.16, 95% CI: 2.17-7.95). Water samples stored in covered containers had lower odds of *E. coli* contamination compared to samples from uncovered containers (aOR: 0.26, 95% CI: 0.10-0.67).

Heavy metals detection in drinking water

Of the 96 primary drinking water samples, half (50%) had detectable levels of arsenic (concentration range from below the average LOD across runs, namely 0.01 µg/L, to above the upper LOD of 25-40 µg/L), two samples (2%) had detectable lead levels (2.31-2.78 µg/L), and one sample had a detectable level of chromium (1.84 µg/L); cadmium was not detected in any sample (Figure 4.3).

Of the 48 drinking water samples positive for arsenic, 24 (50%) had an arsenic concentration exceeding the WHO limit of 10 µg/L. All 24 of these samples were collected from households where the mother had given birth at the Santa Rosa hospital. In univariable analyses (Table 4.4), piped drinking water samples had marginally higher odds of arsenic contamination exceeding 10 µg/L (OR: 3.67, 95% CI: 0.78-17.14), and samples from households in which the caretaker reported insecticides use in the home had significantly higher odds (OR 3.55, 95% CI: 1.35-9.31). As only two variables (enrollment hospital and household insecticide use) met the criteria for inclusion in a multivariable model (Model 2) and all positive samples were from the same hospital, multivariable modeling was not conducted.

Pesticide/herbicide detection in drinking water

Results of pesticide/herbicide detection in drinking water were available for most of the 96 primary water samples, although permethrin concentrations could not be determined for six households and atrazine concentrations were not determined for five households; cypermethrin, pp-DDE, diazinon, and chlorpyrifos levels were missing for one household each. There was at least one pesticide/herbicide detected in 65% of primary water samples from households with available data. Atrazine was most commonly detected (48% of samples; concentration range: 0.06-29.37 µg/L); chlorpyrifos was detected in 15% of samples (range: 3.71-21.41 µg/L), pp-DDE in 14% (range: 0.36-2.15 µg/L), and cypermethrin in 12% (range: 1.19-9.71 µg/L) (Figure 4.4). No drinking water samples had detectable levels of diazinon or permethrin.

In univariable analyses (Table 4.5), the odds of pesticide/herbicide detection were higher in drinking water samples stored in containers in the household (OR 6.55, 95% CI: 2.05-20.96). Enrollees from the Santa Rosa hospital tended to have lower odds of pesticide/herbicide detection in water (OR 0.46, 95% CI: 0.19-1.09). Only drinking water storage met criteria for inclusion in a final multivariable model (Model 3), thus no adjusted estimates are presented.

Characteristics associated with infant diarrhea

Having a primary drinking water sample with arsenic levels ≥ 10 µg/L (collected at Visit 1) was associated with higher odds of caretakers reporting infant diarrhea at Visit 2 (OR: 3.00, 95% CI: 0.90-10.06; Table 4.6). This was the only variable that met screening criteria for a multivariable model of factors associated with infant diarrhea at Visit 2 (Model 4), thus no adjusted estimates are presented.

Based on an *a priori* analysis plan, we considered the association between infant diarrhea at Visit 2 and *E. coli* in the primary drinking water source at Visit 1 (Model 4A) and Visit 2 (Model 4B). *E. coli*

contamination of the primary drinking water source at Visit 1 was associated with marginally higher odds of infant diarrhea at Visit 2 (OR: 1.64, 95% CI: 0.48-5.57); there was a strong cross-sectional association between *E. coli* contamination of the primary water source at Visit 2 and infant diarrhea at that same visit (OR: 14.77, 95% CI: 1.69-129.25; Table 4.6).

No screened household or water characteristics were significantly associated with infants having diarrhea at Visit 1 (Model 4C; not presented).

Characteristics associated with enteropathogen infection

In univariable analyses, factors associated with infant enteropathogen infection (measured at Visit 2) were having a pesticide/herbicide detected in the primary water sample at Visit 1, and caretakers reporting on the Visit 1 survey that infants had been given any drinking water in the past week (Table 4.7). Both variables were retained in the final multivariable model of factors associated with infant enteropathogen infection (Model 5). Infants from households that had a primary drinking water sample positive for any pesticide/herbicide (measured at Visit 1) had higher odds of having an enteropathogen infection at Visit 2 (aOR: 2.93, 95% CI: 1.13-7.61). Compared to 11 infants whose caretakers reported they were not given drinking water in the week prior to the Visit 1 survey, infants given drinking water had higher odds of enteropathogen infection at Visit 2 (aOR: 4.36, 95% CI: 1.11-17.07). There was no significant association between having *E. coli* detected in the primary drinking water sample at Visit 1 (Model 5A), or Visit 2 (Model 5B), and infant enteropathogen infection at Visit 2 (models not presented).

DISCUSSION

In this study we combined chemical and microbiological methods to test drinking water samples in 96 households with infants in Piura, Peru for contamination. The majority of water samples came from a piped water supply and nearly all samples were from improved water sources, yet we found

widespread contamination of drinking water. Arsenic, at least one herbicide or pesticide, and *E. coli* were all found in each type of drinking water source we collected (piped drinking water, protected wells, public water basins, bought/bottled drinking water, and unprotected wells; Figure 4.3).

Drinking water storage was strongly associated with *E. coli* and pesticide/herbicide contamination. At least one enteropathogen infection was detected in 68% of infant stools tested, and caretakers reported 14% of infants had diarrhea at Visit 2; we observed positive associations between both chemical and microbiological water contamination and adverse infant health outcomes. Our results point to the concurrent risks of microbiological and chemical exposure in a low-income setting with high access to piped water, and suggest that safer drinking water storage may reduce health risks.

Microbiological contamination of drinking water and infant diarrhea

E. coli was detected in 37% samples, even though nearly all were from improved sources (316/319), with the majority coming from a piped distribution system. This highlights that improved drinking water sources that are not safely managed (*e.g.*, not available when needed) are prone to contamination.

Our study had the strength of comparing a cross-sectional association between indicator *E. coli* and infant diarrhea with a prospective association. We anticipated that having *E. coli* detected in the primary drinking water sample at Visit 1 would be associated with higher odds of the infant subsequently having diarrhea at Visit 2; the result trended in that direction but was not statistically significant (OR: 1.64, 95% CI: 0.48-5.57). However, having *E. coli* detected in the primary water source at Visit 2 was cross-sectionally associated with the infant having diarrhea reported at that time (OR: 14.77, 95% CI: 1.69-129.25). This finding is consistent with a systematic review that found a positive association between indicator *E. coli* in drinking water and child diarrhea.²⁰ Only one of the 20 studies in that review had a prospective study design with water sampling occurring

before diarrhea surveillance;^{21,22} most analyses employ a cross-sectional design concurrently evaluating diarrhea and water quality. Many waterborne diarrheagenic pathogens cause disease within 3-7 days with symptoms often resolving in a week,²³ thus our Visit 1 water sampling could have fallen in a critical exposure window for risk of diarrhea at Visit 2 (usually 4-10 days after Visit 1, range 4-27 days). Despite a design allowing us to plausibly hypothesize a causal link between *E. coli* in drinking water and subsequent infant diarrhea, we did not find such an association.

Drinking water samples at Visit 2 had higher odds of *E. coli* contamination if the study infant had prior or concurrent diarrhea. This suggests that infants with diarrhea may pose a risk to household drinking water quality, an alternative explanation of a cross-sectional water quality and diarrhea relationship. We did not collect data on disposal of infant feces, but unsafe disposal could pose a risk of contaminating stored drinking water via caretakers' hands. Other research has found a positive association between fecal indicators on hands and in stored household drinking water.²⁴ Our finding of a positive cross-sectional association between *E. coli* in drinking water and child diarrhea differs from a study that found a significant negative cross-sectional association between child diarrhea and microbial contamination of drinking water, perhaps due to caretakers taking precautionary treatment measures for water when children are ill.²⁵

Odds of *E. coli* detection in drinking water were higher for households that kept animals in or around the home (aOR: 2.37, 95% CI: 1.09-5.13). Infants from households with animals also had marginally higher odds of having diarrhea at the second study visit (OR: 2.32, 95% CI: 0.48-11.25). Animals harbor many pathogens capable of infecting humans and producing acute or long-term adverse health outcomes,²⁶ thus attention should be given to separating animal feces from stored drinking water. While improved sanitation may limit exposure to human feces, there may be residual animal feces contamination in households where animal waste is not contained. Unfortunately,

indicator organisms such as those used in our study cannot discern whether microbiological water contamination was a result of human or animal fecal contamination.

Our study found a protective effect of having a toilet connected to piped sewerage on *E. coli* detection in drinking water (aOR: 0.45, 95% CI: 0.21-0.95); however, this result was not statistically significant in the smaller subset of only stored samples. A 2016 review of the effects of sanitation found six studies in which sanitation improvements were not associated with stored drinking water quality, whereas only one study found a protective effect of sanitation for *E. coli* in stored drinking water.²⁷

Chemical/heavy metal drinking water contamination

Arsenic contamination

Others have found high prevalence of arsenic in groundwater^{28,29} and surface water in Peru,²⁸ and suggest sources of arsenic may be from natural deposits, mining activities, or insecticide/pesticide production.²⁸ We identified only one previous study in Peru that considered arsenic in drinking water samples collected in the household, conducted in a small mining/smelting town in central Peru.³⁰ We believe this is the first characterization of arsenic in household drinking water samples in the urban and peri-urban areas of a large Peruvian city; the high prevalence of arsenic in drinking water samples in this region is alarming, especially given the high detection in a piped water system and the percentage of all samples (25%) exceeding the WHO arsenic standard of 10 µg/L. Addressing arsenic contamination of drinking water can be challenging, and have unintended consequences on child health when not considered in conjunction with microbial water quality.³¹

Prior research has found an association between arsenic exposure in drinking water and reduced cognitive development in children.^{32,33} Arsenic exposure *in utero* has also been associated with worse birth outcomes and infant mortality.³² Detection of arsenic in drinking water at Visit 1 trended

toward elevated odds of infant diarrhea at the follow-up visit (OR: 3.00, 95% CI: 0.90-10.06). Prior research has consistently demonstrated increased prevalence of infant diarrhea for those with increased arsenic exposure *in utero*, but the effects of arsenic exposure in infancy on diarrhea have not been characterized.^{34–37} Animal research suggests the association between arsenic exposure and diarrhea may be characterized by increased intestinal motility;³⁸ while little is known about such associations in children, arsenic-induced increased gut motility could explain why an association between arsenic and diarrhea, but not arsenic and enteropathogen infection, was observed in this study. However, animal studies also suggest arsenic exposure may be associated with increased virulence of infections and lowered immune response; little is known about the effect of arsenic exposure on the infant immune system.³⁷

All 24 drinking water samples with arsenic concentration ≥ 10 $\mu\text{g/L}$ came from households where the study infant's mother gave birth at the Santa Rosa hospital, whose patients generally have unstable employment and lower socio-economic status. Two-thirds (67%) of caretakers enrolled at the Santa Rosa hospital reported that they had been concerned about having enough food to eat at some point in the four weeks prior to our first study visit (compared to 49% of José Cayetano Heredia hospital enrollees), thus arsenic exposure is affecting a particularly vulnerable group of children. Previous evidence has suggested lower socio-economic status to be detrimental to child health in Peru, namely lower socio-economic status was found to be associated with increased enteropathogen infections.³⁹ It is possible that the households in the Santa Rosa cohort share a common water provider, although we were unable to evaluate this in our data.

Reported use of insecticides in the home was associated with increased odds of arsenic concentration ≥ 10 $\mu\text{g/L}$ in primary drinking water samples. It is possible that insecticides containing arsenic are contaminating drinking water, although our results do not establish a causal link and such

pesticides are more likely used for agriculture rather than in the home. The use of arsenic-based pesticides has declined since the introduction of DDT;⁴⁰ however use of banned pesticides has been anecdotally reported in Piura and documented elsewhere in Peru.

Pesticides/herbicides

Higher odds of enteropathogen infection were observed in infants from households with pesticides/herbicides detected in drinking water (aOR: 2.93, 95% CI: 1.13-7.61). It has been hypothesized that pesticide exposure may affect human immune response, though epidemiologic data on this association are sparse.⁴¹ There may be commonalities between households in which pesticides/herbicides are found in drinking water and infants with enteropathogens—for example households in rural areas may have both higher pesticide/herbicide use as well as more environmental exposure to enteropathogens—however, we were unable to assess this.

Drinking water storage

While 77% of households had an indoor piped drinking water connection, 55% of these households reported that their water was cut off at least once in the week prior to our first study visit, and household drinking water storage was common. Storing drinking water was associated with substantially higher odds of both *E. coli* and pesticide/herbicide detection. This is consistent with previous research showing post-collection contamination of stored water, with considerable change in quality for water that was relatively uncontaminated at the source.⁴² However, few other studies also highlight the chemical risks of drinking water storage.

Uncovered storage containers and containers on the ground had substantially higher odds of *E. coli* contamination, suggesting that safe drinking water storage--in which water containers have small, covered openings, and a small valve or spigot for pouring--could be beneficial.⁴³ Safe storage messaging may be particularly beneficial in agricultural communities, as odds of detecting a

pesticide/herbicide in drinking water tended to be higher when the mother or father of the study infant worked in agriculture (OR: 2.67, 95% CI: 0.70-10.19). We did not evaluate factors associated with pesticide/herbicide contamination in stored samples only, as there were so few stored drinking water samples (N=4) that did not have pesticide/herbicide contamination.

Enteropathogen infections

Compared to research on child diarrhea, there has been less attention on child enteropathogen infections and their association with drinking water quality measurements. Recent large multi-center studies have shed more light on risk factors for infant enteropathogen infection, including the Global Enteric Multicenter Study (GEMS) and the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study, though these studies have mostly considered caretaker reported water treatment and source types in the absence of household water samples.^{44,45}

We found higher odds of enteropathogen infection in infants coming from households where the primary water source had a pesticide/herbicide detected, but did not find an association between *E. coli* detection in drinking water and subsequent enteropathogen infection. Possible reasons for this lack of an association include (1) an inadequate sample size to assess this association, (2) infants not consistently drinking the water that was sampled, (3) the ability of an indicator organism to establish risk of pathogen contamination of water, or (4) predominant enteropathogen transmission pathways other than drinking water.⁴⁶

Notably, there were 11 infants who were not given drinking water in the week prior to our first survey, and these infants had significantly lower odds of having an enteropathogen isolated in their stool the following week, suggesting that consumption of drinking water may be a risk for infant enteropathogen infection.

We had a limited sample size to evaluate the association between specific enteropathogens and diarrhea, but most enteropathogens were associated with elevated odds of diarrhea, though few of these associations met the threshold of statistical significance. There was an unexpectedly high prevalence of *Clostridium difficile* toxins A and B in stool samples (23.4%); this was the one enteric pathogen that did not tend toward association with higher odds of infant diarrhea (OR: 0.56, 95% CI: 0.11-2.72). Little is known about infant response to *C. difficile* colonization, and clinical disease associated with these toxins may be rare in this age group, as receptor sites for the toxins are not fully developed in infants.⁴⁷⁻⁴⁹ Infants with *Campylobacter* spp. detected in their stool at the follow-up visit had higher odds of having diarrhea reported at that time (OR: 6.76, 95% CI: 1.53-29.83). *Campylobacter* was the leading pathogen to which diarrhea cases in infants (0-11 months) were attributable to at the Peru MAL-ED site (in Loreto, Peru).⁴⁵ Previous research in Peru suggests that the presence of chickens in the household, which was common in our study, may be a risk factor for childhood *Campylobacter* infections.⁵⁰

Limitations

Our study had a small sample size and limited power to detect associations between water quality and health outcomes. Our assessment of microbial water quality as a risk of subsequent infant diarrhea or enteropathogen infection was limited by the fact that not all infants were given drinking water from the household's primary water source, thus contamination of drinking water may not have aligned with a risk of exposure. When considering diarrhea at the second visit, the recall window varied, as we asked caretakers to report whether an infant had had diarrhea since our last study visit, a median of six days earlier but with a range of 4-27 days. We did not have consistent definitions of urban, peri-urban, or rural neighborhoods in our study, making hypotheses about common exposures in these settings challenging.

Conclusions

We found widespread microbiological and chemical contamination of drinking water in Peruvian households with infants, despite most households having access to piped drinking water. Drinking water storage was associated with higher levels of microbiological and herbicide/pesticide contamination, and water was often stored in uncovered containers and/or stored on the ground, which was associated with worse microbiological water quality. The contaminants found in these water samples (including pesticides/herbicides and arsenic) have been previously linked with adverse health outcomes in infants. Arsenic exposure in this setting appears to be affecting more vulnerable populations of infants whose parents may have unstable employment. Despite a limited sample size, we were able to identify risk factors for infant diarrhea and infant enteropathogen infection.

Infants in this study are at high risk of exposure to drinking water contaminants that have previously been linked with impaired cognitive growth, furthermore the majority (68%) of them had an enteropathogen infection at a young age, which is also of concern for cognitive development and adverse health outcomes. The range of drinking water contaminants and enteropathogen exposures suggest that infants may be subject to persistent immune system disruption or gut inflammation during a critical period of development. Our study took a holistic approach to examining a range of drinking water exposures in households with infants, and further follow-up with this cohort to assess health and cognitive outcomes could provide insight into environmental exposures and child development.

FUNDING/DISCLAIMER

This work was supported by the HERCULES: Exposome Research Center [grant number NIEHS: P30 ES0197767] at Emory University and by the National Institute for Allergy and Infectious Diseases [grant number K01AI103544] at the US National Institutes of Health. The content is solely

the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ACKNOWLEDGEMENTS

We acknowledge and thank María del Rosario Jaramillo Ramírez and Alisson Zevallos-Concha for field data collection and survey administration. We also acknowledge and thank Shanon M. Smith, Jeticia R. Sistrunk, Priya E. D'Souza, and Parinya Panuwet of Emory University, and Jackie Knee and Joe Brown from the Georgia Institute of Technology for laboratory analyses. Thank you to Thomas Clasen, Benjamin Lopman, Kyle Steenland, Naser Titu, and Michael Caudle for input on interpreting results.

