PATHOGEN PATHWAYS STUDY FOR CHILDREN UNDER TWO YEARS IN THE USAID FIOVANA INTERVENTION AREAS OF SOUTHEASTERN MADAGASCAR

Final Report

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ACRONYM LIST

ADRA	Adventist Development and Relief Agency
CBT	Compartment bag test
CFU	Colony forming units
CLTS	Community-led total sanitation
CVB	Centre ValBio
DALY	Disability Adjusted Life Years
EED	Environmental Enteric Dysfunction
ENP	Essential nutrition portfolio
EPEC	Enteropathogenic *Escherichia Coli*
E. coli	Escherichia Coli
MAM	Moderate Acute Malnutrition
MPA	Multiphase programmatic approach
MPN	Most probable number
MUAC	Mid-Upper Arm Circumference
NT	Ny Tanintsika
POC	Point of collection
POU	Point of use
QMRA	Quantitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
SAM	Severe Acute Malnutrition
SBC	Social and Behavior Change
WASH	Water, sanitation, and hygiene
WHO	World Health Organization

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1 Executive Summary

Various pathways, such as hand-to-mouth contact, drinking water, food, soil, and fomites (objects that children put in their mouths), can lead to increased fecal pathogen exposure and health risks among young children. In Madagascar, almost 50% of children under five are stunted, with child growth and overall health being negatively impacted by inadequate water, sanitation, and hygiene (WASH). Understanding the degree to which each of these pathways contributes to infection risks is critical in designing targeted interventions that will improve the health of young children. Addressing these challenges also requires a strong understanding of the context and local norms.

The primary objective of this research was to identify the most important pathway(s) for fecal pathogen exposure among children under two in South East Madagascar. The second objective was to identify potential interventions for interrupting these transmission pathways that take into account the specific cultural and social norms of South East Madagascar.

We surveyed a total of 220 households in three regions of South East Madagascar (Fitovinany, Vatovavy, and Atsimo-Atsinanana). We divided fieldwork into two phases: phase I consisted of in-depth observations of 35 children (2-4 hours) and one-hour long surveys of their caregivers, while phase II included shorter caregiver surveys and spot-observations (10-15 minutes) in 185 households. For each household in both phases, we collected environmental samples (caregivers' hand-rinse, children's hand-rinse, cooked food, raw food, drinking water at the point of use (POU), and soil) and analyzed them for *Escherichia Coli (E. coli)* using the Compartment Bag Test (CBT) method. We also collected environmental samples (children hand-rinse, raw food, drinking water at POU, and soil) and fecal samples from animals and children in a subset of 26 households for presence/absence analysis of six pathogens (*Campylobacter, Shigella, Salmonella, Entamoeba Histolytica, Giardia Intestinalis,* and Adenovirus) using Quantitative polymerase chain reaction (QPCR).

Based on our *E. coli* measurements, we found that drinking water and soil were the two compartments most contaminated with fecal matter, with more than 60% of samples in the highest risk category (>100 MPN/100ml or >100 MPN/g). The following two compartments, children and caregivers' hand-rinse, had 25% of samples in the highest risk category.

Following these results, we then performed a Quantitative Microbial Risk Assessment (QMRA) to determine the most important exposure pathways and the risk by age category (0-6 months, 7-12 months, and 13-24 months) using pathogenic *E. coli* O157:H7 as an illustration. We focused on four different exposure pathways: child hand-to-mouth contact (more frequent than caregiver hand-to-mouth contact according to our observations), drinking water, soil ingestion, and food ingestion (we focused on cooked food only as we did not collect enough raw food samples to include in the QMRA).

We found that children's daily *E. coli* intake increased with age (10 Most Probable Number (MPN)/day for children aged 0-6 months, 188 MPN/day for 7-12 months, and 213 MPN/day for 13-24 months), which is consistent with children's development: the more they grow, the more they are in contact with their environment and with potential pathogens if the environment is contaminated. For the 0-6 months category, we found that the primary pathway for *E. coli* intake was through hand-to-mouth contact (representing 70% of total ingestion). For the other two age categories, we found that three pathways played an important role in children's ingestion of fecal matter: for the 7-12 months category hand-to-mouth contact was the primary pathway (41% of total ingestion) followed by drinking water (27% of total ingestion) and soil (21% of total ingestion). For the 13-24 months category, cooked food was the main pathway (34% of total ingestion) followed by drinking water (31%) and hand-to-mouth contact (25%).

Pathogens were present in animal feces more often (67%) than in children's feces (26%). The two compartments with the highest prevalence of pathogens were children's hand-rinse (46%) followed by soil (31%). *Campylobacter* was the pathogen found most frequently (present in 33% of all samples), while we did not detect any *Salmonella* and *Entamoeba Histolytica.* Our results suggested that direct or indirect contact with animal feces was an important pathway for bacterial infections. This interpretation may not apply to viruses, as we only found Adenovirus in drinking water and children's feces. These interpretations are hypotheses to test in future research, as we cannot draw definitive conclusions from this limited number of samples and the non-quantitative nature of results.

In consultation with the USAID-funded FIOVANA Resilience Food Security Activity team working in SE Madagascar and led by the Adventist Development and Relief Agency (ADRA), we developed a number of recommendations to interrupt these four transmission pathways. Some of our recommendations seek to reduce the overall level of fecal contamination present within the environment, such as the construction of animal pens or children playpens, the reduction of open defecation among adults and children, and the development of chlorination systems at the point of collection (POC). Other specific recommendations included the development of low-cost hand-washing stations, better access to soap (including the development of small businesses or community groups to produce locally-made soap), disinfection of storage containers and utensils, and the use of washable piece of fabric ("balotom" in local language) on top of mats to prevent children from being in contact with a contaminated floor. We also recommended developing Social Behavior Change (SBC) programs that would promote good hygiene practices and address detrimental traditional beliefs (e.g., that water is pure and cannot become contaminated). SBC requires understanding social and environmental conditions that facilitate or constrain the adoption of specific behaviors. We recommended that SBC programs include: 1) a barrier analysis to identify bottlenecks in behavior change, 2) community dialogue to identify and promote communities' own solutions, and 3) a household action planning to address each household's specific needs. Additional suggestions from participants who attended the local dissemination workshop included 1) encouraging communities to fence their water source and 2) organizing visits to model households and model villages.

2 Introduction

Exposure to human and animal feces causes enteric infections and can lead to diarrhea, which is the third-leading cause of mortality and morbidity globally among children under five. Diarrhea claims an estimated half a million lives every year among this age group.[1,2] Diarrheal diseases, enteric infections and environmental enteric dysfunction (EED) also contribute to malnutrition and undernutrition via poor nutrient absorption, anemia, and reduction in appetite.[3] Malnutrition is associated with reduced schooling and long-term cognitive impairment.[4,5] Inadequate water, sanitation, and hygiene (WASH) can increase the degree of exposure to fecal contamination. In Madagascar, only 11% of households have access to basic sanitation and almost half of the population practices open defecation. Meanwhile, 43% of households still rely on surface water or other unimproved water sources.[6] These poor levels of access to basic WASH services lead to a high rate of stunting, with 42% of children affected (one of the highest levels in the world).[7] Nearly 50% of children under five tested positive for pathogenic intestinal microorganisms.[8]

Children's exposure to fecal pathogens can occur via various pathways such as contaminated water, food, fingers, and fomites (objects) (**Figure 1**). Fingers represent hand-to-mouth contact, especially among young children exhibiting mouthing behaviors, and fomites (such as toys that children may touch and that may carry pathogens) can also act as an intermediate along this pathway. Flies can come into contact with feces, picking up pathogens, and then carry those pathogens to other places where they land, such as on food and skin. Fields represent contamination of soil with human or animal feces; young children may pick up and ingest soil particles. Fluids refer to water that may be contaminated at the source or during household storage. Finally, each of these pathways can connect to food, for example through dirty hands, contaminated water used to wash raw food, or flies landing on prepared food. People can then ingest pathogens transferred through these pathways by drinking contaminated water, eating contaminated food, and direct hand-to-mouth contact.

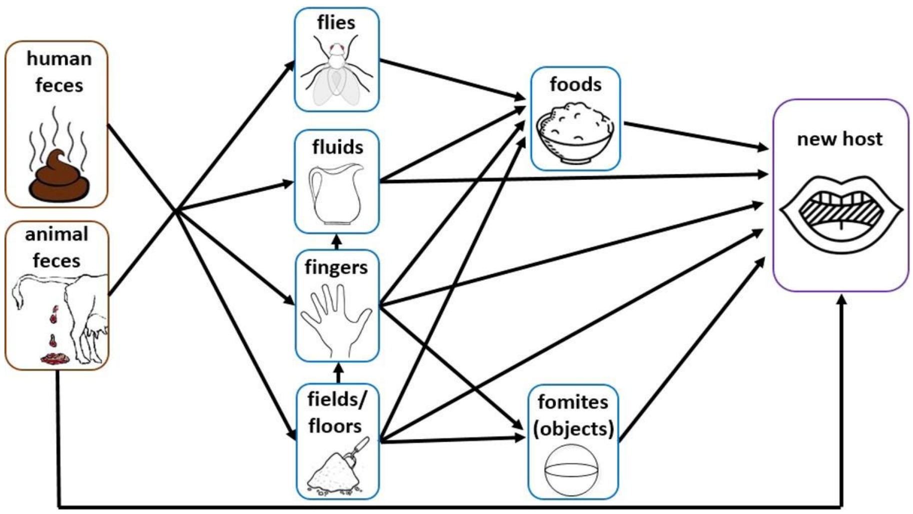

Figure 1: Pathways of exposure to fecal contamination. From Kwong et al., 2020[9] and adapted from Wagner et al., 1958[10]

Understanding the degree to which different exposure pathways contribute to children's ingestion of fecal matter can help prioritize interventions that will improve health among young children. Studies from various contexts have explored the degree to which each of these pathways plays a role in fecal pathogen exposure and transmission.[9,11–15] Several of them highlighted the importance of hand-to-mouth contact, which may often not be addressed by conventional water and sanitation interventions (**Table 1**).[9,12,16–18] Furthermore, the primary transmission pathways are often age-specific, due to differences in behaviors and mobility between age groups. Among children 6 to 35 months, mouthing behaviors, direct soil ingestion, and eating contaminated food were the primary pathways for *E. coli* ingestion in rural Bangladesh, while object-to-mouth contact accounted for the majority (60%) of *E. coli* ingestion among children under six months (**Table 1**).[19] Another study showed that higher measured levels of *E. coli* on hands were strongly associated with reports of diarrheal illness among children under five years.[20] In Tanzania, hand-to-mouth contact similarly resulted in approximately fifty times more ingestion of fecal matter than consumption of stored drinking water (**Table 1**).[16] Likewise, in an informal settlement in Kampala, Uganda, median risks of pathogen infection were highest in samples collected from soil and children's hands, compared with other samples from caregivers' hands and stored drinking water.[21]

Table 1: Main exposure pathway and most contaminated environmental compartments identified in prior studies, with proportions of *E. coli* ingested, if available.

Country	Population	Pathways examined	Main pathways*	Authors
Rural Bangladesh	Children under three	Hand-to-mouth contact, object-to-mouth contact, caregivers' hands, food, water, soil	- for children <6 months: object-to-mouth contact (60% of *E. coli* ingested) - for children 6-24 months: hand-to-mouth contact (~30% of *E. coli* ingested), followed by soil ingestion (~25-30% of *E. coli* ingested) and food (6-27% of *E. coli* ingested).	Kwong et al., 2020[9]
Tanzania	Children under five	Stored drinking water and hand-to-mouth contact	Hand-to-mouth contact (97-98% of pathogens ingested)	Mattioli et al., 2015[22]
Urban Uganda	Children and caregivers	Drinking water, hand-to-mouth contact, soil ingestion	Soil ingestion, followed by hand-to-mouth contact	Byrne et al., 2021[21]
Urban Ghana	Children under five	Drinking water, food, hand-to-mouth contact	Food (>99% of total exposure), followed by hand-to-mouth contact	Wang et al., 2017[23]
Country	**Population**	**Environmental compartments studied**	**Most contaminated compartments**	**Authors**
Rural Bangladesh	Children under five	Tube wells, stored drinking water, pond water, child hand rinses, soil, flies and food	Children's hands	Pickering et al., 2018[12]
Urban Zimbabwe	Adults	Drinking water, soil, hands	Hands, followed by soil and drinking water	Navab-Daneshmand et al., 2018[24]
Urban Kenya	Children and caregivers	Source water, stored drinking water, caregiver hands, child hands, household surfaces, soil, and standing water in open drainage ditches and streams	Open streams, followed by soil, drainage ditches, and floor surfaces	Bauza et al., 2020[25]

* *Only a few studies quantified the exposure pathways*

The majority of studies related to pathogens contamination in Madagascar have focused on pathogen detection in stool samples among humans, while fewer have focused on transmission risks via food or domestic animals.[8,26–29] Different factors affecting the risk or severity of fecal contamination have been highlighted, such as the geographic location,[30] sampling period (during the rainy season diarrheal symptoms are more acute[31]), or living conditions. With regard to living conditions in particular, children living in houses with floors made of solid materials (e.g. cement, tile) and those living in houses containing or surrounded by garbage were more likely to have severe diarrhea. Children who were breastfeeding, were within a family that owned cattle, and lived in a house with electricity were more likely to be healthy.[32]

The primary objective of this research is to identify the most important pathway(s) for fecal pathogen exposure among young children in Southeastern Madagascar. Results will inform the development and refinement of appropriate interventions to improve health. Specifically, this research aims to answer two key research questions:

1) What are the main ingestion pathways of fecal pathogens (both human and animal) for children under the age of two in implementation areas of the FIOVANA project?
2) What interventions could potentially interrupt these transmission pathways, considering the specific implementation challenges (e.g., topography and climate) and unique cultural and social norms of Southeastern Madagascar?

Addressing these questions requires a strong understanding of the contextual factors, structures, and barriers to design appropriate interventions. For example, in the three regions of Southeastern Madagascar targeted for this study (Vatovavy, Fitovinany, and Atsimo Atsinanana), the majority of the rural population live 'ankarenana' (near their fields) and only come to villages for social obligations (e.g., funerals). These isolated conditions increase the complexity of effectively providing WASH services and hygiene education. In addition, traditional beliefs and customs can present obstacles to sanitary behaviors. For instance, in some areas there are taboos around having a toilet in one's house and around male and female household members using the same toilet.[33] In the study areas, the traditional societal and hierarchical structures maintain great importance and power. It is important to design research and intervention strategies that consider these existing structures. Consequently, engagement with local stakeholders and partners is key to developing successful strategies for improving health. Accordingly, this study builds upon the rich formative research and local experience coming out of the FIOVANA program, a five-year (2019-2024) multisectoral project implemented by ADRA, which aims to improve health and nutrition in Southeastern Madagascar. FIOVANA colleagues were consulted on the design of this study, which was aimed to inform refinements to FIOVANA's implementation approach.

3 Methods

Study design

This study consisted of three steps: i) field work, ii) data analysis, and iii) developing recommendations. We divided fieldwork into two phases (**Figure 2**): phase I primarily characterized children's behaviors through in-depth observations; phase II consisted of caregiver surveys, spot observations, and environmental sampling to quantify exposure pathways. Our overall study population consisted of 222 households with children <2 years. In both phases, we stratified our study population into three age categories (0-6 months, 7-12 months, and 13-24 months[19,34]) and used these three groups for all the subsequent analyses. After data collection, we conducted a Quantitative Microbial Risk Assessment (QMRA) to identify the main exposure pathways by age category. Finally, we worked with the FIOVANA team to develop recommendations that took into account the cultural context.

<u>Phase I: In-depth observations</u>. We began with an in-depth study of 35 households (~15% of the total study population) to characterize children's behavior and to start identifying potential enteric pathogen transmission pathways. Phase I was interrupted by two major cyclones in February 2022 that directly affected our three study regions: cyclone Batsirai and cyclone Emnati. We observed 15 households in January 2022 (pre-cyclone) and 20 households in May 2022 after giving the population some time to recover post-cyclone. At each household, we conducted structured observations (lasting 2-4 hours), caregiver surveys (lasting about 1 hour), and environmental sampling for *E. coli* contamination (all described in detail in subsequent sections). We collected a total of 143 environmental samples.

<u>Phase 2: Caregiver surveys and sampling</u>. We conducted caregiver surveys and environmental sampling in an additional 187 households. We used our Phase 1 results to refine data collection protocols for Phase 2 (condensed questionnaires and spot observations). The shorter spot observations (~15 min) enabled our team to rapidly observe the household environment (e.g., sanitation facilities, water storage, and animal proximity). During this phase, we generated 770 environmental samples to test for *E. coli* (approximately five samples per child). A subset of 26 children was randomly selected for pathogen testing. For these, an additional set of environmental and stool samples was collected.

In both phases, enumerator teams worked closely with an FIOVANA staff to collect mid-upper arm circumference (MUAC) data from each child included in the study. These data would inform future FIOVANA follow-ups to assess any changes in child health status. During surveys, our team also asked caregivers about their perceptions of key risks to their children's health, and what ideas they might have to appropriately address these concerns.

Following the two phases of data collection, we performed a QMRA to estimate the risk of infection and illness associated with different exposure pathways. The data collected from surveys, observations, and environmental sampling fed into this risk assessment, which included uncertainty and sensitivity analyses to define how variability in concentrations and children's activities influence their exposure and risk levels.

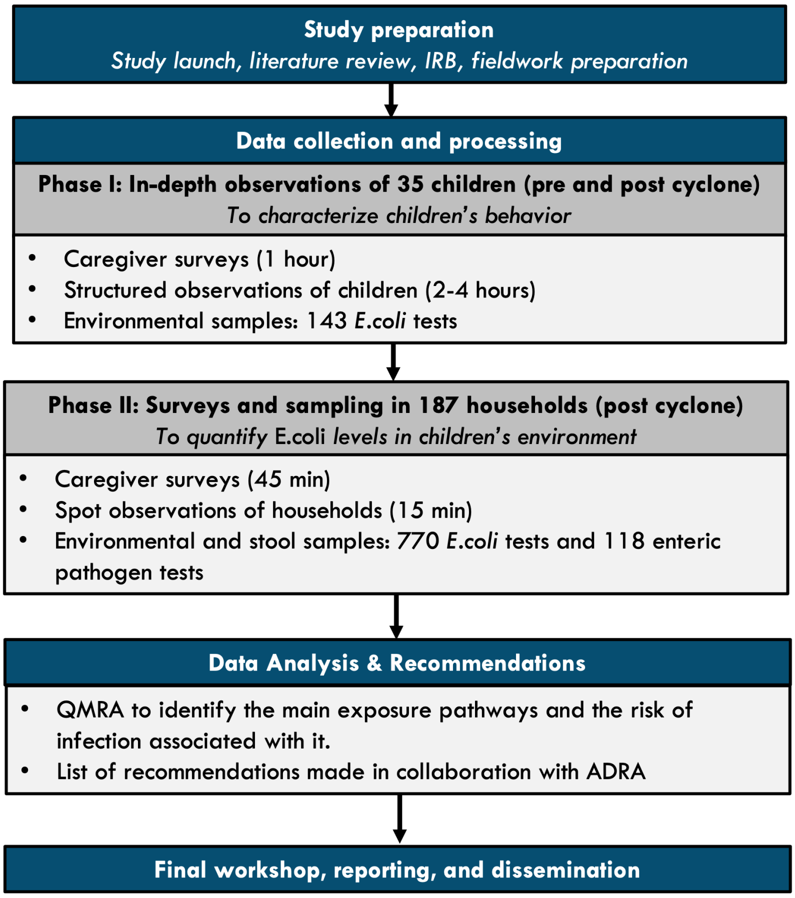

Figure 2: Study Design. We conducted data collection in two phases: (1) an in-depth study of 35 children to identify key exposure pathways and refine data collection tools, and (2) additional surveying and sampling of 187 children to quantify *E. coli* and enteric pathogen exposures.

Study Areas

We focused our study on three regions in South East Madagascar: Vatovavy, Fitovinany, and Atsimo Atsinanana regions (**Figure 3**). Our field laboratory and the main enumerator team were based in Manakara in the Fitovinany region at the office of Ny Tanintsika, a local NGO. We selected different fokontany (a fokontany is a village in the local language) in collaboration with FIOVANA to provide a good representation of different agro-ecological zones (coastal and highland communities), ethnic groups, and different levels of water, sanitation, and hygiene practices. We also took into account the remoteness of the fokontany from district centers and their accessibility by car from our field lab in Manakara: communities were chosen so that they were not more than a 3-hour drive from the lab, as the *E. coli* samples needed to be processed within 6 hours of collection.

Our study areas included a total of 21 different fokontany: 4 Fokontany for phase I pre-cyclone, 10 fokontany for phase I post-cyclone (all different from phase I), and 17 fokontany for phase II (including 7 new fokontany that had not been included in phase I pre- and post-cyclone - **Figure 3**).

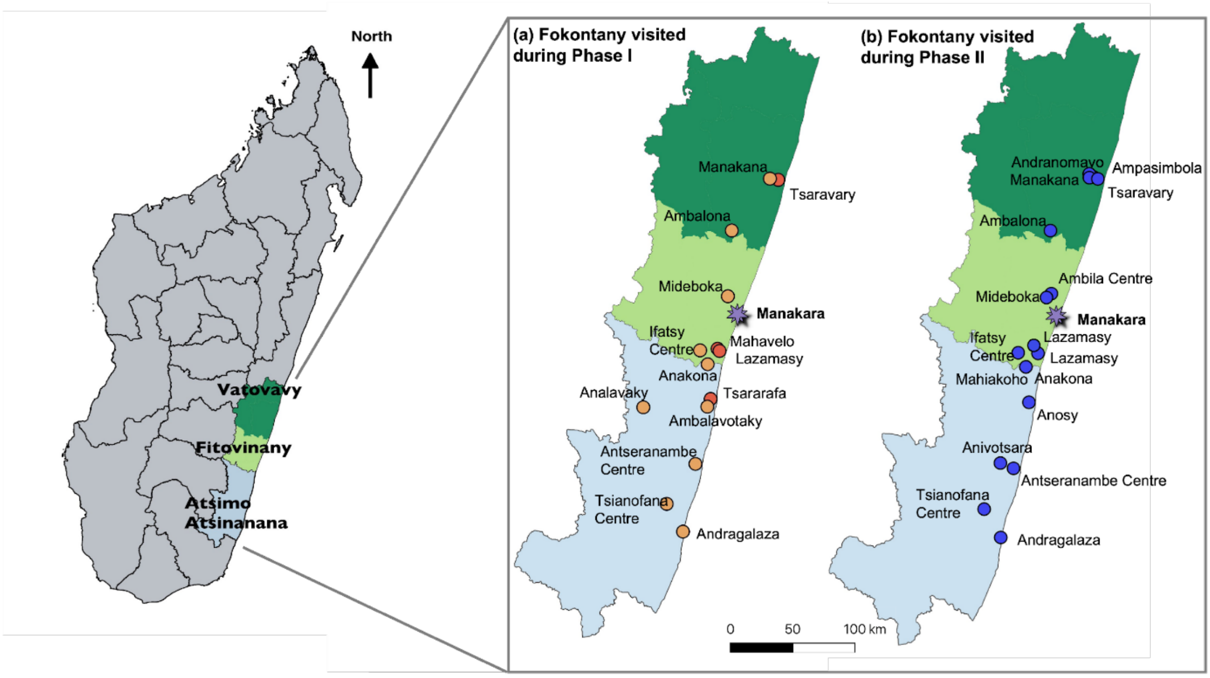

Figure 3: Fokontany visited during the study. (a) fokontany visited during phase I pre and post cyclone (January and May 2022) and (b) fokontany visited during phase II (May and June 2022).

