Identifying pathogen profiles and exposure pathways for children under two years old in Samburu North and Turkana South, Kenya

Project Report

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Innovations for Poverty Action

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CONTENTS

ACRONYMS

APHRC	African Population and Health Research Center
ASAL	Arid and Semi-arid Lands
BHA	Bureau for Humanitarian Assistance
CHC	Centre for Humanitarian Change
EPA	Environmental Protection Agency
FGD	Focus Group Discussion
GAM	Global Acute Malnutrition
IPA	Innovations for Poverty Action
LMIC	Low- and Middle-income Countries
LOD	Limit of Detection
MPN	Most Probable Number
PCR	Polymerase Chain Reaction
PRO-WASH	Practices, Research and Operations in Water, Sanitation and Hygiene
QMRA	Quantitative Microbial Risk Assessment
RT	Reverse Transcription
RTI	Research Triangle Institute
TAC	TaqMan Array Card
USAID	United States Agency for International Development
WASH	Water, Sanitation and Hygiene

EXECUTIVE SUMMARY

Nutrition interventions have been unable to fully alleviate the burden of acute malnutrition in rural, low-income settings, potentially due to repeated exposure to enteric pathogens in the household environment. Fecal contamination is common in household soil, drinking water, in food, and on surfaces in many rural, low-income settings. Animal ownership, and particularly animals living in close proximity to the household, could be an important source of fecal contamination. The study site is in rural, northern Kenya, in Samburu and Turkana counties. These communities are primarily pastoralist and agro-pastoral, with high animal ownership and poverty rates, and have largely been excluded from prior studies due to their remote location and insecurity of the area.

We utilized a mixed-methods approach to quantify enteric pathogen transmission along environmental pathways to children under 2 in rural Kenya. We combined a cross-sectional study design with longitudinal sampling to observe 100 children under 2 years old across two study sites and conduct 2-3 repeated observations of 60 children under 12 months. In a subset of households, we also conducted structured observations to quantify child behavior related to hand contacts and mouth contacts of target children. At each visit, we collected human stool (child and caregiver), animal feces (cow, sheep, chicken, goat, camel, dog, and cat), soil, stored drinking water, source water, child hand rinse, fomite rinse, and food samples. *E. coli* levels in samples were immediately quantified using IDEXX[1] QuantiTray with Colilert media and reported as a most probable number (MPN). To determine the relative importance of each exposure pathway, we use quantitative microbial risk assessment (QMRA) methods to model the *E. coli* dose for each exposure pathway. We also disaggregated the data by age group (0-2 months, 3-5 months, 6-11 months, and 12-23 months) and county (Turkana *versus* Samburu) and conducted separate analyses to determine how key pathways vary with child age and location. In addition, we used TaqMan Array Cards to measure the presence and levels of 39 enteric pathogens in child stool, animal feces, and in soil.

In the study area, 64% of households were below the Kenya poverty line and only 7% households had electricity access. Improved flooring was rare (31%), as were improved wall materials (32%). Among the 100 respondents, 71 caregivers did not finish primary school and only 11 completed secondary school. We also find limited WASH access; while improved water sources are common (main drinking water sources are piped or boreholes), only 6% of households have water access in-home, and 0 households report treating drinking water. Fifty-nine percent of households lack access to a latrine, and only 4% report access to any type of handwashing station.

E. coli contamination levels varied by sample type. The most frequently contaminated sample types were stored drinking water, child hands, and soil. We measured higher *E. coli* levels in stored *versus* source waters, indicating contamination was introduced after collection. We also find that samples collected in Samburu were more likely to contain *E. coli*, and when contaminated, contained higher levels of *E. coli* than observed in Turkana.

Modeled *E. coli* doses were low for children under 6 months (<30 MPN/day) but increased to >10^3 MPN/day in the 6-11 and 12-23 month age groups. Food was the dominant exposure pathway for *E. coli* based on the model, accounting for >40% of the average daily dose for children over 6 months, on average. Drinking water is also an important pathway for these age groups, accounting for approximately 25% of the average daily dose.

[1] The IDEXX QuantiTray Colilert test simultaneously quantifies both total coliforms and *Escherichia coli*. Results are available within 24 hours.