AUTHOR CONTRIBUTIONS

Conceptualization: MJD, MM, RHG, KL; **Data collection:** MJD, RH, FA, LC, AGL, RHG; **Data analysis:** MJD, SH; **Laboratory protocols:** MM, DBB, PBR, KL; **Laboratory (microbiological):** MJD, MM, RH, FA; **Laboratory (chemical):** RH, DBB, PBR; **Methodology (non-laboratory):** MJD, MM, RH, FA, KL; **Project administration:** MJD, MM, CC, LC, AGL, RHG, KL; **Writing (original draft preparation):** MJD; **Writing (review and editing):** MJD, CC, KL

FIGURES AND TABLES

Figure 4.1 Study site (provinces of Piura, Morropon, Paita, Sechura, and Sullana, department of Piura), birth cohort sub-study of water quality contamination in households with infants, 2016

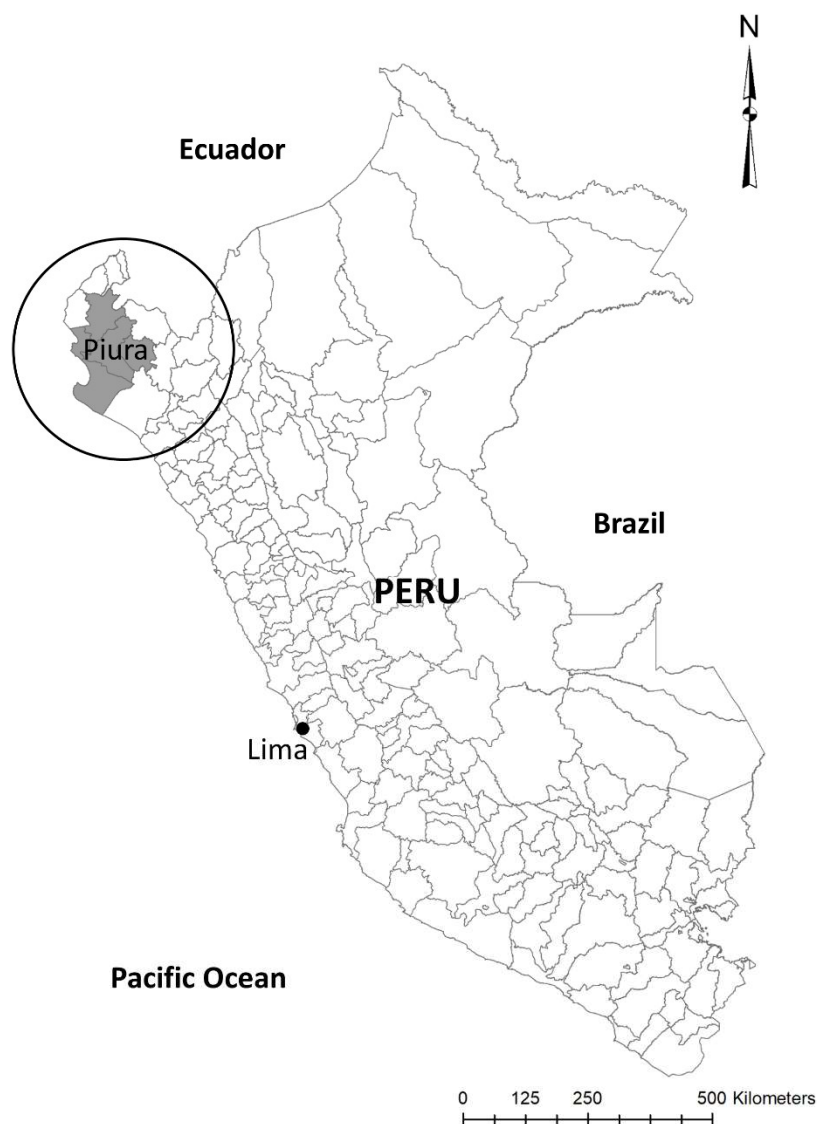
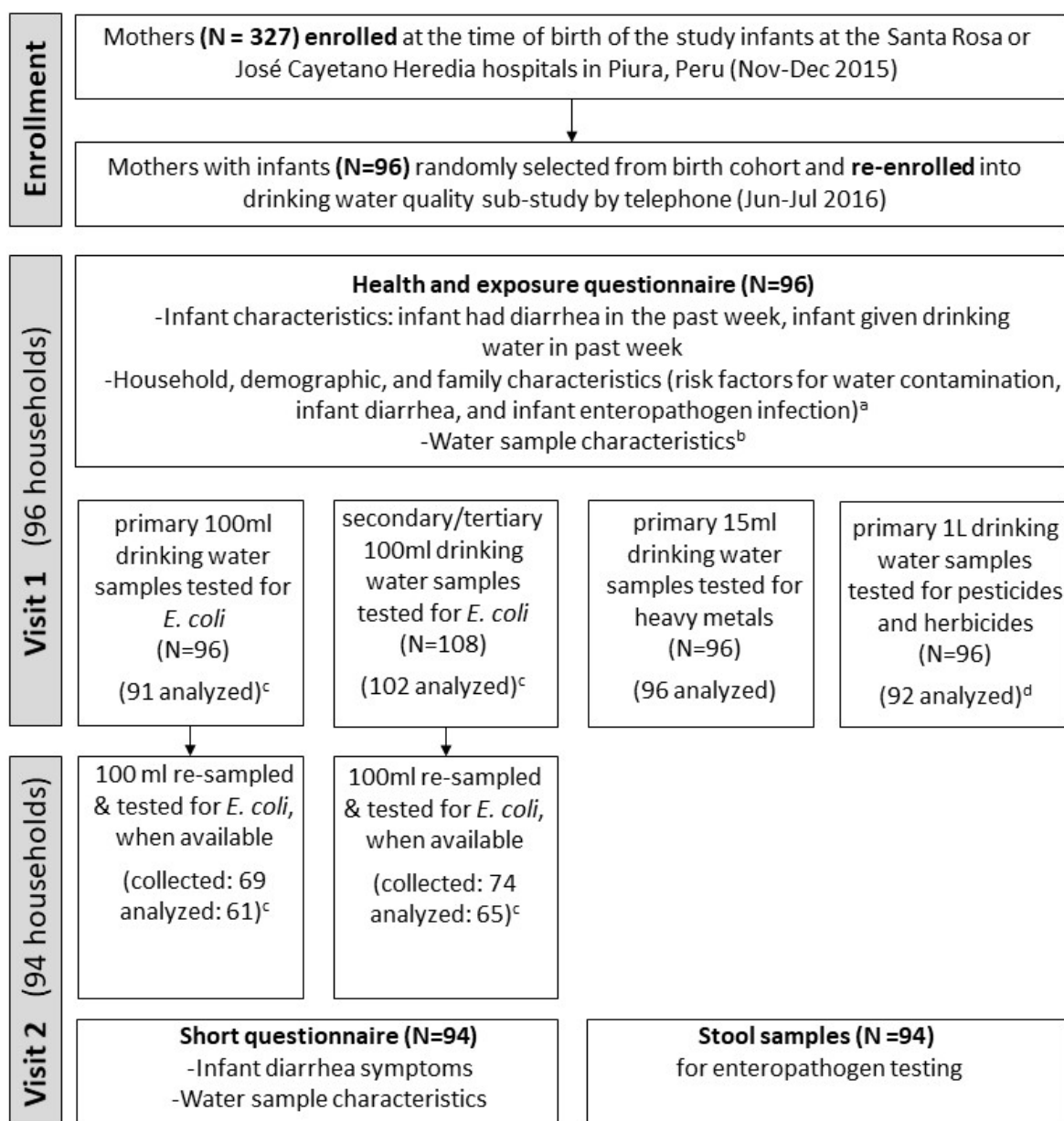


Figure 4.2 Household visits for study of water quality in households with infants, Piura, Peru, 2016



(a.) owns refrigerator, maternal education, parent works in agriculture, home insecticide use, animal presence, sanitation, total number of children under 5 years old residing in household

(b.) water source, water storage (yes/no, stored on ground, stored in container), water treatment

(c.) 28 samples were collected and tested for *E. coli* but not included in models due to potential laboratory contamination on the two days on which these samples were processed.

(d.) Permethrin concentrations not determined for six households; atrazine concentrations not determined for five households; cypermethrin, pp-DDE, diazinon, and chlorpyrifos levels were missing for one household each. We ascertained whether 92 samples were positive/negative for any pesticide/herbicide: 87 samples with complete pesticide/herbicide results, and 5 samples positive for at least one pesticide/herbicide, despite missing information for one pesticide/herbicide each.

Figure 4.3 Detection of *Escherichia coli*, arsenic, and pesticides/herbicides in primary drinking water sources of household with infants (N = 96) in Piura, Peru, 2016

	<i>E. coli</i> [†]	arsenic	atrazine	cyper-methrin	pp-DDE	chlor-pyrifos
Indoor piped water connection	11/68 (16.2%)	38/70 (54.3%)	32/69 (46.4%)	7/70 (10.0%)	7/70 (10.0%)	3/70 (4.3%)
Outdoor piped water connection	2/2 (100%)	1/2 (50.0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	2/2 (100%)
Neighbor's piped water connection	3/4 (75.0%)	2/4 (50.0%)	1/3 (33.3%)	0/4 (0%)	2/4 (50.0%)	1/4 (25.0%)
Protected well	6/7 (85.7%)	2/7 (28.6%)	6/6 (100%)	2/7 (28.6%)	3/7 (42.9%)	4/7 (57.1%)
Public water basin	2/5 (40.0%)	2/5 (40.0%)	1/5 (20.0%)	1/5 (20.0%)	0/5 (0%)	1/5 (20.0%)
Bought water: tanker truck or other bought/bottled water	2/4 (50.0%)	2/7 (28.6%)	4/5 (80.0%)	1/6 (16.7%)	0/6 (0%)	3/6 (50.0%)
Unprotected well	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
TOTAL	27/91 (29.7%)	48/96 (50.0%)	44/91 (48.4%)	11/95 (11.6%)	13/95 (13.7%)	14/95 (14.7%)

<5 samples	no contaminant detected	contaminant detected (<50% of samples)	contaminant detected (≥50% of samples)
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[†]Indicator *Escherichia coli*; limits of detection in most probable number (MPN): 1-2,419.6 MPN/100 mL; range detected: 1-1,299.7 MPN/100mL; median concentration: 10.6 MPN/100mL.

Lower limits of detection (LOD): heavy metals (0.1 µg/L), atrazine (0.05 ng/L), diazinon (0.125 ng/L), chlorpyrifos (1.25 ng/L), p,p'-dichlorodiphenyldichloroethylene (pp-DDE, 0.05 ng/L), permethrin (0.125 ng/L), and cypermethrin (0.125 ng/L). Ranges detected in samples: arsenic (0.01 µg/L to above the upper LOD of 25-40 µg/L), atrazine (0.06-29.37 µg/L); chlorpyrifos (3.71-21.41 µg/L), pp-DDE (0.36-2.15 µg/L), cypermethrin (1.19-9.71 µg/L).

Other contaminants detected included lead (detected in two samples) and chromium (detected in one sample). Cadmium, diazinon, and permethrin were not detected in any sample.

Table 4.1 Demographic and household characteristics, and infant diarrhea/enteropathogen infection, Piura, Peru, 2016

	Percentage of study infants (N = 96)
Demographic characteristics	
Female	42.7%
Age in months: median (range)	7.0 (5.7-8.0)
Mother completed secondary school or above	69.8%
Enrolled at Santa Rosa hospital	46.9%
Enrolled at José Cayetano Heredia hospital	53.1%
Household	
Household has refrigerator	67.7%
Animals reside in/around house	71.9%
Dog	39.6%
Cat	25.0%
Other mammal	8.3%
Chickens or other birds	35.4%
Other child(ren) <5 years old reside in household	40.6%
Breastfeeding and infant feeding	
Ever breastfed	100.0%
Still breastfed	93.8%
Eats solid food	96.9%
Diarrhea and enteropathogen infection	
Caretaker reported diarrhea at Visit 1‡	19.8%
Caretaker reported diarrhea at Visit 2‡	13.8%
Enteropathogen detected in stool at Visit 2	68.1%
Number of enteropathogens detected in stool: median (range)	1 (0-5)

‡Caretakers reported whether infants had diarrhea in the week prior to Visit 1, and whether they had diarrhea between Visit 1 and Visit 2. Visit 2 occurred a median of 6 days after Visit 1, with 94% of second visits occurring within 4-10 days after the first visit (range 4-27 days). Information on Visit 2 diarrhea and enteropathogen detection were available for 94 of the 96 infants.

Table 4.2 Detection of enteropathogens and association with diarrhea in stool samples from 94 infants in Piura, Peru, 2016

	Percent of samples (N=94) positive	Diarrhea odds ratio‡ (95% confidence interval)
Any enteropathogen	64 (68.1%)	2.91 (0.60, 14.03)
<i>Salmonella</i> spp.	24 (25.5%)	1.36 (0.38, 4.88)
<i>Campylobacter</i> spp.	22 (23.4%)	3.48 (1.03, 11.79)
<i>Clostridium difficile</i> (toxins A or B)	22 (23.4%)	0.56 (0.11, 2.72)
Enterotoxigenic <i>Escherichia coli</i> (ST/LT)	9 (9.6%)	6.76 (1.53, 29.83)
<i>Giardia</i> spp.	8 (8.5%)	2.27 (0.41, 12.70)
<i>E. coli</i> O157	5 (5.3%)	1.60 (0.17, 15.59)
<i>Shigella</i> spp.	3 (3.2%)	n/a
shiga toxin-producing <i>E. coli</i> (stx1, stx2)	2 (2.1%)	n/a
Adenovirus (40/41)	2 (2.1%)	n/a
<i>Cryptosporidium</i> spp.	1 (1.1%)	n/a
Norovirus (GI/GII)	1 (1.1%)	n/a

‡Considers odds of infant diarrhea at the time of stool collection.

Odds ratios not calculated for enteropathogens detected in <5 stool samples.

Vibrio cholerae, *Yersinia enterocolitica*, rotavirus, and *Entamoeba histolytica* were not detected in any samples.

Table 4.3 Association between demographic/household & water sample characteristics and *Escherichia coli* detection in drinking water samples (N=319) from households with infants, Piura, Peru, 2016

	Drinking water samples positive for <i>E. coli</i>	OR (95% CI)	aOR (95% CI)
Demographic & household characteristics			
Enrollment hospital			
Santa Rosa	56/143 (39.2%)	1.18 (0.59, 2.38)	--
José Cayetano Heredia	62/176 (35.2%)	ref.	
Household has a refrigerator			
Yes	73/223 (32.7%)	0.55 (0.26, 1.16)	--
No	45/96 (46.9%)	ref.	
Mother's highest level of education:			
Less than secondary school	49/98 (50.0%)	2.20 (1.06, 4.56)*	--
Completed secondary school	69/221 (31.2%)	ref.	
Animals reside in/around household			
Yes	98/225 (43.6%)	2.86 (1.35, 6.06)*	2.37 (1.09, 5.13)
No	20/94 (21.3%)	ref.	ref.
Has a toilet connected to piped sewerage			
Yes	55/201 (27.4%)	0.33 (0.16, 0.67)*	0.45 (0.21, 0.95)
No	63/118 (53.4%)	ref.	ref.
Additional children (<5 years) reside in house			
Yes	55/126 (43.7%)	1.60 (0.78, 3.27)	--
No	63/193 (32.6%)	ref.	
Water Sample Characteristics			
Piped drinking water‡	70/230 (30.4%)	0.37 (0.18, 0.76)*	--
Indoor piped water connection	55/209 (26.3%)	--	--
Outdoor piped water connection	3/4 (75.0%)	--	--
Neighbor's piped water connection	12/17 (70.6%)	--	--
Non-piped drinking water	48/89 (53.9%)	ref.	
Improved sources:			
Protected well	24/32 (75.0%)	--	--
Public water basin	12/22 (54.5%)	--	--
Tanker truck or other	10/32 (31.3%)	--	--
bought/bottled water			
Unimproved sources:			
Surface water	1/1 (100.0%)	--	--
Unprotected well	1/2 (50.0%)	--	--
Water sample is stored			
Yes	105/221 (47.5%)	5.92 (2.79, 12.56)*	4.50 (2.04, 9.95)
No	13/98 (13.3%)	ref.	ref.

E. coli: indicator *Escherichia coli*; (a)OR: (adjusted) odds ratio; CI: confidence interval; ref.: referent group

*Met screening criteria for multivariable model ($p < 0.10$ in univariable analysis); adjusted odds ratio reported only if variable met criteria for final multivariable model ($p < 0.05$ in multivariable analysis)

‡ Piped water compared to non-piped water in models; sub-categories of these sources not considered in models

Table 4.4 Association between demographic/household & water sample characteristics and arsenic detection (exceeding standard of 10 µg/L) in drinking water samples (N=96) from households with infants, Piura, Peru, 2016

	Arsenic concentration ≥10 µg/L	OR (95% CI)
Demographic & household characteristics		
Enrollment hospital		
Santa Rosa	24/45 (53.3%)	n/a
José Cayetano Heredia	0/51 (0.0%)	
Household has a refrigerator		
Yes	15/65 (23.1%)	0.73 (0.28, 1.93)
No	9/31 (29.0%)	ref.
Mother's highest level of education:		
Less than secondary school	7/29 (24.1%)	0.94 (0.34, 2.58)
Completed secondary school	17/67 (25.4%)	ref.
Mother or father works in agriculture		
Yes	4/16 (25.0%)	1.00 (0.29, 3.45)
No	20/80 (25.0%)	ref.
Insecticides are used in the home		
Yes	15/38 (39.5%)	3.55 (1.35, 9.31)*
No	9/58 (15.5%)	ref.
Water Sample Characteristics		
Piped drinking water‡	22/76 (28.9%)	3.67 (0.78, 17.14)
Indoor piped water connection	19/70 (27.1%)	--
Outdoor piped water connection	1/2 (50.0%)	--
Neighbor's piped water connection	2/4 (50.0%)	--
Non-piped drinking water	2/20 (10.0%)	ref.
Improved sources:		
Protected well	1/7 (14.3%)	--
Public water basin	0/5 (0.0%)	--
Tanker truck or other bought/bottled water	0/7 (0.0%)	--
Unimproved sources:		
Unprotected well	1/1 (100%)	--
Water sample is stored		
Yes	9/36 (25.0%)	1.00 (0.39, 2.60)
No	15/60 (25.0%)	ref.

OR: odds ratio; CI: confidence interval; ref.: referent group

* Met screening criteria for multivariable model ($p < 0.10$ in univariable analysis); adjusted odds ratio reported only if variable met criteria for final multivariable model ($p < 0.05$ in multivariable analysis)

‡ Piped water compared to non-piped water in models; sub-categories of these sources not considered in models

Table 4.5 Association between demographic/household & water sample characteristics and pesticide/herbicide detection in drinking water samples (N=92) from households with infants, Piura, Peru, 2016

	Drinking water samples positive for a pesticide or herbicide ^a	OR (95% CI)
Demographic & household characteristics		
Enrollment hospital		
Santa Rosa	24/43 (55.8%)	0.46 (0.19, 1.09)*
José Cayetano Heredia	36/49 (73.5%)	ref.
Household has a refrigerator		
Yes	40/64 (62.5%)	0.67 (0.25, 1.75)
No	20/28 (71.4%)	ref.
Mother's highest level of education:		
Less than secondary school	20/27 (74.1%)	1.79 (0.66, 4.83)
Completed secondary school	40/65 (61.5%)	ref.
Mother or father works in agriculture		
Yes	13/16 (81.3%)	2.67 (0.70, 10.19)
No	47/76 (61.84%)	ref.
Insecticides are used in the home		
Yes	25/38 (65.8%)	1.04 (0.44, 2.50)
No	35/54 (64.8%)	ref.
Water sample characteristics		
Piped drinking water [‡]	45/73 (61.6%)	0.43 (0.13, 1.42)
Indoor piped water connection	40/68 (58.8%)	--
Outdoor piped water connection	2/2 (100.0%)	--
Neighbor's piped water connection	3/3 (100.0%)	--
Non-piped drinking water	15/19 (78.9%)	ref.
Improved sources:		
Protected well	7/7 (100.0%)	--
Public water basin	2/5 (40.0%)	--
Tanker truck or other bought/bottled water	5/6 (83.3%)	--
Unimproved sources:		
Unprotected well	1/1 (100.0%)	--
Water sample is stored		
Yes	29/33 (87.9%)	6.55 (2.05, 20.96)*
No	31/59 (52.5%)	ref.