Sampling Strategy

Many fokontany were spread over a large geographical area and were divided into a several *quartiers* (neighbourhoods) dispersed around one central *quartier*. In addition to sampling the central *quartier*, we also sampled households from these remote *quartiers*, which allowed us to include a more diverse group of households from wider social and economic backgrounds. These *quartiers* were typically spread apart, with some being a long distance away from each other and some not feasibly accessible to the group given daily time constraints. The team randomly selected accessible *quartiers* (less than 15 minutes away from the central *quartier* by foot or by car) to survey after consulting with the community health workers and FIOVANA staff. We selected households randomly within a community: enumerators used pre-generated random directions (e.g., Northwest, South, etc.) and distances to walk (50 meters, 100 meters…) to find a household from a random starting point in the fokontany. For phase I, we sampled between one and four households per fokontany, and between 3 to 24 households per fokontany during phase II depending on the number of enumerators present per fokontany.

Data Collection

Caregiver surveys and observations

For this study, we targeted households with a child under 2 years of age and an available caregiver who was at least 18 years old. We employed a team of four enumerators and two supervisors, who were recruited locally by Ny Tanintsika and trained by Aquaya for a full week prior to going to the field. The training focused on how to conduct the household survey, structured observations, and environmental and fecal sampling ethically and scientifically. We used the CommCare data collection app to conduct caregiver surveys and observations (structured child observations during phase 1 and spot household observations in phase 2).

The surveys for both phases contained questions concerning household characteristics and demographics, food preparation habits, eating and breastfeeding habits, water access and treatment, sanitation access and toilet use, pathogen risk sources (e.g., domestic animals, open defecation behaviors, exposure to surface water, etc.), personal hygiene, household cleaning, and child soil ingestion behaviors (the full survey is in Appendix B-1).

The purpose of the structured observations was to link children's behavior to potential pathogen ingestion pathways. During the observation period, an enumerator recorded how long the child spent in each environment (the general site of their activity; e.g., outside/inside the house, in livestock areas), setting (the child's specific location within the environment; e.g., improved/non-improved ground, off ground) and activity (e.g., playing, sleeping, eating, bathing - **Table 2**). Enumerators also counted the number of times children put their hands in their mouth, the number of times the caregiver put their hands in the child's mouth, and the number of times an object was placed in the child's mouth. The full structured observation survey is in Appendix B-2.

Table 2: List of the different environments, settings, and activities used to characterize children's behaviors.

Environment	Setting	Activity
Inside the house	Unimproved ground[b]	Playing or sitting
Outside the house[a]	Improved ground[c]	Sleeping
In the kitchen	Off ground	Washing hands
In the livestock area	In or next to open water/drain	Bathing
In the fields	Trash / Rubbish area	Defecating
		Eating or drinking

[a] *Outside the house meant in close proximity to the house*
[b] *Unimproved ground included non-solid floors like soil or dirt.*
[c] *Improved ground included hard floors (such as wooden or cement floors), mats, and carpets*

In phase II, we replaced in-depth observations with spot observations: enumerators directly observed housing infrastructure, WASH infrastructure such as toilets, handwashing stations, water sources (if accessible within 10 minutes by foot), the presence of feces around the house, and the presence of standing water around the house. The full spot observation guide is in Appendix B-3.

We used spot checks and back checks for quality control during surveying. A field supervisor conducted spot checks on 40% (6/15) of the surveys during phase 1. We did not conduct back checks during phase I: we deemed that after 2-4 hours of observations, revisiting households to ask a subset of survey questions again was unethical. During phase II, we conducted spot checks on 20% (41/210) of the surveys and back checks on 19% of surveys (39/210).

E. coli and pathogen sampling

During both phases, we collected environmental samples that were then tested for *E. coli* in our field laboratory in Manakara. We collected drinking water, soil, cooked food, raw food, child hand rinse, and caregiver hand rinse samples (see Appendix C for the field sampling protocol). Upon collection, samples were immediately placed in coolers with ice packs for transport and were kept on ice until processing in the lab within six hours.

We collected drinking water at the point of use (POU) using sterile WhirlPak sample bags that included sodium thiosulfate to neutralize any potential chlorine. Water samples either were raw (i.e., untreated water) or had been boiled to make rice tea (*ranonampango*). To collect drinking water samples, enumerators asked respondents to fetch the water out of the storage container (typically a bucket or jerry can for unboiled water and a pot for rice tea) with the same cup that their child would use, and to pour it into a plastic sample bag held by the enumerator.

We collected soil using a sterile spoon (approximately 5 grams of soil from 2-3 scoops) and placed the sample in a sterile plastic sample bag. We sampled within an area where the caregiver said the child played, which was typically immediately in front of the house. We were unable to dry and measure the moisture content of the soil samples during analysis: accordingly, the results present *E. coli* MPN values relative to the total mass of soil, as opposed to the dry mass. Values of *E. coli* MPN per dry gram would be higher than what we reported. We collected food by asking participants to place one or two spoonfuls of the food (approximately 3 to 5 grams) in a sterile plastic sample bag with the same utensils that they would use to feed their child. Finally, we collected hand rinse samples by having the enumerator rub each of the fingers on both hands of the child or caregiver in sterile plastic sample bags filled with 100 mL of distilled water. Each hand was rubbed and rinsed in the sample bag for 10 to 20 seconds.

Our main enumerator team was based in Manakara, where we had set up our field laboratory. A separate team traveled to the southern Atsimo Atsinanana region to survey fokontany that were too far to be accessed from Manakara within the day. The remote team only performed tests on drinking water, child hand rinse, and caregiver hand rinse samples because they were limited by the amount of time, staff, and materials that they could bring to the field. This team incubated *E. coli* samples at ambient temperature because they were not within driving distance of a location with electricity that could have served as a lab.

In addition to *E. coli* samples, we collected in a subset of 26 households samples for pathogen testing from soil, drinking water, child hand rinse, raw food, child feces, and animal feces in the child's environment. To limit cultural resistance due to some taboos about defecation, we organized 30-45-minute community meetings to formally introduce the project, so that potential participants felt more comfortable with participating and providing children's fecal samples. Community health workers, fokontany presidents, or chiefs convened mothers with children under 2 years for our team to meet. A consultant from Centre ValBio with experience in collecting human fecal samples in rural Malagasy communities gave an explanation about the study and explained how to collect fecal samples from their children. Mothers could volunteer at the end of the meeting, if they wished, and participants were randomly selected from the list of volunteers. Participants were given sample containers, a sterile spoon, soap, and disposable gloves, and they were instructed to take a small sample of their child's feces the next time they defecated after sundown that evening (about 6:00 pm) and store the sample in a cool place for collection the next day. After collection, we froze the samples on arrival at the laboratory in Manakara, within 20 hours of the child's defecation. In addition to child feces, enumerators collected unboiled drinking water, child hand rinse, soil, raw food, and animal feces samples for pathogen testing, which were frozen within approximately 6 hours of collection, after carrying the samples to the lab in a cool box. Soil and animal feces were collected directly from the environment within or around the child's household with a sterile spoon. Raw food was collected directly from the household. For drinking water and hand rinse samples, we filtered the samples with membrane filtration and then froze the filter in a sterile centrifuge tube.

We handed over 118 frozen samples to Institut Pasteur in Antananarivo (Madagascar) to be tested for 6 different pathogens: *Campylobacter, Shigella, Salmonella,* Adenovirus, *Entamoeba Histolytica,* and *Giardia Intestinalis.* The RNA of our samples was too damaged to be analyzed for Rotavirus.

Ethics

This study protocol was reviewed and approved by the Independent Review Board and the Ethics Review Board HML-IRB (study ID 2011). In each study household, we obtained written informed consent from the caregiver after describing the study (Appendix A-1, A-2, and A-3). Each caregiver had to be above 18 years old to participate in the study.

The survey team conducted community entry with FIOVANA staff, engaging in introductory meetings with local leaders, and worked with community health workers to approach selected households appropriately.

Data Analysis

E. coli analysis

We used the *Aquagenx Compartment Bag Test* to test study samples for *E. coli* (protocol in Appendix D). This test relies on presence/absence tests in 5 different compartments (holding 1 mL, 3mL, 10 mL, 30 mL, and 56 mL, respectively) to estimate the most probable number (MPN) of *E. coli* per sample. For drinking water and hand rinse samples, growth media was added and dissolved in 100 mL of sample water, and then the sample was poured into a compartment bag. For soil and food samples, we mixed one gram of sample with 100mL of distilled water in a sterile Whirlpack bag, poured the growth media into the bag, and transferred the sample into a compartment bag. Samples brought to the laboratory in Manakara were incubated at 37°C for 20 to 24 hours in a laboratory incubator. Samples collected and analyzed in the field by the team that traveled to the southern Atsimo Atsinanana region were incubated at ambient temperature (between 17°C and 25°C) for 43 to 61 hours, depending on fluctuations in ambient temperature. The manufacturer recommends an incubation time greater than 48 hours if the temperature falls below 20°C. After incubation, a color change in a compartment indicated that compartment was positive for *E. coli*. Depending on which compartments were positive for *E. coli*, the most probable number (MPN) of *E. coli* was estimated based on look-up tables provided by the manufacturer.

A major advantage of the compartment bag test method is that it can be used with solid materials like soil or food and can also perform well when testing turbid water. This was particularly important because water sources included highly turbid rivers, while hand rinse water sometimes captured a lot of dirt from the hands of the subjects.

For quality control, field teams also analyzed blank samples daily. These included field blanks (distilled water processed as a sample in the field), which help identify whether field procedures introduced contamination into the samples. The field team based in Manakara also processed a laboratory blank (distilled water processed as a sample in the lab) daily to see if laboratory procedures were introducing contamination. No laboratory or field blanks tested positive for *E. coli*, so we do not believe that our procedures introduced contamination to the samples.

Pathogen analysis

Institut Pasteur in Madagascar tested the pathogen samples for presence/absence. We sent all the samples frozen (few grams of fecal matter, food, and soil). For water samples (drinking water and hand-rinse), we sent the filters collected after membrane filtration (also frozen). We did not have information from the laboratory about the specific methods used to analyze the pathogens, except for *Salmonella,* which was tested using the VIDAS method. [35] We did not know whether they quantified PCR inhibition in the different environmental compartments.[36,37]

Quantitative Microbial Risk Assessment (QMRA)

We performed a quantitative microbial risk assessment (QMRA) to identify the relative contributions of four fecal exposure pathways among children in three age categories – 0-6 months, 7-12 months, 13-24 months. A QMRA involves four steps: hazard identification, exposure assessment, dose-response analysis, and risk characterization. We summarize these four steps below.

- ***Hazard-identification***. Measuring a comprehensive suite of fecal pathogens in all our field samples was beyond the scope of this study. Instead, we measured *E. coli* as its concentration is closely linked to that of pathogenic *E. coli* O157:H7, the strain with the most severe public health outcomes and with available dose-response models.[38] Based on the literature, we assumed that 8% of the total *E. coli* population was pathogenic.[39–42] *E. coli* can also serve as a proxy for fecal matter more generally.

- ***Exposure assessment.*** We selected four exposure pathways: ingestion via drinking water, soil, hand-to-mouth contact, and food. We ignored object-to-mouth and fly exposures, as previous studies in rural Bangladesh and other low-income settings have shown that i) the frequency of object-to-mouth contact is typically lower than hand-to-mouth contact,[43] ii) hands played a more important role than objects in pathogen ingestion,[14] and iii) flies were not associated with diarrhea in rural Bangladesh.[12] Also, capturing flies required additional resources[12] that were difficult to acquire in remote areas. The equations to compute the daily *E. coli* intake per day and per exposure pathways are provided in Appendix G-1; Equations 1-2. For the modeled quantity of *E. coli* ingested per day (dose), we computed geometric means and expressed uncertainties as the geometric standard deviation. All exposure values are presented in Appendix G-2.
- ***Dose-response (probability of infection)***: we used a dose-response model to estimate the daily probability of infection ($P_{(inf,\ daily)}$) by *E. coli* O157:H7. A dose-response model describes the reaction (e.g., the magnitude of an infection) to an exposure (e.g., a certain quantity of pathogens). We applied the ß-Poisson model to determine the risk associated with *E. coli* O157:H7 (Appendix G-1; Equation 3).[44]
- ***Risk Characterization:*** we first determined the annual probability of illness from *E. coli* O157:H7 (Appendix G-1; Equations 4-5), and we then characterized the risk using DALYs (Disability-adjusted life years) per person and per year (Appendix G-1; Equations 6-7). We included three different infection outcomes: watery diarrhea, bloody diarrhea, and death from diarrhea.[40] All equations were from Byrne et al., 2021.[21]

We conducted an uncertainty analysis using 1,000 Monte-Carlo simulations, where we replaced single input values from our field results with ranges from the literature. We performed this analysis with the MonteCarlo package in R.

4 Results

Caregiver surveys

We extracted general statistics from the caregiver surveys to better understand living conditions and habits in terms of nutrition, food behavior, access to WASH and perception of health in the three studied regions (**Table 3)**.

Mobility and feeding behaviors
Among the 220 children we surveyed during both phases (we removed two households from the analysis because we had concerns about the quality of their answers), more than 50% were between 13 and 24 months, and approximately 60% were girls (**Table 3)**. The vast majority of children under 6 months were exclusively breastfed and not mobile. Most of the children between 7 and 12 months consumed other food in addition to breastfeeding, and most of them (64%) were crawling. More than 80% of children above 13 months were still breastfeeding but were also eating solid food (primarily rice), and most were walking (~80%).

Diarrhea prevalence
According to the caregiver surveys, 26% of children had diarrhea (i.e., three or more loose or watery stools in a day) in the past two weeks. Children under 6 months were less prone to diarrhea (11% of the caregivers replied that their children experienced a diarrhea episode in the past two weeks), compared to the two other categories (36% of children 7-24 months and 26% of children 13-24 months). Both of these rates were slightly higher than those reported in rural areas at national scale (two-week prevalence of diarrhea of 16% for children below one year and 21% for children 12-23 months).[45] Our results and the literature suggested that the risk of fecal exposure increased as children grow. When children can move around and have more interaction with their environment, they are more likely to ingest pathogens, leading to more frequent diarrhea episodes.
Before the cyclones, 53% (8/15) of caregivers reported an episode of diarrhea in the last two weeks, compared with 24% (49/205) after the cyclones. However, the number of households interviewed before the cyclones (N=15) was too low to be representative and directly compared with values post-cyclones.

Nutrition
We also measured the mid-upper arm circumference (MUAC) for children above 6 months. The median MUAC was 14.5 cm for both the 7-12 months and 13-24 months categories, while the minimum MUAC value observed was 11.5 cm for the 7-12 months category and 12.0 cm for the 13-24 months category. None of the children observed were classified under Severe Acute Malnutrition (SAM; MUAC < 11.5 cm), 7% of observed children were classified under Moderate Acute Malnutrition (MAM; MUAC of 11.5-12.4 cm), and the rest (92%) had a normal MUAC (≥12.5) as per WHO standards. The prevalence of MAM was higher than that reported by FIOVANA for children under 5 (4% in the three studied regions – personal communication – August 2022), but corroborates previously reported malnutrition rates of 10-12% in these three regions.[46] According to our observations, the prevalence of MAM among children between 13-24 months (9%, 10/117) was higher than that among children in the 7-12 months category (4%, 2/48). These observations were consistent with the literature, which showed that stunting was associated with increasing child age in the Vatovavy region.[47]
We did not observe any statistical differences in MUAC before and after the cyclones ($p>0.05$, Wilcoxon test), though our sample size pre-cyclone was likely too small to detect such differences.

Hygiene practices
Regarding hygiene, the vast majority of households (176/220 – 80%) had a wooden floor covered by a mat made out of natural fibers, while only 11% (24/220) of households had a dirt floor. The remaining 9% had a cement floor or fitted carpets. Nearly all households (212/220 – 96%) reported cleaning their house every day without using any disinfectant products. Handwashing stations were relatively rare

(present at 10% of households; **Table 3**), but 65% (141/220) of caregivers reported washing their hands more than three times a day with water only (50%, 111/217). The rest used soap (31%, 68/217) or ashes (17%, 38/217). Between 80% and 90% of the caregivers reported washing their children's hands between one and three times a day.

Livestock were present in the vast majority of surveyed households (91%), with most livestock being poultry (60%) or cows (20%). Around 80% (171/220) of the households stored food that is not consumed immediately, primarily in covered containers inside the house. Almost all households (169/171) reported keeping cooked food no longer than one day.

Sanitation
Open defecation was very common, with 50% of households practicing open defecation (**Table 3**). Among children, 51% (112/220) defecated in re-usable diapers and 44% (98/220) defecated outside the house (in a hole, in a bush, in water, or in a trash area). Among children using re-usable diapers, 85% of the caregivers (95/112) cleaned the diapers in the river, and 64% (142/217) of caregivers washed their hands all the time or most of the time after handling children's feces. Less than 10% (17/217) reported never washing their hands after handling children's feces.

Water access
In term of water access, only 30% (63/220) of the surveyed households had access to improved drinking water sources, but 70% (155/220) of all households reported treating water before giving it to the child. Almost all of those treating water reported that they boiled water (154/155), although 12% (18/155) added non-boiled cold water to the prepared/cooked food or boiled water they gave to children.

Table 3: General statistics of the caregiver surveys during phase I pre-cyclone, phase I post-cyclone, and phase II.

	Category	Phase I pre-cyclone (N=15)	Phase I post-cyclone (N=20)	Phase II (N=185)
Children's age	0-6 months	N=6 (40%)	N=2 (10%)	N=45 (24%)
	7-12 months	N=2 (13%)	N=4 (20%)	N=44 (24%)
	13-24 months	N=7 (47%)	N=14 (70%)	N=96 (52%)
Gender	Female	N=10 (67%)	N=12 (60%)	N=104 (56%)
	Male	N=5 (33%)	N=8 (40%)	N=81 (44%)
Mobility	0-6 months			
	Not mobile	N=6 (100%)	N=2 (100%)	N=45 (100%)
	Crawling			
	Cruising[a]			
	Walking			
	7-12 months			
	Not mobile	N=1 (50%)		N=11 (25%)
	Crawling	N=1 (50%)	N=3 (75%)	N=27 (61%)
	Cruising[a]		N=1 (25%)	N=5 (12%)
	Walking			N=1 (2%)
	13-24 months			
	Not mobile			
	Crawling		N=1 (7%)	N=6 (6%)
	Cruising[a]			N=19 (20%)
	Walking	N=7 (100%)	N=13 (93%)	N=71(74%)
Breastfeeding	0-6 months			
	Only breastfeed	N=4 (67%)	N=2 (100%)	N=41 (91%)
	Breastfeed + other food	N=2 (33%)		N=4 (9%)
	No breastfeeding			
	7-12 months			
	Only breastfeed			N=3 (9%)

	Category	Phase I pre-cyclone (N=15)	Phase I post-cyclone (N=20)	Phase II (N=185)
	Breastfeed + other food No breastfeeding	N=2 (100%)	 N=4 (100%)	N=41 (91%)
	13-24 months Only breastfeed Breastfeed + other food No breastfeeding	 N=5 (71%) N=2 (29%)	 N=8 (57%) N=6 (43%)	 N=83 (86%) N=13 (14%)
House with dirt floor		N=10 (67%)	N=3 (15%)	N=11 (5.9%)
Open Defecation		N=7 (47%)	N=15 (75%)	N=105 (57%)
Improved drinking water[b]		N=5 (33%)	N=2 (10%)	N=56 (30%)
Presence of handwashing station		N=2 (13%)	N=2 (10%)	N=18 (10%)
MUAC[c]	0-6 months 7-12 months % MAM[d] % normal MUAC 13-24 months % MAM[d] % normal MUAC	NA N=1 (50%) N=1 (50%) N=1 (1%) N=6 (90%)	NA N=0 (0%) N=3 (100%) N=4 (29%) N=10 (71%)	NA N=1 (2%) N=42 (98%) N=5 (5%) N=91 (95%)
Diarrhea frequency	0-6 months More than once a month Once every 2-3 months 2-3 times per year Once per year or less Never	 N= 1 (17%) N= 1 (17%) N= 1 (17%) N= 3 (50%)	 N= 2 (100%)	 N= 3 (7%) N= 2 (4%) N= 1 (2%) N= 39 (87%)
	7-12 months More than once a month Once every 2-3 months 2-3 times per year Once per year or less Never	 N= 1 (50%) N= 1 (50%)	 N= 1 (25%) N= 1 (25%) N= 1 (25%) N=1 (25%)	 N= 1 (2%) N= 10 (23%) N= 12 (27%) N= 6 (14%) N= 15 (34%)
	13-24 months More than once a month Once every 2-3 months 2-3 times per year Once per year or less Never	 N= 1 (14%) N= 5 (72%) N= 1 (14%) 	 N=3 (21%) N=1 (7%) N=5 (36%) N=2 (14%) N=3 (21%)	 N= 10 (10%) N= 11 (11%) N= 36 (38%) N= 14 (15%) N= 25 (26%)

[a] Cruising: the child is walking while holding on to furniture or other structures, prior to walking independently

[b] Improved drinking water includes piped water, boreholes, protected sources, protected wells, rainwater, and bottled/sachet water

[c] MUAC: Mid-Upper Arm Circumference

[d] MAM: Moderate Acute Malnutrition

Structured observations

In total, we collected approximately 95 hours of structured observations, with children 13-24 months being the most observed (around 62 hours of observations for this category, versus 19 hours for the 0-6 months category and 15 hours for the 7-12 months category). Most of the structured observations (85%) started in the late morning (usually after 10 am) and ended in the middle of the afternoon (typically before 3 pm). Less than 10% of the structured observations started before 10 am or ended after 3 pm, meaning that we missed the early morning and late afternoon activities. We visualized the time spent within each environment, within each setting, and doing each activity (**Figure 4**) to help us understand how children in each age category were typically spending their time.