For the younger age groups, which exclusively consume breastmilk and other liquids, the dominant pathway was child hands.

We detected frequent pathogen carriage in child and caregiver stool and animal feces, and in soil. Caregiver stool contained a greater number of pathogens, on average, than child stool. A higher pathogen burden was detected in dogs and chickens, as compared to ruminants cattle, goats, sheep, and camels. The high pathogen loads in dogs and chickens are particularly concerning due to close proximity of these animals to child activity areas. The most commonly detected pathogens overall were enterovirus, adenovirus, norovirus GII, pathogenic *E. coli*, *Giardia duodenalis*, and *Campylobacter* spp. The most commonly detected pathogens in child stool were enterovirus, pathogenic *E. coli* (specifically Enteroaggregative *E. coli* and Enteropathogenic *E. coli*), *Giardia duodenalis*, and adenovirus.

Given that from our *E. coli* exposure assessment modeling, we determined that key pathways for child fecal exposure in the study sites were food and drinking water (pathogen results were not yet available at the time of focus group discussions). Focus group discussions with 13-15 study participants in each sub county provided insight on potentially preferred interventions to prevent food and drinking water contamination, and improve management of animal feces. Knowledge of fecal-oral transmission pathways among community members was high. Participants identified that food insecurity among nursing mothers leads to early weaning, which causes children to be exposed to contaminated food at an early age. They also mentioned inadequate drinking water infrastructure in the region, limited access to chlorine for water treatment, and not enough water available to be used for cleaning and hygiene purposes. Participants also identified that animals are important in the Turkana and Samburu cultures, and keeping animals separate from children may not be feasible; interventions could instead focus on prevention or removal of animal feces from child activity areas.

By rigorously evaluating the transmission pathways for enteric pathogens, we provide context-specific evidence to serve as a foundation for potential intervention development. It is recommended that the Resilience Food Security Activity engages communities in co-creating interventions to address important exposure pathways in the study communities: poultry and dog feces, and consuming contaminated food and water. In order to increase the acceptability and feasibility of these recommendations, it is suggested that a human-centered design approach is utilized. However, our results also suggest that comprehensive infrastructure upgrades at the community-level are likely needed to effectively curb pathogen transmission. Considering the reality that most household-level interventions place the burden of implementation on women caregivers, available resources may be better spent supporting the government in investing in services and infrastructure identified as top priorities by the study communities and their leadership.

INTRODUCTION

Acute malnutrition remains an important child health issue in many low- and middle-income countries despite numerous nutritional interventions. Globally, malnutrition occurs primarily between birth and 5 years of age and is associated with higher mortality and decreased developmental outcomes.[1, 2] Malnutrition overall is a factor in 45% of deaths of children under 5, and Africa experiences the second-highest rate of acute malnutrition.[3,4] Nutrition interventions, including supplemental feeding programs, have been unable to substantially reduce child malnutrition in rural, low-income settings.[5]

Enteric infections have been linked to acute malnutrition. Chronic exposure to enteric pathogens can contribute to acute malnutrition by reducing the ability of the gut to absorb nutrients.[6] Malnutrition in turn can worsen frequency and severity of diarrhea.[7] Prior work suggests that the first two years of life are vital to long-term child nutrition, cognitive development, and later health and educational outcomes.[3,8] Forms of child malnutrition such as stunting and wasting that develop in the first two years of life have been shown to persist into adolescence and adulthood.[3,9] One mechanism for the persistence of malnutrition into older ages may be through disruption of the gut microbiome development in young children. The microbiome develops in the first years of life and stabilizes around age three. Chronic exposure to pathogens and systemic diarrhea at these ages, and specifically under 2, disrupts microbiome development and may limit the ability of the gut microbiome to resist pathogens later in life.[10]