OR: odds ratio; CI: confidence interval; ref.: referent group

(a.) Target pesticides/herbicides: atrazine, diazinon, chlorpyrifos, p,p'-dichlorodiphenyldichloroethylene, permethrin, and cypermethrin.

*Met screening criteria for multivariable model ($p < 0.10$ in univariable analysis); adjusted odds ratio reported only if variable met criteria for final multivariable model ($p < 0.05$ in multivariable analysis)

‡ Piped water compared to non-piped water in models; sub-categories of these sources not considered in model

Table 4.6 Association between demographic/household, infant (N=94), & water sample characteristics and infant diarrhea at follow-up Visit 2, Piura, Peru, 2016

	Infant diarrhea at Visit 2	OR (95% CI)
Demographic & household characteristics		
Enrollment hospital		
Santa Rosa	9/45 (20.0%)	2.82 (0.80, 9.88)
José Cayetano Heredia	4/49 (8.2%)	ref.
Household has a refrigerator		
Yes	8/64 (12.5%)	0.71 (0.21, 2.40)
No	5/30 (16.7%)	ref.
Mother's highest level of education:		
Less than secondary school	3/28 (10.7%)	0.67 (0.17, 2.66)
Completed secondary school	10/66 (15.2%)	ref.
Animals reside in/around household		
Yes	11/68 (16.2%)	2.32 (0.48, 11.25)
No	2/26 (7.7%)	ref.
Has a toilet connected to piped sewerage		
Yes	7/61 (11.5%)	0.58 (0.18, 1.91)
No	6/33 (18.2%)	ref.
Additional children (<5 years) reside in house		
Yes	7/39 (17.9%)	1.79 (0.55, 5.80)
No	6/55 (10.9%)	ref.
Infant characteristics		
Caretaker reported giving the study infant drinking water in the week before enrollment		
Yes	11/83 (13.3%)	0.69 (0.13, 3.61)
No	2/11 (18.2%)	ref.
Water sample characteristics (primary source)		
Any pesticide/herbicide detected		
Yes	8/59 (13.6%)	1.06 (0.29, 3.84)
No	4/31 (12.9%)	ref.
Arsenic concentration ≥ 10 µg/L		
Yes	6/24 (25.0%)	3.00 (0.90, 10.06)*
No	7/70 (10.0%)	ref.
<i>E. coli</i> detected in primary drinking water sample at Visit 1		
Yes	5/26 (19.2%)	1.64 (0.48, 5.57)‡
No	8/63 (12.7%)	ref.
<i>E. coli</i> detected in primary drinking water sample at Visit 2		
Yes	7/25 (28.0%)	14.77 (1.69, 129.25)‡
No	1/39 (2.6%)	ref.

E. coli: indicator *Escherichia coli*; (a)OR: (adjusted) odds ratio; CI: confidence interval; ref.: referent group

*Met screening criteria for multivariable model ($p < 0.10$ in univariable analysis).

‡ It was decided *a priori* to include *E. coli* detection in the primary drinking water sample at Visit 1 and Visit 2 in separate multi-variable models with the other factors significantly associated with infant diarrhea.

Table 4.7 Association between demographic/household, infant (N=94), & water sample characteristics and infant enteropathogen infection at follow-up Visit 2, Piura, Peru, 2016

	≥1 enteropathogen detected in stool	OR (95% CI)	aOR (95% CI)
Demographic & household characteristics			
Enrollment hospital			
Santa Rosa	29/45 (64.4%)	0.73 (0.30, 1.73)	--
José Cayetano Heredia	35/49 (71.4%)	ref.	
Household has a refrigerator			
Yes	43/64 (67.2%)	0.88 (0.34, 2.25)	--
No	21/30 (70.0%)	ref.	
Mother's highest level of education:			
Less than secondary school	19/28 (67.9%)	0.99 (0.38, 2.54)	--
Completed secondary school	45/66 (68.2%)	ref.	
Animals reside in/around household			
Yes	48/68 (70.6%)	1.50 (0.58, 3.87)	--
No	16/26 (61.5%)	ref.	
Has a toilet connected to piped sewerage			
Yes	44/61 (72.1%)	1.68 (0.69, 4.12)	--
No	20/33 (60.6%)	ref.	
Additional children (<5 years) reside in house			
Yes	26/39 (66.7%)	0.90 (0.37, 2.15)	--
No	38/55 (69.1%)	ref.	
Infant characteristics			
Caretaker reported giving the study infant water in the week before enrollment			
Yes	60/83 (72.3%)	4.57 (1.22, 17.08)*	4.36 (1.11, 17.07)
No	4/11 (36.4%)	ref.	ref.
Water sample characteristics (primary source)			
Any pesticide/herbicide detected			
Yes	45/59 (76.3%)	3.01 (1.20, 7.60)*	2.93 (1.13, 7.61)
No	16/31 (51.6%)	ref.	ref.
Arsenic concentration ≥10 µg/L			
Yes	13/24 (54.2%)	0.44 (0.17, 1.15)	--
No	51/70 (72.9%)	ref.	

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; ref.: referent group

*Met screening criteria for multivariable model ($p < 0.10$ in univariable analysis); adjusted odds ratio reported only if variable met criteria for final multivariable model ($p < 0.05$ in multivariable analysis)

APPENDIX

Appendix Table 4.1 Multivariable models considered for study of water quality and infant health in Piura, Peru, 2016

Model	Description	Samples/subjects included	Variables included in multivariable model‡
Model 1 (Table 4.3)	Factors associated with presence of <i>E. coli</i> in all household drinking water samples collected for microbiological testing	319 primary, secondary, and tertiary drinking water samples collected from 96 households at Visit 1 and Visit 2	- animals live in or around household (yes/no) - household has toilet connected to piped sewerage (yes/no) - water sample is stored (yes/no)
Model 1A (Appendix Table 4.2)	Model 1 extended to consider the prospective association between infant diarrhea at Visit 1 and subsequent <i>E. coli</i> contamination of drinking water at Visit 2	126 primary, secondary, and tertiary drinking water samples collected from 64 households at Visit 2	- variables selected for multivariable Model 1 (based on screening criteria/backward selection) - infant diarrhea at Visit 1 (based on <i>a priori</i> decision to include)
Model 1B (Appendix Table 4.3)	Model 1 extended to consider the cross-sectional association between infant diarrhea at Visit 2 and concurrent <i>E. coli</i> contamination of drinking water at Visit 2	126 primary, secondary, and tertiary drinking water samples collected from 64 households at Visit 2	- variables selected for multivariable Model 1 (based on screening criteria/backward selection) - infant diarrhea at Visit 2 (based on <i>a priori</i> decision to include)
Model 1C (Appendix Table 4.3)	Model 1 extended to consider the cross-sectional association between infant diarrhea at Visit 1 and concurrent <i>E. coli</i> contamination of drinking water at Visit 1	193 primary, secondary, and tertiary drinking water samples collected from 91 households at Visit 1	- variables selected for multivariable Model 1 (based on screening criteria/backward selection) - infant diarrhea at Visit 1 (based on <i>a priori</i> decision to include)
Model 1D (Appendix Table 4.5)	Factors associated with presence of <i>E. coli</i> in stored household drinking water samples	221 primary, secondary, and tertiary drinking water samples collected from 80 households at Visit 1 and Visit 2	- animals live in or around household (yes/no) - stored water is covered (yes/no) - stored water is on the ground (yes/no)
Model 2 (Table 4.4)	Factors associated with arsenic detection (≥ 10 µg/L) in primary household drinking water samples	96 primary household drinking water samples collected from 96 households at Visit 1	insecticide use in the household and enrollment hospital met screening criteria; adjustment by hospital/multivariable modeling not performed as all samples with arsenic ≥ 10 µg/L came from households from the same enrollment hospital

‡variables that remained in the multivariable model after screening; variables that did not meet screening criteria are presented in univariable analyses in the model results table

(continued)

Appendix Table 4.1 Multivariable models considered for study of water quality and infant health in Piura, Peru, 2016 (continued)

Model	Description	Samples/subjects included	Variables included in multivariable model [‡]
Model 3 (Table 4.5)	Factors associated with detection of any herbicide/pesticide in primary household drinking water	92 primary household drinking water samples collected from 92 households at Visit 1	multivariable model not presented: water sample storage was the only variable to meet screening and backward selection criteria
Model 4 (Table 4.6)	Factors associated with infant diarrhea at Visit 2	94 infants who were available for Visit 2	only arsenic concentration ≥ 10 $\mu\text{g/L}$ (in primary water source, measured at Visit 1) met screening criteria; multivariable model not presented
Model 4A (Table 4.6)	Model 4 extended to consider the prospective association between <i>E. coli</i> contamination in the primary drinking water sample at Visit 1 and subsequent caretaker report of infant diarrhea at Visit 2	91 infants who were available at Visit 2 and had primary drinking water sample results from Visit 1	-no Model 4 variable met both screening ($p < 0.10$) and backward selection ($p < 0.05$) criteria - <i>E. coli</i> contamination of the primary water sample at Visit 1 (based on <i>a priori</i> decision to include)* *not significant
Model 4B (Table 4.6)	Model 4 extended to consider the cross-sectional association between <i>E. coli</i> contamination in the primary drinking water sample at Visit 2 and concurrent caretaker report of infant diarrhea at Visit 2	61 infants who were available for Visit 2 and had available primary drinking water results for Visit 2	-no Model 4 variable met both screening ($p < 0.10$) and backward selection ($p < 0.05$) criteria - <i>E. coli</i> contamination of the primary water sample at Visit 2 (based on <i>a priori</i> decision to include)
Model 4C (not presented)	Factors associated with caretaker reporting infant diarrhea at Visit 1	91 infants who were available at Visit 1 and had primary drinking water sample results from Visit 1	- no variables met screening criteria - <i>E. coli</i> contamination of the primary water sample at Visit 1 (based on <i>a priori</i> decision to consider)* *not significant; model not presented

[‡]variables that remained in the multivariable model after screening; variables that did not meet screening criteria are presented in univariable analyses in the model results table
(continued)

Appendix Table 4.1 Multivariable models considered for study of water quality and infant health in Piura, Peru, 2016 (continued)

Model	Description	Samples/subjects included	Variables included in multivariable model[‡]
Model 5 (Table 4.7)	Factors associated with infant enteropathogen infection (measured in stool at Visit 2)	Stool sample results from 90 infants available at Visit 2 who had available pesticide/herbicide data from water collected at Visit 1	- caretaker reported at Visit 1 that the study infant had/had not been given any drinking water in the past week - any pesticide/herbicide was detected in the primary drinking water sample collected at Visit 1
Model 5A (not presented)	Model 5 extended to consider the prospective association between <i>E. coli</i> contamination in the primary drinking water sample at Visit 1 and subsequent infant enteropathogen infection (measured in stool at Visit 2)	Stool sample results from 85 infants available at Visit 2 who had available primary drinking water sample results (<i>E. coli</i> and pesticide/herbicide results) from Visit 1	- Model 5 variables (based on screening criteria/backward selection) - <i>E. coli</i> contamination of the primary water sample at Visit 1 (based on <i>a priori</i> decision to consider)* *not significant; model not presented
Model 5B (not presented)	Model 5 extended to consider the cross-sectional association between <i>E. coli</i> contamination in the primary drinking water sample at Visit 2 and infant enteropathogen infection (measured in stool at Visit 2)	Stool sample results from 62 infants available at Visit 2 who had available primary drinking water sample <i>E. coli</i> results from Visit 2 and available pesticide/herbicide results from Visit 1	- Model 5 variables (based on screening criteria/backward selection) - <i>E. coli</i> contamination of the primary water sample at Visit 2 (based on <i>a priori</i> decision to consider)* *not significant; model not presented

Appendix Table 4.2 Model for *E. coli* detection in follow-up Visit 2 drinking water samples (N = 126), considering infant diarrhea at Visit 1, households with infants, Piura, Peru, 2016

	Drinking water samples positive for <i>E. coli</i>	adjusted odds ratio (95% CI)
Animals reside in/around household		
Yes	44/91 (48.4%)	1.77 (0.56, 5.57)
No	9/35 (25.7%)	ref.
Has a toilet connected to piped sewerage		
Yes	24/76 (31.6%)	0.48 (0.16, 1.43)
No	29/50 (58.0%)	ref.
Water sample is stored		
Yes	50/91 (54.9%)	13.53 (2.95, 62.09)
No	3/35 (8.6%)	ref.
Study infant had diarrhea (Visit 1)		
Yes	11/21 (52.4%)	3.45 (1.20, 9.96)
No	42/105 (40.0%)	ref.

E. coli: indicator *Escherichia coli*; CI: confidence interval; ref.: referent group

Appendix Table 4.3 Model for *E. coli* detection in follow-up Visit 2 drinking water samples (N = 126), considering infant diarrhea at Visit 2, households with infants, Piura, Peru, 2016

	Drinking water samples positive for <i>E. coli</i>	adjusted odds ratio (95% CI)
Animals reside in/around household		
Yes	44/91 (48.4%)	1.62 (0.51, 5.13)
No	9/35 (25.7%)	ref.
Has a toilet connected to piped sewerage		
Yes	24/76 (31.6%)	0.52 (0.17, 1.56)
No	29/50 (58.0%)	ref.
Water sample is stored		
Yes	50/91 (54.9%)	27.08 (2.89, 253.76)
No	3/35 (8.6%)	ref.
Study infant had diarrhea (Visit 2)		
Yes	12/13 (92.3%)	73.03 (4.75, >999.99)
No	41/113 (36.3%)	ref.

E. coli: indicator *Escherichia coli*; CI: confidence interval; ref.: referent group

Appendix Table 4.4 Model for *E. coli* detection in Visit 1 drinking water samples (N = 193), considering infant diarrhea at Visit 1, households with infants, Piura, Peru, 2016

	Drinking water samples positive for <i>E. coli</i>	adjusted odds ratio (95% CI)
Animals reside in/around household		
Yes	54/134 (40.3%)	2.68 (1.07, 6.76)
No	11/59 (18.6%)	ref.
Has a toilet connected to piped sewerage		
Yes	31/125 (24.8%)	0.43 (0.19, 0.99)
No	34/68 (50.0%)	ref.
Water sample is stored		
Yes	55/130 (42.3%)	3.05 (1.37, 6.77)
No	10/63 (15.9%)	ref.
Study infant had diarrhea (Visit 1)		
Yes	12/38 (31.6%)	1.05 (0.43, 2.55)
No	53/155 (34.2%)	ref.

E. coli: indicator *Escherichia coli*; CI: confidence interval; ref.: referent group

Appendix Table 4.5 Multivariable model for factors associated with *E. coli* detection in stored water samples (N = 221), Piura, Peru 2016

	Drinking water samples positive for <i>E. coli</i>	adjusted odds ratio (95% CI)
Animals reside in/around household		
Yes	91/166 (54.8%)	3.02 (1.24, 7.33)
No	14/55 (25.5%)	ref.
Water storage container is covered		
Yes	84/191 (44.0%)	0.26 (0.10, 0.67)
No	20/27 (74.1%)	ref.
Water storage container is on the ground		
Yes	75/117 (64.1%)	4.16 (2.17, 7.95)
No	30/104 (28.8%)	ref.

E. coli: indicator *Escherichia coli*; CI: confidence interval; ref.: referent group

Chapter 4 References

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Chapter 5 Conclusion

The strengths and limitations of each chapter are detailed in their discussion sections, in which I also contextualize our findings in reference to the broader literature. In this concluding chapter, I discuss more generally the research findings, strengths, and limitations of the dissertation, and give suggestions for policy recommendations and future research.

Main conclusions

This dissertation evaluated factors associated with diarrheal disease in Peruvian children under five years old in a decade during which several health and infrastructure improvements took place. It also assessed challenges that Peru may face in reducing the burden of diarrheal diseases as climate change progresses. We compiled several national datasets to explore long-term trends in clinic visits for childhood diarrhea, as well as short-term variations in meteorological conditions that may influence the childhood diarrhea rate. We also demonstrate barriers in reducing waterborne health risks, even in areas with high piped water access, using data from an infant birth cohort.

Chapter 2 focused on long-term trends in clinic visits for childhood diarrhea in Peru and evaluated how improvements to piped water access and sewerage access, lower poverty rates, and the introduction of rotavirus vaccination were associated with the childhood diarrhea rate. Introduction of the rotavirus vaccination was associated with a significantly lower rate of childhood clinic visits for diarrhea; however, the benefits of vaccination were not fully realized among those provinces with the lowest levels of access to piped water and sewerage connections, perhaps due to poor vaccine coverage and/or performance. Nationally, the rate of clinic visits for childhood diarrhea decreased steadily throughout 2005-2015, both before and after the introduction of the rotavirus vaccine. While myriad factors may have contributed to this reduction, we did not identify increased access to piped water, increased access to sewerage, or declines in poverty as being significantly

associated with the downward trend in the pre-rotavirus vaccine era. The high correlation between these factors led us to examine them in isolation and therefore limited our ability to differentiate between their possible effects. After the introduction of the rotavirus vaccine, higher access to piped water was associated with a significantly lower rate of clinic visits for childhood diarrhea, and higher access to sewerage also tended to be associated with a lower diarrhea rate; possible explanations for this finding are detailed in Chapter 2.

Although the focus of Chapter 3 was on the short-term effects of meteorological factors on the childhood diarrhea rate, several variables considered in Chapter 2 were utilized in the analysis, allowing the long-term trend in the rate of clinic visits for diarrhea to be examined through a different lens. This chapter lacked the granularity of levels of piped water and sewerage coverage that were examined in Chapter 2 but had the advantage of controlling for province in models, thereby accounting for other provincial-level factors that may have changed over the long course of the time series. In Chapter 2, the provinces that were in the lowest quartile of access to piped water in the country, namely those provinces in which <40% of households had a piped water connection, did not have lower rates of childhood clinic visits for diarrhea in the post-rotavirus vaccine era (2010-2015) compared to the pre-vaccine era (2005-2009), accounting for long-term trend. In Chapter 3, analyses were stratified by provinces that consistently had lower access to piped drinking water (<60% of households in the province had access) compared to those that had higher access, or transitioned from lower to higher access. Again, in Chapter 3, the provinces in the lowest group of piped water access did not have significantly lower rates of clinic visits for childhood diarrhea in the post-vaccine era, accounting for secular trend. Considering sewerage, in Chapter 2, those in the lowest group of sewerage access (<17% of households in the province having access to a toilet connected to the sewerage network) did not have significantly lower rates of childhood diarrhea in the post-vaccine era; but the lowest group considered in Chapter 3 (<30% of households having

access to sewerage) did. This could reflect a shift between the two cut-off points or could result from methodological differences.