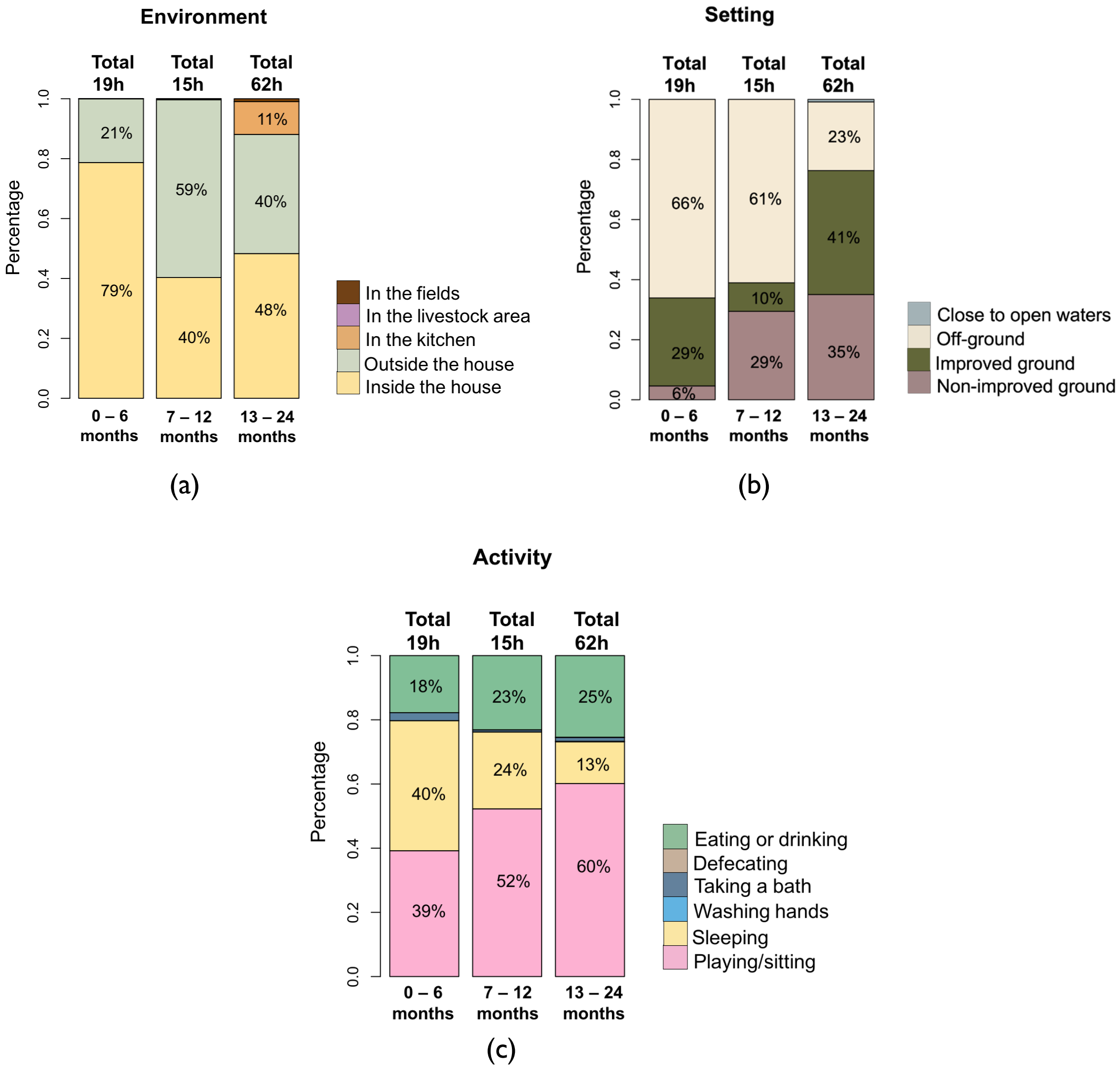

Figure 4: Findings from structured observations: time spent per environment (a), per setting (b), and per activity (c). Note that "outside the house" refers to locations that are in close proximity to the house.

Environment. Observed children typically spent their time around the house: babies under 6 months spent around 80% of their time inside the house and about 20% outside the house, while children between 7 and 24 months spent more time outside the house (59% of the time between 7-12 months, and 40% between 13-24 months). Children between 13-24 months spent more time in the kitchen (11%) than the other categories (1% for the 7-12 months category and 0% for children under 6 months). All observed children spent a limited amount of time in the field (<1%, which can be explained by the late morning observations: when we arrived, people had already left to their fields). We observed only one child (1/35) playing in the livestock area.

Setting. Babies below 6 months were usually off ground (66% of the time because caregivers were usually holding them) or on improved ground (29%; usually mat or wood flooring) and spent limited time on unimproved ground (6%). Children 7-12 months spent most of their time off ground (61%) and on unimproved ground (29%), and a limited amount of time on improved ground (10%). Children between 13 and 24 months spent most of their time on improved ground (41%) and unimproved ground (35%), followed by off ground (23%). Time spent around open water or open drains was very limited (around 1%).

Activity. Most children spent their time playing (from 39% of the time for the youngest up to 60% for the oldest), sleeping (40% of the time for the youngest down to 13% for the oldest) and eating (18-25% of the time). Bathing represented only 1% of the children's time, and hand washing was observed only among the 13-24 months category for 3 out of 39 observed children.

Improved ground was usually fairly dirty (80% of the observations). Enumerators reported the presence of children feces in 17% (6/35) of the households observed, either inside the house (52%) or outside but close to the house (43%). The presence of animal feces was reported in 66% (23/35) of the households observed, outside the house (60%) more often than inside the house (37%).

E. coli and pathogens

E. Coli

Across phases 1 and 2, we obtained a total of 835 *E. coli* samples for analysis (we removed 78 samples that were duplicates or that had been collected at the water source instead of the point of use). We found no statistically significant differences in contamination before and after the cyclones for most pathways ($p>0.05$, Wilcoxon-test), with the exception of soil: the geometric mean for soil before the two cyclones was 9 MPN/g, which increased to 83 MPN/g after the cyclones ($p<0.001$, Wilcoxon test). In the QMRA, we used the after-cyclone value (i.e., 83 MPN/g) to reflect the potentially higher risk levels associated with post-cyclone conditions, when most of our data collection occurred. For the other pathways, we used the overall geometric mean combining pre- and post-cyclone data.

Soil and drinking water were both highly contaminated, with more than 60% of samples having *E. coli* concentrations above 100 MPN per gram (for soil) or per 100 mL (for water) (**Figure 5**). Note that we cannot directly compare *E. coli* concentrations across all compartments due to differences in units (but our QMRA, presented below, will allow for direct comparisons). Children and caregivers' hand rinse samples can be compared, however, and these showed similar trends, with around 30% of samples classified as safe (0 MPN/2 hands) and 25% of samples classified as highly contaminated (above 100 MPN/2 hands). The number of child hand-rinse samples was lower than caregivers' because child samples were sometimes difficult to collect. In some instances, caregivers refused to allow infants to have their hands rinsed due to a belief that it was not healthy to bathe them in anything but warm water. Food samples were the most difficult to collect (**Figure 5** shows that we were not able to collect as many of these), because of food shortages after the two cyclones and the resulting reluctance from respondents to share even small amounts. Raw food was even more difficult to collect because eating raw food is uncommon in the regions: according to our survey, 60% of children never eat raw food, while 34% eat raw food only between one and five times per week. The vast majority of the cooked food we collected was rice (56/58), and half of the samples were safe (0 MPN/g).

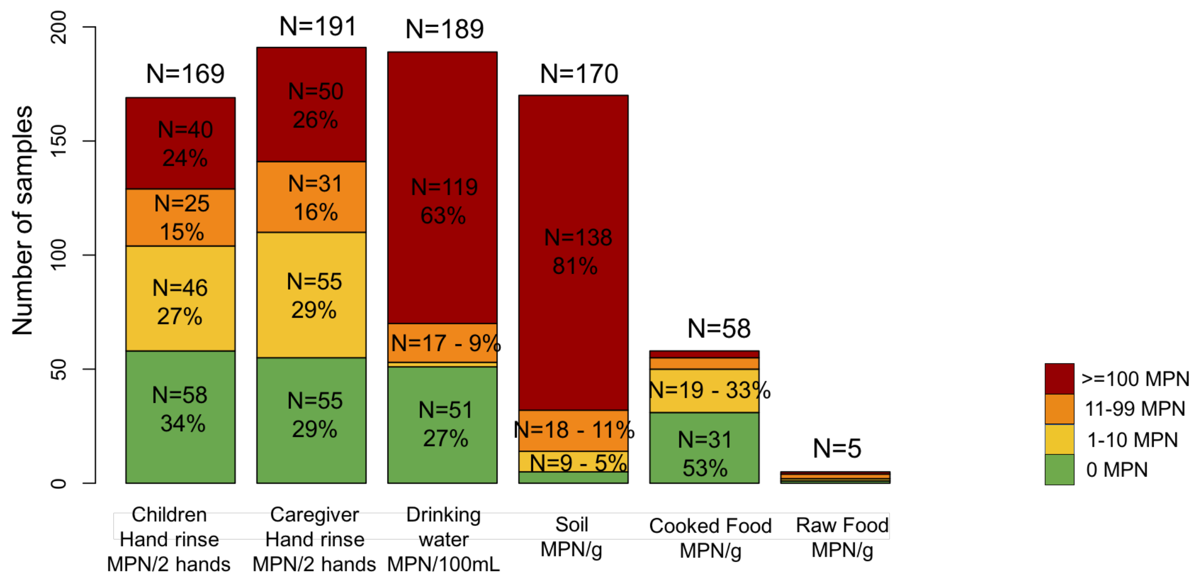

Figure 5: Level of *E. coli* contamination among the six different environmental compartments. Only the samples collected after the two cyclones are presented in this figure.

We also compared contamination levels across the different regions and did not observe any major differences (Appendix F – Figure F.1): no particular region was more contaminated than another.

We also checked for differences in contamination levels across water source types: even though we collected water at the POU, we wanted to see whether the source type had an influence on contamination at the POU. We observed that drinking water was highly contaminated across all source types (Appendix F- Figure F.2), and fetching water from an improved source versus a non-improved source did not necessarily provide increased protection against degraded water quality during household storage (contamination between improved and non-improved source was not statistically different, p=0.05 Wilcoxon test). However, boiling water seemed to efficiently reduce contamination: for the 148 water samples where we had information about whether the water was boiled, the geometric mean for the boiled water samples (N=44) was 16 MPN/100mL, compared with 121 MPN/100 mL for the un-boiled water samples (N=104; p<0.05 Wilcoxon test). We did not find any statistically significant differences in *E. Coli* contamination between households with livestock and households without livestock (p>0.05 Wilcoxon test), but the sample size of households without livestock might be too low to see such differences (N=20/220).

Pathogens

We collected a total of 118 samples representing two potential sources of contamination: (children and animal feces) and four environmental compartments (Figure 6). For animal feces, 14/24 samples came from poultry, 9/24 from cows, and 1/24 from a dog. It is important to note that children's feces are not a natural source of enteric pathogens, therefore the presence of pathogens is a marker of infection.

Animal feces were more contaminated with pathogens than any other sample type (16/24 – 67% of samples were positive for at least one pathogen), consistent with the nature of this sample (a source as opposed to an environmental compartment). Children's feces were less contaminated than some environmental compartments: the prevalence of pathogens was 26% (6/23) in children's feces, versus 46% (11/24) for children's hand-rinse and 31% (8/26) for soil. We found the same prevalence in children's feces as in drinking water 26% (5/19). Finally, we did not find any pathogens in raw food, likely due to the very small sample size (N=2). These results were slightly different from what we found for *E. coli:* the two compartments most contaminated with *E. coli* were soil and drinking water, while we found that hand rinse and soil were the compartments with the highest prevalence of enteric pathogens.

The most common pathogen was *Campylobacter,* found in 33% (39/118) of samples, followed by *Shigella* (8% - 8/118), *Giardia Intestinalis* (3% - 4/118), and finally Adenovirus (1% - 2/118). Among all samples positive for any pathogen (36% - 43/118), *Campylobacter* was present in most (90% - 39/43), followed by *Shigella* (32% - 14/43), *Giardia Intestinalis* (9% - 4/43), and finally Adenovirus (5% - 2/43). We did not find *Salmonella* or *Entamoeba Histolytica* in any samples.

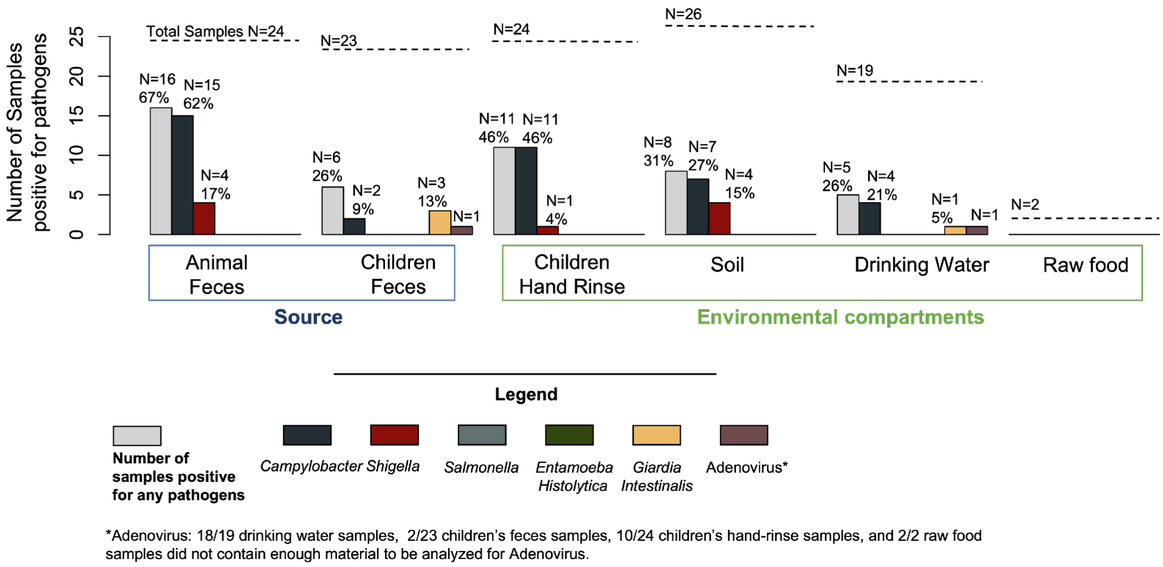

Figure 6: Results of presence/absence analysis for six pathogens across six sample types. All samples were collected between May and June 2022.

Quantitative Microbial Risk Assessment (QMRA)

Exposure estimates

We estimated exposure levels from four pathways: drinking water, soil to mouth, children's fingers to mouth, and cooked food. We did not include the raw food exposure pathway, as the number of collected samples was very low (N=5) and thus not representative. We did not include the caregiver's fingers to child's mouth exposure pathway either, as the observed frequency of caregivers' hands touching their child's mouth during structured observations was only 0.1 contacts/hour for the 7-12 months category and 0 contacts/hour for the two other age categories (Appendix G-2– Table G.3).

The daily amount of *E. coli* ingested by a child increased with age, going from 10 MPN/day for the 0-6 months category to 188 MPN/day for the 7-12 months category and to 213 MPN/day for the 13-24 months category (**Figure 7**).

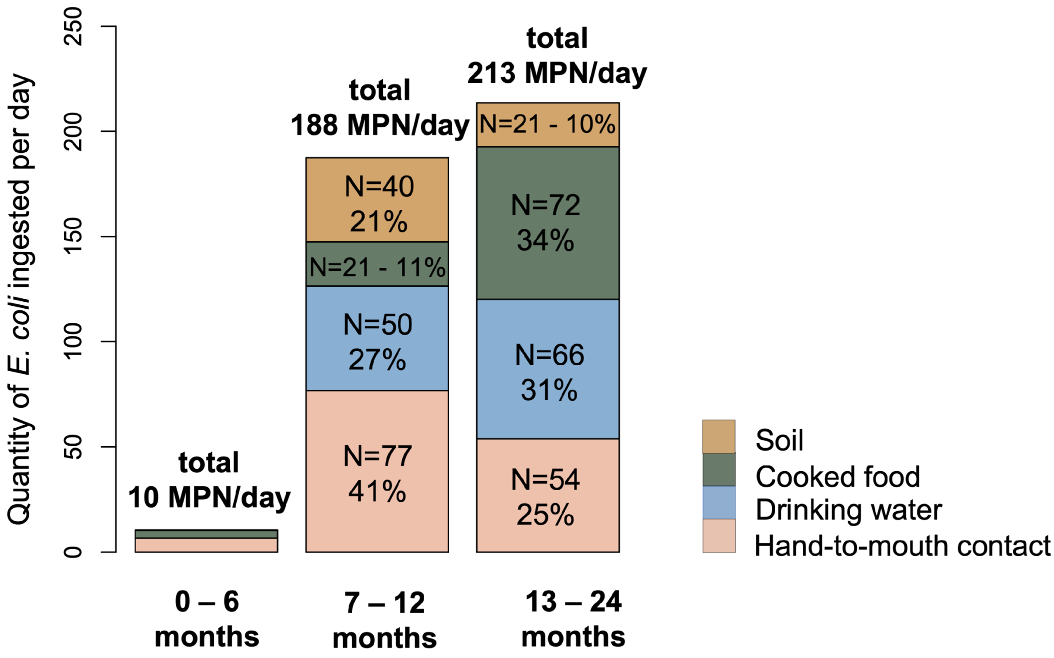

Figure 7: Quantity of *E. coli* ingested by a child per day by age category and by exposure pathway.

The main pathway for *E. coli* intake among children under 6 months (**Figure 7**) was hand-to-mouth contact (7 MPN/day or 70% of total ingestion), followed by cooked food (3 MPN/day or 30% of total ingestion). Drinking water was a negligible pathway because most children in this age group were breastfeeding exclusively, and soil was also negligeable for this category, as only one caregiver (1/53) reported seeing their child eating soil in the previous three days.

For the 7-12 months category (**Figure 7**), the main pathway for *E. coli* intake was also through hand-to-mouth contact (77 MPN/day or 41% of total ingestion), followed by drinking water (50 MPN/day or 27% of total ingestion), soil (40 MPN/day or 21% of total ingestion), and food (21 MPN/day or 11% of the total ingestion).

Finally, the main pathways for *E. coli* intake among the 13-24 months category were through food (72 MPN/day or 34% of total ingestion), drinking water (66 MPN/day or 31% of total ingestion), and hand-to-mouth contact (54 MPN/day or 25% of total ingestion). *E. coli* intake through soil ingestion represented 21 MPN/day or 10% of total ingestion (**Figure 7**).

The uncertainty analysis enabled us to quantify the impact of model assumptions on the resulting exposure estimates. **Figure 8** represents the range of exposure estimates that we could have obtained if we had used model inputs from the literature (all exposure values are presented in Appendix G-2) rather than values that we measured in the field (e.g., for contamination levels and exposure times). The distribution of exposure estimates was wide-ranging for almost all pathways, indicating high uncertainties.

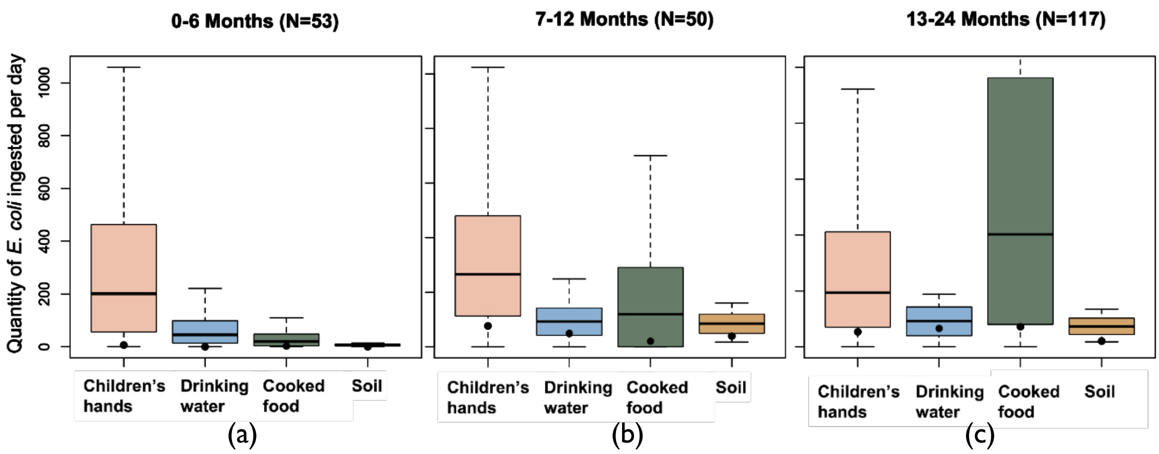

Figure 8: Daily E. coli intake (MPN/day) for each exposure pathway and by age category: (a) 0-6 months, (b) 7-12 months, (c) 13-24 months. The box plots represent the distributions of results obtained by 1,000 Monte-Carlo simulations (the whiskers represent "reasonable" extreme values [1.5*Inter-Quartile Range] excluding outliers, the boxes represent the first and the third quartiles, and the black line represents the median). The black dots show the values estimated in this study.

Exposure estimates were particularly wide-ranging for the children's fingers-to mouth pathway (**Figure 8**). This is because the frequency of hand-to-mouth contact measured in this study was substantially lower than frequencies reported in the literature (Appendix G-2 – table G.3). Across the three age categories, hand-mouthing frequencies observed in this study ranged from 1.8 to 5.4 contacts/hour, while frequencies observed for children in the US ranged from 14 to 23 contacts/hour,[48,49] and from 28.2 to 43.6 for children in rural Bangladesh.[43] We observed the same trend for object-to-mouth frequencies (Appendix G-2 – table G.3). The lower frequencies we observed may be due to cultural practices: in rural Madagascar, children are commonly carried on the caregiver's back and tightly wrapped in a cloth that limits hand-to-mouth contacts. Observational bias is another explanation: observations usually took place between 10 am and 3 pm, when children spent a substantial part of their time sleeping, thus limiting hand-to-mouth contact. Exposure estimates were also wide-ranging for the cooked food pathway (**Figure 8**). This is because food intake values found in the literature were highly variable[9] (see Appendix G-2 – table G-8). The intake of cooked food in the 7-24 months category is highly dependent on the amount of breastfeeding: some children eat more solid food than others, which varies from setting to setting depending on different education and cultural practices.

Our estimates of pathogenic *E. coli* ingestion were always on the low end of the spectrum (**Figure 8**), suggesting that actual intakes might be higher than what we found. The uncertainty analysis suggested that for the 0-6 months category, hand-to-mouth contact may be more important than our field estimates indicated (the quantity of pathogenic *E. coli* ingested via this pathway was significantly higher than the three other pathways, $p<0.001$ – Wilcoxon test), possibly dwarfing all other pathways (**Figure 8a**). The uncertainty analysis also confirmed that hand-to-mouth contact is one of the most important pathways for the 7-12 months category (the amount of pathogenic *E. coli* ingested via this pathway was also significantly higher than the other pathways $p<0.001$ – Wilcoxon test; **Figure 8b**), and that cooked food is increasingly important as children grow (it became the most important pathway for the 7-12 months category, $p<0.001$ – Wilcoxon test; **Figure 8a-b-c**).

Risk of infection and illness from pathogenic E. coli

To estimate the risks and impacts of pathogenic *E. coli* illness associated with these pathways, we converted daily *E. coli* intakes into DALYs, focusing on outcomes of watery diarrhea, bloody diarrhea, and death from diarrhea. The DALY values we obtained (**Figure 9**) followed the same trend as daily *E. coli* intakes (**Figure 7**). They all fell below the WHO reference level of tolerable risk for drinking water[50] (this threshold has also been used in literature as a reference for other exposure pathways,[11,51] and it ranges from 10^{-5} to 10^{-4} DALYs per person per year, represented by the hatched area in **Figure 9**).