Recent trials and studies demonstrate that rural, low-income households can be highly contaminated with fecal contamination and enteric pathogens. Findings from several large-scale randomized controlled trials (the WASH Benefits and SHINE trials) in Kenya, Bangladesh, and Zimbabwe demonstrate pervasive enteropathogen exposure and persistent high enteric pathogen burdens among the children, even after improved WASH interventions had been delivered.[11–13] One hypothesis for the persistent contamination in these household environments is that a substantial source of fecal contamination and enteric pathogens in the domestic setting is animal feces. Both the human health risk associated with animal ownership and mechanism of bacterial transmission between humans and animals is poorly understood. Animal husbandry is a common source of income in many low- and middle- income countries (LMICs).[14] Particularly in urban communities where animal husbandry is practiced, the close proximity between humans and livestock can result in adverse health outcomes, including increased risk of diarrheal illness and growth faltering in children.[15,16] Many enteric pathogens, such as pathogenic *E. coli*, can be transmitted zoonotically from human exposure to animal feces.[15] Human exposure to enteropathogens primarily occurs in the household environment, particularly for young children, the age group most vulnerable to severe enteric infections.

Previous research by members of this investigative team has shown that living in close proximity to animals is associated with increased exposure to fecal contamination in the household environment.[17,18] Host-specific fecal markers from animals (dogs, birds, and ruminants) were detected in multiple household reservoirs (soil, on hands, and in stored drinking water) in rural/urban Bangladesh.[18,19] In all sample types tested, animal fecal markers were more prevalent than human fecal markers. In rural Bangladesh, increased concentration of BacCow, an animal-specific fecal marker, was associated with increased prevalence of pathogenic shiga toxin-producing *E. coli* (STEC) in environmental samples.[18] This previous work has shown that living in close proximity to animals puts households at elevated risk of contact with animal fecal contamination, which can harbor enteropathogens, though a mechanistic understanding of transmission is still missing. Understanding animal hosts for enteric pathogens is critical, as without rigorous assessment it remains difficult to implement intervention strategies to prevent the spread of zoonotic enteropathogens. Interventions specifically targeting the human-animal interface, such as use of chicken corralling and improved floor material (enabling washing of

poultry feces from inside the household), could be strategies to reduce child exposure to enteric infections and reduce child stunting.[11]

Intervention design and development to reduce the diarrheal disease burden would ideally be informed by identification of dominant exposure pathways to enteric pathogens. The relative comparison of different transmission routes would provide insight on which pathway(s) should be targeted to have the largest health effect. The complexity of environmentally-mediated transmission of enteric pathogens has made it such that very few studies have been able to comprehensively assess household transmission of enteric pathogens. Further, many rely on proxy indicators, such as *E. coli*, for the comparison, rather than pathogen-specific data. Mattioli et al. compared exposure to *E. coli* between hand-to-mouth contacts and ingestion of drinking water based on measurements of contamination of female care-giver hands and stored drinking water in peri-urban and rural Tanzania. They found that, generally, hand-to mouth contacts result in more exposure to *E. coli* than drinking water.[20] Wang et al. compared water, food, and soil ingestion and hand-to-mouth contacts and flies to identify the dominant exposure pathway to fecal contamination in urban Ghana. They found that 99% of a child's exposure to *E. coli* came from food ingestion.[21] A recent study by Kwong et al., found that child mouthing of objects and their hands along with direct ingestion of soil were dominant exposure pathways to *E. coli* for children under 3 years old in rural Bangladesh. Ingestion of food became a more dominant pathway as children got older.[22,23]

Although limited, the variation in findings between existing studies on pathways of feces exposure highlight how dominant transmission pathways are likely context-specific. This could be the case for many reasons. Transmission of enteric pathways can be influenced by a location's climate, as temperature, humidity, and rainfall patterns can influence the fate and transport of pathogens in the environment. Also, human behavior patterns can be unique to different cultures and complicated by age and gender differences. Object and hand mouthing of toddlers and infants, diet, hydration practices, geophagy and coprophagy all could vary based on cultural norms, including proximity of households and livestock, child rearing and care practices and environmental conditions (e.g., temperature, housing structure, household assets). Only one of the three studies described above used context-specific behavior data to model exposure. And finally, the sources and reservoirs of the contamination may be site specific. Variation in domestic animals presence and animal husbandry practices, sanitation infrastructure and flooring materials, would influence the dominant transmission pathways. Further, the health risks associated with different host feces has been poorly evaluated and could be dependent on location and environmental context.