The goal of Chapter 3 was to focus on shorter-term associations between environmental factors—namely meteorological factors—and the rate of clinic visits for childhood diarrhea. Consistent with previous research globally and in Peru,^{1–3} higher temperatures were associated with higher rates of clinic visits for childhood diarrhea. Nationally, there was no overall difference in the temperature-diarrhea association from the pre- to post-rotavirus vaccine era. Curiously, the one context in which the temperature-diarrhea relationship did significantly change from the pre- to post-vaccine era was in provinces with lower access to piped drinking water and sewerage, where there was no association between temperature and diarrhea in the pre-rotavirus vaccine era, but there was a significant positive association after the widespread introduction of the rotavirus vaccine. This type of shift is in line with what might be expected if the viral diarrheal disease burden, typically associated with cooler temperatures,⁴ was reduced. This suggests the rotavirus vaccination may have had an impact on clinic visits for viral diarrhea in these areas, despite the lack of an overall lower rate of childhood diarrhea in the post-rotavirus vaccine era beyond the secular trend.

Contrary to the hypothesis that better access to piped water and sewerage might mitigate the effects of temperature on diarrhea, we did not find evidence of a weaker temperature-diarrhea association in areas with better access to piped water and sewerage. In fact, the opposite tended to be true, though we were limited in establishing causal relationships, and data limitations led to spatial confounding in this assessment. Yet Chapter 3 highlighted other meteorological factors that may be a risk for childhood diarrhea in areas with lower access to piped water and sewerage, namely El Niño events and the dry season. Provinces with lower access to piped water and sewerage were generally located in areas where El Niño tends to be associated with droughts, not flooding. In Chapter 3, we detail

further how those with worse water/sanitation infrastructure may be more affected by dry weather conditions.

In Chapter 4, laboratory and survey data were used to characterize drinking water quality and infant diarrhea and enteropathogen infection in households located in four coastal provinces in the department of Piura, Peru in 2016. Despite most samples coming from improved water sources, and most households having a piped water connection (77%), there was extensive microbiological, chemical, and heavy metal contamination of the drinking water, including high arsenic levels, demonstrating barriers in reducing waterborne health risks even in areas with high piped water coverage. Household drinking water storage was common, perhaps because the majority of respondents with a piped water connection (55%) reported that there had been a service cut in the last week.

Drinking water storage was strongly associated with both microbiological and pesticide/herbicide contamination; microbiological contamination was more common when stored samples were uncovered or stored on the ground. Drinking water samples coming from households with animals had higher odds of *E. coli* detection. Anecdotally, we observed animals perched on or near open drinking water containers while collecting survey data. While our laboratory methods did not differentiate between possible human vs. animal fecal contamination of drinking water, the possibility of animal fecal contamination is plausible and is of concern, given the many pathogens harbored by animals that are capable of infecting humans (Appendix Chapter 6). Most (68%) of the infants, who were only 6-8 months old, had at least one enteropathogen isolated in their stool, and the prevalence of diarrhea ranged from 14-20% at two study visits. Even asymptomatic enteropathogen infections can be harmful to child growth and development.^{5,6}

This study added context to some of the national results of this dissertation. Chapter 2 and Chapter 3 illustrated that provinces with better access to piped drinking water did not always have lower rates of childhood diarrhea, especially in the pre-rotavirus vaccine era. While results from a small cohort in four provinces are not necessarily generalizable to the other provinces of Peru, Chapter 4 does demonstrate the potential for piped drinking water to pose health risks for children in Peru. Notably, all of the infants in the Piura cohort had received at least the first dose of the rotavirus vaccine, and rotavirus was not detected in any stool sample.

Strengths

Duration and timing of national evaluation

Chapters 2 and Chapter 3 utilized a temporally and spatially rich dataset to analyze long- and short-term trends in the childhood diarrhea rate. We had ample data from both the pre- and post-rotavirus vaccine eras, allowing us to appropriately control for the secular trend throughout the study period (2005-2015). The data come from a decade characterized by health and infrastructure improvements in Peru, and we were able to compare these improvements both across space and time. We conducted the first national characterization of the association between the rotavirus vaccine era and the childhood diarrhea rate and were able to highlight contextual factors that modified vaccine impact.

The analyses from 2005-2015 utilized in this dissertation also complement previous literature on the association between temperature, El Niño events, and diarrhea in Peru. Previous research examining the effects of temperature and/or El Niño events on diarrhea in Peru was all conducted on data from the 1990s, a decade during which there were two El Niño events occurring simultaneously with cholera epidemics.^{2-3, 7-11} Thus, we provide an update to the temperature-diarrhea association in

Peru that better reflects the landscape of endemic diarrhea in Peru in the early 21st century, both before and after the introduction of the rotavirus vaccine.

Geographic scope

Much of the previous research on childhood diarrhea in Peru cited throughout this dissertation was conducted in the capital of Lima (and for El Niño analyses, in the coastal city of Piura), with the notable exception of the MAL-ED site (see Chapter 1) in the Peruvian Amazon (Loreto, Peru). While a high proportion of the Peruvian population lives in Lima, less consideration has been given to non-coastal provinces, especially communities in the Andes. However, we found many of the provinces with lower access to piped water and sewerage were located in the Andes and Peruvian Amazon, and that these communities may be particularly vulnerable: these provinces generally had higher rates of clinic visits for diarrhea, realized fewer gains in decreasing the childhood diarrheal disease burden in the rotavirus vaccine era, and were particularly vulnerable to increased diarrhea in the dry season and during moderate/strong El Niño events.

While this work spanned the country of Peru and included a variety of settings in terms of geography and infrastructure, the work may lack generalizability to other settings. While contaminated drinking water and diarrhea morbidity are of concern in Peru, there are few deaths associated with diarrheal diseases, thus this work may be less generalizable to settings with high childhood diarrhea mortality. While El Niño is a global phenomenon, its effects on weather pattern can be highly localized, and our results from Peru may not be applicable to other countries. Nevertheless, this work provides an analysis model that can be utilized by other researchers to explore similar research questions in different settings.

This dissertation also includes data from a cohort study in the coastal department of Piura, Peru from the year 2016. Piura suffered catastrophic flooding, infrastructure damage, and loss of life the

following year during a strong El Niño event.¹² Research is now ongoing that compares child health indicators before and after this El Niño event, and our study contributes a sample of baseline water quality measurements prior to the 2017 infrastructure damage. To our knowledge, we also provide the first characterization of arsenic in household drinking water samples in a larger Peruvian city. I identified only one previous study in Peru that considered arsenic in drinking water samples collected in the household, conducted in a small mining/smelting town in central Peru.¹³

Consideration of water and sanitation factors

Throughout this study, we considered water and sanitation factors not only as main effects, but as possible effect modifiers of the association between other variables and the childhood diarrhea rate. A 2017 review of the global impact of rotavirus vaccination identified 26 studies of rotavirus vaccine impact conducted in LMICs; none of these explicitly considered vaccine impact at varying levels of water and sanitation access.¹⁴ National analyses of the impact of rotavirus vaccination stand to benefit from including local factors related to water and sanitation infrastructure, as they are potentially protective factors, and also possible effect modifiers when considering rotavirus vaccine and childhood diarrhea (Introduction and Chapter 2). We also considered water/sanitation infrastructure in our analysis of temperature and diarrhea; the studies that contributed to a review of global estimates of the temperature-diarrhea association gave little consideration to water and sanitation factors,¹ despite the importance of considering these factors for future climate projections and mitigation strategies.¹⁵ Unfortunately, there were several limitations in our analyses of water and sanitation coverage, described in more detail below. However, the consideration of these variables in such research remains a strength of this dissertation.

Government partnerships and outputs

We collaborated with several government agencies to compile a national dataset for Chapter 2 and Chapter 3. We worked alongside, and shared our findings, with the Ministry of Health and Ministry of the Environment, both in small meetings including key stakeholders, as well as to large audiences, which will hopefully raise awareness of policy considerations resulting from our work. I also personally participated in training courses in environmental health, to share knowledge with my counterparts in Peru.

I compiled information from several sources to make a large national database for Chapter 2 and Chapter 3 that has been heavily cleaned and is currently in a user-friendly format. Currently, the province-level poverty estimates constructed for 2005-2015 are being used in an analysis of temperature and mortality in Peru, and the entire dataset has potential to be used in several future research studies, discussed further in the “Policy implications and future research” section.

Limitations: Aim 1 and Aim 2

Assessing long-term trend

Importantly, the rate of clinic visits for childhood diarrhea is not necessarily reflective of the rate of childhood diarrhea cases experienced. For a childhood diarrhea case to result in a clinic visit, a caretaker must believe the severity of the case warrants medical attention, and the caretaker must have the access and means to visit a clinic. It is possible that fewer cases of diarrhea, even if equally severe, result in clinic visits in high poverty and/or remote areas. High levels of clinic avoidance have been observed in the Peruvian Amazon, where respondents cite distance and wait times as barriers to visiting a clinic.¹⁶

Nationally, several factors may have contributed to a higher proportion of diarrhea cases resulting in clinic visits in the decade over which our study was conducted. Firstly, poverty declined considerably in Peru between 2005 and 2015.¹⁷ Caretakers may have been more able to afford the costs associated with clinic visits. Secondly, the rural population decreased in the study period as people moved to urban areas.¹⁸ As urban populations may have fewer barriers to visiting clinics, such as distance and transportation, this may have increased care-seeking.

Two factors may have influenced the observed rate of childhood clinic visits for diarrhea that represent limitations in our data, rather than actual changes in incidence of clinic visits for diarrhea. Firstly, diarrhea reporting may have improved over the course of the study. We did not have data on the number of clinics that sent surveillance reports each week; cases were aggregated to the level of the district of residence of the patients. While reporting is obligatory for public clinics, we excluded one province from our analyses based on a lack of reporting in the pre-rotavirus vaccine era, indicating that even when reporting is mandated, it is not always practiced. Private clinics can opt into the diarrhea surveillance system, but we do not know what percentage of private clinics reported regularly, and whether this increased over the 11-year time series. Secondly, for the denominator of our childhood diarrhea rate, we used an interpolated, census-derived population of children under five years old in each province, assuming linear change between censuses. It is worth noting that the assumption of a linear change is rather simplistic, given complex population and migration dynamics. Furthermore, while difficult to ascertain, there were some indications that the child population may have been underestimated in the latter half of the study period. The number of rotavirus vaccine doses given to children often far exceeded the estimated infant population (Chapter 2). Furthermore, the census-estimated child population declined in most provinces between 2005 and 2015, which contrasts with data we received on birth records, that showed

increasing births over the same period. It is difficult to determine whether census or birth records can better capture child population trends, but noteworthy that the trends differed.

While we cannot confirm the following suggestions, all of these scenarios could contribute to increases in reports of clinic visits for diarrhea throughout the study period, even in the absence of there being a higher rate of childhood diarrhea cases that would prompt care-seeking under ideal circumstances: (1) an increase over time in the proportion of community diarrhea cases that result in clinic visits, due to increasing affordability/accessibility of visiting clinics, (2) a higher proportion of urban residents seeking care at clinics for diarrhea (compared to rural counterparts), coupled with rural to urban migration, (3) improved reporting of diarrhea cases from clinics, and (4) an underestimation in the child population that decreasingly reflected the true population from 2005-2015. In summary, childhood diarrhea rates may have appeared artificially high toward the end of the study period compared to the beginning, which could bias our assessment of the impact of the rotavirus vaccine toward a null effect. This might explain why our estimate of the impact of the rotavirus vaccination era was much lower than it was in other countries in Latin America,¹⁹⁻²⁵ although we also considered a broader age group of children, as discussed in Chapter 2. The potential sources of bias here also have implications for comparing the diarrhea rate in areas with better and worse access to piped water and sewerage, as there may be differential diarrhea reporting between urban and rural areas.

These possible sources of bias in the long-term trend do not necessarily bias our interpretation of the differential vaccine performance between areas with low and high piped water/sewerage coverage, assuming that the potential bias occurred non-differentially between these different areas. Potential flaws in assumptions about the population at risk should not have a large effect on the shorter-term meteorological trends, as the population should remain relatively stable week to week.

The length of the time series may also mean other unmeasured factors changed over the study period that could affect the childhood diarrhea rate. For example, hygiene practices may have improved in response to a large national handwashing promotional campaign.²⁶

Ecologic study design and spatial confounding

The analysis utilized an ecologic study design, which is well-suited to study large-scale impacts of population-level interventions;²⁷ however, the results can be subject to ecologic bias and limit causal inference. While this is problematic for interpreting results for an individual or household level, the study design is more appropriate for making provincial level policy inferences.

Chapter 2 and Chapter 3 were subject to spatial confounding. Most provinces with lower access to piped water and sewerage were located in the interior of Peru (*i.e.*, away from the Pacific Ocean) in the Amazon region in northern Peru and near Lake Titicaca in southern Peru. These provinces tended to have lower population densities and have mountainous or rainforest landscapes, compared to the densely-populated arid coastal regions where much of the population of Peru lives. Therefore, in Chapter 2 and Chapter 3, findings specific to areas with higher or lower water/sanitation coverage may reflect an impact of these specific conditions or could be explained by other differences between provinces.

Variables not considered

We did not consider a number of factors that may have been relevant to our study. Notably, we did not consider any measures of hygiene, an important method of interrupting enteric pathogen transmission. Handwashing metrics may not be meaningful at a provincial level, yet the lack of inclusion of any hygiene data is a limitation of this research. We also did not consider maternal education. In Chapter 2, we considered poverty as a possible effect modifier of the association between the rotavirus vaccine era and the rate of childhood clinic visits for diarrhea, as the rotavirus

vaccine tends to perform sub-optimally in lower-income settings. However, some of the hypotheses around poorer performance of the vaccine in lower-income areas point to environmental enteric dysfunction and/or malnutrition as the underlying reason for differences. Metrics of malnutrition may have been more suitable than poverty measures; however, we did not consider childhood malnutrition in this study.

In analyzing meteorological factors associated with childhood diarrhea in Chapter 3, analyses controlled for the wet/dry season based on general definitions, but did not include precipitation data. Rainfall patterns tend to be highly localized and more spatially variable than temperature estimates; use of typical rainfall datasets can lead to large bias in analyses of waterborne disease.²⁸ The association between rainfall and diarrhea is complex: though heavy rainfall can be associated with increased diarrhea, this relationship may depend on antecedent conditions.²⁹ While I therefore think it was appropriate to not consider precipitation in a province-level analysis, the lack of specific rainfall information may still be considered a shortcoming of the analysis.

Interpretability of access to piped water and sewerage

While a strength of this research was the consideration of water/sanitation infrastructure as possible effect modifiers of the rotavirus vaccine association with diarrhea, and the temperature-diarrhea association, our measurements of piped water and sewerage coverage were subject to several limitations. Firstly, provinces that had low access to water also tended to have low access to sewerage, making it difficult to distinguish between potential effects of piped water vs. sewerage access. Secondly, these variables were not only highly correlated with one another, but they were correlated with other factors, such as geographic region of the country. It was therefore difficult to interpret whether differences observed at varying levels of water and sewerage coverage were due to the water/sanitation infrastructure, or to other inherent differences between these provinces.

Nationally we did not consider the quality or availability of the piped drinking water. By the WHO/UNICEF JMP definitions (see Chapter 1),³⁰ we considered water that was “improved”, but we could not distinguish whether it was “safely managed”. Chapter 4 demonstrated in Piura, Peru the possibility of having high availability of improved drinking water that fails to meet several criteria of safely managed drinking water (*e.g.*, available when needed and free of contamination).

Limitations: Aim 3

Our study of an infant cohort in Piura, Peru had a small sample size that limited inferences about the association between water quality measurements and health outcomes. While Piura tends to be arid and hot for most of the year, our study was conducted in a single season. In this study, I excluded microbiological water quality measurements from two days in which negative controls tested positive for total coliforms; this reflects an overall high percentage of negative controls testing positive for total coliforms (6.7%). There may have been a problem with the sterile water we used in these negative controls, or this could reflect laboratory contamination during processing of samples.

Policy implications and future research

Based on this research, maintaining high levels of rotavirus vaccine coverage is important to reducing the childhood diarrhea rate in Peru. There were several theories for why there was no significant reduction in the rate of clinic visits for childhood diarrhea in the post-rotavirus vaccine era (compared to the pre-vaccine era, controlling for secular trend) in provinces with lower access to piped drinking water and sewerage (Chapter 2). For example, there may have been worse seroconversion of the vaccine in these settings, or the percentage of infants receiving both doses of the vaccine may have been lower in these provinces. It is also possible that the rotavirus vaccine was successful at reducing viral diarrhea in these provinces (Chapter 3), but that other factors contributed to a different long-term trend in the childhood diarrhea rate in these provinces. Further

exploring hypotheses about differential vaccine impact in Peru is recommended using household data and/or infectious diseases models. An SIR or other compartmental infectious disease model could allow for more detail on rotavirus transmission dynamics to be accounted for (*e.g.*, the within-subject clustering of rotaviruses cases that has been observed for infants in Peru,³¹ changing immunity in the population, or seasonal dynamics of vaccine effectiveness³²). More advanced spatial techniques could also be utilized to account for the high level of spatial correlation of variables considered in this research.

While Chapter 2 and Chapter 3 of this dissertation focused on national trends in the rate of childhood diarrheal disease, we have constructed a dataset that can be used to explore how changes in access to piped water and sewerage, introduction of rotavirus vaccination, and meteorological conditions have been associated with the childhood diarrhea rate within each province, or in specific geographic regions of the country. Notably several provinces in the Puno department of southern Peru neighboring Lake Titicaca had low piped water and sewerage coverage, but also low rates of childhood diarrhea. It would be interesting to learn what factors contribute to low diarrhea reporting in this area. As mentioned, a limitation of this study was that we could not assess clinic access, and provinces with lower clinic access may appear to have lower rates of childhood diarrhea when analyzed using clinic visits. Peru collects information on caretaker-reported diarrhea in the Encuesta Demográfica y de Salud Familiar (Demographic and Family Health Survey). While these data are not suitable for analysis in geographic regions smaller than the department level (*i.e.*, they are not appropriate for province-level analyses), these data could be analyzed to give a sense of regions of Peru where the childhood rate of clinic visits for diarrhea does not necessarily reflect the burden of diarrheal disease morbidity.