For the 0-6 months category, only the 75th percentile value of the hand-to-mouth exposure pathways exceeded the upper end of the tolerable risk range (**Figure 9**a). For the two other age categories, the 75th percentile value exceed the upper tolerable risk for all four exposure pathways (except for cooked food for the 7-12 months category) (**Figure 9**b & c). However, it is important to note that the DALYs presented here are likely underestimates of actual morbidity, as we only accounted for one pathogen (pathogenic *E. coli*, because it was the only one we could adequately characterize using our field *E. coli* data); our DALY estimates thus do not account for other common pathogens such as *Campylobacter*, *Shigella*, and others.

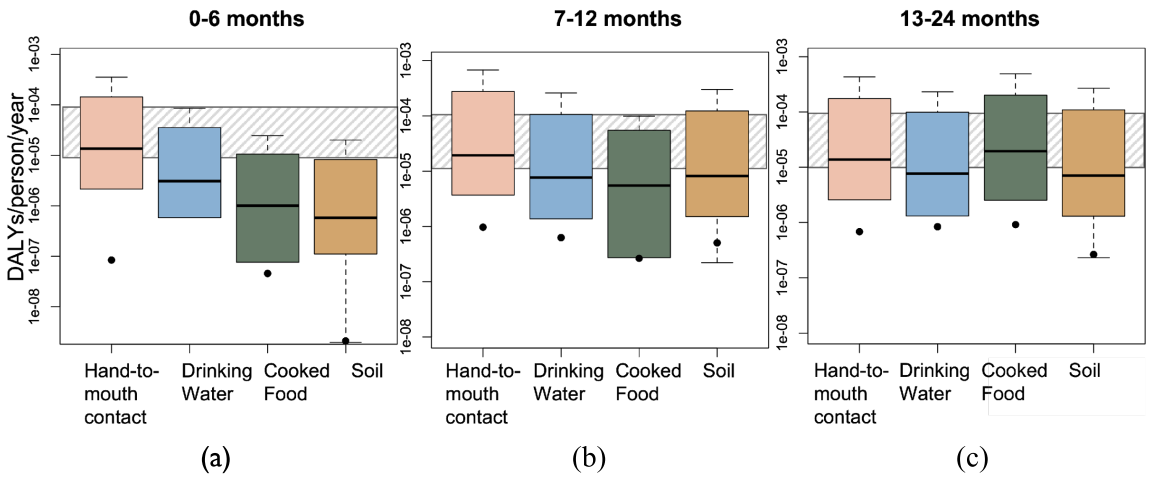

Figure 9: Estimated DALYs due to pathogenic *E. coli* per person per year for each exposure pathway and by age category: (a) 0-6 months, (b) 7-12 months, (c) 13-24 months. The box plots represent the distributions of results obtained by 1,000 Monte-Carlo simulations (the whiskers represent "reasonable" extreme values [1.5*Inter-Quartile Range] excluding the outliers, the boxes represent the first and the third quartiles, and the black line represents the median. For most of the boxplots, the lower whiskers had the same values as the first quartiles and did not appear on the graph. The black dots show the single values computed using the results of this study (the single value for drinking water among the 0-6 months category was 0, and thus was not represented on the y-log axis of **Figure 9**). The hatched area represents the WHO reference level of tolerable risk for drinking water of 10^{-5} to 10^{-4} DALYs per person per year.

Caregivers' risk perception

During the household survey, we asked the caregivers what they perceive as being the main risk for their children's health. Caregivers thought that contaminated water and contaminated food (**Figure 10**) were the main risks, followed by touching/playing with soil and touching animals. Interactions with animal or human feces, as well as with contaminated objects, were usually not perceived as the main risk for children's health (**Figure 10**). Among the caregivers who replied "other risks" to this question, 14/75 replied that poor hygiene practices were a risk, 10/75 mentioned contaminated food or malnutrition, 9/75 highlighted climate change, and 7/75 reported the cold and the humidity being a risk for their children's health.

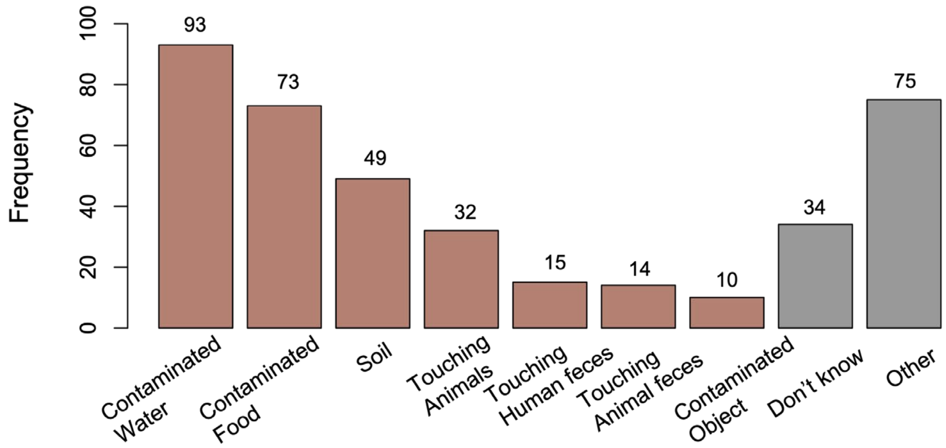

Figure 10: Responses to the multiple-choice question "According to you, what are the main risks for your children's health?"

These results suggested that caregivers' perceptions about what makes their children sick were somewhat in agreement with our findings. Drinking water, contaminated food, and soil were the top three risks identified by the caregivers, consistent with the main exposure pathways found previously. But additional awareness efforts should be made to emphasize the risk associated with feces, which should be perceived as the main risk to children's health.

5 Discussion

Age trends
The estimated daily intakes of fecal matter changed depending on children's age, with older children likely ingesting more fecal matter (188-213 MPN *E. coli* per day at 7-24 months) compared to babies below 6 months (who ingested less than 10 MPN *E. coli* per day). Our results were consistent with children's development: as children grow, they interact more with their environment, eat more food, and drink more water, increasing the chance of ingesting fecal matter if these different environmental compartments are contaminated. Our observations indeed showed that children below 6 months of age spent most of the time sleeping (40%) off ground and inside the house, while children above 7 months spent most of the time playing, typically off ground for the 7-12 months category and on improved or non-improved ground for the 13-24 months category.

Environmental contamination (E. coli and pathogens)
We found that drinking water and soil were most contaminated with *E. coli,* with more than 60% of the samples being in the highest risk category (>100 MPN/100ml or >100 MPN/g), followed by children's and caregivers' hand-rinse (with 25% of samples being in the highest risk category).

According to our observations, improved ground (usually mat or wood flooring) was usually quite dirty, while human and animal feces were observed at 17% and 66% of households, respectively. Feces (human and animal) were observed inside the house in 50% of these cases.

Regarding pathogen contamination, we detected pathogens more often in animal feces (with 67% of samples positive for at least one pathogen) than in children's feces (26%). Children's hand-rinse (46%) and soil (31%) samples had the highest prevalence of pathogens, followed by drinking water (26%). Finding pathogens more frequently in children's hand-rinse, soil, and animal feces than in children's feces suggested that i) animals – not children – were the primary reservoir of pathogens, at least bacterial pathogens, and ii) children became infected primarily via hands and soil contact.

This interpretation may not apply to viral pathogens, of which we found very few in our samples (perhaps due in part to our sample collection method). We found Adenovirus only in drinking water and in children's feces, which might imply that viral contamination (Adenovirus) in drinking water comes primarily from human feces. In contrast, other studies in sub-Saharan Africa found Adenovirus mainly in soil and on children's hands, and rarely in drinking water.[17,21,25]

Some of our results could be directly compared with the literature. For example, the prevalence of *Shigella* in our animal feces samples was higher than previous findings from the Vatovavy region in Madagascar (17% in our study versus 7% found previously in livestock samples that included pigs and cattle), but we did not find any *Salmonella* (0% versus 9%).[52] Regarding children's feces samples, we found the same prevalence of *Campylobacter* (10%) and Adenovirus (1/21 – 5%) as previous studies on children under five in various areas of Madagascar.[27,53,54] In addition, the prevalence of *Shigella, Salmonella,* and *Entamoeba Histolytica* was low in these three studies (0.5% up to 2%), similar to our results for these three specific pathogens.

The pathogens found on children's hands varied across different settings studied in the literature. For example, the prevalence of Adenovirus on children's hands was reported to be 5% in Tanzania,[55] 13% in Uganda,[21] and 22% in Kenya,[25] while we did not find any in our studied regions of Southeastern Madagascar. The prevalence of *Shigella* varied from 2% in Uganda[21] up to 19% in Kenya,[25] and was 4% in our study. Finally, the prevalence of *Campylobacter* was higher in our study than literature: we found 46% of children's hand-rinse samples positive for *Campylobacter*, versus 2% and 12% in Uganda and Kenya, respectively.[21,25] The absence of Adenovirus in children's hands in our study could be due to the filtration method: we used membrane filtration (0.45-µm filters) and then transported the filters for analysis, but viruses could have passed through the membrane.

Existing literature has typically reported low pathogen prevalence in drinking water (less than 2% of samples were contaminated with Adenovirus, *Campylobacter,* and *Shigella* in Kenya, Uganda and Tanzania[21,25,55]). Our results were consistent with literature values for *Shigella,* but the prevalence of *Campylobacter* was higher (21%). Like hand-rinse samples, we filtered the drinking water samples, potentially resulting in underestimation of Adenovirus.

Finally, contamination in soil was typically high for Adenovirus, *Campylobacter,* and *Shigella:* prevalence was between 18% and 25% in Uganda,[21] and between 50% and 86% in Kenya.[25] Our results were similar to the Uganda study for *Shigella* and *Campylobacter* (prevalence between 15% to 27%), but we did not find any Adenovirus in our soil samples.

Our pathogen results showed that pathogen contamination is context-specific and cannot be generalized. These results also complemented the *E. coli* analysis, suggesting that testing only for indicator organisms such as *E. coli* might not be sufficient to fully characterize pathogen contamination from fecal matter. For example, children's hands were not the most contaminated compartment with *E. coli* but were the most contaminated compartment with pathogens. However, further investigations are needed, as we cannot draw definitive conclusions from this limited number of samples and the non-quantitative nature of results.

Exposure pathways

We found that the main exposure pathway for children under 6 months was hand-to-mouth contact, representing 70% of total ingestion. For the 7-12 months category, we identified three main pathways: hand-to-mouth contact (41% of total ingestion), drinking water (27% of total ingestion) and soil ingestion (21% of total ingestion). We also found three main exposure pathways for the 13-24 months age category: cooked food was the highest (34% of total ingestion), followed by drinking water (31% of total ingestion) and hand-to-mouth contact (25% of total ingestion). Soil represented only 10% of the total ingestion of *E. coli.*

Our results were somewhat consistent with the literature. Hand-to-mouth contact tended to play a large role in other studies, in agreement with our results. For example, a study on children under 36 months in rural Bangladesh found that hand-to-mouth contact was the primary pathway for *E. coli* ingestion followed by soil, whereas drinking water was least important.[9] Another study in rural Bangladesh also found that hand-to-mouth contact was positively associated with diarrhea,[12] while in low-income urban areas of Accra, food has been shown to be the primary exposure pathway for children under five years, followed by hand-to-mouth contact.[14] In our study, hand-to-mouth contact was the primary pathway for the 0-6 months and for the 7-12 months categories, but the frequency of hand-mouthing was very low compared to the literature (see Appendix G-2; table G-3). In some cases, the frequency of hand-mouthing may be low if the study did not include lunch time, when children may eat with their hands, depending on the cultural context. We included lunch time in our observations, which represented 18-25% of children's time. However, in Madagascar, children usually eat with a spoon, reducing hand-to-mouth contact frequency. In some countries, children use pacifiers, which can also reduce hand-to-mouth contact frequency,[56] but we did not observe any pacifier use among our study population. The main explanation might be that 85% of our observations took place between 10 am and 3 pm, which is not the most active period for children, as sleep represented 13-40% of children's activity. In addition, children spent between 22% and 66% of the time off ground, including being wrapped in a cloth and being carried by the caregiver, both of which can reduce the frequency of hand-to-mouth contact.

We found that drinking water was an important infection pathway for children in the 7-24 months age categories, while in the literature, drinking water's contribution to pathogen ingestion was usually low.[19,21–23] Finally soil ingestion was an important pathway in diverse studies: it was the main pathway in a study in urban Ghana,[21] and represented 25-35% of the *E. coli* ingested among children between 6-24 months in rural Bangladesh. [57] While it played a role for children in the 7-24 months age categories, it was never the main pathway in our study (10% to 21% of total *E. coli* ingestion for these two age categories). Overall, these results highlighted that the importance of different exposure pathways is often context-specific and should not be generalized.

Malnutrition

We did not observe any children with SAM during this field campaign, although 7% of children had MAM. These results were consistent with the last survey that monitored the achievement of the Millennium Development Goals in Madagascar, which revealed that malnutrition was between 10% and 12% in the three studied regions.[58] Children with MAM are at three times greater risk of death than well-nourished children and face greater risk of morbidity from infectious diseases and delayed physical and cognitive development.[59–61] For example, poor linear growth and short stature are consistently seen when children are followed systematically during and after recovery from both MAM and SAM,[59,62–64] and even after successful treatment of MAM, there is a strong statistical association between poor linear growth and relapse to acute malnutrition.[65] Prior studies suggest that malnutrition episodes may be particularly harmful to children if they take place after the age of 6 months. Specifically, children who were wasting in the first 6 months of their life did not seem to have a linear growth deficit compared with children who were wasting during the 12 to 17 months period. Children who were wasting between 6 and 17 months were at an elevated risk for stunting between 18 to 24 months.[66–68] In addition, we saw higher MAM among the 13-24 months, along with higher fecal exposure, but we don't have evidence to support a definitive relationship between these two parameters. These findings reinforce that preventing malnutrition through different interventions focused on nutrition as well as hygiene is critical to improve children's health, especially for growing children who are more at risk for MAM, but are also more exposed to pathogens.

Climatic conditions

We found that soil was more contaminated after the two cyclones than before (9 MPN/g before versus 83 MPN/g after the cyclones, $p<0.001$, Wilcoxon test). No other pathways exhibited a statistically significant difference in *E. coli* contamination levels before and after the cyclones. The intense rains might have spread fecal contamination (originating, for example, from open defecation) across the soil, increasing the level of *E. coli*. Accordingly, specific attention to fecal exposure through soil might be needed after intense rainfall episodes (i.e., after the rainy season or after extreme climatic events like storms or cyclones).

6 Recommendations

In this section, we suggested recommendations to interrupt the four studied pathways: drinking water, hand-to-mouth contact, ingestion of soil, and contaminated cooked food. We identified one main pathway for the 0-6 months category (hand-to-mouth contact) and four main pathways for the 7-24 months category (child hand-to-mouth contact, drinking water, cooked food, and soil). We recommended prioritizing prevention of exposure among the 7-12 and the 13-24 months categories because: i) the daily *E. coli* intake was low for the 0-6 months category (<2 MPN/day), and ii) the 7-12 months category recovers more slowly than the 0-6 months category after a malnutrition episode.[66] Notably, reducing fecal exposure for these age categories may involve interventions prior to a child reaching 7 months, to prepare caregivers and communities when exposure risks begin to rise.

USAID FIOVANA staff had already engaged in a number of awareness campaigns to improve and develop good hygiene practices among households. Based on detailed consultations with the local ADRA team, we recommended developing future interventions to strengthen their work and to induce sustainable behavior change, focusing on:

- Identifying bottlenecks in behavior change: for example, our survey showed that caregivers seemed to be aware of the importance of washing their children's hands, with more than 80% of caregivers stating they washed their children's hands more than three times a day. However, among the 35 children we observed, we saw only 3 children washing their hands at any time during the entire observation period. This example highlights the fact that people may be aware of good hygiene practices, but they do not implement them in their day-to-day lives. This can be done by using the RANAS (Risk, Attitudes, Norms, Abilities, and Self-regulation) method,[69] which was developed to design and evaluate behavior change strategies.
- Promoting communities' own solutions as well as "model households" to encourage other households to adopt safer hygiene practices. One approach recommended by behavioral scientists is public commitment.[70] For example, this can be done through regular community-consultation meetings led by a field agent or by local leaders, and where people can discuss ideas regarding what they can do to improve hygiene at their home. This approach to change behavior should be applied for each of the four pathways (soil, drinking water, hand-to-mouth contact and cooked food).
- Household action planning[70]: field workers can visit households and help them develop a detailed action plan that will be tailored based on the household needs. For example, it can include using a latrine or an improved water source, building a handwashing station, or developing specific hygiene practices such as using re-usable diapers for children and disinfecting water containers regularly.

These three recommendations should be incorporated into a Social Behavior Change (SBC) program designed to help support adoption of new practices and sustain changes in individual behaviors and social norms. SBC programs operate at three levels: advocacy to increase resources and political commitment, community mobilization for wider participation, and individual behavior change communication.[71] Properly implementing a SBC program requires following five systematic steps: [72]

- Understand the context (e.g., issues, audience targeted)
- Program/strategy design
- Create tools, materials, and interventions
- Implement and monitor
- Evaluation and re-planning

This research supported and illustrated the first step. Based on our results, we invited implementers to follow the remaining four steps to develop their own SBC program and to respect SBC best practices.[71] For example, a review of SBC methods and approaches[71] recommended abandoning the language of message dissemination and focusing on dialogue and transformational adult learning through group facilitation. They also recommended perceiving people as active agents rather than "beneficiaries", with the local culture being seen as an asset rather than an obstacle. In South East Madagascar, local leaders (such as mayors, local chiefs, and kings) are influential and should be involved in SBC programs to mobilize communities to ensure wider participation and to initiate collective actions. Another recommendation from the SBC review was to encourage focus group discussions, such as women's groups, to talk about collective and individual actions to put in place to change a specific behavior. Finally, because the two cyclones likely increased soil contamination, we would recommend strengthening awareness prior to cyclones and intensely focusing on hygiene actions after intense rainfall episodes.

In addition to the three general recommendations listed above, we provide below some specific actions and objectives to prioritize within future programs, based on workshop discussions with the FIOVANA team and their stakeholders.

RECOMMENDATIONS FOR DRINKING WATER

Actions

- ***Water treatment at the point of collection:*** Water treatment is one of the most effective interventions for limiting pathogen ingestion through drinking water.[50] We usually differentiate water treatment at the Point of Collection (POC) and at the Point of Use (POU). Even though POU water treatment has been shown to be efficient at improving household water quality, sustainability and scalability are low[73,74] and highly dependent on the training and education of users.[75] In low-resources settings, POC-water treatment might be more appropriate as it has been shown to require minimal behavioural change for users,[76–78] and has demonstrated long-term improvements in water quality leading to reductions in diarrhea.[76] One strategy for POC-water treatment would be to use passive in-line chlorination systems: they can operate without electricity and are capable of delivering drinking water that meets the WHO guidelines for free chlorine residual and *E. coli* contamination.[79] They can be installed in a wide variety of water distribution systems, can be adapted to different flow rates and regimes, and are also compatible with intermittent water supplies.[77–80] A study that reviewed 27 passive chlorinators showed an average cost of 140 USD, with some devices as low as 3 USD, as they can be constructed using affordable and locally available materials such as PVC pipe.[79] However, their success depends on the local availability of chlorine and the strength of supply chains,[79] which can be a long-term challenge in South East Madagascar. In addition, advancements are needed to improve chlorine dosing accuracy and better develop their compatibility with handpumps.[77–79,81] Users may also be averse to the natural taste and odor of chlorine (in the case of high concentration).[82,83] Despite these limitations, passive chlorinators would be highly recommended in the three studied regions, as they will disinfect the water at the POC and provide a residual to the POU. If installing in-line passive chlorinators on handpumps is too challenging, the chlorine dispensers distributed by Evidence Action could be a safe and sustainable alternative, as they are low-cost and easily scalable.[84] If developing a supply chain for chlorine is not appropriate in the local context, the production of in-situ chlorine could be a solution: local projects have developed techniques to produce chlorine electrochemically from salty water using solar panels or grid electricity (SOLEA company,[85] and Sandrandano company – Personal communication 2021).

Behavior changes to include within a SBC program

- ***Perception of water contamination:*** Fitovinany (one of the studied regions) means seven rivers in the local language. Rivers and water play an important role in the local culture. For people living close to the river, many of their activities occur in the river (e.g., washing clothes, bathing, fetching water, playing). The belief that water never gets dirty or contaminated is prevalent, and addressing this belief through a SBC program is a priority. This program could visually reinforce knowledge about water flow, with emphasis on concepts of upstream and downstream contamination. For example, small models showing a water stream where colored dyes illustrate water contamination could help develop awareness. Defecating or washing reusable diapers in the river should no longer be viewed as safe practices by the communities. To stop this belief, communities and local leaders should be involved and encouraged to engage in dialogues to understand cultural resistances and find solutions that would be acceptable and easy to implement for the entire community. Another recommendation that came from the participants of the local workshop was to develop in each fokontany best water management practices specific to each community water source (e.g., fencing the water source).

- ***Developing and encouraging access to improved water sources:*** After group discussions about cultural resistance, people should be encouraged by the community and the local leaders to use improved water sources (30% of surveyed households were fetching water from the river). Where no improved water sources are available or where improved water points are too far away (>30 minutes for a round-trip), developing water infrastructure (building new water points or rehabilitating old ones) should be a priority.
- ***Developing best practices for water transport, storage and handling:*** Another important contamination vector concerns drinking water at the POU. Developing awareness about contamination in storage containers would be helpful, as well as promoting safe storage practices. For example, FIOVANA could help communities to identify one or two types of containers that should only be used for water, distribute advice cards to help households develop good hygiene practices such as cleaning containers and utensils regularly, encourage safe storage of water (i.e., for less than 24 hours and in a container with a lid and a small opening, such as a jerry can, to encourage pouring water instead of scooping), and cleaning hands before manipulating drinking water or utensils used to fetch water. FIOVANA should also encourage the practice of boiling water, especially for water given to children, and explain why cold (non-boiled) water should not be added to the preparations given to children. Finally, women's group discussions and household action planning can support positive behavior change at the household level.