A main limitation of existing literature on this topic stems from the use of fecal indicators, like *E. coli*, to compare the contribution of different exposure pathways to negative health outcomes. *E. coli* is a widely used microbial indicator for fecal contamination in environmental media; however, there are many known limitations of this indicator. In particular, it is known that *E. coli* can exhibit different fate and transport in the environment compared to other enteric pathogens, such as viruses and protozoa.[24] Ideally, enteric pathogen presence and concentrations would be assessed to characterize the health risks with different transmission pathways.[25] Enumerating a wide-array of enteric pathogens in environmental media can be time consuming and costly. However, a new method, using TaqMan Array Card technology, has made it more feasible to expand testing enteric pathogens in environmental media, including feces, water, soil, and food.[26,27] These tests use polymerase chain reaction (PCR) assays that are embedded into cards that allow for multiple targets to be included. After extracting nucleic acids from the environmental sample, multiple targets can be evaluated in a single sample run.

STUDY OBJECTIVES

Our main goal was to identify the dominant environmentally-mediated transmission pathways of enteric pathogens in Nawiri intervention areas of Samburu North and Turkana South for children under 2 years old. We had the following specific objectives:

- Quantify *E. coli* presence and contamination levels in the household environment, including in soil, food, water, fomites, and on child hands
- Observe child behavior (frequency of potential exposure events) and use quantitative microbial risk assessment to identify dominant child exposure pathways to *E. coli*, including differences by child age and study site
- Determine enteric pathogen profiles of child stool, animal feces, and soil samples
- Present study results to community members and understand community perceived needs

Another objective for this study was to suggest, with community input, potential strategies for interrupting these transmission pathways, taking into account the specific implementation challenges related to the unique cultural and social norms of Northwestern Kenya.

METHODS

Study Site

This project was conducted in collaboration and within the program area of the USAID Nawiri project. Nawiri is a USAID Bureau for Humanitarian Assistance (BHA) funded Development Food Security Activity designed to reduce persistent acute malnutrition in four counties of northern Kenya including Turkana, Samburu, Marsabit and Isiolo. In Turkana and Samburu, the program is led by Mercy Corps in close collaboration with several consortium members including: Save the Children, Research Triangle Institute (RTI), Centre for Humanitarian Change (CHC), the BOMA Project, African Population and Health Research Center (APHRC), Caritas Lodwar, and Caritas Maralal.

This study was conducted between January and May 2022 in pastoralist and agro-pastoral communities in the Turkana South and Samburu North sub counties of arid and semi-arid lands (ASAL) regions of Kenya. The primary occupation in the study areas is pastoralism and agro-pastoralism. Access to WASH is limited in these regions. The USAID NAWIRI project has documented that less than 10% of households have access to improved sanitation, and 60% still rely on surface water or shared improved water points for drinking water. Further, the water fetching burden is high in these regions, with 1 in 10 households reporting walking 1 hour or more to the water source. Water insecurity is exacerbated by droughts and flooding.[28] Acute malnutrition is higher in these regions than has been documented in recent WASH trials in Kenya, Bangladesh, and Zimbabwe, with global acute malnutrition (GAM) levels of 16% in Samburu and 26% in Turkana. Children also have high rates of reported gastrointestinal illnesses (ranging from 10-15% two-week prevalence in 2018).[29,30]

Study Design

We utilized a mixed-methods approach in this study (Fig 1). The sample frame consisted of villages that were part of the USAID Nawiri longitudinal project conducted by APHRC, RTI International, and Mercy Corps titled “Examining the Complex Dynamics Influencing Persistent Acute Malnutrition in Turkana and Samburu Counties – a Longitudinal Mixed Methods Study to Support Community Driven Activity Design”. Within each sub county, we selected 10-15 villages for study based on travel feasibility (proximity to the field lab) and field staff safety. Random selection of villages was not feasible due to needing to ensure the safety of our field staff, and to ensure the integrity of our microbiological samples and field lab analyses. The samples collected needed to be kept on ice and the *E. coli* cultured within 6 hours of collection, meaning that selecting villages far from our