The dataset constructed for this project, as well as our estimates between meteorological factors and the childhood diarrhea rate, could be used for vulnerability assessments for future climate scenarios and El Niño events. The unique landscape and diversity of microclimates in Peru contribute to a range of climate change projections within the country, both in terms of temperature and precipitation changes.^{33,34} Downscaling approaches could be utilized to create relatively local future temperature projections within Peru; these projections could be combined with the coefficients of our temperature-diarrhea analysis to identify those provinces of Peru most vulnerable to increased cases of childhood diarrhea under future climate scenarios. Our work identified certain provinces with historically larger associations between El Niño events and the childhood diarrhea rate, namely those with lower access to piped water and sewerage. Many of those provinces were located in areas more prone to drought during El Niño. Local research aimed at understanding the potential contributions of water scarcity and lack of WASH infrastructure and their associations with childhood diarrhea during El Niño events could be useful for El Niño and climate mitigation planning in those areas.

Chapter 3 underscored the risk posed by drier conditions on the childhood diarrhea rate. Future research on drought and childhood diarrhea is recommended. Peru declared a state of emergency and spent >\$20 million on flood and drought prevention upon the forecast of a strong El Niño for 2015, but was not prepared for the catastrophic 2017 El Niño that rapidly developed.^{35,36} Building overall resilience to drought conditions and other extreme weather events may benefit the country even outside of El Niño periods, and will be useful when El Niño predictions fail to forecast major events.

The Peruvian Ministry of Health has an impressive wealth of surveillance data, and data on other health metrics such as vaccination. One barrier to maximizing the utility of this extensive

surveillance network is the difficulty in capturing the child population, which is the denominator for the percentage of children receiving vaccines, as well as the denominator, or population at risk, for disease rates. Based on personal communications, the Ministry of Health is currently constructing district-level registers of their child populations to be utilized in health decision making. Based on this dissertation, I am very supportive of this effort and think it could have great benefits for evidence-based health decision making in Peru.

In Chapter 4, we identified a cohort of infants highly exposed to enteropathogens that risks being exposed to arsenic and pesticides/herbicides in drinking water. Further follow-up in this cohort could assess whether such exposures are associated with immune function or other adverse health outcomes. Nationally, piped drinking water supplies should undergo routine chemical and microbiological testing, and should be resilient to extreme weather events. Another recommendation from this dissertation is that the provision of piped drinking water in Peru should be accompanied by messaging on safe storage. Attention should also be given to reducing service interruptions in the piped drinking water supply, and to ensuring adequate provision of water during drought conditions.

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Chapter 6 (Appendix Chapter) Pathogens transmitted in animal feces in low- and middle-income countries

Miranda J. Delahoy,¹ Breanna Wodnik,¹ Lydia McAliley,¹ Gauthami Penakalapati,¹ Jenna Swarthout,¹

Matthew C. Freeman,¹ Karen Levy¹

Author Affiliations: (1) Department of Environmental Health, Emory University, Atlanta, GA, United States Department of Environmental Health, Emory University Rollins School of Public Health, 1518 Clifton Road NE, Atlanta, GA, USA

Note: this manuscript is published in the *International Journal of Hygiene and Environmental Health* as Delahoy, M. J. *et al.* Pathogens transmitted in animal feces in low- and middle-income countries. *Int. J. Hyg. Environ. Health* **221**, 661–676 (2018). It is formatted for the requirements of that journal.

ABSTRACT

Animals found in close proximity to humans in low-and middle-income countries (LMICs) harbor many pathogens capable of infecting humans, transmissible via their feces. Contact with animal feces poses a currently unquantified—though likely substantial—risk to human health. In LMIC settings, human exposure to animal feces may explain some of the limited success of recent water, sanitation, and hygiene interventions that have focused on limiting exposure to human excreta, with less attention to containing animal feces.

We conducted a review to identify pathogens that may substantially contribute to the global burden of disease in humans through their spread in animal feces in the domestic environment in LMICs.

Of the 65 potentially pathogenic organisms considered, 15 were deemed relevant, based on burden of disease and potential for zoonotic transmission. Of these, five were considered of highest concern based on a substantial burden of disease for which transmission in animal feces is potentially important: *Campylobacter*, non-typhoidal *Salmonella* (NTS), Lassa virus, *Cryptosporidium*, and *Toxoplasma gondii*. Most of these have a wide range of animal hosts, except Lassa virus, which is spread through the feces of rats indigenous to sub-Saharan Africa. Combined, these five pathogens cause close to one million deaths annually. More than half of these deaths are attributed to invasive NTS. We do not estimate an overall burden of disease from improperly managed animal feces in LMICs, because it is unknown what proportion of illnesses caused by these pathogens can be attributed to contact with animal feces.

Typical water quantity, water quality, and handwashing interventions promoted in public health and development address transmission routes for both human and animal feces; however, sanitation interventions typically focus on containing human waste, often neglecting the residual burden of disease from pathogens transmitted via animal feces. This review compiles evidence on which pathogens may contribute to the burden of disease through transmission in animal feces; these data will help prioritize intervention types and regions that could most benefit from interventions aimed at reducing human contact with animal feces.

Keywords: zoonotic pathogens; animal feces; diarrhea; enteropathogens; water sanitation & hygiene

INTRODUCTION

Many human pathogens can be found in animal feces, yet the feces from these animals pose a currently unquantified—though likely substantial—risk to human health (Dufour et al., 2012). Insufficient separation of animal feces from human domestic environments can lead to fecal-oral transmission of zoonotic pathogens through direct contact with animal feces or soil, or fecal contamination of fomites, food, or water sources (Penakalapati et al., 2017). In addition to the acute gastrointestinal symptoms that can arise from contact with animal feces, people—particularly children, pregnant women, and the immunocompromised—may experience severe sequelae after a zoonotic infection (Checkley et al., 1998; Dufour et al., 2012). There is currently no estimate of the burden of poorly managed animal feces on human health.

Systematic reviews have demonstrated a 30-40% decrease in childhood diarrhea after the introduction of improved sanitation in low- and middle-income countries (LMICs) (Freeman et al., 2017; Wolf et al., 2014), but several recent water, sanitation, and/or hygiene interventions have failed to find consistent evidence of decreasing rates of childhood diarrhea, decreasing fecal contamination of household stored water, decreasing soil-transmitted helminth (STH) infections, and/or improving anthropometric indicators of malnutrition (Clasen et al., 2014; Null et al., 2018; Patil et al., 2014). One possible conclusion is that these interventions failed to interrupt a critical pathway in pathogen transmission: exposure to animal feces.

Improvements to water quality, water quantity, and handwashing could reduce exposure to both human and animal pathogens. However, providing access to improved sanitation by definition involves separating humans from contact with their own excreta, but not from animal waste (WHO/UNICEF, 2015). Less attention has been given to the role of contact with animal feces in causing human illness (Dufour et al., 2012). Animals are often present in the domestic environment

in LMICs, and people in these countries may have frequent contact with them (Dione et al., 2011; Harvey et al., 2003; Zambrano et al., 2014). Thus, even as sanitation efforts may reduce the quantity of human excreta in the environment, contamination from animal feces may still contribute to substantial burden of disease in humans.

Understanding the burden of disease from animal feces would provide critical information to direct public health investments, yet consolidated information on which pathogens may be transmitted in animal feces is lacking, making it difficult to assess the importance of animal feces exposure to human health. In this review, we identify pathogens that may substantially contribute to the global burden of disease in humans through their spread in animal feces in the domestic (household) environment in LMICs, and identify the animals that transmit these pathogens. Understanding the contribution of animal feces to the burden of disease involves identifying pathogens that can be transmitted in animal feces, understanding the extent of zoonotic transmission, and establishing that such zoonotic transmission can give rise to illness in humans. This review accumulates evidence on these topics from recent research in LMICs.

METHODS

2.1. Pathogen inclusion criteria

We evaluated bacteria, protozoa, viruses, and helminths for inclusion in this review using a multistep process. As the majority of reviewed literature on human health impacts from exposure to animal feces has focused on health outcomes related to exposure to enteric pathogens or helminths (including both short- and long-term sequelae such as diarrhea and malnutrition) (Penakalapati et al., 2017), we first considered all enteric pathogens and STHs that were included in two recent large studies describing an array of enteric pathogens/STHs: the Global Enteric Multicenter Study (GEMS) and MAL-ED (The Etiology, Risk Factors, and Interactions of Enteric Infections and

Malnutrition and the Consequences for Child Health Study) (Houpt et al., 2014; Panchalingam et al., 2012). Animal feces also pose a risk to human health by contaminating water sources (Dufour et al., 2012). Thus, we next considered waterborne excreted pathogens listed in the Global Water Pathogen Project as of August 29, 2017 (Rose and Jiménez-Cisneros, 2017), which provides an extensive compilation of waterborne pathogens. Third, we identified potential pathogens through our systematic review of the health impacts of exposure to poorly managed animal feces (see Penakalapati et al., 2017 for detailed methods), as this review was specifically focused on health outcomes (including pathogen infection) resulting from exposure to animal feces, and was not restricted to literature on waterborne or enteric pathogens. We considered pathogens detected in studies that met the inclusion criteria of that review, as well as pathogens from additional papers marked as relevant during the title and abstract assessment, specifically if the abstract mentioned pathogens not previously considered or new pathogen-animal host pairs from research in an LMIC.

Pathogens were categorized based on burden of disease and potential for transmission in animal feces. The transmission of pathogens via animal feces in LMICs was considered “potentially important” if (1) most transmission to humans resulted from exposure to the feces of specific animals, rather than human feces (e.g., rats for Lassa virus) or (2) the feces of a broad range of animal hosts pose a risk of transmitting the pathogen and causing illness. Transmission via animal feces in LMICs was considered to be “of limited importance, or insufficient evidence of importance” for pathogens that (1) were predominantly transmitted from human-to-human and, while transmitted in animal feces, did not have a wide range of animal hosts or (2) had insufficient epidemiologic and/or molecular evidence of contributing to the human disease burden through zoonotic transmission. If zoonotic species of an organism were less pathogenic to humans than anthropogenic strains (and thus zoonotic strains were not thought to account for a large proportion of the symptomatic illness caused by that pathogen), the role of animal fecal transmission in the

burden of disease was also considered “of limited importance, or insufficient evidence of importance”.

Pathogens had to meet the following criteria to be included. Exclusions to these criteria are noted where applicable:

1. **Found in animal feces in domestic settings.** Species of the pathogen capable of infecting humans can be found in the feces of animals that are common to domestic settings. Pathogens that are only transmitted by a small number of wild animal species (e.g., pathogens transmitted only by primates) or aquatic species were excluded.
2. **Cause illness in humans.** The pathogen is linked to illness in humans. Pathogens that primarily cause illness in animals with humans serving only as incidental dead-end hosts were excluded. Organisms generally considered commensals that occasionally give rise to sporadic opportunistic infections were excluded.
3. **Substantial contribution to disease burden.** The pathogen contributes substantially to the global burden of disease in humans. We considered pathogens responsible for either a minimum of one million disability-adjusted life years (DALYs), or at least 5,000 deaths annually to have a “substantial” contribution to the burden of disease in humans. Pathogens with an unquantified burden of disease were included, whereas pathogens with a quantified burden of disease that was not “substantial” were excluded.

Once identified for inclusion, pathogens were classified into the following categories. Category I pathogens contribute substantially to the burden of disease and the role of animal feces in transmission is potentially important. Category II pathogens have an unquantified burden of disease,

and the role of animal feces is also potentially important. Category III and Category IV pathogens have limited or insufficient evidence of transmission via animal feces. Category III pathogens have a substantial burden of disease, whereas the burden for Category IV pathogens is unquantified.

2.2. Literature review extraction

Information for this review was gathered from our team's literature review of the impact of animal feces on human health (Penakalapati et al., 2017), reference texts (Heymann and American Public Health Association, 2015; Rose and Jiménez-Cisneros, 2017), and from targeted journal searches. For the included pathogens, all literature uncovered in our review of the impact of animal feces on human health was included if it reported an association between exposure to animals or animal feces and the detection of a human pathogen infection. Information on burden of disease was primarily collected from the mortality analysis of the 2015 Global Burden of Disease Study (GBD) (Wang et al., 2016); however, targeted searches were used to assess burden for pathogens not included in the GBD.

For Category I pathogens, which were considered the pathogens of highest concern, we extracted information on the burden of disease, clinical manifestation, known animal hosts, transmission routes/burden associated with zoonotic transmission, relevant species, control options proposed in existing literature, and other information relevant to host susceptibility and transmission. Shorter descriptions of Category II-IV pathogens covering similar topics are also included. For excluded pathogens, we documented references to support exclusion. Proposed control options and other information on host susceptibility and transmission were included when identified in the literature we reviewed, but are not ordered based on any specific criteria and do not constitute a comprehensive list. While the transmission of antimicrobial resistant bacteria may be of concern when considering exposure to animal feces, it was not specifically a focus of this review.

RESULTS

Approximately 65 potentially pathogenic species or groups ("potential pathogens") were considered for inclusion (Figure 6.1 and Table 6.1). The exact number of potential pathogens depends on how they are grouped; for example, several different *Escherichia coli* pathotypes were counted as distinct pathogens. We excluded 50 potential pathogens, and outline the reasons for these exclusions in Table 6.1. Classification grouping of the remaining 15 pathogens is shown in Figure 6.1 and discussed below for each category, ordered by bacteria, viruses, protozoa, then helminths. The five pathogens of highest concern (Category I) are *Campylobacter*, non-typhoidal *Salmonella*, Lassa virus, *Cryptosporidium*, and *Toxoplasma gondii*.

Of the 62 papers included in our team's literature review of the impact of animal feces on human health (Penakalapati et al., 2017), 15 reported an association between exposure to animals or animal feces and human infection with one of the pathogens included in this review. The findings from each paper are integrated into the following pathogen descriptions.

3.1. Category I: pathogens with a substantial burden of disease with potentially important transmission in animal feces

3.1.1. *Campylobacter* spp.

In 2015, *Campylobacter* caused an estimated 37,500 deaths from acute diarrhea ("diarrhea deaths") globally, most of these (30,900) in children under five years old (Wang et al., 2016). *Campylobacter* is endemic in Africa, Asia, and the Middle East, and has been found in approximately 5-25% of gastrointestinal cases across a number of studies in LMICs (Kaakoush et al., 2015). The incidence and prevalence of *Campylobacter* have increased globally in the last decade, though deaths have decreased (Kaakoush et al., 2015; Wang et al., 2016). MAL-ED identified *Campylobacter* as a

significant contributor to community diarrhea in children under two years old (Platts-Mills et al., 2015).

The main symptoms of *Campylobacter* infection are fever, diarrhea, and abdominal pain (Butzler, 2004). There are several potential long-term sequelae of *Campylobacter* infections, including reactive arthritis and Guillain-Barré syndrome (Butzler, 2004; Wilson et al., 2008).

Campylobacter is most often found in poultry and cattle; other animals—including young dogs and cats, other pets, pigs, rodents, and birds—may also be reservoirs of *Campylobacter* capable of infecting humans (Heymann and American Public Health Association, 2015). *Campylobacter coli* and *C. jejuni* are the primary species of importance to human health; *Campylobacter concisus* and *Campylobacter ureolyticus* are emerging species that may also be important to human health (Kaakoush et al., 2015).

Campylobacter transmission occurs through foodborne and waterborne routes, as well as through exposure to chicken feces (Butzler, 2004; Kaakoush et al., 2015; Pitkänen, 2013). Exposure to live chickens has been identified as an important transmission route of *Campylobacter* in studies spanning multiple LMICs (Butzler, 2004). In a recent meta-analysis, exposure to domestic poultry was significantly associated with higher odds of campylobacteriosis (Zambrano et al., 2014). In a study in Lima, Peru, exposure to chickens in the household was strongly associated with *Campylobacter jejuni* infection; no specific foods were associated with *C. jejuni* infection in the same study (Grados et al., 1988). Other research in Lima, Peru reported that children often come into contact with chicken feces, that many chickens are infected with *Campylobacter*, and that *Campylobacter* survives well on the patios of households where children may come into contact with animal feces (Marquis et al., 1990). Having chickens infected with *C. jejuni* was identified as a risk factor for *C. jejuni* detection in children with backyard poultry in Egypt (El-Tras et al., 2015). Transmission of *C. jejuni* between animals (chickens, dogs, guinea pigs, and rabbits) and children was demonstrated in a study in

Ecuador, though the children were asymptomatic (Vasco et al., 2016). *C. jejuni* can survive in chicken feces for nearly a week (Ahmed et al., 2013; Kaakoush et al., 2015). Outside of a host, *Campylobacter* survives best in cold, moist environments (Wilson et al., 2008).

Proposed control options include appropriate food preparation and handling, water treatment, and keeping chickens out of the household (though chicken corralling may be ineffective in reducing exposure (Oberhelman et al., 2006)). Providing clean water for livestock can reduce infection in cattle (Ellis-Iversen et al., 2009; Kaakoush et al., 2015).

Since the detection of fluoroquinolone-resistant clinical *Campylobacter* isolates in Africa and Asia in the early 1990s, antimicrobial resistance to *Campylobacter* has become increasingly prevalent in both high-income countries and LMICs, with macrolide resistance also of increasing concern (Luangtongkum et al., 2009).

3.1.2. Non-typhoidal *Salmonella*

The GBD estimates that non-typhoidal *Salmonella* (NTS) causes 90,300 annual diarrhea deaths globally, with 38,500 of these deaths occurring in children under five years old (Wang et al., 2016). Of the diarrheal pathogens considered in the GBD, NTS had the third-highest population attributable fraction for all-age diarrhea mortality, surpassed only by rotavirus and *Shigella* (Wang et al., 2016).

The GBD estimate is limited to diarrheal deaths and does not include deaths from invasive NTS (iNTS), a severe form of NTS with a high case fatality rate that is often not associated with diarrhea/typical NTS presentation (Ao et al., 2015). In 2010, there were an estimated 3.4 million cases of iNTS, with iNTS estimated to cause more than 650,000 annual deaths, approximately half of which occur in Africa (Ao et al., 2015).

Common symptoms of NTS include bloody diarrhea, nausea, and vomiting; other more severe complications (such as hepatomegaly and appendicitis) are less frequently observed (Sánchez-Vargas et al., 2011). iNTS is a major cause of bloodstream infection in Africa (Ao et al., 2015; Feasey et al., 2012). Symptoms of iNTS resemble enteric fever and are life-threatening; they include fever and respiratory complications, often without gastrointestinal symptoms (Gal-Mor et al., 2014). There is a positive correlation between iNTS and HIV, malaria, and malnutrition (Ao et al., 2015). Malaria may also be associated with non-invasive NTS in children (Crump et al., 2015).

NTS has a broad range of animal hosts, including poultry, cattle, swine, and domestic animals, as well as wild animals, reptiles, rodents, and insects (Hilbert et al., 2012). Transmission routes have been studied in developed countries; less is known about transmission where NTS is endemic (Crump et al., 2015; Morpeth et al., 2009). In developed countries, consumption of produce contaminated with animal feces and contact with animals have been described as risk factors (Crump et al., 2015; Williams et al., 2016). NTS can multiply in food and can survive for months in soil, insect feces, or rodent feces (Lynch and Tauxe, 2009; Mitscherlich and Marth, 1984).