RECOMMENDATIONS FOR SOIL INGESTION

Actions

- ***Animal husbandry interventions and play-pens for children:*** Prior studies showed that low-cost, household-level WASH interventions focused on water treatment, latrine usage, and caregivers' hand cleanliness may be insufficient to substantially reduce fecal exposure in settings where all environmental compartments are highly contaminated with feces and where there are many opportunities for direct soil ingestion. Animal husbandry interventions, such as confining animals to areas where children would be discouraged from playing, may more effectively reduce fecal contamination of the different environmental compartments than addressing human sanitation.[52,53] In South East Madagascar, livestock are not fenced and usually roam around houses freely. Developing systematic animal fencing will be challenging, as cultural practices often lead people to let their livestock walk around freely to find food. However, according to FIOVANA colleagues, it may be possible within a multisectoral activity like FIOVANA, particularly with the integration of a SBC program to limit cultural resistance. Providing support to the most vulnerable families to help them find local fencing materials would be a key part of this type of intervention. As deforestation is high in Madagascar, an aspect of the program could be to help people plant trees that will be used later as local, sustainable materials for fencing. If animal fencing is too ambitious, developing safe playpens or areas for children free from animals and cleaned regularly could help separate them from animal feces. In addition, playpens are usually well accepted by communities.[88–91]

Behavior changes to include within a SBC program

- ***Reducing open defecation:*** Along with reducing contamination via livestock feces, additional efforts should be made to reduce open defecation (practiced by 50% of households). A study across four countries (Mali, India, Indonesia, and Tanzania) showed that the best approach to significantly reduce open defecation is to combine intensive health promotion campaigns (like Community-Led Total Sanitation, CLTS) with increased resources.[92] This hybrid approach (sensitization combined with subsidies for improved sanitation facilities) is highly recommended in Madagascar where cultural beliefs are strong, education access is restricted,[93] and the level of poverty is high: 81% of the population in 2020 is below the poverty line of 1.90USD/person/day.[94] Health promotion campaigns aim to change behaviors through informational messages combined with nudges such as helping to develop specific plans, reminders, and financial incentives.[62,92,95,96] For example, the CLTS approach could be a starting point for sensitization. However, the sustainability of CLTS is sometimes questionable due to the poor quality of latrines built with local materials,[97] and some programs are now training local masons to build hygienic floor slabs.[98] Subsidies to support the construction of safe and sustainable latrines might contribute to long-term behavior change, as subsidies can support households or fokontany that are willing to engage in healthy behaviors but are liquidity-constrained and thus unable to invest in health products.

- ***Clean floors:*** To reduce children's exposure to contaminated soil, encouraging the systematic use of improved flooring and hygienic play pens have shown mixed results: in Mexico, replacing dirt floors with cement floors led to a substantial decrease in diarrhea and in parasitic infestations,[99] while it was ineffective at preventing enteric infections in rural Zimbabwe.[100] In the three studied regions of Madagascar, cementing floors will be difficult to implement and to scale up, as most people live in traditional houses on stilts with an already improved floor (the floor is usually made out of wood and covered by traditional mats), and because this intervention is usually expensive in remote areas due to poor access to materials. Instead, we suggest focusing on improving the utilisation of mats that are easy to clean. Caregivers usually have their children sit on mats (observed children spent between 13% and 44% of their time on improved ground), but mats were usually quite dirty. Encouraging households to regularly disinfect mats might help reduce pathogen ingestion, although mat disinfection might not be easy to implement on a regular basis: mats are usually large and made out of natural fibers, and are thus not easy to wash, particularly when access to water is restricted. Engaging community discussions will help find contextually appropriate ways of cleaning and disinfecting mats, and ensuring that play areas are clean more generally. Another solution worth studying and exploring could be to promote the use of "balotom" – a small piece of fabric that is easier to wash – which can be placed on top of the mats to prevent children from being in contact with a contaminated environment.

For all the above recommendations, participants to workshop suggested identifying a model village and organizing neighboring villages to visit to learn about good practices and scale up solutions.

RECOMMENDATIONS FOR HAND-TO-MOUTH CONTACT

- ***General recommendations:*** More hand-washing can reduce the hand-to-mouth exposure pathway, but proper hygiene behaviors require large amounts of water,[55,101] a requirement that is often not attainable in low-income countries without further investment in water infrastructures[102]. For example, in the three studied regions, 72-97% of households did not have water on premises, and approximately 25% of households spent 30-60 minutes to fetch water,[45] limiting the quantity of water available within the house. In addition, handwashing promotion for children under two years old would likely have little success at reducing their exposure as low compliance is to be expected, and even for children that would wash hands more often, their hands would quickly become dirty again through contact with a contaminated household environment.[17] Instead, interventions aimed at reducing the fecal contamination of a child's household environment, such as improved human or animal waste management interventions and proper hygiene practices by the primary caregiver after defecation and food handling,[55] are likely to have greater success at reducing young children's exposure to feces from hand-to-mouth contacts.[16]

Actions

- ***Development of handwashing stations:*** In addition to sensitisation, it will be crucial to develop access to handwashing stations, as only 10% of surveyed households had one: supporting and promoting the construction of low-cost handwashing stations (also called tippy-taps) with local materials (such as jerry cans or plastic buckets) would increase handwashing coverage.[103] Village committees and local leaders should also be encouraged to follow up to make sure that handwashing stations are functioning and are being used. Access to soap is usually restricted because of prohibitive prices in local markets, so developing local women-led businesses to produce soap might increase access and generate revenue for vulnerable households enrolled in the program. If soap is not available, ashes can also be promoted for handwashing. Even though handwashing might have limited results with respect to certain pathways, improving access to soap and reinforcing handwashing practices for children are still important for limiting pathogen ingestion, as restricted access to soap is associated with a higher risk of stunting.[104]

Behavior changes to include within a SBC program

- ***Limit child mouthing***: support communities to develop relatively safe mouthing behavior by limiting the load of pathogens in children's hands (for example reduce open defecation among children, improve waste and feces management and increase handwashing at critical times). Developing group discussions (such as women or mothers groups) to talk about appropriate solutions to interrupt this pathway, putting in place visualization tools (such as glitter or puppets) to represent contamination, and identifying model households might increase and support positive behavior change.

RECOMMENDATIONS FOR COOKED FOOD

Behavior changes to include within a SBC program

- ***Encouraging good hygiene practices:*** Potential mechanisms of food contamination include contamination by dust, flies, and contaminated hands.[105–108] Food hygiene interventions that focus on handwashing before food preparation and covering cooked food during storage could reduce food contamination and diarrhea.[106,108] Awareness campaigns to improve hygiene in the kitchen can also help to reduce pathogen ingestion. For example, these campaigns could highlight that no animals should enter the kitchen (and the house more broadly), promote handwashing (for caregivers and children), and encourage disinfection of utensils and containers before food preparation. Based on FIOVANA's experience, distributing advice cards for good hygiene and safe storage practices during meal preparation can support sustainable behavior change.

7 Conclusions and Limitations of the Study

In this study, we estimated the relative importance of various fecal exposure pathways among 220 children in three regions in South East Madagascar. We conducted in-depth observations, caregiver surveys, and *E. coli* analysis from six different types of samples (children's hand rinse, caregivers' hand rinse, cooked and raw food, drinking water, and soil). We also collected additional environmental and fecal samples (118 samples) from 26 households for pathogen analysis.

Based on our *E. coli* results, we found that soil was the environmental compartment most contaminated with fecal matter, followed by drinking water, caregivers' and children's hand rinse, and finally food (cooked and raw). We compared *E. coli* contamination before and after two major cyclones (Batsirai and Emnati) for all the environmental compartments and only found a statistical difference for soil: the geometric mean of *E. coli* in soil samples increased by a factor of nearly 10 after the cyclones. The intense rains that came with the cyclones might have remobilized surface contaminants and caused flooding of contaminated water bodies, with the contaminants then absorbed and stored by soils.

According to our pathogen results, we found that animal feces may be a more important source of fecal pathogens (at least bacterial pathogens) than children feces. The two environmental compartments with the highest prevalence of pathogens were children's hand-rinse and soil, followed by drinking water. *Campylobacter* was the pathogen found most frequently (in 33% of samples), and we found Adenovirus only in drinking water. Generally, our pathogen analysis suggested that direct or indirect (soil ingestion, hand-to-mouth) contact with feces, particularly animal feces, in their environment was an important infection pathway for children. However, these possibilities require further research, as we cannot draw definitive conclusions from this limited number of samples and the non-quantitative nature of results.

We conducted a QMRA for pathogenic *E. coli* and found that the daily *E. coli* intake per day increased with children's age: for children below six months, the daily intake was below 10 MPN/day, compared to 188 MPN/day for the 7-12 months category and 213 MPN/day for the 13-24 months. These results were consistent with child development: as children grow, they have more contact with their environment and are more likely to ingest pathogens through different exposure routes. We identified hand-to-mouth contact as being the main pathway for the 0-6 months category (representing 70% of total *E. coli* ingestion per day). For the 7-12 months and 13-24 months categories, we identified three main pathways in each category. For 7-12 months, the main pathway was hand-to-mouth contact (41% of total ingestion) followed by drinking water (27%) and soil (21%). For 13-24 months, the main pathway was cooked food (34%) followed by drinking water (31%) and hand-to-mouth contact (25%).

To block these exposure pathways efficiently, we recommended focusing on the 7-24 months categories, as children in the 0-6 months category ingested less than 10 *E. coli* MPN/day. Based on global WASH findings, we also recommended general measures including animal pens, water chlorination at the point of collection, and campaigns to reduce open defecation. Finally, with specific focus on the local context and culture, we recommended developing Sustainable Behavior Change (SBC) programs, identifying bottlenecks in behavior change and then promoting local solutions via "model households" to sustainably increase good hygiene practices within communities. Behavioral science strategies such as public commitment or action plans may also help. In addition, we recommended using visual demonstrations during awareness campaigns, such as the use of glitter, to illustrate the spread of contamination on hands and on objects, or the use of colored dyes to visually portray the spread of contamination through water. Increasing the number of low-cost handwashing stations and promoting small soap-making businesses may also help to increase access to handwashing infrastructure and materials. Additionally, we highlight the importance of promoting regular disinfection of containers and utensils.

Finally, our findings suggested that the intense rains resulting from cyclones (which may become more frequent due to climate change) may considerably increase fecal contamination of soil, which can then potentially contaminate other environmental compartments such as floors, hands, and water. Further research focused on how seasonal patterns affect contamination among fecal exposure pathways may be particularly useful.

This study had several limitations. The remoteness of our study areas did not allow us to conduct longer structured observations, and we thus captured only behaviors linked to a limited portion of the day, typically between 10 am and 3 pm. Additionally, we did not observe children while in the fields. Another limitation was that our QMRA focused on the *E. coli* O157:H7 strain only and did not include other common pathogens. Our risk results are thus likely an underestimate of actual infection risks. Some parameters in the QMRA also relied on literature values derived from other contexts outside of Madagascar, meaning that the results may not fully reflect local conditions. We could not collect *E. coli* samples from all the compartments in the Atsimo-Atsinanana region: we only sampled drinking water, children and caregivers' hand-rinse. We did not have information about *E. coli* contamination for soil and food in this region. We also faced some laboratory limitations. We filtered drinking water and hand-rinse samples using membrane filtration, and thus the presence of Adenovirus may be underestimated, as viruses are small enough to pass through the membrane. Additionally, we were not sure whether the local laboratory didquantify PCR inhibition in the different compartments, which may bias our comparison of pathogen prevalence in different types of samples.[36,37] Finally, we did not conduct any analyses of adult feces, which may have also been a source of contamination in this context.

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10 Supplementary Information

Appendix A: Informed consent and description of the study

A-1 Information note for participant

INFORMATION NOTE FOR PARTICIPANTS

Hello, my name is _________. I am a staff member at Ny Tanintsika working with the Aquaya Institute based in Nairobi and United States, and we are carrying out a research study about exposure risks associated with sanitation for young children. We are working with ADRA/FIOVANA and NT, with funding from USAID.

The purpose of our research is to understand the exposure risks associated with water and sanitation for young children in this area, because these exposures can affect child growth and overall health. In particular, we aim to answer two questions:

1. What are the risks that children under two years of age are exposed to germs associated with feces through different pathways?
2. What are potential interventions for blocking these pathways and reducing risks, which are appropriate in this area of Madagascar?

This research will be conducted over 6 months, and involves surveys with caregivers of children under two years of age, structured observations of children, their caregivers, and the surrounding environment, and the collection of samples associated with different pathways of exposure. Collected samples will be shipped and analyzed in Madagascar and the United States to determine levels of exposure and risk to germs associated with feces.

There are no personal risks or benefits to participation. Everything from the surveys, observations, and sampling will be confidential, and we will not use real names or any identifying information in any of our reports or papers. The aggregated results will be used to inform local institutions in developing sanitation intervention strategies appropriate for this area.

Participants have the right to review, edit, or erase any information they have provided. **Participation in this research is completely voluntary.** Participants can decline to answer any questions and can withdraw from the study at any time for any reason. Participants will not receive any monetary payment for participation. An alternative is not to participate in this study.

If you have any questions or concerns about the research, please feel free to contact Ny Tanintsika at 034172------ or 034090------.

A-2 Informed consent for phase I

INFORMED CONSENT: Phase 1 Participants

Introduction
Hello, my name is ________. I am a staff member at Ny Tanintsika working with the Aquaya Institute based in Nairobi and United States. I would like to invite you to participate in our research study. The purpose of our research is to understand the exposure risks associated with sanitation for young children in this area, to propose appropriate sanitation interventions that minimize those risks. The study will be conducted over 6 months. You are being asked to participate in this study because you live in one of the areas selected for the present study.

Is this a good time to talk?

Thank you for your interest in our research study. May I begin?

Pre-screening

1) Do you live in this household, and are you over 18 years of age? *If yes to both, continue. If not, ask to speak with someone who meets these criteria.*
2) Does this household have a child under 2 years of age, and is this child here now? *If yes, continue.*
3) Are you the primary caregiver of this child? *If yes, skip to informed consent section. If not, continue to question 4.*
4) Is the primary caregiver over 18 years of age and available now? *If yes, ask to speak with the primary caregiver and continue to the informed consent section. If the caregiver is not available but over 18 years of age, ask if the caregiver will be available later. If the primary caregiver is not available later ask to talk to the secondary caregiver (if above 18 years old). Then thank them again for their time and interest.*

Informed consent
The purpose of our research is to understand the exposure risks associated with sanitation for young children in this area. If you agree to participate, we will conduct an interview and a structured observation, and collect samples to help us assess possible exposure to germs associated with feces.

The interview will include questions about living conditions, sanitation facilities and practices, and typical behaviors of you and your child. The discussion should last no longer than 1 hour or until you feel you have told me everything you want me to know. There are no right or wrong answers, so please be honest and tell us what is true for you.

With your permission, after the interview, I will observe you and your child for about 4/5 hours and take notes. Please feel free to act as if I am not here. During this time, I will also take 6 samples for E.Coli analysis, which will include:

- drinking water,
- soil,
- child hand rinse,
- caregiver hand rinse,
- cooked food, and
- raw food.

We will analyze these samples in Madagascar to determine levels of exposure and risk to germs associated with feces. We will also take child arm measurement.

There are no personal risks or benefits to your participation. Everything from the interview, observation, and sampling will be confidential, and we will not use your real name or any identifying information in any of our reports or papers. Our team may sometimes look at your record for research purposes. The aggregated results will be used to inform local institutions in developing sanitation intervention strategies appropriate for this location.

You have the right to review, edit, or erase any information that you do not want documented or written down. This will always be done in a way to protect your identity (e.g. your name and the exact location will not be used). Any other material or information generated by you, such as ideas written down on paper, will be subject to the same strict controls. The information you provide will be strictly confidential and never connected to you. Other people will not know if you are in this study or what you have said. We will put information we learn from you together with information we learn from other people in the study. No one will be able to tell what information came from you. When we tell other people about this research, we will never use your name, and no one will ever know what answers you gave. Only a few researchers will have access to this information, and all information will be stored safely and destroyed under the care of the lead researcher.

Your participation in this research is completely voluntary. If you don't want to be in the study, it is OK. If you want to be in the study now and change your mind later, that's OK too. You can stop at any time. If you agree to participate, you can decide not to answer any question and can stop at any time. Your decision about whether to participate in this study or to answer any specific questions will in no way affect any services that you receive. If you do choose to participate, please answer the questions honestly and openly, so that we can understand your experience and find out what you really think and have experienced.

If you have any questions or concerns about the research, please feel free to contact Ny Tanintsika at 034172---- or 034090----.

If you agree to participate, please say so. Do you have any questions or concerns now?
Do you understand everything I have explained?
Do you agree to participate in this interview?

[ALL QUESTIONNAIRES WILL BE SAVED BY THE INTERVIEWER REGARDLESS OF THE RESPONDENT'S DECISION TO PARTICIPATE OR NOT TO PARTICIPATE.]

Participant Consent Declaration

I, ________________________, sincerely agree to participate in this research, entitled "Pathogens pathway study for children under two years old in the FIOVANA intervention areas of Southeastern Madagascar," which will be conducted by a Research team led by Dr. Rachel Peletz, the head investigator, who will work together with FIOVANA and NT, and it is being funded by USAID. I have read and fully understand all the details of this research that have been provided to me, including the objectives, the reason for inviting me as a participant, methods to be applied, risks, benefits, obligations, responsibilities of researchers and their partners, contact information, and answers to any questions I have asked. I understand that my research contribution is voluntary, not paid, but free of charge. I understand that I have a choice to participate, I am not forced, and I can stop participating at any time. A copy of this form has been provided to me. I freely and voluntarily consent to participate in this research, and I assent to my child's participation in this research.

________________________	________________________	______________________
Caregiver Name	Caregiver Signature	Date

________________________	________________________
Caregiver Age (years)	Caregiver Gender

Child Name	Caregiver Signature	Date
Child Age (months)	Child Gender	
Interviewer Name	Interviewer Signature	Date

A-3 Informed consent for phase II

INFORMED CONSENT: Phase 2 Participants

Introduction

Hello, my name is ________. I am a staff member at Ny Tanintsika working with the Aquaya Institute based in Nairobi and United States. I would like to invite you to participate in our research study. The purpose of our research is to understand the exposure risks associated with sanitation for young children in this area, to propose appropriate sanitation interventions that minimize those risks. The study will be conducted over 6 months. You are being asked to participate in this study because you live in one of the areas selected for the present study.

Is this a good time to talk?

Thank you for your interest in our research study. May I begin?

Pre-screening

5) Do you live in this household, and are you over 18 years of age? *If yes to both, continue. If not, ask to speak with someone who meets these criteria.*
6) Does this household have a child under 2 years of age, and is this child here now? *If yes, continue.*
7) Are you the primary caregiver of this child? *If yes, skip to informed consent section. If not, continue to question 4.*
8) Is the primary caregiver over 18 years of age and available now? *If yes, ask to speak with the primary caregiver and continue to the informed consent section. If the caregiver is not available but over 18 years of age, ask if the caregiver will be available later. If the primary caregiver is not available later ask to talk to the secondary caregiver (if above 18 years old Then thank them again for their time and interest.*

Informed consent

The purpose of our research is to understand the exposure risks associated with sanitation for young children in this area. If you agree to participate, we will conduct an interview and a structured observation, and collect samples to help us assess possible exposure to germs associated with feces.

The interview will include questions about living conditions, sanitation facilities and practices, and typical behaviors of you and your child. The discussion should last no longer than 30 minutes or until you feel you have told me everything you want me to know. There are no right or wrong answers, so please be honest and tell us what is true for you.

With your permission, after the interview, I will observe the surrounding environment for approximately 15 minutes and take notes. Please feel free to act as if I am not here. During this time, I will also take approximately 6 samples for E.coli analysis, which may include:

- drinking water,
- soil,
- child hand rinse,
- caregiver hand rinse,
- cooked food, and
- raw food.

We will analyze these samples in Madagascar by our team.

If your house is selected for pathogens sampling, in addition of the above samples we will also collect child stool, and livestock stool. These samples will be analyzed in the United States by UNC (The University of North Carolina at Chapel Hill) to determine levels of exposure and risk to germs associated with feces. We will also take child arm measurement.

There are no personal risks or benefits to your participation. Everything from the interview, observation, and sampling will be confidential, and we will not use your real name or any identifying information in any of our reports or papers. Our team may sometimes look at your record for research purposes. The aggregated results will be used to inform local institutions in developing sanitation intervention strategies appropriate for this location.

You have the right to review, edit, or erase any information that you do not want documented or written down. This will always be done in a way to protect your identity (e.g. your name and the exact location will not be used). Any other material or information generated by you, such as ideas written down on paper, will be subject to the same strict controls. The information you provide will be strictly confidential and never connected to you. Other people will not know if you are in this study or what you have said. We will put information we learn from you together with information we learn from other people in the study. No one will be able to tell what information came from you. When we tell other people about this research, we will never use your name, and no one will ever know what answers you gave. Only a few researchers will have access to this information, and all information will be stored safely and destroyed under the care of the lead researcher.

Your participation in this research is completely voluntary. If you don't want to be in the study, it is OK. If you want to be in the study now and change your mind later, that's OK too. You can stop at any time. If you agree to participate, you can decide not to answer any question and can stop at any time. Your decision about whether to participate in this study or to answer any specific questions will in no way affect any services that you receive. If you do choose to participate, please answer the questions honestly and openly, so that we can understand your experience and find out what you really think and have experienced.

If you have any questions or concerns about the research, please feel free to contact Ny Tanintsika at 0341------ or 034090------.

If you agree to participate, please say so. Do you have any questions or concerns now?
Do you understand everything I have explained?
Do you agree to participate in this interview?

[ALL QUESTIONNAIRES WILL BE SAVED BY THE INTERVIEWER REGARDLESS OF THE RESPONDENT'S DECISION TO PARTICIPATE OR NOT TO PARTICIPATE.]

Participant Consent Declaration

I, ______________________, sincerely agree to participate in this research, entitled "Pathogens pathway study for children under two years old in the FIOVANA intervention areas of Southeastern Madagascar," which will be conducted by a Research team led by Dr. Rachel Peletz, the head investigator, who will work together with FIOVANA and NT, and it is being funded by USAID. I have read and fully understand all the details of this research that have been provided to me, including the objectives, the reason for inviting me as a participant, methods to be applied, risks, benefits, obligations, responsibilities of researchers and their partners, contact information, and answers to any questions I have asked. I understand that my research contribution is voluntary, not paid, but free of charge. I understand that I have a choice to participate, I am not forced, and I can stop participating at any time. A copy of this form has been provided to me. I freely and voluntarily consent to participate in this research, and I assent to my child's participation in this research.