field lab might jeopardize the typical 6-hour time window for processing. Thus, we selected villages where samples could feasibly be transported to the lab early enough in the day to ensure immediate analysis. The Nawiri project has reported the study areas are fairly homogeneous and so despite not being able to do random sampling, purposeful sampling is likely sufficient to give the Nawiri project team actionable information to improve programming. In each village, we enrolled all households with a child under 2 years of age until we reached our target numbers in each child age group. In total, we enrolled 50 households in each sub county for n = 100 total households. Twenty-five households with a child in each target age group (0-<3 months, 3-<6 months, 6-<12 months, and 12-<24 months) were enrolled across both sub counties. Target age groups were selected based on Environmental Protection Agency (EPA) child development stages and to allow for the detection of first exposures to pathogens for young children. Within each household, we conducted a range of activities including a household survey, stool and environmental sample collection, and, in a subset of households, structured observations of child behavior (n = 60 households and n = 76 observations).

In a subset of participating households with children under 12 months of age (n = 60), we conducted 3-4 rounds of longitudinal data collection. Visits were 1-2 weeks apart and at each visit we repeated household surveys and sample collection. Structured observations were conducted only at the first and last round of data collection.

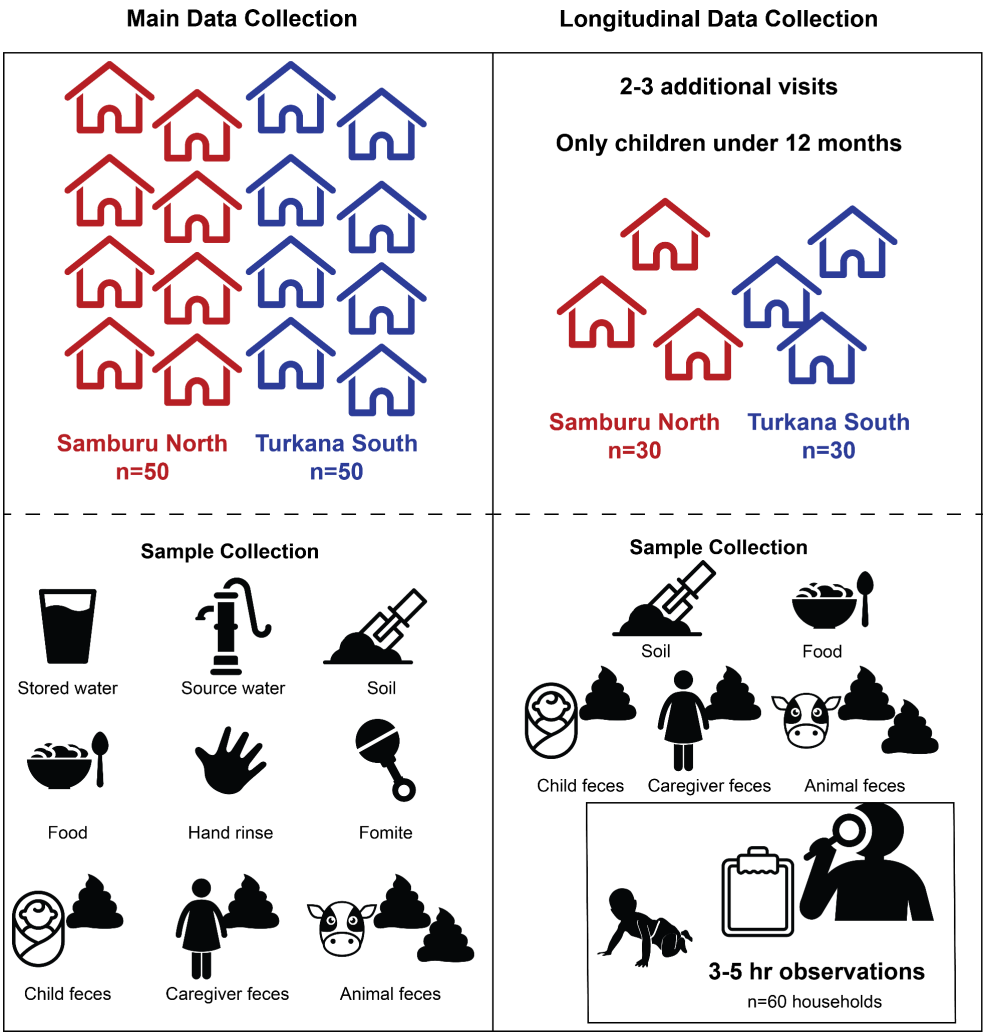

Figure 1. Schematic of study design.