Much of the transmission of NTS is considered zoonotic, with little sustained human-to-human transmission (Okoro et al., 2012). However, one study of risk factors for NTS infection in Bangladesh did not find an association between NTS infection and presence of animals in the home (Leung et al., 2013). Foodborne transmission likely accounts for a high proportion of global NTS cases (Majowicz et al., 2010).

Human-to-human transmission has been postulated as more important than zoonotic transmission of iNTS, though it has been demonstrated that *Salmonella* Typhimurium ST313, a dominant cause of iNTS in sub-Saharan Africa, is not host-specific and can infect chickens (Kariuki, 2006; Okoro et al., 2012; Parsons et al., 2013). Other zoonotic serovars of NTS have been associated with invasive NTS

infection, e.g., *Salmonella enterica* serovar Choleraesuis, which can be difficult to treat because of antimicrobial resistance (Chiu et al., 2005).

There are more than 2,500 serovars of *Salmonella*; however, only a small subset of these serovars (especially serovars Typhimurium and Enteritidis) are of importance to human health (Crump et al., 2015; Hendriksen et al., 2009).

Improvements in safe handling of food and reductions in malaria prevalence are thought to be effective control measures for reducing NTS; however, evidence on control options for LMICs remains limited, as little data exists on risk factors for endemic transmission (Crump et al., 2015). An NTS vaccine has proved efficacious in poultry; however, there is no vaccine for other animal hosts or for humans (Desin et al., 2013; Gal-Mor et al., 2014). While antibiotics are recommended for invasive disease and for non-invasive disease in elderly and immunocompromised individuals, antibiotics can be harmful and prolong NTS symptoms in previously healthy individuals (Gal-Mor et al., 2014). The prevalence of antibiotic resistance in NTS has been increasing in the past decades and is of public health concern; this topic has been reviewed elsewhere (Crump et al., 2015).

3.1.3. Lassa virus

The United States Centers for Disease Control and Prevention (CDC) reports that surveillance for Lassa fever is not well established. The best current estimates suggest there are 100,000-300,000 cases of Lassa fever annually, resulting in approximately 5,000 deaths, almost all in West Africa (CDC Viral Special Pathogens Branch, 2015). Earlier reports estimated 2-3 million annual Lassa virus cases, resulting in 5,000-10,000 annual deaths (Fichet-Calvet and Rogers, 2009; Saluzzo and Dodet, 1999). Many people infected with Lassa virus are asymptomatic, but symptoms can be severe, resulting in high mortality rates for children and pregnant women. Lassa virus may present

similarly to Ebola virus, with common symptoms including fever, headache, sore throat, vomiting, and bleeding (Richmond and Baglole, 2003).

The reservoir for Lassa virus is multimammate rats (*Mastomys natalensis*), which are found in sub-Saharan Africa (Bonwitt et al., 2017). Lassa fever is predominantly identified in West Africa, where rodents may enter the household or food storage area, and where rodents are hunted and consumed (CDC Viral Special Pathogens Branch, 2015; Richmond and Baglole, 2003; Yun and Walker, 2012). People may become infected upon ingestion or inhalation of rodent feces, or by ingesting rodent meat. Person-to-person transmission is also possible, including through sexual transmission, though the contribution of this latter transmission route is unknown (Richmond and Baglole, 2003).

Food hygiene measures, such as keeping rodents from entering food storage areas, have been recommended for Lassa virus prevention (World Health Organization, 2017a). Isolation of patients may reduce spread in health care facilities; coupled with surveillance and contact tracing, this may help curb epidemics (World Health Organization, 2017a). Vaccine research has been proposed, though problems may arise with delivery, as vaccine uptake is already low in the regions most affected (Richmond and Baglole, 2003). In a 2017 meeting, the World Health Organization (WHO) identified Lassa fever as a priority disease for increased focus, based on high epidemic potential with few existing medical countermeasures (World Health Organization, 2017b).

3.1.4. *Cryptosporidium* spp.

Cryptosporidium causes an estimated 64,800 diarrhea deaths annually, almost all in children under five years old (Wang et al., 2016). The GBD ranks *Cryptosporidium* second in diarrheal pathogens to which the most deaths in children under five are attributed (Wang et al., 2016). In another burden analysis based on GEMS data, *Cryptosporidium* was estimated to contribute to approximately 200,000 annual

deaths in children under two years old in sub-Saharan Africa, India, Pakistan, Bangladesh, Nepal, and Afghanistan (Sow et al., 2016).

Cryptosporidium causes acute watery diarrhea, and can cause vomiting and fever (Heymann and American Public Health Association, 2015). *Cryptosporidium* is also significantly associated with prolonged and persistent diarrhea (Baqui et al., 1992; Cruz et al., 1988; Mølbak et al., 1993; Moore et al., 2010) and with growth faltering, the deficits of which may not be recovered by children infected in infancy (Checkley et al., 1998). GEMS identified *Cryptosporidium* as a major cause of moderate-to-severe diarrhea (MSD) in young children, with an increased risk of death among toddlers aged 1-2 years (Kotloff et al., 2013). MAL-ED research further implicated *Cryptosporidium* as a significant contributor to community diarrhea in infants (Platts-Mills et al., 2015). *Cryptosporidium* infection is particularly harmful for immunocompromised individuals (Hunter and Nichols, 2002).

Cryptosporidium infection occurs through waterborne, foodborne, person-to-person, and zoonotic transmission routes. *Cryptosporidium* has been identified in more than 150 mammalian species (Fayer et al., 2000), as well as in birds, reptiles, fish, and amphibians (Fayer, 2004). *Cryptosporidium hominis* is transmitted from person-to-person (with the only non-human reservoir being primates), whereas *Cryptosporidium parvum* has a number of animal hosts, predominantly ruminant animals (Cacciò et al., 2005). The two most common species of *Cryptosporidium* found in humans are *C. parvum* and *C. hominis* (Cacciò et al., 2005; Huang et al., 2004) and other important species identified in children in developing countries include *Cryptosporidium canis*, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, and *Cryptosporidium muris* (Cama et al., 2008; Xiao and Feng, 2008; Xiao and Ryan, 2004), which are generally considered dog, avian, cat, and mouse species, respectively. While it is thought that anthropogenic strains of *Cryptosporidium* predominate in LMICs, zoonotic strains are still commonly

isolated in both symptomatic and asymptomatic individuals in LMICs (Gatei et al., 2006; Xiao et al., 2001; Xiao and Feng, 2008).

One study in Cambodia found an association between having birds in the household and *Cryptosporidium* infection in children (Moore et al., 2016). A study among HIV/AIDS patients in Kenya found a significant positive association between contact with farm animals and *Cryptosporidium* infection (Wanyiri et al., 2014). A study in Ghana did not, however, find an association between presence of animals and *Cryptosporidium* infection among children (Adjei et al., 2004). Research in rural India suggests that animals may play a role in contaminating water sources with *Cryptosporidium* oocysts (Daniels et al., 2016, 2015).

Cryptosporidium has a very low infectious dose: even a very small number of oocysts can cause symptomatic infection in humans (Xiao and Ryan, 2004). *Cryptosporidium* oocysts are shed in large quantities by infected humans and animals and are immediately infectious upon shedding, contributing to ease of transmission (Dillingham et al., 2002). *Cryptosporidium* oocysts can persist for months in the environment (Fayer, 2004; Xiao and Ryan, 2004).

Control options include boiling or filtering water. If filtering, the pore size must be adequately small to capture the relatively small oocysts (Fayer et al., 2000). Chlorine water treatment alone is not effective for elimination of *Cryptosporidium* because the oocysts are highly chlorine tolerant (Dillingham et al., 2002). Access to sanitation and handwashing, especially for infected individuals and those in contact with livestock, are thought to be meaningful prevention/control measures (Heymann and American Public Health Association, 2015).

3.1.5. *Toxoplasma gondii*

As of 2013, there was very little information on the overall burden of disease of toxoplasmosis (Hotez et al., 2014); however, the burden of congenital toxoplasmosis (CT) alone is estimated to be

1.2 million DALYs annually, resulting from approximately 200,000 cases (Torgerson and Mastroiacovo, 2013). CT occurs in infants after their mothers become infected during pregnancy (Robert-Gangneux and Darde, 2012).

The majority of cases of toxoplasmosis are likely asymptomatic (Robert-Gangneux and Darde, 2012), though case presentation can include a wide range of symptoms including fever, lymphadenopathy, headaches, and visual impairment (Hill and Dubey, 2002). Symptoms are often more severe and life-threatening in the immunocompromised (Robert-Gangneux and Darde, 2012). CT can result in miscarriage, or in visual impairment or mental retardation in children whose mothers were infected during pregnancy (Batz et al., 2013). *T. gondii* infections have been linked with schizophrenia and other psychiatric conditions (Batz et al., 2013).

Cats are the definitive hosts of *T. gondii*. A range of other animals (hundreds of species, including cattle, pigs, sheep, chickens, and other mammals and birds) can serve as intermediate hosts (Robert-Gangneux and Darde, 2012). Transmission can occur through contaminated food, water, or soil (Heymann and American Public Health Association, 2015). Drinking unfiltered water has been associated with toxoplasmosis infection (Bahia-Oliveira et al., 2003). Direct contact with cat feces is also a route of exposure. Most non-congenital cases likely occur through ingestion of cat feces (e.g., on contaminated food) or from ingesting tissue cysts in undercooked meat (Dubey, 1996). Cats can shed large quantities of oocysts (Pena et al., 2006). Oocysts found in moist soil or water can be infectious for up to a year after they are shed (Heymann and American Public Health Association, 2015). Oocysts thrive in moist environments, which may explain high prevalence in the tropics. At least one epidemiologic study found a correlation between rainfall and toxoplasmosis infections (Robert-Gangneux and Darde, 2012).

Control options include filtering water, safe processing and handling of meat, washing produce, washing hands after exposure to cat feces or after handling soil, and reducing pregnant women's contact with cat feces (Heymann and American Public Health Association, 2015; Robert-Gangneux and Darde, 2012). Oocysts are resistant to chlorination (Robert-Gangneux and Darde, 2012).

3.2. Category II: pathogens with an unquantified burden of disease with potentially important transmission in animal feces

3.2.1. Shiga toxin-producing *E. coli*

The 2015 GBD does not estimate a burden of disease for shiga toxin-producing *E. coli* (STEC). A recent study estimates that STEC is the cause of 2.8 million acute illnesses and 230 deaths annually, which are likely conservative approximations (Majowicz et al., 2014). Despite the available estimate of STEC mortality and prevalence, this pathogen was classified as having an unquantified burden of disease, as a DALY estimate was not available. Surveillance and availability of diagnostics for STEC are lacking in LMICs (Croxen et al., 2013). STEC diagnostics were available for GEMS and MAL-ED, but STEC was not detected at any site in either study (Kotloff, 2017).

STEC can cause mild or bloody diarrhea that is often accompanied by fever and vomiting. Severe complications can include hemolytic uremic syndrome (HUS), end-stage renal disease, and death (Croxen et al., 2013; Majowicz et al., 2014). STEC infections usually resolve within a week and are self-limiting, but there is currently no protocol to prevent development of HUS after an infection (Croxen et al., 2013). General case management techniques for STEC have been proposed and discourage the use of antibiotics (Croxen et al., 2013; Holtz et al., 2009).

Most descriptions of the routes of transmission of STEC come from developed countries.

Transmission via contaminated food (especially beef) and water, contact with animals and their feces, and environmental transmission via soil have been recorded (Croxen et al., 2013). Cattle are

considered a primary reservoir for STEC (especially O157 strains), and they usually display asymptomatic carriage (Persad and LeJeune, 2014). STEC has also been isolated from a variety of other animals and insects (Croxen et al., 2013). Exposure to ruminant feces is thought to be important to the burden of disease in humans (Croxen et al., 2013; Gyles, 2007). Many STEC isolates carry antibiotic resistance genes (Croxen et al., 2013). Vaccine development is underway, focused both on vaccines for humans and animals (Croxen et al., 2013).

3.2.2. *Toxocara canis* and *Toxocara cati*

Toxocara canis and *Toxocara cati* are nematodes carried by dogs and cats, respectively, and are the causative agents of toxocariasis in humans. A 2014 review of the burden of disease from neglected tropical diseases lists toxocariasis as a condition for which limited or no burden information is available (Hotez et al., 2014), despite evidence that seroprevalence is quite high in a number of developing countries (Macpherson, 2013).

Toxocariasis is asymptomatic in many, but can manifest in a number of different symptoms, dependent on which organ the parasite migrates to (Fan et al., 2013). Visceral larva migrans is one of the more common outcomes of repeat *T. canis* infections and can result in a number of symptoms including headache, fever, abdominal pain, vomiting, diarrhea, fatigue, and weight loss (Fan et al., 2013; Macpherson, 2013). Ocular larva migrans is less common and can result in vision impairment (Fan et al., 2013). Exposure to dogs was significantly associated with *Toxocara* seropositivity in children in a study in Sri Lanka (Fernando et al., 2007).

3.3. Category III: pathogens with a substantial burden of disease with transmission in animal feces of limited importance, or with insufficient evidence of importance

3.3.1. Enteropathogenic *E. coli*

Enteropathogenic *E. coli* (EPEC) causes an estimated 12,000 global diarrhea deaths annually, almost all in children under five years old (Wang et al., 2016). Symptoms may include diarrhea (which can be watery and/or contain mucus), vomiting, dehydration, and fever (Croxen et al., 2013; Heymann and American Public Health Association, 2015). EPEC can result in persistent diarrhea and a failure to respond to rehydration therapy (Croxen et al., 2013).

There are two pathotypes of enteropathogenic *E. coli*: typical EPEC (tEPEC) and atypical EPEC (aEPEC), with important differences between transmission routes and illness severity; however, these two types are grouped together in estimations of disease burden. While tEPEC is transmitted only by humans, there are animal reservoirs of aEPEC, including dogs, sheep, rabbits, pigs, cattle, and non-human primates (Croxen et al., 2013; Vasco et al., 2016). Transmission of aEPEC between animals (pigs, dogs, and chickens) and children was demonstrated in a study in Ecuador, though the children were asymptomatic (Vasco et al., 2016). The pathogenicity of aEPEC may be low: in some studies aEPEC has been isolated as often in asymptomatic controls as in diarrhea cases (Ochoa and Contreras, 2011). GEMS did not find a significant association between aEPEC and MSD (Kotloff et al., 2013). Some evidence suggests that aEPEC might be more common than tEPEC in developing countries (Croxen et al., 2013; Ochoa and Contreras, 2011). Because the pathogenicity of the strain carried by animals (aEPEC) remains unclear, animal feces may have a limited role (or insufficient evidence of a potentially important role) in contributing to the overall burden of disease from EPEC.

3.3.2. Hepatitis E virus

Hepatitis E virus (HEV) is estimated to cause 26,700 deaths per year (Wang et al., 2016). Generally, the case fatality rate of HEV is low (~1%); however, case fatality rates in pregnant women are much higher, at around 20% (Dalton et al., 2008). Increased fatality may also occur among those with underlying liver disease (Kamar et al., 2014). Normally, HEV infections are self-limiting, with symptoms including fever, abdominal pain, and vomiting (Kamar et al., 2014).

There are four main genotypes of HEV found in mammals (genotypes 1-4); there is also a fifth genotype found in birds that is not thought to infect humans (Purcell and Emerson, 2008).

Genotypes 1 and 2 are transmitted between humans without zoonotic reservoirs, whereas genotypes 3-4 can have animal hosts. Genotype 3 is capable of infecting humans, but is less virulent to humans than genotypes 1 and 2 (Purcell and Emerson, 2008). There is limited evidence of whether genotype 4 infects humans (Dalton et al., 2008). Genotypes 1 and 2 predominantly circulate in developing countries with poor sanitation (Kamar et al., 2014). Genotypes 3 and 4 are mostly thought to infect humans in developed countries, although the distribution of genotype 3 in swine is widespread globally (Dalton et al., 2008). Despite genotype 3's widespread global prevalence in swine, most reported human infections have occurred in the United States, Europe, and Japan (Purcell and Emerson, 2008). The importance of HEV transmission from swine to humans is thought to differ geographically, being important in eastern and western China, though of lesser importance in central China and India (Kamar et al., 2014).

Pigs appear to be the main animals responsible for zoonotic transmission of HEV to humans (Kamar et al., 2014). Other animals that may have caused human infections include deer, rabbits, and wild boar (Kamar et al., 2014). The proportion of HEV transmission that is zoonotic is unknown; however, consumption of undercooked meat may contribute most to zoonotic

transmission (Kamar et al., 2014). A study in rural Bangladesh did not find an association between animal exposure and HEV (Labrique et al., 2013).

The role of animal feces in the transmission of HEV in LMICs was classified as “of limited importance, or insufficient evidence of importance” as anthropogenic genotypes appear to be more common in LMICs and non-human hosts of HEV are limited. However, given the potential for zoonotic transmission and the high prevalence of HEV genotype 3 in swine worldwide, this pathogen is worthy of attention when considering the burden of disease from improperly managed animal feces. Control of swine feces may be an important control measure for vulnerable groups, such as pregnant women, who have a high case fatality rate from HEV (Dalton et al., 2008).

3.3.3. *Ascaris* spp.

Ascariasis is a helminth infection that can contribute to poor nutritional status in humans. While there are few overt clinical symptoms until the passing of worms in feces, there can be serious complications of infection, such as bowel obstruction (Heymann and American Public Health Association, 2015). The GBD estimates that ascariasis is responsible for 2,700 annual deaths and approximately 1 million DALYs (Kassebaum et al., 2016; Wang et al., 2016).

Ascaris is transmitted through ova-contaminated soil—either through direct ingestion or through ingestion on produce (Heymann and American Public Health Association, 2015). Humans and pigs are the main reservoirs of *Ascaris*; there is also evidence that dogs may act as reservoirs for human infection (Shalaby et al., 2010). While previously *Ascaris lumbricoides* was thought to be the human form of *Ascaris*, and *Ascaris suum* was thought to be specific to pigs, there is current controversy as to whether these two species should be considered distinct (Alves et al., 2016; Betson et al., 2014). Zoonotic transmission of *Ascaris* has been given much attention recently. There is mounting evidence that *Ascaris* from pigs (usually *A. suum*) can infect humans, though establishment of

infection via this route may be less probable than infection from humans (Betson et al., 2014). It is thought that human-to-human transmission of *Ascaris* is the main transmission route in developing countries, whereas zoonotic transmission may be more common in developed countries (Betson et al., 2014). A recent large-scale molecular analysis of *Ascaris* from both human and pigs in Europe, Asia, Latin America, and Africa suggested that the isolates from humans and pigs were indeed distinguishable; however, there was evidence of transmission between humans and pigs. While this route of transmission was very common in Europe, it was uncommon (only “sporadic”) in areas where *Ascaris* infections are endemic (Betson et al., 2014). Thus, the role of animal feces in the transmission of *Ascaris* in LMICs was classified as “of limited importance, or insufficient evidence of importance”.