Caregiver Name	Caregiver Signature	Date
Caregiver Age (years)	Caregiver Gender	
Child Name	Caregiver Signature	Date
Child Age (months)	Child Gender	
Interviewer Name	Interviewer Signature	Date

Appendix B: Caregiver surveys and observation guides

B-1Caregiver survey questionnaire

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
A0	Enumerator:	Enumerator 1 Enumerator 2 Enumerator 3	1 2 3		
A1a	Region:	Atsimo Atsinanana Vatovavy Fitovinany	1 2 3		
A1b	Municipality:	Municipality 1 Municipality 2 Municipality 3 Municipality 4 Municipality 5	1 2 3 4 5		
A2	What number is this household of those you have surveyed IN THIS COMPOUND ?	1st household 2nd household 3rd household 4th household 5th household 6th household 7th household 8th household 9th household 10th household	1 2 3 4 5 6 7 8 9 10		
ID	CommCare generates unique household ID				
A3	Is somebody available in this house?	Yes No	1 0	>>A4 >>Note	
A4	Please collect the GPS location of the house				
A5.a	Is the primary caregiver of the child available?	Yes No	1 0	>>A6 >>A5.b	
A5.b	Is the secondary caregiver of the child available?	Yes No		>>A6.a >>Note	

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
Note	Return to this compound later today. SAVE THIS FORM AS INCOMPLETE				
A6.a	READ CONSENT FORM FOR HOUSEHOLD SURVEY Are you willing to participate in this study?	Yes No	1 0	>>A7 >>Ineligible	
Ineligible	This household is ineligible for the Household survey. Thank them for their time.			>>A6.b	
A6.b	Why is this household ineligible?	No one was home after 3 attempts Household was not willing to participate Caregiver not available and it's the last day of surveying Other	1 2 3 96	>>End >>End >>End >>End	
A7	Ask the caregiver for the family/last name of the child you will be observing.	______			
A8	Ask the caregiver for the child's first name.	_______			
A9	Child's gender	Male Female	1 2		
A10	Child's age	Less than 6 months 6-11 months 12-23 months Don't know	1 2 3 99		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
A10a	How mobile is the child?	Not mobile Crawling Cruising (walking while holding on to people or things for support) Walking independently	0 1 2 3		
A11	Caregiver's gender	Male Female	1 2		
A12	Caregiver's first name	______			
Household Characteristics *I am going to ask you information about you and about your household*					
H1	What is your age?	______ Do not know	99		
H2	What is your marital status?	Traditionally married Legally married Living together Separated Divorced Never married / single Widowed Other	1 2 3 4 5 6 7 96		
H3	What is the main occupation of the household head?	Agriculture, fishing, forestry Business/trader (Selling produce goods -market or kiosk) Teacher Craftsperson Cooperatives Other Private Sector: Self-empl Other Private Sector: Employed Government sector NGOs (local & international) Student No occupation, stay home Other Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 0 96 98 99		Question removed from phase 2 survey

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
H4	How many people are living in this household? (People who eat and sleep more than 50% of the time or 6 months in year).	____ Don't know	 99		
H5	How many children under 2 years old are living in the household?				
H6	What is your familial connection with the child?	Mother/Father Aunt/Uncle Other relative Non-relative Other	1 2 3 4 96		
H7	Is the child breastfed (only nursing or does the child eat other food)?	Yes, only breast fed Yes, and eats other food No, not breastfed	1 2 0		
H8	What is the main construction material used for the dwelling's outer walls?	Ravinala leaves/cane/palm/trunk Mud/landcrete Bamboo or wood with mud Stone with mud Uncovered adobe / mud brick Reused wood Cement Stone with lime/cement Kiln-fired bricks Cement blocks Covered adobe (plastered) Wood planks/shingles No walls Other Don't know	1 2 3 4 5 6 7 8 9 10 11 12 0 96 99		Question moved to observation section of phase 2 survey

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
H9	What is the main material used for the dwelling's roof?	Thatch/palm leaf Mud/sod Palm/bamboo Wood planks (rudimentary roo Cardboard Zinc/aluminum Wood (finished roofing) Calamine/cement fiber Ceramic/brick tiles Cement Roofing shingles Asbestos/slate roofing sheets No roof Other Don't know	1 2 4 5 6 7 8 9 10 11 12 13 0 96 99		Question moved to observation section of phase 2 survey
H10	What is the main construction material used for the dwelling's floor?	Earth/sand Palms/trunks (rapaka) Dung Wood planks Palm/bamboo Parquet or polished wood Vinyl or asphalt strips Tiles (ceramic, marble, porcelai terrazo) Cement Carpet (woolen or synthetic) Linoleum/rubber carpet Other Don't know	1 2 3 4 5 6 7 8 9 10 96 99		Question moved to observation section of phase 2 survey
H11	How many rooms are in the households?				
H12	Are there domestic animals IN the compound or AROUND the house? (including outside and under the house)	Yes No	1 0	>>H13a >>H14	

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
H13a	What kind of domestic animals are in the compound or AROUND the house? *Select all that apply. Focus on domestic animals only.*	Cats Dogs Cow Pigs Goats Poultry Others	1 2 3 4 5 6 96		Question removed from phase 2 survey
H13b	How many animals does the household own?	Cats: Dogs: Cows: Pigs: Goats: Poultry: Others:	1 2 3 4 5 6 96		Question removed from phase 2 survey
H14	Are there domestic animals WITHIN the house?	Yes No	1 0	>>H15 >>H16	Question removed from phase 2 survey
H15	What kind of animals are within the house? *Select all that apply*	Cats Dogs Cow Pigs Goats Poultry Others	1 2 3 4 5 6 96		Question removed from phase 2 survey
H16	Did it rain in the past week?	Yes No	1 0		
H17	Do you ever experience flooding in/around your house or your compound?	Yes No	1 0	 >>F1	
H18	How many times do you experience flooding in a year?	Once per year Between 2 and 5 times per year More than 5 times per year Never Don't know	1 2 3 0 99		Question removed from phase 2 survey
Food and raw produce					

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
F1	How often do you eat raw produce in a typical week? (any produce that does not grow on a tree, and that does not have a peel or shell. Think both about the produce you eat whole and produce you prepare but eat raw, such as tomato, cucumber, or lettuce)	More than 10 times in the past week 6 to 10 times in the past week 1 to 5 times in the past week Never Do not know	1 2 3 0 99		Question removed from phase 2 survey
F2	How often does the child eat raw produce in a typical week? (any produce that does not grow on a tree, and that does not have a peel or shell. Think both about the produce you eat whole and produce you prepare but eat raw, such as tomato, cucumber, or lettuce)	More than 10 times in the past week 6 to 10 times in the past week 1 to 5 times in the past week Never Do not know	1 2 3 0 99	>>D1 (Never)	
F3	What kind of raw produce does the child eat?	Tomato Lettuce Sugarcane Guava Makoba (pomme canaque) Zamboarizano Do not eat raw produce Other (specify)	1 2 3 4 5 6 0 96		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
F4	Do you wash the produce before giving it to the child?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0	 >>F6	
F5	What do you use to wash the produce?	Water and soap Water and bleach Water only Other: _______	1 2 3 96		
F6	For food that you cook, do you wash it before cooking?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0	 >>F8	
F7	What do you use to wash food before cooking it?	Water and soap Water and bleach Water only Other: _______	1 2 3 96		
F8	What do you do with food that you have cooked but that is not consumed immediately?	Dispose of it Feed it to animals Store it to be eaten later Other: _______	1 2 3 96	>>D1 >>D1 >>D1	
F9	Where do you store the cooked food?	In covered containers/plates outside In covered containers/plates inside In uncovered containers/plates outside In uncovered containers/plates inside In a refrigerator Other: _______	1 2 3 4 5 96		
F10	How long do you typically keep food that has been cooked and then stored?	Less than one day 1-2 days 3-6 days A week or more	1 2 3 4		
F11	Do you give cooked food that you have stored to your child?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
F12	Typically, where is the child while you are cooking?	With another caregiver With me Sitting/playing on the floor around me Outside the house In his/her bed Other (specify)	1 2 3 4 5 96		
Drinking water					
D1	Does your household use the same water source for drinking and other uses such as cooking, cleaning, washing, etc.?	Yes No	1 0		
D2	What is the main source of **drinking** water for members of your household?	Piped water inside dwelling Piped water to yard/plot (on co Piped water to neighbor Piped water to public tap/stand Tube-well or borehole (handpu Protected dug well Unprotected dug well Protected spring Unprotected spring Rainwater Tanker truck Cart with small tank Surface water (river, dam, lake, stream, canal) Bottled water Other (specify) Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 11 12 13 14 96 98 99		
D2ObsA	Ask how far the water source is. If the water source is less than 5 minutes away ask to see it.	___________			Question moved to observation section of phase 2 survey

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
D2ObsB	Is the source closer than 5 minutes by foot?	Yes No	1 0	>>D2ObsC	Question moved to observation section of phase 2 survey
D2ObsC	What is the water source type?	Piped water inside dwelling Piped water to yard/plot (on co Piped water to neighbor Piped water to public tap/stand Tube-well or borehole (handpu Protected dug well Unprotected dug well Protected spring Unprotected spring Rainwater Tanker truck Cart with small tank Surface water (river, dam, lake, canal) Bottled water Sachet water Other (specify) Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 96 98 99		Question moved to observation section of phase 2 survey
D3	Is the water you give to the child (directly or for mixing formula) from this source?	Yes No My child exclusively breastfeeds	1 0 2	>>D5.a >>D4 >>D6	

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
D4	Where does the water you give to your child come from?	Piped water inside dwelling Piped water to yard/plot (on co Piped water to neighbor Piped water to public tap/stand Tube-well or borehole (handpu Protected dug well Unprotected dug well Protected spring Unprotected spring Rainwater Tanker truck Cart with small tank Surface water (river, dam, lake, canal) Bottled water Sachet water Other (specify) Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 96 98 99		Question removed from phase 2 survey
D5.a	How many cups / spoons / does your child drink per day?	Number:			
D5.b	Unit (glasses / cups / spoons / pots / bowls)				
D6	Do you apply any treatment to the water that you drink before drinking it? (filter – boiling - adding chlorine - rice tea)	Yes No	1 0	 >>D8.a	Question removed from phase 2 survey
D7	What kind of treatment do you apply?	Filtering water Boiling water Chlorine Rice tea Solar disinfection (SODIS) Other (specify)	1 2 3 4 5 96	>>D8.a >>D8.a >>D8.a >>D8.a >>D8.a	Question removed from phase 2 survey
D7a	Do you boil the water when preparing the rice tea?	Yes No	1 0	>>D7c	Question removed from phase 2 survey

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
D7c	Do you add cold water to the rice tea when you drink it?	Yes No	1 0		Question removed from phase 2 survey
D8.a	*[If D3 != 2]* Do you apply any treatment to the water your child drinks before giving it to your child? (filter – boiling - adding chlorine …)	Yes No	1 0	 >>D9	
D8.b	What kind of treatment do you apply?	Filtering water Boiling water Chlorine Solar disinfection (SODIS) Other (specify)	1 2 3 4 96		
D8.c	Do you add cold water to the rice tea/boiled water/rice porridge/plumpy nut/milk you give to your child?	Yes No	1 0		
D9	*[If D1=0]* What is the main source of water used by your household for other purposes besides drinking, such as cooking, cleaning, washing, etc.?	Piped water inside dwelling Piped water to yard/plot (on co Piped water to neighbor Piped water to public tap/stand Tube-well or borehole (handpu Protected dug well Unprotected dug well Protected spring Unprotected spring Rainwater Tanker truck Cart with small tank Surface water (river, dam, lake, stream, canal) Bottled water Sachet water Irrigation/field water Other (specify) Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 96 98 99		Question removed from phase 2 survey

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
Sanitation					
S1	Where do members of your household usually defecate?	 Toilet, latrine Dig & bury, bush, refuse dum body, beach/sand Other (specify) Refuse to answer Don't know	1 2 96 98 99	 >>S4 >>S4 >>S4 >>S4	
S2	What kind of toilet facility is it?	 Flush to manhole/septic tank Flush to pit latrine Flush to somewhere else VIP latrine (with vent pipe) Pit latrine with slab Pit latrine without slab Composting toilet Bucket toilet Hanging toilet No toilet, open defecation Other	1 2 3 4 5 6 7 8 9 10 11 96		Question moved to observation section of phase 2 survey
S2c	Does the latrine have a lid?	Yes No	1 0		
S3a	Is this a public toilet facility?	Yes No Don't know	1 0 99		
S3b	Do you have a different toilet facility for men and women?	Yes No Don't know	1 0 99		Question removed from phase 2 survey
S4	How many households are using the toilet facility?	Only 1 2 to 5 more than 5 Don't know	1 2 3 99		Question removed from phase 2 survey
S5	Where does your child usually defecate?	Single usage Diapers Re-usable Diapers Toilets/small potty Dig & bury, bush, refuse dump, water body Other (specify)	1 2 3 4 96	 >>S7	
S6	How do you handle the child's feces?	Feces/diapers in the latrines Feces/diapers in a drain Feces/diapers in a trash pile Feces/diapers buried Feces/diapers left outdoors Others (specify)	1 2 3 4 5 96		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
S7	Where is the child when you use the toilet or the bush when you are around the compound?	I take the child with me The child is waiting around the toilet / defecation area I give the child to another caregiver I give the child to someone else from my community The child stays at home Other (specify)	1 2 3 4 5 96		
S8	Where is the child when you use the toilet or the bush when you are at the market or in the field?	I take the child with me The child is waiting around the toilet / defecation area I give the child to another caregiver I give the child to someone else from my community I give the child to someone else outside my community I don't use toilets or the bush when in the field or at the market Other (specify)	1 2 3 4 5 0 96		
S8 Observation	Enumerator asks whether he/she can see the toilet – What is the condition of the toilet? *Observe*	Latrine functional (pit is not full or collapsed) Latrine pit is full Pit is collapsed Other	1 2 3 96		Question removed from phase 2 survey
S9 Observation	What is the condition of the latrine superstructure? *Observe*	No superstructure Full superstructure (4 walls and roof) Partial superstructure (partial walls/roof) Collapsed superstructure	0 1 2 3		Question moved to observation section of phase 2 survey
Hygiene					
Y1	Do you have a handwashing station with water and/or soap in the house or in the compound?	Yes in the compound Yes in the house No	1 2 0		
Y2	How often do you wash your hands?	1 time per day Between 1 and 3 times per day More than 3 times per day Never	1 2 3 0	 >>Y8	

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
Y3	Do you use soap or ashes to wash your hands?	Yes, soap Yes, ashes No, water only	1 2 0	 >>Y5	
Y4	How often do you use soap or ashes to wash your hands?	All the time Most of the time Sometimes/half of the time Rarely	1 2 3 4		
Y5	Do you wash your hands before feeding the child?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0		
Y6	Do you wash your hands before changing the child's diapers?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0		Question removed from phase 2 survey
Y7	Do you wash your hands after changing the child's diapers / after handling feces?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0		
Y8	How often do you wash the child's hands?	1 time per day Between 1 and 3 times per day More than 3 time per day Never	1 2 3 0	 >>Y10	
Y9	Do you use soap/ashes to wash the child's hands?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0		
Y10	How often do you bath / clean the child per week with water?	Once per week or less 2 to 5 times per week More than 5 times per week	1 2 3		
Y11	How often do you use soap to bath / clean the child?	All the time Sometimes Never	1 2 0		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
Y12	What type of water do you use to bath/clean the child?	Piped water inside dwelling Piped water to yard/plot (on co Piped water to neighbor Piped water to public tap/standp Tube-well or borehole (handpu Protected dug well Unprotected dug well Protected spring Unprotected spring Rainwater Tanker truck Cart with small tank Surface water (river, dam, lake, stream, canal) Bottled water Sachet water Other (specify) Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 96 98 99		
Y13	How often do you wash/change the child's clothes?	Less than once per week Once per week 2 to 5 times per week every day Don't know	1 2 3 4 99		
Y14	How often do you clean (sweep, mop, etc.) the interior of the house?	Every day 2 to 3 times per week 3 to 5 times per week Never	1 2 3 0		
Y15	Do you use any disinfectant (like soap) to clean the house?	Yes, all the time Yes, most of the time Yes, sometimes/half of the time Yes, rarely No, never	1 2 3 4 0		
Y16 Observation	Enumerator ask whether he/she can see the handwashing station What type of hand washing station is it? *Observe*	Fixed hand washing station Mobile hand washing station	1 2		Question moved to observation section of phase 2 survey

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
Y17 Observation	Are soap/ashes and water available at the handwashing station? *Observe and select all that apply*	Hand washing station has water Hand washing station has soap Hand washing station has ashes Hand washing station has none of these	1 2 3 0		Question moved to observation section of phase 2 survey
Surface water / open drains					
W1	Think about whether you go into **rivers, ponds, or lakes** in your neighborhood to wade, swim, splash around, fish, do laundry, or to defecate. How many times in the past month did you go into the rivers, ponds, or lakes for any of these reasons?	More than 10 times in the past month 6 to 10 times in the past month 5 times or less in the past month Never Do not know	1 2 3 0 99		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
W2	Now think about whether your children (<2 years old) go into **the rivers, ponds, or lakes** in your neighborhood to wade, swim, splash around, fish, help with laundry, or to defecate. How many times in the past month did your children go into the rivers, ponds, or lakes for any of these reasons?	More than 10 times in the past month 6 to 10 times in the past month 5 times or less in the past month Never Do not know	1 2 3 0 99		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
W5	Think about whether you ever come into contact with **floodwater** during the rainy season, including to pick up something that fell into floodwater, to walk through floodwater in the street, or to clean your house after it floods. How many times total every week did you come into contact with floodwater during the rainy season?	More than 10 times total every week during the rainy season 6 to 10 times total every weekduring the rainy season 5 times or less total every week during the rainy season Never Do not know	1 2 3 0 99		Question removed from phase 2 survey

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
W6	Now think about whether your children ever come into contact with floodwater during the rainy season, including to pick up something that fell into floodwater, to play in the floodwater, to walk through floodwater in the street, or to clean your house after it floods. How many times total every week did your children come into contact with floodwater during the rainy season?	More than 10 times total every week during the rainy season 6 to 10 times total every week during the rainy season 5 times or less total every week during the rainy season Never Do not know	1 2 3 0 99		Question removed from phase 2 survey
Soil ingestion					
I1	Have you observed your child putting soil into their mouth in the past 3 days?	Yes No	1 0	 >>Z1	
I2	How many times in the past 3 days did you observe your child putting soil into their mouth?	___________			

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
I3	About how much soil did you watch your child put into their mouth each time?	The amount of dirt normally on fingers The amount they could hold between two fingers Half of a handful A handful More than a handful Other	1 2 3 4 5 96		
Reported diarrhea/illness					
B1	Has the child had diarrhea in the last 2 weeks, that is three or more loose or watery stools in a day?	Yes No Don't know	1 0 99		
B2	How often does your child have diarrhea?	More than once a month Once every 2-3 months 2-3 times per year Once a year or less Never	1 2 3 4 0		
B3	Has your child had other illnesses in the last 2 weeks?	Malaria/fever Cold/flu Headache Stomach ache None Other: _______	1 2 3 4 0 96		
B4	How often does your child get sick?	More than once a month Once every 2-3 months 2-3 times per year Once a year or less Never	1 2 3 4 0		
Caregiver recommendations and comments					
Z1	What do you think are the biggest risks to your child's health? *Select all that apply*	Contaminated water Contaminated food Touching/playing in contaminated soil Touching/playing with animals Touching human feces Touching animal feces Touching contaminated toys or other objects Other: _______ Don't know	1 2 3 4 5 6 7 96 99		

No.	Consent and basic information	Answer Choices	Code	Logic	Phase 2 question removal
Z2	What ideas do you have to help protect your child from these risks?	______________			
Z3	This concludes our survey. Thank you for your time				
Z4	Any final comments or notes from the enumerator	______________			

B-2 Structured observation guide for phase 1

In part, this guide is modeled after the structured observation approach described in Teunis et al. (2016), which was part of the SaniPath study in Ghana.[109] Kwong et al. (2016) also informed the questions related to hand-to-mouth contact.[110] The structured observation will occur after the caregiver survey.

No.	Consent and basic information	Answer Choices	Code	Logic
S6c	Measure the mid-upper arm circumference (MUAC) if the child is above 6 months old	___		
S6d	Does the child seem underweight?	Yes No Don't know	1 0 99	
S7	Time of day of the structured observation	Morning (starting before 9 am) Middle of day (starting at 9 am-1 pm Afternoon/evening starting after 1 pm)	1 2 3	
No.	**Structured observation**	**Answer Choices**	**Code**	**Logic**
The enumerator will input a sequence of "observation records" for a given child in CommCare. Each record will represent a distinct combination of the **environment**, **setting**, and **activity** (see below for details on these terms). The following questions (S8-S19, which make up a single observation record) will all appear on a single screen, and will be repeated as many times as necessary until the structured observation time is complete. When the child's environment, setting, or activity changes (e.g., a change in environment/location, or a change to a different activity, or both), the enumerator continues to the next screen to input a new observation record.				
S8	Record the start time of this observation record	Time: ___________		
S9	**Environment** *General site of child's activity*	Inside the house Outside, near the house Kitchen area Livestock area Crop area Other: ___________	1 2 3 4 5 96	
S10	Do you observe any human feces present in this environment?	Yes No	1 0	
S11	Do you observe any animal feces present in this environment?	Yes No	1 0	

No.	Consent and basic information	Answer Choices	Code	Logic
S12	**Setting** *Specific category of child's location within a given environment*	Unimproved ground (e.g., soil, dirt) Improved ground (e.g., floor, veranda, woven mat) Off ground (e.g., held by caregiver, sitting on a chair) In or next to open water or open drain Trash/rubbish area Other: ___________	1 2 3 4 5 96	>>S13 >>S13 >>S13 >>S13 >>S13
S12a	How dirty is the improved ground?	Extremely dirty Somewhat dirty Slightly dirty Clean	1 2 3 4	
S13	**Activity** *Behavior child is engaged in (the* caregiver *may be involved as well)*	Playing or sitting Sleeping Washing hands Bathing Defecating Eating or drinking Other: ___________	1 2 3 4 5 6 96	>>S14 >>S14 >>S13a >>S13a >>S13b >>S13c
SC1	*[If S13 = 1 or 2]* What is the caregiver doing while the child is playing, sitting, or sleeping?	Cooking Working in the house Working outside Talking/interacting with others Sleeping Washing their own hands Defecating Other: _______	1 2 3 4 5 6 7 96	
SC1.b	*[If SC1=9]* What did the caregiver use when washing their hands?	Water and soap Water and ash Water only Ash only Other: ________	1 2 3 4 96	
S13a	*[If S13 = 3 or 4]* Is the child using soap to wash hands or bathe?	Yes, soap Using another cleansing agent No	1 2 0	>>S14 >>S14 >>S14
S13b	*[If S13 = 5]* How are the child's feces managed?	Caregiver deposits in toilet Caregiver deposits in a rubbish area or similar Left in the open Other: __________	1 2 0 96	>>S14 >>S14 >>S14 >>S14

No.	Consent and basic information	Answer Choices	Code	Logic
S13c	*[If S13 = 6]* What types of foods or drinks is the child consuming? *Select all that apply*	Water Sugary water Ricewater tea/liquid Breastmilk Condensed milk with water Infant formula Cow/goat milk Tea Uncooked fruits/vegetables Cooked food Local brew Other drink: _________ Other food: _________	1 2 3 4 5 6 7 8 9 10 11 95 96	
S13d	*[If S13c = 3]* How is the formula prepared? *Select all that apply*	With milk With untreated water With water that is boiled or treated in another way As part of the family meal Other: _________	1 2 3 4 96	
S13e	*[If S13 = 3 or 4, or S13c = 1, or S13d = 2 or 3]* What water source did the water for this activity come from?	Piped water inside dwelling Piped water to yard/plot (on compound) Piped water to neighbor Piped water to public tap/standpipe Tube-well or borehole (handpump) Protected dug well Unprotected dug well Protected spring Unprotected spring Rainwater Tanker truck Cart with small tank Surface water (river, dam, lake, pond, stream, canal) Bottled water Sachet water Other (specify) Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 96 97 99	

No.	Consent and basic information	Answer Choices	Code	Logic
S13f	*[If S13c = 6]* What kind of raw produce is the child eating? *Select all that apply*	Tomato Lettuce Sugarcane Guava Makoba (pomme canaque) Zamboarizano Other: _________	1 2 3 4 5 6 96	
S14	Is the child touching or playing with any toys or other objects during this activity?	Yes No	1 0	 >>S17
S15	What objects is the child touching? *Select all that apply*	Toys Plates/cups/utensils Food Soil (picking it up) Rocks/stones/bricks Sticks/leaves/other vegetation Human feces Animal feces Cloth/clothing Other: __________	1 2 3 4 5 6 7 8 9 96	
S15a	*[If S15 = 7]* What type of human feces was the child touching? *Select all that apply*	The child's own feces Other human feces	1 2	
S15b	*[If S15 = 8]* What type of animal feces was the child touching? *Select all that apply*	Poultry Goat/sheep Cow Cat/dog Other: _______	1 2 3 4 96	
S16	How many times during this observation record has the child put an object in their mouth? *Keep a tally in your notebook while this activity is going on, and fill in this question before continuing to the next activity.*	__________		

No.	Consent and basic information	Answer Choices	Code	Logic
S17	How many times during this observation record has the child put their fingers or hands in their mouth? *Keep a tally in your notebook while this activity is going on, and fill in this question before continuing to the next activity.*	__________		
SC3	How many times during this observation record has the caregiver put their fingers or hands into the child's mouth? *Keep a tally in your notebook while this activity is going on, and fill in this question before continuing to the next activity.*	__________		
SC4	*[If SC3 > 0]* Did the caregiver wash their hands before putting them in the child's mouth?	Yes No	1 0	
S18	Please record any comments or notes related to this specific observation record.			
S19	Record the end time of this observation record	Time: ______________		
S20	Is the structured observation time completed? *Have 5 hours elapsed since the beginning of the structured observation?*	Yes No	1 0	>>SS1 >>Repeat beginning at S8

No.	Consent and basic information	Answer Choices	Code	Logic
SS1	Did you observe the child sleeping during any part of this structured observation?	Yes No	1 0	>>S99
SS2	Ask the caregiver if you can see the place where the child normally sleeps. What type of location is it?	Bed Woven mat Improved ground (e.g., cement floor) Unimproved ground (e.g., soil, dirt) Other: ______	1 2 3 4 96	
SS3	How dirty is the sleeping location?	Extremely dirty Somewhat dirty Slightly dirty Clean	1 2 3 4	
S99	This structured observation is complete. Please record any general comments or notes.			