Data Collection Methods

A variety of environmental and fecal samples were collected at each household and were analyzed for total *E. coli* in the field. A subset of samples were analyzed by TaqMan Array Card (TAC). Finally, we used quantitative microbial risk assessment (QMRA) modeling to determine key transmission pathways (Fig 2).

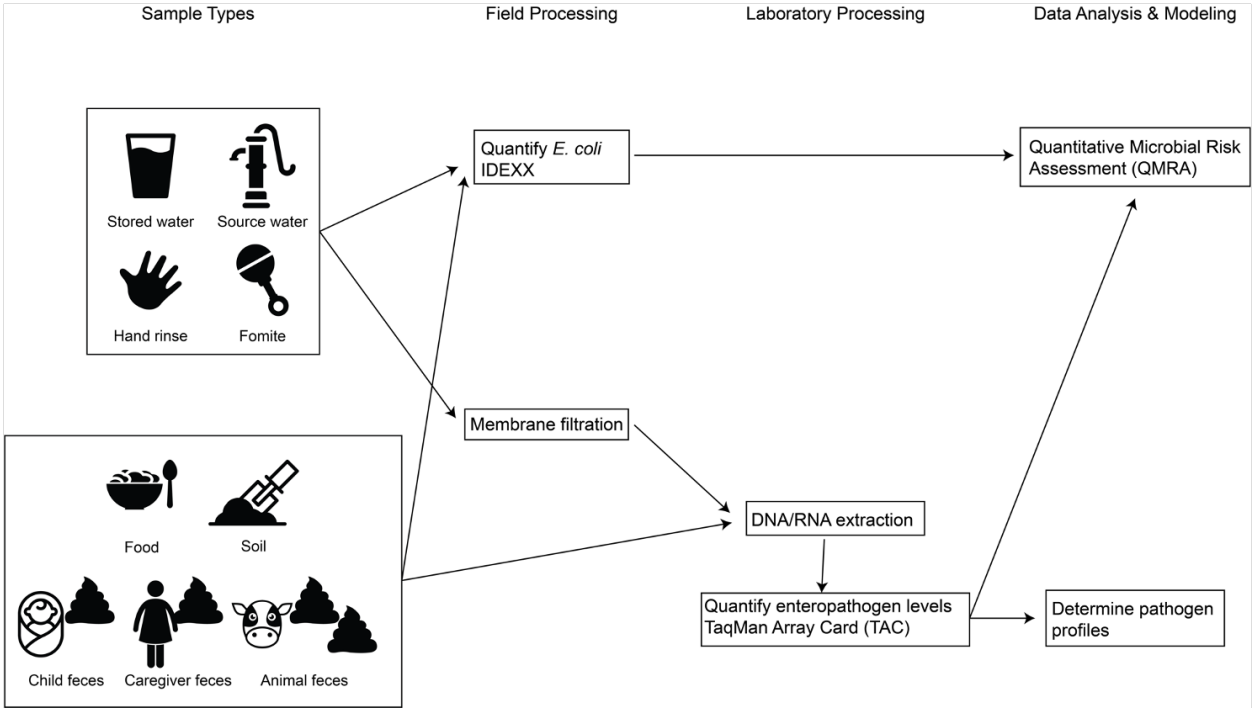

Figure 2. Schematic of study activities, including sample collection, field processing, laboratory processing, and data analysis and modeling.

Child and mother fecal sampling

Stool samples were collected from the target child under the age of two years old and each mother/caregiver in eligible households. Mothers were given two stool collection kits consisting of a 50ml sterile container, gloves, aluminum foil, and a spatula. Caregivers were instructed to fill both containers. Field staff revisited the household the following morning and up to three times in total to collect the stool samples. Stool samples were kept in cooler boxes on ice packs until they arrived at the local field lab, where aliquots were made and stored at -20 degrees Celsius until they were shipped on dry ice to KEMRI in Nairobi for storage at -80 degrees Celsius. Stool samples were later on shipped in dry ice to UC Berkeley in the US for further analysis detailed below.