3.3.4. *Ancylostoma* spp. and *Necator americanus*

Hookworms (*Ancylostoma* spp. and *Necator americanus*) cause anemia and growth shortfalls, though may be asymptomatic in those with light infections. The GBD estimates hookworm infections to be responsible for approximately 1.8 million DALYs, but does not attribute any deaths to hookworm disease (Kassebaum et al., 2016; Wang et al., 2016).

The predominant species of hookworm in humans is *Necator americanus*, which is exclusively transmitted between humans (Tang et al., 2014), and another important species to public health is *Ancylostoma duodenale*, which is also not zoonotic. *Ancylostoma ceylanicum*, however, is zoonotically-transmitted by cats and dogs and may be of public health importance. This species predominates in humans in southeast Asia (Heymann and American Public Health Association, 2015), and in certain settings in this region its prevalence may rival that of *N. americanus* (Inpankaew et al., 2014).

A. ceylanicum and other zoonotic hookworms may also cause cutaneous larva migrans upon dermal penetration with their larvae (Reichert et al., 2016). Presence of animal feces in the compound was

associated with hookworm-related cutaneous larva migrans in Brazilian children (Reichert et al., 2016). Because of the unknown burden of disease specifically from *A. ceylanicum*, and the limited geographic distribution of this species of hookworm, a limited proportion of the global burden of hookworm infection may arise from zoonotic infections, though transmission in animal feces may be of higher importance in specific regions.

3.4. Category IV: pathogens with an unquantified burden of disease with transmission in animal feces of limited importance, or with insufficient evidence of importance

3.4.1. *Arcobacter* spp.

Arcobacter is a genus of bacteria closely related to *Campylobacter*, but distinguished as its own genus in the 1990s (Collado and Figueras, 2011). There is limited knowledge on the impact of *Arcobacter* on human health, though *Arcobacter butzleri* has been associated with persistent watery diarrhea and has been isolated in diarrheagenic stools in a small number of studies globally, with prevalence as high as 13% in diarrheagenic stools, from a single study in South Africa (Collado and Figueras, 2011; Lehner et al., 2005). Transmission routes of *Arcobacter* are not well understood, though it is thought that humans are infected via contaminated food or water (Collado and Figueras, 2011). Those in the food safety community have identified *Arcobacter* as a potentially serious foodborne hazard (Collado and Figueras, 2011). *A. butzleri* can be found in the stools of animals such as pigs, cattle, and horses (Collado and Figueras, 2011; Lehner et al., 2005).

3.4.2. *Yersinia enterocolitica*

Yersinia enterocolitica can cause diarrhea and abdominal pain, often in young children (Heymann and American Public Health Association, 2015). Symptoms can also be more severe and mimic appendicitis (Gupta et al., 2015). *Y. enterocolitica* has been isolated from the feces of a number of animals such as cows, sheep, goats, dogs, monkeys, deer, and rabbits; however, swine are the major

reservoir of importance to human health (Rahman et al., 2011; Wang et al., 2009). Foodborne transmission, often through consumption of pork, is thought to be the main source of yersiniosis cases (Bancerz-Kisiel and Szweda, 2015; Bucher et al., 2008). *Yersinia pseudotuberculosis* has been less well described, though may also be acquired through contact with animal feces and may contribute to the burden of yersiniosis (Nuorti et al., 2004). *Yersinia* infections are associated with cooler climates and were not detected in GEMS or MAL-ED (Kotloff, 2017).

3.4.3. *Giardia duodenalis*

The pathogen *Giardia duodenalis*, synonymous with *Giardia lamblia* or *Giardia intestinalis* (Muhsen and Levine, 2012), can give rise to giardiasis in humans, which may manifest in acute diarrhea and can have longer-term effects on malnutrition and malabsorption of nutrients (Anuar et al., 2014; Ortega and Adam, 1997). *Giardia* is often present in asymptomatic carriers; in fact, *Giardia* was significantly negatively associated with MSD in some age groups in GEMS, having been isolated in more asymptomatic controls than children with MSD (Kotloff et al., 2013). In a systematic review and meta-analysis of the association between *G. duodenalis* and diarrhea in children, it was found that *Giardia* was not significantly associated with pediatric acute diarrhea (and in several studies, was negatively associated with diarrhea), though it was associated with persistent diarrhea (Muhsen and Levine, 2012). The association between *Giardia* and markers of environmental enteric dysfunction (EED) was inconsistent in the MAL-ED study. *Giardia* was positively associated with a measure of increased intestinal permeability, but showed no significant association (and for one marker, a significant negative association) with measures of intestinal inflammation (Rogawski et al., 2017). Higher exposure to *Giardia* was associated with worse physical growth outcomes (length- and weight-for-age) in MAL-ED (Rogawski et al., 2017).

A review of the global burden of disease of *Giardia* is currently underway (Torgerson et al., 2014). Preliminary results of this review suggest that *Giardia* prevalence is high; however, there is limited or no information available on health outcomes that could be used to estimate the DALYs attributable to *Giardia* infections (Torgerson et al., 2014). Estimates of the burden of disease from giardiasis are not given in the 2015 GBD (Wang et al., 2016).

G. duodenalis can be found in the feces of humans, livestock, dogs, cats, rodents, non-human primates, and wild animals (Cacciò et al., 2005); however, of the eight known assemblages of *G. duodenalis*, only two (Assemblages A and B) are associated with giardiasis in humans, with Assemblage A more commonly being associated with symptomatic cases (Cacciò, 2015; Feng and Xiao, 2011). Assemblage A has been commonly isolated in animals such as livestock, dogs, and cats, whereas Assemblage B is less commonly found in these mammals (Feng and Xiao, 2011). While Assemblage A infects animals and can give rise to symptomatic giardiasis in humans, animals are more commonly infected with subtype AI whereas humans are mostly infected with subtype AII (Cacciò, 2015). In an analysis of risk factors for *G. duodenalis* infection in Malaysia, close contact with household pets was identified as a significant risk factor for *G. duodenalis* Assemblage A, but not Assemblage B (Anuar et al., 2014). The MAL-ED study found having a dirt floor and owning chickens to be significantly associated with *Giardia* infection in children under two years old (Rogawski et al., 2017). Being infected with *Giardia* was strongly correlated with being infected with *Campylobacter* in this study, indicating these pathogens may share transmission routes or susceptibility patterns (Rogawski et al., 2017).

While *Giardia* has a wide range of animal hosts, we classified it as a pathogen for which transmission in animal feces is “of limited importance, or insufficient evidence of importance” as evidence is lacking that verifies that zoonotic transmission significantly contributes to the overall burden of

disease from *Giardia*. The zoonotic potential of *Giardia* has been described elsewhere in detail and it has generally been concluded that the public health risk posed by zoonotic transmission of *Giardia* may be minimal (Cacciò, 2015; Feng and Xiao, 2011). In their comprehensive review of the zoonotic potential of *Giardia* spp., Feng and Xiao state that “subtyping data accumulated so far do not support a widespread occurrence of zoonotic transmission” (Feng and Xiao, 2011), which is also a conclusion of another review of the zoonotic potential of *Giardia* (Cacciò, 2015). Epidemiologic data also do not support widespread zoonotic transmission, though these data are largely from high-income countries (Xiao and Fayer, 2008).

3.4.4. *Strongyloides* spp.

Strongyloides stercoralis is a roundworm that can infect humans when its larvae penetrate the skin. *S. stercoralis* is estimated to infect 30-100 million people, although difficulties in diagnosing infection may lead to underestimates in prevalence and difficulty in assessing the burden of disease (Bethony et al., 2006; Engels and Savioli, 2006; Schär et al., 2013). Possible clinical symptoms include nausea, abdominal pain or discomfort, diarrhea, larva currens, skin eruptions, and weight loss (Olsen et al., 2009; Siddiqui and Berk, 2001; Viney, 2015). *S. stercoralis* can be life-threatening for the immunocompromised (Viney, 2015). *S. stercoralis* can replicate in humans, potentially causing hyperinfection that can last for years (Olsen et al., 2009; Siddiqui and Berk, 2001). Such chronic cases may contribute substantially to morbidity in a way that is yet unmeasured (Engels and Savioli, 2006). The species of *Strongyloides* that infect humans include *S. stercoralis* and *S. fulleborni*. While *S. stercoralis* has a high prevalence in humans and can be transmitted by dogs, *S. fulleborni* is transmitted by primates, has a limited geographic scope, and is a less common human infection (Olsen et al., 2009; Schär et al., 2013; Viney, 2015). It is currently unclear how much of transmission to humans is anthropogenic vs. zoonotic.

DISCUSSION

This review compiles evidence on which pathogens may contribute to the burden of disease through transmission in animal feces, which will help prioritize intervention types and regions that could most benefit from interventions aimed at reducing human contact with animal feces. Five pathogens were considered of highest concern based on a substantial burden of disease for which transmission in animal feces is potentially important: *Campylobacter*, non-typhoidal *Salmonella*, Lassa virus, *Cryptosporidium*, and *Toxoplasma gondii*. Combined, these five pathogens cause close to one million deaths annually. The four enteropathogens with GBD diarrhea mortality estimates that have the potential to be transmitted in animal feces (NTS, EPEC, *Campylobacter* spp., and *Cryptosporidium* spp.) are responsible for 28.3% of the ~500,000 estimated annual global diarrhea deaths in children under five years old (Figure 6.2) (Wang et al., 2016). While improvements to water quantity, water quality, and handwashing typically promoted in public health address some secondary transmission routes for both human and animal feces (Penakalapati et al., 2017), a residual burden of disease from these pathogens would be expected even under scenarios of improved sanitation coverage targeted at containing human feces.

While childhood diarrhea, helminth infection, and child growth have been areas of focus in previous reviews of the impact of animal feces on human health (Penakalapati et al., 2017; Zambrano et al., 2014), we found there may be a substantial burden of disease associated with fecal-oral transmission of pathogens found in animal feces but not related specifically to these outcomes (Section 4.2).

4.1. Zoonotic origin and intervention approaches

The pathogens that contribute to the burden of disease through their spread in animal feces are found in a broad range of animals (Sections 3.1-3.4). Cattle and other ruminants, poultry and other birds, dogs, cats, and rodents are all hosts of zoonotic pathogens that are carried in feces and

capable of infecting humans (Sections 3.1-3.4). Swine also harbor many of the pathogens that contribute to the burden of disease through transmission in animal feces, and are the primary non-human hosts of HEV and *Ascaris suum* (Sections 3.3.2-3.3.3). Many pathogens that can be transmitted in animal feces have also been isolated from horses, amphibians, and insects (Sections 3.1-3.4). Interventions to reduce human exposure to animal feces in LMICs will need to consider this broad range of hosts. Some interventions identified in our review of the impact of animal feces on human health (Penakalapati et al., 2017), such as corralling chickens or providing veterinary care to animals, would not sufficiently cover the range of animal hosts that contribute to the burden of disease by contaminating the environment with their feces.

Relative to other animals, rodents have received little attention in studies addressing exposure to animal feces in LMICs (Penakalapati et al., 2017). Preventing rodents from entering the household and food storage areas could be beneficial in the areas of West Africa where Lassa fever is endemic. Presence of rodent feces in the village compound (though outside of households) was associated with higher odds of moderate-to-severe diarrhea in a GEMS analysis of animal exposures (Conan et al., 2017).

We found that iNTS contributes significantly to the overall burden of disease represented by the pathogens included in this review, although information is limited on the zoonotic potential of NTS serovars with higher propensity to cause invasive infections. Improved understanding of the transmission routes of serovars of NTS causing invasive disease is needed (Clemens, 2009; Feasey et al., 2012). Additionally, with approximately half of the iNTS deaths and all Lassa fever deaths occurring Africa, this is a continent deserving of attention for interventions aimed at reducing the burden of disease from improperly managed animal feces.

Sanitation programs focused exclusively on containment of human waste may have limited ability to combat illness due to zoonotic transmission of the pathogens identified in this review. Similarly, some water treatment interventions, such as chlorinating water, would do little to reduce the burden from pathogens such as *Cryptosporidium* and *T. gondii* (Section 3.1). The average diameters of *Cryptosporidium* and *T. gondii* oocysts are approximately 3-5 μm and 10-12 μm , respectively (Isaac-Renton et al., 1998). Though some conventional point-of-use or small-scale water filtration systems may not be adequate for removal of such small oocysts (Jones and Dubey, 2010), the ability of point-of-use water filters to achieve high removal efficacy for *Cryptosporidium* has been demonstrated (Abebe et al., 2015).

A vaccine for Hepatitis E is available in China (Zhang et al., 2015); however, human vaccines are not available for most of the pathogens included in this review. Thus, control efforts will require alternate strategies, including environmental and/or behavioral interventions. Strategies to reduce the burden of disease from pathogens in this report likely need to focus on safe handling/disposal of animal feces; handwashing with soap after contact with animals or animal feces; and safe handling, preparation, and storage of food (Penakalapati et al., 2017). Pathogen infections in infancy and early childhood can have long-term consequences on growth and development (Checkley et al., 1998; The MAL-ED Network Investigators et al., 2014). Exclusive breastfeeding of infants could interrupt foodborne and waterborne transmission of the pathogens of highest concern identified in this review. Interventions should also account for the susceptibility of different individuals to severe sequelae from infections. Many pathogens identified in this review are particularly harmful to pregnant women, the immunocompromised, or those with underlying co-morbidities including malaria, HIV, liver disease, and malnutrition.

4.2. Burden of disease associated with pathogens transmitted in animal feces

Improperly managed animal feces contribute to an unknown proportion of the approximately one million annual deaths caused by the four pathogens of highest concern with available death estimates: *Campylobacter*, non-typhoidal *Salmonella*, *Cryptosporidium*, and Lassa virus, with iNTS contributing significantly to this figure. This represents an upper limit of the deaths from these pathogens attributable to improperly managed animal feces globally, as many of these infections may occur through other transmission routes. Contact with animal feces in soil or through other direct routes is important to the transmission of *T. gondii*, which is responsible for 1.2 million annual DALYs from congenital infections as well as an unquantified burden from non-congenital infections (Torgerson and Mastroiacovo, 2013). In addition to these pathogens of highest concern, other pathogens may contribute to the burden of disease from transmission in animal feces in LMICs (Sections 3.2-3.4).

The estimate of one million annual deaths is comprised of ~200,000 global diarrhea deaths each year from *Campylobacter*, NTS, and *Cryptosporidium* that are captured in the GBD estimates, an additional >100,000 deaths from *Cryptosporidium* above GBD estimates, >650,000 annual iNTS deaths, and 5,000-10,000 annual Lassa virus deaths (Section 3.1). The GBD estimates for diarrheal pathogens capture acute diarrheal deaths, and do not consider deaths that may occur after acute symptoms have subsided, or non-diarrheal deaths caused by the same pathogens. The burden of *Cryptosporidium* in children under two years old only in sub-Saharan Africa and certain regions of Asia exceeds GBD diarrhea estimates by more than 100,000 deaths (Section 3.1.4), when deaths from longer-term follow-up are counted (Sow et al., 2016).

The compiled burden estimates reflect morbidity/mortality from all transmission routes combined. There is limited information on what proportion of the burden of disease caused by these pathogens

is attributable to poor management of animal feces in LMICs. The WHO conducted a structured expert elicitation on transmission routes of foodborne hazards (Hald et al., 2016). The study indicated that animal contact is most important to the transmission of *Campylobacter*, NTS, STEC, and *Cryptosporidium* in WHO geographic regions containing LMICs (~10-20% of transmission), while animal contact was estimated to account for much less of the transmission of pathogens such as *Giardia*, EPEC, and enterotoxigenic *E. coli* (ETEC) (Hald et al., 2016). While this expert elicitation did not specifically identify animal contact as a major transmission route for *T. gondii*, it did find transmission in soil to account for ~20-40% of *T. gondii* transmission, indicating the importance of animal fecal contamination of soil in the spread of this pathogen (Hald et al., 2016). These findings are consistent with our review, though the WHO focused on direct contact with animals, rather than exposure to animal feces in the domestic environment.

The proportion of pathogen infections that is attributable to contact with animals in the United States has been estimated using outbreak data (Hale et al., 2012), but such estimations are more difficult where pathogens are endemic and surveillance of pathogens found in animal feces is limited. In the absence of an estimate of what proportion of transmission of pathogens in this review was in fact a result of contact with animal feces, we were not able to establish an estimate of the burden of disease resulting from contact with improperly managed animal feces in LMICs.

4.3. Human viral enteric pathogens

Viral enteropathogens—which as a group contribute substantially to the burden of childhood diarrhea—are generally host-specific, and we did not find evidence of animal feces contributing to the burden of disease caused by fecal-oral exposure to enteric viruses. One exception is that swine feces can pose a risk for Hepatitis E virus infection, though transmission through this route was considered of limited importance (or insufficient evidence of importance) in LMICs. While animal

feces play a minimal or non-existent role in the transmission of some high-burden viral enteropathogens such as rotavirus, there is potential for viral reassortment to occur and potentially change transmission dynamics of such viruses over time (Cook, 2004).

Rotavirus vaccination has been introduced in many countries since 2010, after investigators conducted the diarrhea etiology study that formed the basis of the attribution of diarrheal deaths to various enteropathogens in the GBD (GEMS, conducted 2008-2011). Global rotavirus vaccine coverage is now approximately 25% (PATH, 2016; World Health Organization, 2017c). As rotavirus deaths decline, pathogens such as NTS and *Cryptosporidium* may begin to account for a greater proportion of overall diarrheal deaths.

4.4. Limitations

This review considered the contribution of feces from animals that are common to domestic settings in contributing to the burden of disease; however, uncontained animal excreta can contribute to the burden of disease through other means. Animal feces can contaminate water, which is relevant for some pathogens included in this review (e.g., *Cryptosporidium*), but may also contribute to the transmission of other pathogens such as *Schistosoma* spp., for which animal feces are important in sustaining transmission, even though they do not pose a risk in the household environment (Table 6.1). Animal feces may also attract flies that can transmit infectious diseases, such as trachoma. Animal urine can transmit pathogens such as *Leptospira* spp., which is responsible for a high number of annual deaths (Costa et al., 2015). Pathogens transmitted only by primates were excluded; however, primates may live in proximity to humans in certain settings (Ghai et al., 2014).

Comparisons of the burden of disease between pathogens were not always possible. Some pathogens (such as the causative agents of echinococcosis and cysticercosis) were excluded based on the comparatively low burden of disease, although the burden from such pathogens could be higher

than that of some pathogens that were included in the review with an unquantified burden of disease (e.g., *Arcobacter* spp. or *Yersinia* spp.). Furthermore, estimates of disease burden were based on death or DALY estimates, which may not adequately capture the burden from long-term sequelae such as stunting or EED.