B-3 Spot observation guide for phase 2

This guide was used after the survey by the enumerators for direction observation of the environmental surrounding of the children in the study.

No.	Consent and basic information	Answer Choices	Code	Logic
O_pre	With your permission I will begin the observation. This observation will last about 10 minutes and I will take notes. You can act like I'm not there. Can I start?	Yes No	1 0	
O_preNote	If this household cannot participate in structured observation. Thank people for their time.	______________		
O_07	Time of day when structured observation takes place	Morning (starting before 9 am) Middle of day (starting at 9 am-1 pm Afternoon/evening starting after 1 pm)	1 2 3	
OH_08	What is the main construction material used for the dwelling's outer walls?	Ravinala leaves/cane/palm/trunks Mud/landcrete Bamboo or wood with mud Stone with mud Uncovered adobe / mud brick Reused wood Cement Stone with lime/cement Kiln-fired bricks Cement blocks Covered adobe (plastered) Wood planks/shingles No walls Other Don't know	1 2 3 4 5 6 7 8 9 10 11 12 0 96 99	
OH_09	What is the main material used for the dwelling's roof?	Thatch/palm leaf Mud/sod Palm/bamboo Wood planks (rudimentary roofing) Cardboard Zinc/aluminum Wood (finished roofing) Calamine/cement fiber Ceramic/brick tiles Cement Roofing shingles Asbestos/slate roofing sheets	1 2 4 5 6 7 8 9 10 11 12	

No.	Consent and basic information	Answer Choices	Code	Logic
		No roof Other Don't know	13 0 96 99	
OH_10	What is the main construction material used for the dwelling's floor?	Earth/sand Palms/trunks (rapaka) Dung Wood planks Palm/bamboo Parquet or polished wood Vinyl or asphalt strips Tiles (ceramic, marble, porcelain, terrazo) Cement Carpet (woolen or synthetic) Linoleum/rubber carpet Other Don't know	1 2 3 4 5 6 7 8 9 10 96 99	
O_12a	How clean is the improved floor?	Very dirty Dirty A little dirty Clean	1 2 3 4	
OS_08Observation	ENUMERATOR: ask to see the toilets			
OS_08Observationb	What kind of toilet facility is it?	Flush to manhole/septic tank Flush to pit latrine Flush to somewhere else VIP latrine (with vent pipe) Pit latrine with slab Pit latrine without slab Composting toilet Bucket toilet Hanging toilet No toilet, open defecation Other	1 2 3 4 5 6 7 8 9 10 11 96	
OS_09Observation	What is the condition of the latrine superstructure? *Observe*	No superstructure Full superstructure (4 walls and roof) Partial superstructure (partial walls/roof) Collapsed superstructure	0 1 2 3	
OS_02c	Does the toilet have a cover?	Yes No	1 0	
OY_16Observation	Enumerator ask whether he/she can see the handwashing station What type of hand washing station is it? *Observe*	Fixed hand washing station Mobile hand washing station	1 2	

No.	Consent and basic information	Answer Choices	Code	Logic
OY_17Observation b	Are soap/ashes and water available at the handwashing station? *Observe and select all that apply*	Hand washing station has water Hand washing station has soap Hand washing station has ashes Hand washing station has none of these	1 2 3 0	
OW_01Observation	Ask how long ago the water source is. If the water source is less than 5 minutes away ask to see it.	__________		
OW_03Observation	Is the source closer than 5 minutes by foot?	Yes No	1 0	
OW_02Observation	What is the water source type?	Piped water inside dwelling Piped water to yard/plot (on compound) Piped water to neighbor Piped water to public tap/standpipe Tube-well or borehole (handpump) Protected dug well Unprotected dug well Protected spring Unprotected spring Rainwater Tanker truck Cart with small tank Surface water (river, dam, lake, pond,stream, canal) Bottled water Sachet water Other (specify) Refuse to answer Don't know	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 96 98 99	
OC_5	Does the parent/guardian wear sandals?	All the time The majority of the time Sometimes Rarely	1 2 3 4	
OZ_2	Ask the parent/guardian if you can see the place where the child is usually there. What type of sleeping location is it? (multiple answers)	Mattress Woven mat Improved ground (for example, concrete) Unimproved ground (for example, soil) Other	1 2 3 4 99	

No.	Consent and basic information	Answer Choices	Code	Logic
OZ_3	How clean is the place where the child sleeps?	Very dirty Dirty A little dirty Clean	1 2 3 4	
OZ_4	Did you observe human feces in the exterior of the house?	Yes No	1 0	
OZ_5	Did you observe animal feces in the exterior of the house?	Yes No	1 0	
OZ_6	Did you observe stagnant water outside the house?	Yes No	1 0	
OZ_7	Did you observe animals outside the house?	Yes No	1 0	
OZ_8a	How clean are the parent/guardian's hands?	Clean Dirty Very dirty	1 2 3	
OZ_8b	How clean are the child's hands?	Clean Dirty Very dirty	1 2 3	
O_06c	Does the child appear underweight?	Yes No Don't know	1 0 99	
O_06b	Measure the child's mid-upper arm circumference			
OZ_99	This structured observation is complete. Record comments or notes here and thank the parent/guardian for their time			

Appendix C: Field sampling protocol

Field sampling protocol

Adapted from [87,111–113]

To evaluate fecal and pathogen contamination along various possible exposure pathways, we will collect environmental samples for each of the 20 children observed during Phase 1, as well as the 180 children from Phase 2. These samples will represent several different pathways through which pathogen exposure may occur, as described below.

During or immediately after the caregiver survey, the enumerator who will be collecting samples can go through the following procedures. During Phase 1, samples will be taken from all compartments described below, and these samples will be used to quantify *E. coli* contamination. During Phase 2, we will select a subset of compartments based on the most important pathways identified during the first phase. Phase 2 samples will be used to quantify *E. coli* contamination, and a subset (from approximately 20 children) will also be used for pathogen testing. During Phase 2, we will also be collecting child feces samples from the subset of 20 children.

Gloves should be worn during sampling, and facemasks should be worn at all times in the field. All samples will be collected in sterile Whirl-Pak Thio-Bags[1], which contain a sodium thiosulfate tablet to neutralize any chlorine in the sample (e.g., from water treatment) that may inactivate *E. coli* or pathogens. See Figure C1 for a visual depiction of the proper procedures for opening and sealing Whirl-Pak bags to avoid contamination of the sample. Samples should be stored in a cooler with ice packs during transport to the laboratory.

Sampling procedures by compartment

Drinking water.

- Ask the caregiver to provide a glass of water from their primary drinking water storage container, as if preparing it to give to the child. (If they normally heat the water, before giving it to the child, use unheated/room temperature water.)
- Open the sample bag by tearing off the perforated seam and pulling apart the white tabs. Avoid touching the inside of the bag.
- Pour 100 ml of water from the glass into the sample bag.
- Seal the sample bag by rolling down the top of the bag, or by holding the long tabs and spinning it 3-4 times to create a seal. Bring the long tabs together and twist tie them.
- If the household is selected for pathogen testing, fill a second sample bag with another 100 ml from the glass, and seal the bag.

Child hands.

- Open the sample bag by tearing off the perforated seam and pulling apart the white tabs. Avoid touching the inside of the bag.
- Fill the sample bag with 100 ml of distilled water.
- Ask the caregiver to place the child's hands, one at a time, in the sample bag filled with water.
- Massage each hand (from outside the bag) for approximately 30 seconds to transfer any material from the hand to the water in the bag.
- Seal the sample bag by rolling down the top of the bag, or by holding the long tabs and spinning it 3-4 times to create a seal. Bring the long tabs together and twist tie them.
- If the household is selected for pathogen testing, repeat this procedure using a second sample bag.

[1] https://whirl-pak.com/wp-thio-4oz100ml-stand-up-box-100

Caregiver hands (can be sampled anytime, though collecting just before preparing a meal or feeding the child would be most ideal, if possible).

- Open the sample bag by tearing off the perforated seam and pulling apart the white tabs. Avoid touching the inside of the bag.
- Fill the sample bag with 200 ml of distilled water.
- Ask the caregiver to place her hands, one at a time, in the sample bag filled with water.
- Massage each hand (from outside the bag) for approximately 30 seconds to transfer any material from the hand to the water in the bag.
- Seal the sample bag by rolling down the top of the bag, or by holding the long tabs and spinning it 3-4 times to create a seal. Bring the long tabs together and twist tie them.
- If the household is selected for pathogen testing, repeat this procedure using a second sample bag.

Soil.

- Ask the caregiver to specify a nearby outdoor location where the child commonly plays or spends time.
- Go to the location and open the sample bag by tearing off the perforated seam and pulling apart the white tabs. Avoid touching the inside of the bag.
- Take a sterile spoon from the pack, and scoop approximately 10 grams of soil (2-3 spoonfuls with a 5-ml spoon; will adapt as needed depending on spoon size) from an area of approximately 10 cm x 10 cm into the sample bag.
- Seal the sample bag by rolling down the top of the bag, or by holding the long tabs and spinning it 3-4 times to create a seal. Bring the long tabs together and twist tie them.
- If the household is selected for pathogen testing, repeat this procedure using a second sample bag.

Food.

- Ask the caregiver to provide a small amount of food that is commonly eaten by the child, to be given in the same way they would feed it to the child.
- Open the sample bag by tearing off the perforated seam and pulling apart the white tabs. Avoid touching the inside of the bag.
- With a sterile spoon, scoop approximately 10 grams of the food (2-3 spoonfuls) into the sample bag.
- Seal the sample bag by rolling down the top of the bag, or by holding the long tabs and spinning it 3-4 times to create a seal. Bring the long tabs together and twist tie them.
- If the household is selected for pathogen testing, repeat this procedure using a second sample bag.

Child feces (pathogen testing only).

- Provide the caregiver with a sterile (unopened) sample bag, spoon, and gloves.
- If the child has recently defecated, collect a fecal sample using the following steps. Alternatively, the caregiver can be asked to collect a sample later, after the child has defecated. In this case, explain the following steps to the caregiver.
    - Put on the gloves.
    - Open the sample bag by tearing off the perforated seam and pulling apart the white tabs. Avoid touching the inside of the bag.
    - Take a sterile spoon from the pack, and scoop approximately 10 grams of feces (a few spoonfuls) into the sample bag.
    - Seal the sample bag by rolling down the top of the bag, or by holding the long tabs and spinning it 3-4 times to create a seal. Bring the long tabs together and twist tie them.
- If the caregiver is collecting the sample, return to the household later to collect the sample.

Animal feces (pathogen testing only).

- Go to a location with animal feces and open the sample bag by tearing off the perforated seam and pulling apart the white tabs. Avoid touching the inside of the bag.
    - If there are multiple locations with animal feces, choose a location closest to where the child spends most time.
- With a sterile spoon, scoop approximately 10 grams of feces (2-3 spoonfuls) from an area of approximately 10 cm x 10 cm into the sample bag.
- Seal the sample bag by rolling down the top of the bag, or by holding the long tabs and spinning it 3-4 times to create a seal. Bring the long tabs together and twist tie them.

Labelling instructions (for each sample collected)

- Date: DD/MM/YYYY
- Region (code)
    - Atsimo Atsinanana (AA)
    - Vatovavy (VV)
    - Fitovinany (FV)
- Municipality name
- Household ID
- Type of sample (code)
    - Drinking water (DW)
    - Child Hands (CH)
    - Caregiver Hands (GH)
    - Soil (S)
    - Food (F, and specify the type of food)
    - Surface / Objects (O, and specify the type of surface/object)
    - Child feces (CF)
    - Animal feces (AF)
- Name of the person who collected the sample

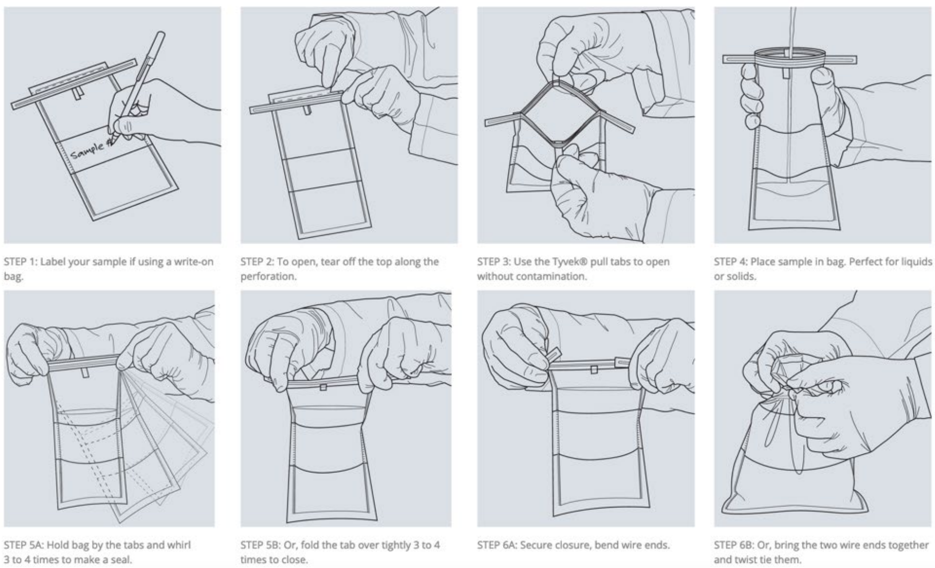

Figure C1: Guidance on opening and sealing Whirl-Pak bags to ensure the sample is not contaminated inside the bag[2].

[2] https://whirl-pak.com/whirl-pak-bags-general-information?gclid=CjwKCAjw7rWKBhAtEiwAJ3CWLH6zcSWjAdNR22XIjp4GFeXeA_MriWiJMaHgk9Ed4aCvbZjGxvAFiBoCSQ8QAvD_BwE

Appendix D: *E. coli* testing protocol

E. coli testing protocol using Compartment Bag Tests

Adapted from Aquagenx Compartment Bag Test for E. coli and total coliform instructions for use [114]

All collected environmental samples will be tested for *E. coli* using the compartment bag test (CBT) method. This method involves adding a growth medium to the collected 100-ml sample, mixing, and then transferring the sample into a compartment bag and incubating, as described below.

After collection, samples should ideally arrive at the laboratory for processing within 6 hours. During processing, gloves should be worn to prevent sample contamination and minimize health risks. Before starting the procedure, sanitize the laboratory work area using disinfectant.

Sample preparation

For water and hand rinse (i.e., child or caregiver hands rinsed in a bag of water) samples:

- Dilution may be needed, depending on the *E. coli* concentrations measured previously. Standard dilutions can be determined from samples collected during piloting.
- We will dilute samples of a certain type (water, child hands, caregiver hands) if:
    - At least 25% of previous samples of that type are measured to have >100 MPN / 100 ml, OR
    - At least 50% of previous samples of that type are measured to have at least 10 MPN / 100 ml.
- If one of these conditions is met, we will use a 1:10 dilution for that sample type. This would require mixing 10 ml of sample with 90 ml of distilled water.
- If the 1:10 dilutions continue to produce results that meet one of the dilution conditions above (2.a or 2.b), we will increase to a 1:100 dilution (1 ml of sample with 99 ml of distilled water).
- During data collection, we will revise dilution procedures at least once per week, as needed.

For solid samples (e.g., soil, food):

- Mix the sample and weigh out 2.00 grams.
    - A different sample mass may be needed, depending on the *E. coli* concentrations measured previously. Standard masses can be determined from samples collected during piloting.
    - We will decrease the mass to be tested from samples of a certain type (soil, food) if:
        - At least 25% of previous samples of that type are measured to have >100 MPN / 100 ml, OR
        - At least 50% of previous samples of that type are measured to have at least 10 MPN / 100 ml.
    - If one of these conditions is met, we will halve the weighed mass, to 1.00 gram. If samples continue to produce results that meet one of the conditions above (1.b.i or 1.b.ii), we will reduce the mass by half again (0.50 grams).
    - We will increase the mass to be tested from samples of a certain type if:
        - At least 50% of previous samples of that type are measured to have 0 MPN / 100 ml, AND
        - No previous samples of that type are measured to have at least 10 MPN / 100 ml.
    - If one of these conditions is met, we will double the weighed mass, to 4.00 grams. If samples continue to produce results that meet both of the conditions above (1.d.i and 1.d.ii), we will double the mass again (8.00 grams).
- Transfer the weighed sample to a bag and mix with 100 ml of distilled water.
- During data collection, we will revise weighing procedures at least once per week, as needed.

***E. coli* Testing Procedure (summarized below in Figure D1):**

- Open growth medium packet (with scissors) and pour the full contents of the packet into the sample bag. Avoid touching the growth medium or the inside of the sample bag with bare fingers.
- Roll down the top of the bag to seal it, and then gently swirl the bag until the growth medium is dissolved.
- Prepare a compartment bag by labeling it with the sample information and tearing off the perforated seam at the top of the bag.
- Rub top and sides of the compartment bag together to ensure that all individual compartments are open to receive the sample.
- Open the compartment bag using the white tabs at the top. Avoid touching the inside of the bag with bare fingers.
- Slowly pour the sample into the compartment bag, while slightly tilting and squeezing the compartment bag to help distribute the sample across all five individual compartments. Ensure all five compartments are filled evenly, up to the fill line.
- Seal compartment bag shut by rolling down the Whirl-Pak seal at the top and attaching a seal clip to prevent leakage. Place the U-shaped part of the clip across the width of the bag along the fill line and below the compartment openings. Place the rod-shaped part of the clip on the opposite side of the bag and snap into the U-shape to lock in place.
- Incubate the bag. Ideally, incubation should be at 35-37°C for 24 hours. However, less stringent temperature ranges can also be used (but the temperature should remain above 25°C):
    - 31-34°C for 24-30 hours
    - 25-30°C for 48 hours
- After incubation, compartments that are positive for *E. coli* will have changed color from yellow to blue (Figure D2). Compartments with any trace of blue or blue-green coloring, including colored specks or sediment at the bottom of the compartment, should be treated as positive. Match the color pattern of the five compartments with one of the rows in the MPN Table (Table D1). Record the MPN value and the upper 95% confidence level for *E. coli*.
- To decontaminate sample for disposal, add 4 ml of liquid bleach. After 30 minutes, the contents can safely be poured into a drain or disposal site. The seal clip can be retained and reused.

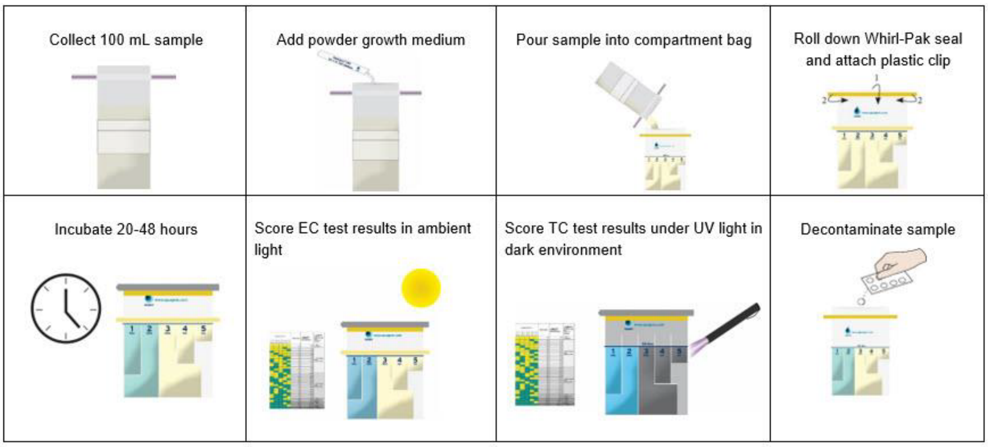

Figure D1: Summary of compartment bag test procedure.

Color of compartment in Compartment Bag	Yellow/Yellow Brown in ambient light and does not fluoresce blue under UV light	Yellow/Yellow Brown that … fluoresces blue under UV light	Blue/Blue Green in ambient light	Blue/Blue Green that… fluoresces blue under UV light
E. coli	Negative	Negative	Positive	Positive
Total Coliforms	Negative	Positive	Positive	Positive

Figure D2: Coloring of compartments indicating positive or negative *E. coli* presence.

Row Number:	Compartment Number 1 10mL	2 30mL	3 56mL	4 3mL	5 1mL	MPN/100mL	Upper 95% Confidence Level/100mL	WHO Health Risk Category Based on MPN and Upper 95% Confidence Level
1						0.0	2.87	Low Risk/Safe
2						1.0	5.14	Intermediate Risk/ Probably Safe
3						1.0	4.74	
4						1.1	5.16	
5						1.2	5.64	
6						1.5	7.81	
7						2.0	6.32	
8						2.1	6.85	
9						2.1	6.64	
10						2.4	7.81	
11						2.4	8.12	
12						2.6	8.51	
13						3.2	8.38	
14						3.7	9.70	
15						3.1	11.36	Intermediate Risk/ Possibly Safe
16						3.2	11.82	
17						3.4	12.53	
18						3.9	10.43	
19						4.0	10.94	
20						4.7	22.75	
21						5.2	14.73	
22						5.4	12.93	
23						5.6	17.14	
24						5.8	16.87	
25						8.4	21.19	
26						9.1	37.04	
27						9.6	37.68	
28						13.6	83.06	High Risk/Possibly Unsafe
29						17.1	56.35	
30						32.6	145.55	High Risk/Probably Unsafe
31						48.3	351.91	
32						>100	9435.10	Unsafe

Figure D3: MPN Table for matching the color pattern of the compartments.