Animal fecal sampling

We collected fresh animal feces from the following types of animals when they were available in the household of the target child: chickens, cattle, goats, dogs, donkeys, sheep, and camels. The field staff observed animals defecating and collected feces as soon as possible after defecation when possible; otherwise, our team asked respondents to point out where the freshest animal feces was located and the sample was collected from the ground. Veterinary technicians on the field team performed rectal palpation on goats, sheep, and cattle to obtain freshly defecated animal feces when possible. Fecal samples were collected with sterile scoops into sterile 50 ml containers. Fecal samples were placed in a cooler box with ice packs until

transported to the local field lab, and then shipped to KEMRI on dry ice after aliquoting. Animal fecal samples were shipped in dry ice to UC Berkeley in the US for further analysis detailed below.

Drinking water
Field staff asked the respondent to provide a glass of water as if giving it to their < two year-old child. When the water was provided from a storage container, the sample was collected by asking the respondent to pour the water from the glass into a sterile Whirlpak bag. Field staff collected > 250 mL of drinking water at each household. Water samples were transported to the local field lab on ice, processed using analysis techniques detailed below, and then shipped to KEMRI on dry ice.

Source water
Field staff asked the respondent to identify where they collect their drinking water from. Field staff decanted a 500 mL sample into a sterile WhirlPak bag. When source waters were shared between enrolled households, we sampled the source waters only once and tracked this in the survey.

Child hand rinses
Field staff asked the respondent to place the target child's left hand into a sterile Whirlpak bag pre-filled with 250 mL of distilled water. The hand was massaged from the outside of the bag for 15 seconds, followed by 15 seconds of shaking. The same procedure was repeated with the right hand in the same bag, and the rinse water was preserved in the Whirlpak bag.[31] Clean gloves were worn by field staff during hand rinse sample collection.

Fomite rinses
Field staff asked respondents to provide objects that children frequently interact or play with. Field staff placed the object into a sterile Whirlpak bag pre-filled with 250 mL of distilled water and massaged it for 30 seconds. Sterile gloves were worn by field staff during this procedure.

Soil samples
The soil surrounding the entrance to the home (<2 meters away from the entrance) was sampled as this location has been found to frequently contain high levels of fecal bacteria, as well as soil transmitted helminths in Kenya and Tanzania during previous studies conducted by this team.[19,32,33] Field workers marked a 30 cm by 30 cm area using a disposable sterile stencil and scraped the top layer of soil within the stencil into a sterile Whirlpak bag using a sterile disposable plastic scoop; the sample area was scraped once vertically and once horizontally to collect approximately 50 g of soil from the ground surface. Field staff prioritized sampling locations that were shaded. Soil samples were transported on ice to the local field lab, where they were stored at -20 degrees Celsius prior to final shipment to KEMRI.

Food samples
Field workers identified stored weaning food to be served to children <2 years in the household and asked the respondent to provide a small amount of food in the same manner they feed their children, including serving it on a plate/bowl using any common utensils. Food types varied between porridge, chapati, potatoes, cassava, beans, rice, and others. Food was scooped to fill a 50mL sterile plastic tube using a sterile spoon attached to the lid of the tube. Food samples were transported on ice to the local field lab, where they were stored at -20 degrees Celsius prior to final shipment to KEMRI.

Enumerating *E. coli* from environmental and fecal samples
All samples were preserved on ice and transported to the field lab to be processed within 6 hours of sample collection using the IDEXX most probable number (MPN) method with Colilert media to detect *E. coli* and fecal coliform. Aliquots of the feces, food and soil samples were preserved at -20 degrees Celsius in the local field

lab before being shipped to KEMRI on dry ice for storage at -80 degrees Celsius for further enteric pathogen molecular analysis. Stored water, hand rinse, and fomite rinse samples were processed by first diluting the sample with distilled water when necessary. Dilutions ensure that the bacteria enumerated will be within the detection limit of the IDEXX MPN method. We conducted pilot work prior to data collection to determine the appropriate dilution factor for each type of sample based on the level of contamination, with heavily contaminated samples requiring larger dilution factors. Food and soil samples required a homogenization step before processing with IDEXX. These samples were aliquoted upon arrival in the lab (10g for food; 20g for soil) and placed into a 50 mL falcon tube with 40 mL distilled water and vortexed for several minutes before analysis with IDEXX. An additional aliquot (5g) from the original un-homogenized food and soil samples were weighed and placed in a drying oven overnight for determining the sample moisture content in order to calculate bacterial counts per dry weight of each sample.