The aggregation of zoonotic and non-zoonotic pathotypes of certain pathogens in the GBD made comparisons difficult between the burden of zoonotic and non-zoonotic species of the same genus. The burden of disease from *Cryptosporidium* spp. is substantial; however, the majority of this burden in LMICs is likely from the transmission of *C. hominis* (for which humans are the primary hosts), which is more prevalent than zoonotic strains of *Cryptosporidium* in many developing countries and may also be responsible for more severe symptoms (Mbae et al., 2015; Xiao and Feng, 2008). Similarly, there is a single estimate for the burden of disease from tEPEC and aEPEC combined, despite only one of these pathotypes being zoonotic.

While the transmission of antimicrobial resistant bacteria and antimicrobial resistant genes from animals to humans is an area deserving of attention when considering the burden of disease resulting from human exposure to animal feces (Dufour et al., 2012), this topic was not specifically considered in this review.

We used a systematic process to identify pathogens for inclusion in this review and to identify research from our team's prior review of the impact of animal feces on human health. However, when data or information were not identified as part of our systematic search, we conducted a targeted search of the literature to identify information for the review.

4.5. Research priorities

While we identify key pathogens that may contribute to the burden of disease through transmission in animal feces in LMICs, in this review we were unable to develop an overall estimate of the burden

of poorly managed animal waste on human health. Yet doing so would provide important guidance for policy makers and practitioners for the control of acute and chronic sequelae of enteric infection such as diarrhea, EED, and stunting. Estimates of what proportion of the burden of disease from the pathogens listed in this report is a result of zoonotic transmission would be required to produce such an estimate.

Identifying the proportion of infections with the pathogens considered in this review that are attributable to zoonotic transmission is a key research need. Microbial source tracking methods and genome sequencing of human, animal, or environmental samples present an opportunity to attribute pathogen infections to a human or zoonotic source. Such sequencing could provide new information on pathogens lacking evidence on the attribution of zoonotic transmission to the burden of disease, or provide insights on sources of environmental contamination (Schriewer et al., 2015). For example, our recent systematic review found *Giardia* to be the most commonly-reported pathogen outcome in studies assessing the impact of animal feces on human health in LMICs (Penakalapati et al., 2017); however, information on the zoonotic potential of *Giardia* remains limited for LMICs (Xiao and Fayer, 2008). Though the current state of the literature does not suggest animal feces to be of potentially high importance for *Giardia* transmission, epidemiologic studies and further investigations on host specificity of *Giardia* assemblage subtypes in LMICs could be useful in furthering the evidence on the zoonotic potential of *Giardia* (Feng and Xiao, 2011; Xiao and Fayer, 2008).

Quantifying concentration and shedding rates of pathogens found in animal feces, survival of these zoonotic pathogens in the environment, factors influencing fate and transport of such pathogens, dose responses to these pathogens, and human ingestion/contact patterns with animal feces is important to parameterizing quantitative microbial risk assessment (QMRA) models that could be

used to estimate the burden of disease associated with human exposure to pathogens transmitted in animal feces (Dufour et al., 2012). While QMRA methods have been used to assess health impacts from exposure to animal feces in high-income countries (e.g., Soller et al. 2010), we found little evidence of application of this methodology for exposure to animal feces in LMICs; however, identifying such research was not the main focus of the review and it is possible that we have overlooked some work in this area. Metrics such as concentration of common enteric pathogens in the feces of livestock, swine, and poultry (Graham and Nachman, 2010), as well as other domestic and wild animals (Cox et al., 2005), have been estimated in high-income countries and could be used to parameterize QMRA models. Such concentration estimates are available for most of the pathogens of highest concern identified in this review (*Campylobacter*, *Salmonella*, and *Cryptosporidium*) for high-income settings (Graham and Nachman, 2010). Although utilizing data from high-income countries would provide an opportunity to conduct QMRA modelling in LMICs, estimations of key QMRA parameters specifically for LMICs would be useful given differing conditions in these settings. LMICs have geographically unique and understudied pathogens, as well as vulnerable populations with varying susceptibilities to severe sequelae from pathogen infections (see Section 4.1). Estimating these parameters and conducting QMRA analyses for LMICs is a key research priority.

4.6. Conclusions

Through this review, we identified key pathogens that may substantially contribute to the global burden of disease in humans through their transmission in animal feces in the domestic environment in LMICs, and several other pathogens that should continue to be monitored for their potential contribution to human illness associated with contact with animal feces. This review fills an important gap in assessing which pathogens may have the potential to substantially contribute to the

burden of disease through transmission via animal feces in the household environment in LMICs, and reviews burden estimates for these pathogens. Improved understanding of which pathogens are transmitted in animal feces and the burden from these pathogens provides insight into the residual burden of disease that may be attributable to fecal contamination in the environment under improved human sanitation scenarios in the absence of animal feces control, and can elucidate meaningful ways to reduce the burden of disease via the safe management of animal feces.

Role of the funding source

This work was supported by the Bill & Melinda Gates Foundation (grant OPP1157522 to Emory University). Karen Levy is supported by the National Institute for Allergy and Infectious Diseases, NIH (grant 1K01AI103544). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders.

Acknowledgements

We thank Dr. Thomas Clasen for his advice in the early stages of this work.

Declaration of interest

Conflicts of interest: none.

Figure 6.1 Classification of pathogens by burden of disease and potential for transmission in animal feces in domestic/household settings in low- and middle-income countries (LMICs)

BURDEN OF DISEASE				
		High/substantial [‡]	Unquantified	Low [‡]
TRANSMISSION IN ANIMAL FECES (in LMICs)		Category I <u>bacteria</u> <i>Campylobacter</i> spp. non-typhoidal <i>Salmonella</i> <u>viruses</u> Lassa virus (West Africa) <u>protozoa</u> <i>Cryptosporidium</i> spp. <i>Toxoplasma gondii</i>	Category II <u>bacteria</u> shiga toxin-producing <i>E. coli</i> <u>helminths</u> <i>Toxocara canis</i> / <i>Toxocara cati</i>	Excluded [§]
Potentially important*				
Of limited importance/insufficient evidence of importance*		Category III <u>bacteria</u> atypical enteropathogenic <i>E. coli</i> <u>viruses</u> Hepatitis E virus <u>helminths</u> <i>Ascaris</i> spp. Hookworms	Category IV <u>bacteria</u> <i>Arcobacter</i> spp. <i>Yersinia enterocolitica</i> <u>protozoa</u> <i>Giardia duodenalis</i> <u>helminths</u> <i>Strongyloides stercoralis</i>	Excluded [§]
Minimal/none*		Excluded [§]	Excluded [§]	Excluded [§]
‡ High: >5,000 deaths or >1 million disability-adjusted life years (DALYs) annually; if quantified and not meeting the criteria for "high/substantial" then burden was "low" and the pathogen was excluded * Considered "potentially important" if (1) most transmission to humans resulted from contact with specific animals or (2) the feces of a broad range of animal hosts pose a risk of transmitting the pathogen and causing symptomatic illness. Considered to be "of limited importance, or insufficient evidence of importance" for pathogens if: (1) they were predominantly transmitted from human-to-human with few animal hosts, (2) zoonotic species were less pathogenic to humans and thus zoonotic strains were not thought to account for a large proportion of the symptomatic illness, or (3) there was insufficient epidemiologic and/or molecular evidence of contributing to the disease burden through zoonotic transmission. ¥ Animal feces in the domestic environment; pathogens are included in this category if exposure is not in domestic environment (e.g., exposure to animal feces in the aquatic environment); also included in this category are pathogens for which zoonotic strains do not cause symptomatic illness in humans. § Reasons for exclusion listed in Table 1				

Figure 6.2 Diarrhea deaths by attributable fraction, children under five years old, Global Burden of Disease Study (2015)

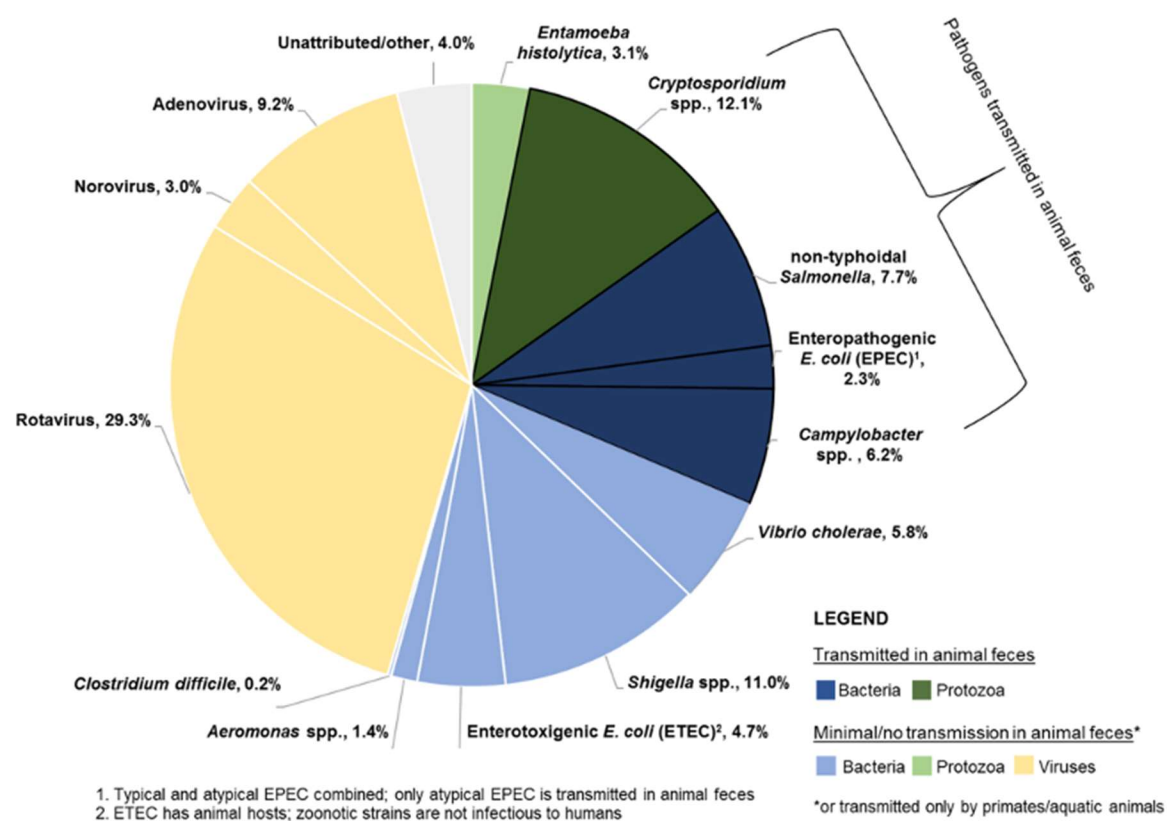


Table 6.1 Reasons for excluding potential pathogens from the list of pathogens that potentially substantially contribute to the burden of disease via transmission in animal feces in the household setting in low-and middle-income countries

Potential pathogen	Exclusion reason*
Bacteria	
<i>Aeromonas</i> spp.	Reservoirs are primarily humans and aquatic environments/organisms (Janda et al., 1983); pathogen has been isolated from other animals but not linked to human health outcomes (Parker and Shaw, 2011)
<i>Brucella</i> spp.	Most zoonotic transmission occurs when humans assist in birthing animals, or from eating unpasteurized milk products; these bacteria do not survive well in the environment; while animal contact may be an important risk factors, exposure to animal feces in the domestic setting does not seem to be an established exposure route to humans (Atluri et al., 2011)
<i>Chlamydia trachomatis</i>	Transmitted human-to-human via <i>Musca sorbens</i> ; while animal feces may provide a breeding area for this vector, the preferred breeding area is human feces (Emerson et al., 2001); exposure to animal feces in the household not considered the transmission route
Diffusely adherent <i>E. coli</i> (DAEC)	Hosts unknown and may not include animals (Croxen et al., 2013)
Enteroaggregative <i>E. coli</i> (EAEC)	Isolated in animals but this is not thought to be a source of human infections (Okhuysen and DuPont, 2010)
Enteroinvasive <i>E. coli</i> (EIEC)	Limited animal hosts (primates) (Croxen et al., 2013)
Enterotoxigenic <i>E. coli</i> (ETEC)	Zoonotic strains not pathogenic to humans: adhesion factors are species-specific (Torres, 2010; Wasteson, 2002)
<i>Helicobacter pylori</i>	Generally human-to-human transmission, the role of animals and food is controversial (Safaei et al., 2011; Vale and Vitor, 2010)
<i>Klebsiella</i> spp.	Generally considered commensals, but may cause opportunistic infections in the immunocompromised; however, given low pathogenicity unlikely to contribute substantially to burden of disease (Ristuccia and Cunha, 1984)
<i>Leptospira</i>	Transmitted in urine (Bharti et al., 2003)
<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>	May contribute to Crohn's disease (the burden of which is largely unknown in developing countries), but link to human illness is not well-defined (Economou and Pappas, 2008)
<i>Plesiomonas shigelloides</i>	Animal feces not implicated as transmission route; non-human hosts are mostly aquatic animals (Janda et al., 2016)
<i>Salmonella paratyphi</i>	Not transmitted in animal feces (Crump et al., 2015; Gal-Mor et al., 2014)
<i>Salmonella typhi</i>	Not transmitted in animal feces (Crump et al., 2015; Gal-Mor et al., 2014)
<i>Shigella</i> spp.	Limited animal hosts (primates) (Heymann and American Public Health Association, 2015)
<i>Vibrio cholerae</i>	Limited/no transmission by animal feces (Heymann and American Public Health Association, 2015; Nelson et al., 2009)
<i>Yersinia pestis</i>	Transmitted to humans through flea bites (Achtman et al., 1999)
Viruses	
Astrovirus	No documented cases of zoonotic transmission (De Benedictis et al., 2011)
Enteric adenovirus 40/41	Enteric adenovirus strains 40/41 are human-specific (Ghebremedhin, 2014)
Hepatitis A virus	Limited animal hosts (primates) (Heymann and American Public Health Association, 2015)
Human enteroviruses (A-D)	Not considered zoonotic (Taylor et al., 2001); includes polioviruses, Coxsackievirus, and echoviruses
Human papillomaviruses	Human viruses that are predominately transmitted sexually or from mother to child (Syrjanen and Puranen, 2000)

Potential pathogen	Exclusion reason*
Norovirus (GI/GII)	Humans are known hosts of norovirus GI/GII (Glass et al., 2009)
Picobirnavirus	Human pathogenicity is debated; little evidence of cross-species transmission (Ganesh et al., 2012)
Polyomavirus	Human polyomaviruses are host-specific to humans (Bofill-Mas et al., 2013)
Rotavirus	Strains found in animals unlikely to infect humans (Cook, 2004)
Sapovirus	Has been isolated in swine feces but not a major route of transmission to humans, if a route at all (Bank-Wolf et al., 2010)
Protozoa	
<i>Balantidium coli</i>	Low prevalence in humans; low virulence; public health significance thought to be low as most cases are asymptomatic (Ponce-Gordo and Jirků-Pomajbíková, 2017; Schuster and Ramirez-Avila, 2008)
<i>Cyclospora cayentanensis</i>	Not transmitted in animal feces (Mansfield and Gajadhar, 2004)
<i>Endolimax nana</i>	Pathogenicity and host specificity remain debated; likely a commensal in humans (Poulsen and Stensvold, 2016)
<i>Entamoeba</i> spp.	Zoonotic transmission not thought to contribute significantly to burden of disease (Thompson and Smith, 2011)
<i>Trichomonas hominis</i>	Low pathogenicity; unlikely to contribute significantly to the burden of disease (Li et al., 2015; Rüttgers, 1983)
Helminths	
<i>Ascaridia galli</i>	Pathogenic to poultry, not humans (Höglund and Jansson, 2011)
<i>Clonorchis sinensis</i> , <i>Metorchis</i> spp., <i>Opisthorchis</i> spp. (human liver flukes)	Transmitted to humans by ingestion of fish (Murell and Pozio, 2016)
Diphyllobothriidea	Though some may be transmitted in animal feces, transmission to humans is associated with eating contaminated fish (Waeschenbach et al., 2017)
<i>Echinococcus granulosus</i> / <i>Echinococcus multilocularis</i>	Excluded based on burden of disease (<5,000 deaths and <1 million disability-adjusted life years (DALYs) annually) (Kassebaum et al., 2016; Wang et al., 2016)
<i>Enterobius</i> spp.	<i>Enterobius vermicularis</i> , responsible for infections in humans, are human pinworms without non-primate animal hosts (Knopp et al., 2012)
<i>Fasciola</i> spp.	Exposure to aquatic environment and plants is a main source of transmission to humans, though there are mammals that can also harbor these pathogens; burden estimated as <1 million DALYs (Fürst et al., 2012)
Heterophyidae and Echinostomatidae	These intestinal flukes are mostly transmitted to humans through ingestion of fish (Traub and Dalsgaard, 2016)
<i>Oesophagostomum bifurcum</i>	Limited to primates; limited evidence of cross-species transmission (Gasser et al., 2005)
<i>Paragonimus</i> spp.	Humans become infected by ingesting crustaceans (Vélez et al., 2003)
<i>Schistosoma</i> spp.	There are many mammalian species that shed eggs in their feces that are important to the transmission cycle; however, humans become infected by coming into contact with cercariae in water, not by coming into contact with mammalian feces (Colley et al., 2014)
<i>Spirometra</i> spp.	There are animal hosts that can shed <i>Spirometra</i> in their feces but animal feces in the domestic environment is not the major source of transmission to humans (Liu et al., 2015)
<i>Taenia</i> spp.	The burden of disease from cysticercosis was not considered substantial (<5,000 deaths and <1 million DALYs annually) (Kassebaum et al., 2016; Wang et al., 2016)
<i>Trichuris</i> spp.	<i>Trichuris</i> spp. tend to be host-specific with human infections acquired from other primates (Ghai et al., 2014); responsible for <1 million annual DALYs (Hotez et al., 2014)
Other	
<i>Blastocystis</i> spp.	Pathogenicity for humans is still debated (Tan, 2008)

Potential pathogen	Exclusion reason*
<i>Colonic spirochetosis</i>	Pathogenicity for humans is still debated (Calderaro et al., 2007)
<i>Enterocytozoon bienersi</i> /microsporidia	<i>E. bienersi</i> and other microsporidia mostly infect immunocompromised hosts; pathogenicity for immunocompetent hosts is not well defined and scope may be limited; of note, most infections appear to be zoonotic in origin (Didier, 2005; Matos et al., 2012)
Isospora	Animals do not serve as hosts for <i>Isospora belli</i> , which is responsible for most human cases; opportunistic infection in immunocompromised (Lindsay et al., 1997; Pierce and Kirkpatrick, 2009)
Mycetoma	Group of fungal infections not transmitted in animal feces (though not all transmission routes are known) (Zijlstra et al., 2016)
* Details on inclusion/exclusion criteria are outlined in manuscript methods (Section 2.1)	

Chapter 6 References

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