Example recording sheet for water and hand rinse samples:

Sample type	Sample ID	Date sampled	Date read	Dilution	MPN value	Upper 95% confidence level
Water	4/11-DW-HH1	4 Nov 2021	5 Nov 2021	1:1 (none)	13.6	83.06
Child hands	4/11-CH-HH1	4 Nov 2021	5 Nov 2021	1:10	4.0	10.94
Adult hands	4/11-GH-HH1	4 Nov 2021	5 Nov 2021	1:10	2.0	6.32
Water	4/11-DW-HH2	4 Nov 2021	5 Nov 2021	1:1 (none)	2.1	6.85
Water	5/11-DW-HH1	5 Nov 2021	6 Nov 2021	1:1 (none)	3.2	11.82

Example recording sheet for soil and food samples:

Sample type	Sample ID	Date sampled	Date read	Sample mass (g)	Water volume (ml)	MPN value	Upper 95% confidence level
Soil	4/11-S-HH1	4 Nov 2021	5 Nov 2021	2	100	13.6	83.06
Food	4/11-F-HH1	4 Nov 2021	5 Nov 2021	5	100	4.0	10.94
Soil	4/11-S-HH2	4 Nov 2021	5 Nov 2021	2	100	2.1	6.85
Soil	5/11-S-HH1	5 Nov 2021	6 Nov 2021	2	100	3.2	11.82

Appendix E: Number of household surveys and environmental samples

E-1 Phase I pre-cyclone

Table E.1a: summary of household surveys and structured observations conducted during phase I pre-cyclone

Household Surveys and Structured observations - Phase 1 pre-cyclone			
District	**Fokontany**	**Household Surveys**	**Number of children Observed**
Mananjary	Tsaravary	4	4
Vohipeno	Mahavelo	3	3
Vohipeno	Lazamasy	2	2
Farafangana	Tsararafa	6	6
TOTAL		**15**	**15**

Table E.1b: summary of environmental samples collected for *E. coli* analysis during phase I pre-cyclone

Environmental Samples - Phase 1 pre-cyclone								
District	**Fokontany**	**Drinking Water**	**Soil**	**Caregiver Hand Rinse**	**Child Hand Rinse**	**Cooked Food**	**Raw Food**	
Mananjary	Tsaravary	3	4	0	2	3	0	
Vohipeno	Mahavelo	3	2	2	3	1	0	
Vohipeno	Lazamasy	2	1	2	1	2	0	
Farafangana	Tsararafa	4	5	7	4	3	1	
								TOTAL
TOTAL		**12**	**12**	**11**	**10**	**9**	**1**	**55**

E-2 Phase I post-cyclone

Table E.2a: summary of household surveys and structured observations conducted during phase I post-cyclone

Household Surveys and Structured observations - Phase 1 post-cyclone			
District	**Fokontany**	**Household Surveys**	**Number of children Observed**
Farafangana	Anakona	3	3
Vohipeno	Ifatsy Centre	3	3
Manakara	Mideboka	3	3
Mananjary	Ambalona	3	3
Mananjary	Manakana	3	3
Vondrozo	Analavaky	1	1
Farafangana	Ambalavotaky	1	1
Farafangana	Antseranambe centre	1	1
Vangaindrano	Tsianofana Centre	1	1
Vangaindrano	Andrangalaza	1	1
TOTAL		**20**	**20**

Table E.2b: summary of environmental samples collected for *E. coli* analysis during phase I post-cyclone

Environmental Samples - Phase 1 post-cyclone								
District	**Fokontany**	**Drinking Water**	**Soil**	**Caregiver Hand Rinse**	**Child Hand Rinse**	**Cooked Food**	**Raw Food**	
Mananjary	Tsaravary	3	4	0	2	3	0	
Vohipeno	Mahavelo	3	2	2	3	1	0	
Vohipeno	Lazamasy	2	1	2	1	2	0	
Farafangana	Tsararafa	4	5	7	4	3	1	
Farafangana	Anakona	3	3	3	3	2	1	
Vohipeno	Ifatsy Centre	3	3	3	3	3	0	
Manakara	Mideboka	3	3	3	3	2	1	
Mananjary	Ambalona	3	3	3	3	3	0	
Mananjary	Manakana	3	3	3	2	2	0	
Vondrozo	Analavaky	1	0	1	1	0	0	
								TOTAL
TOTAL		**20**	**15**	**20**	**19**	**12**	**2**	**88**

E-3 Phase II

Table E.3a: summary of household surveys and spot-checks conducted during phase II

Household Surveys and Spot Checks - Phase II		
District	**Fokontany**	**Household Surveys**
Mananjary	Ambalona	24
Manakara	Ambila Centre	8
Mananjary	Ampasimbola	10
Farafangana	Anakona	18
Vangaindrano	Andrangalaza	5
Mananjary	Andranomavo	9
Farafangana	Anivotsara	3
Vohipeno	Anosy	10
Farafangana	Antseranambe Centre	4
Vohipeno	Fenoarivobe	16
Vohipeno	Ifatsy Centre	8
Vohipeno	Iazamasy	12
Farafangana	Mahiakoho	4
Mananjary	Manakana	13
Manakara	Mideboka	20
Mananjary	Tsaravary	18
Vangaindrano	Tsianofana Centre	5
TOTAL		**187**

Table E.3b: summary of environmental samples collected for *E. coli* analysis during phase II

Environmental Samples - Phase I post-cyclone							
District	**Fokontany**	**Drinking Water**	**Soil**	**Caregiver Hand Rinse**	**Child Hand Rinse**	**Cooked Food**	**Raw Food**
Mananjary	Ambalona	18	18	20	13	5	0
Manakara	Ambila Centre	8	8	6	6	0	0
Mananjary	Ampasimbola	10	10	10	10	2	0
Farafangana	Anakona	17	16	18	15	7	0
Vangaindrano	Andrangalaza	5	0	4	3	0	0
Mananjary	Andranomavo	9	9	8	9	2	1
Farafangana	Anivotsara	3	0	3	3	0	0
Vohipeno	Anosy	7	10	10	9	6	0

Environmental Samples - Phase I post-cyclone								
District	**Fokontany**	**Drinking Water**	**Soil**	**Caregiver Hand Rinse**	**Child Hand Rinse**	**Cooked Food**	**Raw Food**	
Farafangana	Antseranambe Centre	3	0	0	3	1	0	
Vohipeno	Fenoarivobe	14	16	16	14	5	0	
Vohipeno	Ifatsy Centre	8	8	8	8	1	1	
Vohipeno	Iazamasy	11	11	11	8	0	0	
Farafangana	Mahiakoho	4	4	4	4	1	0	
Mananjary	Manakana	13	12	13	10	4	0	
Manakara	Mideboka	19	18	20	14	8	0	
Mananjary	Tsaravary	16	17	17	18	5	1	
Vangaindrano	Tsianofana Centre	5	0	5	5	0	0	
Unknown *(duplicate or additional samples or issues with labelling)*		15	12	22	11	8	0	
								TOTAL
TOTAL		**185**	**169**	**195**	**163**	**55**	**3**	**770**

Table E.3c: summary of environmental samples collected for pathogens analysis during phase II

Pathogen Samples - Phase II								
District	**Fokontany**	**Child Feces**	**Drinking Water**	**Soil**	**Animal Feces**	**Child Hand Rinse**	**Raw Food**	
Farafangana	Antseranambe	6	7	8	6	8	0	
Vohipeno	Ifatsy Centre	7	6	8	8	6	0	
Mananjary	Ambalona	10	6	10	10	10	2	
								Total
Total		**23**	**19**	**26**	**24**	**24**	**2**	**118**

Appendix F: *E. coli* contamination per region, environmental compartment, and source type

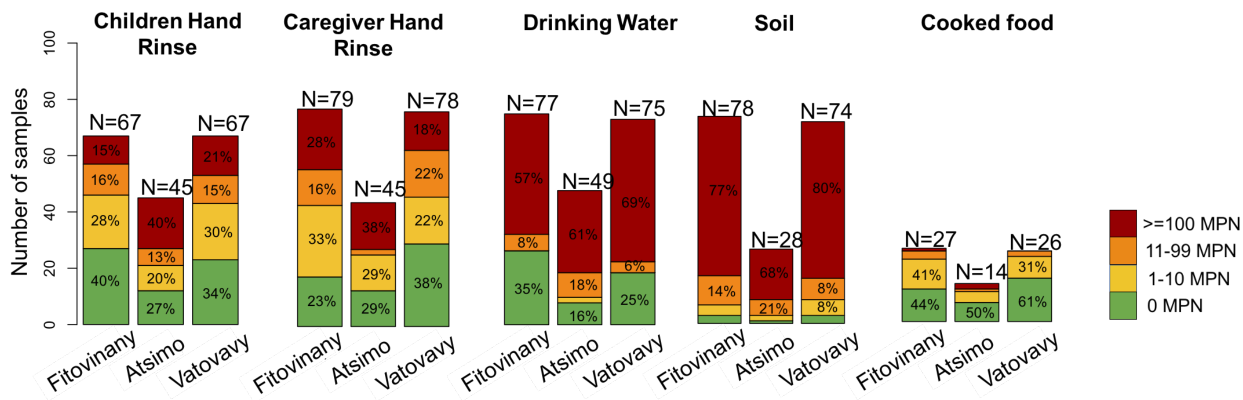

Figure F.1: *E. coli* contamination per region and per environmental compartments

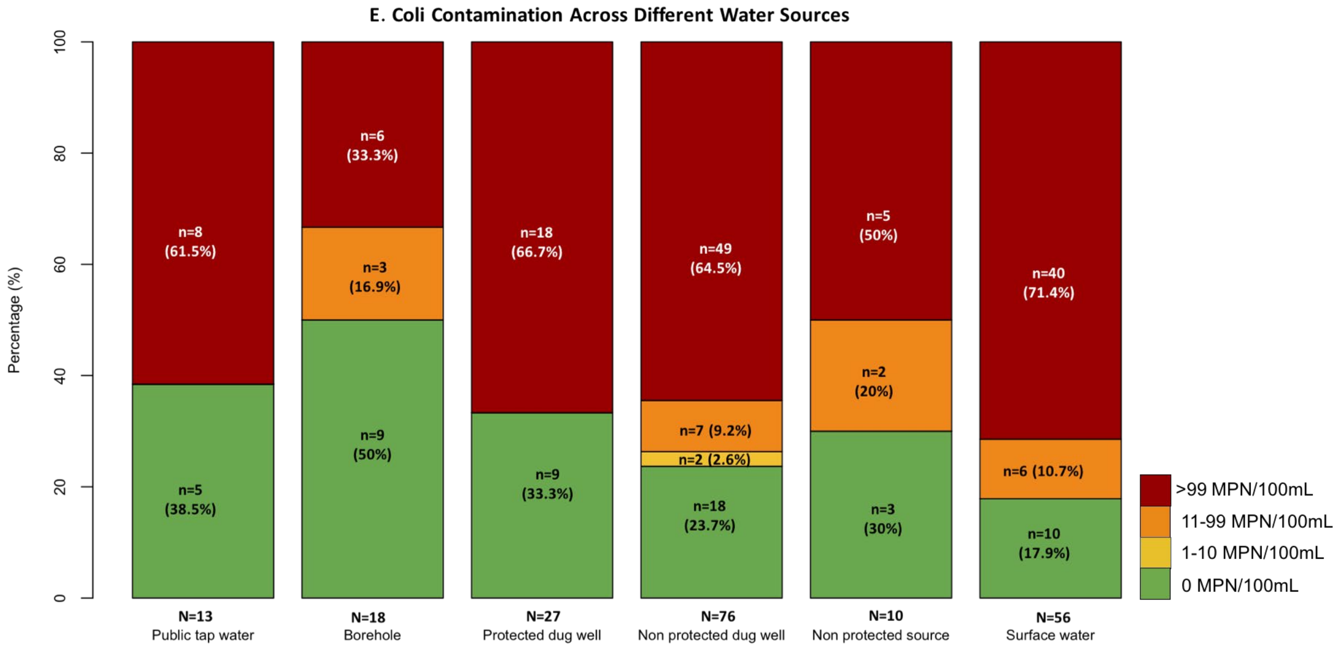

Figure F.2: *E. coli* contamination per water source type

Appendix G: Equations and exposure variables used for the QMRA for the different exposure pathways

G-1 Equations used for the QMRA

a) Equations used for the exposure assessment:

- *Ingestion via drinking-water*: to quantify the amount (dose) of pathogenic *E. coli* ingested per day via drinking water we used Equation 1[38,40,115]

$$Dose = c \times q \qquad \text{(Equation 1)}$$

Where *c* is the concentration of *E. coli* O157:H7 in drinking water (MPN/100mL) and q is the quantity of drinking water (mL) ingested per day. We used the results of the caregiver surveys (question: how many cups does your child drink per day?) to quantify the daily amount of water ingested by the child, and we used results from literature for the sensitivity analysis.[116]

- *Ingestion via soil:* we used Equation 1 to quantify the amount of pathogenic *E. coli* ingested per day via soil: *c* is the concentration of *E. coli* O157:H7 in 1 g of soil (MPN/g) and q is the quantity of soil ingested per day (g). We used the results of the caregiver surveys (question: how many times over the last three days did you see your child putting soil in their mouth? And in which quantity?) to quantify the daily amount of soil ingestion among children. We used data from literature for the sensitivity analysis. [111,117]

- *Ingestion via hand to mouth contact:* we used Equation 2 to quantify the amount of pathogenic *E. coli* ingested daily via hand to mouth contact.[9,22,115]

$$dose = EC_{hands} \times Freq \times hours_{awake} \times prop_{hands} \times t_{eff} \quad \text{(Equation 2)}$$

where:
EC_{hands} = *E. coli* contamination on hands (MPN/hands - from our study)
Freq = frequency of hand to mouth contact per hour ($N_{contact}$/hour - from structured observations, where the enumerators recorded the number of objects to mouth contact and fingers to mouth contact per activity. We took values from literature[48] for the sensitivity analysis)
$Hours_{awake}$ = duration child is awake (Hour - from literature[118] – Appendix G-2; table G.4)
$prop_{hands}$ = fraction of hands mouthed in oral contact (% - from literature[117] – Appendix F; table F.4)
t_{eff} = hand to mouth transfer efficiency (67% - from literature[117])

- *Ingestion via food:* we used Equation 1 to compute the quantity of pathogenic *E. coli* ingested per day via food: in this case, *c* is the concentration of *E. coli* O157:H7 in 1 g of food (MPN/g) and q is the quantity of food ingested per day (g). We used values from literature[9,119,120] for the quantity of food ingested per day per age category and for the sensitivity analysis).

b) Equations used for the dose response model:

To estimate the probability of infection we used a ß-Poisson model (Equation 3).

$$P_{(inf,daily)} = 1 - \left[1 + dose\frac{2^{\frac{1}{a}-1}}{N^{50}}\right]^{-a}$$ *Equation (3)*

Where, $P_{(inf,\ daily)}$ is the probability of infection per day, *dose* is the quantity of pathogens ingested per day, α is a pathogen infectivity constant and N^{50} is the number of pathogens required to infect 50% of the population.[39] We took the optimized parameters used by Katukiza et al., 2014 for *E. coli* [11]: α=0.49 and N^{50}= 596,000.

c) Equations used for the Risk characterization:

Annual Probability of infection: $P_{Inf,annual=}1 - (1 - P_{inf,daily})^{365}$ *Equation (4)*

Annual Probability of illness: $P_{ill,annual} = P_{inf,annual} \times P_{ill|inf}$ *Equation (5)*

$P_{ill|inf}$: probability of illness given infection

DALYs per case: $DALY \therefore case = \sum \quad P_{ill|inf} \times s \times d$ *Equation (6)*

$P_{ill|inf}$ =probability of illness given infection
s=severity of illness
d=duration of illness

DALYs per person per year $DALY \therefore person \therefore year = P_{ill,annual} \times S \times DALY \therefore case$ *Equation (7)*

S=percentage of population susceptible of being infected (100%)

G2 Exposure values used for the QMRA

Table G.1: variables used to compute the DALY

Disease outcome	Probability of outcome[a]	Severity[b]	Duration[b]		DALY
			Days	Year	
Watery Diarrhea	53%	0.067	3.4	0.009	$3.19.10^{-4}$
Bloody Diarrhea	47%	0.39	5.6	0.015	$2.75.10^{-3}$
Death from Diarrhea	0.70%	1	/	67	$4.69.10^{-2}$

[a] based on Havelaar and Melse 2003[121], WHO 2004[122], Howard and Pedley 2004[123], Howard et al., 2006[38], Machdar et al., 2013[40]
[b] severity and duration based on Havelaar and Melse 2003[121], Howard and Pedley 2004[123] and Machdar et al., 2013[40]

Table G.2: exposure values used in the QMRA for the drinking water exposure pathway

Age category	Geometric mean[a]	Geometric SD[a]	Water intake from our study [a]	Water intake from the literature[b]	Water intake uncertainties[c]
0-6 months	25.5 MPN/100mL	46.8 MPN/100mL	0 mL	184-362 mL	0 – 362 mL
7-12 months			195 mL	360 mL	195 – 360 mL
13-24 months			260 mL	271 mL	260 – 271 mL

[a] geometric mean and median from our study taken to compute the dose ingested per day for the exposure assessment (i.e., the point estimate associated with our results)
[b] from the exposure factors handbook[116]
[c] The uncertainties represented the minimum and the maximum values found either in the literature or in our study (used for the uncertainty analysis)

Table G.3: frequency of objects-to-mouth contact, children's fingers-to-mouth contact, caregiver's fingers-to-mouth contact observed in this study through structured observations

Age category	Frequency objects-to-mouth contact	Frequency children's fingers-to-mouth	Frequency caregiver's fingers-to-mouth
0-6 months	0.9 contact/hour	1.8 contact/hour	0.0 contact/hour
7-12 months	3.9 contact/hour	5.4 contact/hour	0.1 contact/hour
13-24 months	1.8 contact/hour	3.5 contact/hour	0 contact/hour

Table G.4: variables used to compute the dose of *E. coli* ingested per day via hand-to-mouth contact (Equation 2)

Age category	Hours awake[a]	Fraction of hand mouthed in oral contact[b]	Hand to mouth transfer efficiency[b]
0-6 months	11h15	0.58 Sensitivity: 0.25-0.75	0.67
7-12 months	11h43		
13-24 months	12h01		

[a] from Galland et al., 2012[118]
[b] from Kwong et al., 2019.[117] This study computed the hand-to-mouth transfer efficiency for children in rural Bangladesh, where conditions were similar to our study areas.

Table G.5: variables used in the QMRA for the hand-rinse exposure pathway

Age category	Geometric mean[a]	Geometric SD[a]	Frequency fingers to mouth form our study [a]	Average Frequency fingers to mouth from different studies[b]	Frequency uncertainties[c]
0-6 months	0.8 MPN/100mL	13.3 MPN/100mL	1.8 contact/hour	23.0[48]-43.6[43] contact/hour	0.9 – 23 contact/hour
7-12 months	3.2 MPN/100mL	18.2 MPN/100mL	5.4 contact/hour	14.0[48]-31.6[43] contact/hour	3.9 – 14 contact/hour
13-24 months	3.3 MPN/100mL	15.8 MPN/100mL	3.5 contact/hour	14.0[48]-28.2[43] contact/hour	1.8 – 14 contact/hour

[a] values from our study taken to compute the dose ingested per day for the exposure assessment (i.e., the point estimate associated with our results)
[b] from Kwong et al., 2016[40] and Xue et al., 2007[48]
[c] as the frequency values found in our study were very low compared to the literature, we took the frequency of object-to-mouth contact found in our study as minimum values, and we took the median frequency found by Xue et al., 2007[48] as a maximum value for the sensitivity analysis.

Table G.6: soil intake observed in our study

Age category	N observed[a]	Soil ingestion[b]	Quantity[c]	N[d]	Quantity g/day[e]
0-6 months	N=1 (<1%)	0.04 freq/day	The amount of dirt normally on fingers (0.5g) The amount they could hold between two fingers (0.5g) Half of a handful (1.25g) A handful (2.50g) More than a handful (3g)	N=1	0.00 g/day
7-12 months	N=24 (48%)	1.14 freq/day	The amount of dirt normally on fingers (0.5g) The amount they could hold between two fingers (0.5g) Half of a handful (1.25g) A handful (2.50g) More than a handful (3g)	N=9 N=10 N=1 N=3 N=1	0.48 g/day
13-24 months	N=54 (46%)	0.81 freq/day	The amount of dirt normally on fingers (0.5g) The amount they could hold between two fingers (0.5g) Half of a handful (1.25g) A handful (2.50g) More than a handful (3g)	N=22 N=25 N=4 N=3	0.25 g/day

[a] Number of children being observed by their caregiver putting soil in their mouth during the last 3 days
[b] We derived the daily frequency from the questionnaire
[c] Descriptions of each quantity were included in the questionnaire; estimates of the mass of soil associated with each description were taken from Bauza et al., 2018[111]
[d] Number of children observed putting a certain amount of soil in their mouth, from structured observations
[e] Quantity (g/day) = Soil ingestion (Freq/day) * mean (Quantity) * ($N_{\text{Children observed eating soil}}$ / $N_{\text{Children total}}$)

Table G.7: exposure values used in the QMRA for the soil exposure pathway

Age category	Geometric mean[a]	Geometric SD[a]	Quantity g/day from our study[a]	Quantity g/day from the literature [b]	Values for sensitivity analysis[d]
0-6 months	83.3 MPN/g	5.1 MPN/g	0.12 g/day	0.162 g/day[117]	0.12-0.162 g/day
7-12 months			1.01 g/day	0.224[117] – 1.90[111]g/day	0.224 – 1.90 g/day
13-24 months			0.54 g/day	0.234[117] – 1.54[111]g/day	0.234 – 1.54 g/day

[a] values from our study taken to compute the dose ingested per day for the exposure assessment (i.e., the point estimate associated with our results)
[b] values from Kwong et al., 2019[117] and Bauza et al., 2018[111] used for comparison and sensitivity analysis
[c] for the sensitivity analysis we used the minimum and maximum values of our study or from the literature

Table G.8: exposure values used in the QMRA for the cooked food exposure pathway

Age category	Geometric mean[a]	Geometric SD[a]	Average Quantity g/day[b]	Values for sensitivity analysis[b]
0-6 months	0.56 MPN/g	9.64 MPN/g	6.45 g/day	0.00-12.91 g/day
7-12 months			37.42 g/day	0.03 – 74.82 g/day
13-24 months			129.42 g/day	0.39 – 258.46 g/day

[a] values from our study taken to compute the dose ingested per day for the exposure assessment (i.e., the point estimate associated with our results)
[b] values from Kwong et al., 2020[9]

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