Samples were incubated at 35° Celsius for 24 hours to quantitatively enumerate *E. coli* and fecal coliforms. After incubation, IDEXX trays were counted by trained lab technicians for the presence of *E. coli* and fecal coliform. In addition, field and lab blanks were processed, and 5% of samples were processed in duplicate. All used IDEXX trays and any other infectious materials were placed in biohazard bags and disposed of by incineration.

Structured Observations

In a subset of 30 households in each study area (n=60 in total), target children under two years of age were randomly selected and invited to participate in a structured observation on the first household visit. Any of these households also enrolled in the longitudinal cohort received an additional structured observation on the 4th and final visit. Children from all age groups (0-<3 months, 3-<6 months, 6-<12 months, and 12-24 months) were invited to participate. Field staff were trained prior to data collection and conducted structured observations in pairs during piloting to assess and correct for any discrepancies across field staff. Following caregiver consent, trained staff conducted a structured observation of the target child for three consecutive hours in the presence of the caregiver. To observe children's activities spanning the daylight hours, observation start time varied from 7 am to 10 am. The structured observation tool captured information on hand-to-object contacts and recorded every object-to-mouth and hand-to-mouth contact of the target child. Observers also recorded information on each behavior, defined as hand or mouth contact with one or more of the following: own or mother's hand/skin, soil, feces, animals, cloth, toys and food, including food waste. For each of the behaviors, the enumerator recorded the number of times a hand is put in the child's mouth after touching one of these objects. In addition to recording information regarding specific behaviors, the observers also recorded child-specific information, such as mobility and sleeping times, as well as household-specific information, such as the time certain foods were cooked, the time water was collected, and presence and type of feces in the courtyard area where the child spent time.

TaqMan Array Card Methods

The ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research) was used to extract nucleic acids from all animal feces and human stool samples. Lysis buffer volume was increased to 1:5 to ensure complete lysis in all samples. For soil and food samples, we used the RNeasy PowerSoil Total RNA Kit with the DNA Elution Kit add-on (Qiagen) to co-extract RNA and DNA. For both kits, we eluted in 100 µL and stored extracts at -80°C until TAC analysis.

To detect pathogens in feces and environmental samples, we designed a custom TaqMan Array Card (TAC; Fisher) containing enteric pathogen targets. The TAC is a form of quantitative real-time PCR that enables simultaneous detection of up to 48 targets per sample in one run. We selected enteric pathogen targets that are associated with diarrhea, particularly in children, and have been detected in Kenya or similar settings. In

brief, we used 2-step reverse transcription followed by TAC to detect and quantify pathogens in nucleic acid extracts. Reverse transcription (RT) was performed on 16 µL of nucleic acids using the Invitrogen SuperScript IV VILO Master Mix with the following cycling parameters: 25°C for 10 min, 50°C for 10 min, and 85°C for 2 min. The RT output was diluted to a total volume of 55 µL and mixed 1:1 with 55 µL of Applied Biosystems TaqMan Fast Advanced Master Mix. 100 µL of this solution was loaded onto the TAC and processed per TAC guidelines.

Sample Viability

To determine the viability of the Turkana samples, which underwent freeze-thaw cycles in the field due to power losses to our freezer, we compared results of the 18S (total eukaryotes) and *uidA* (total *E. coli*) assays between the two sub counties (Table 1). We find similar amplification in the 18S and total *E. coli* assays between the counties. These results suggest there was no significant nucleic acid degradation during power losses and potential freeze-thaw cycles during sample storage in Turkana, potentially due to the fact that we preserved all samples in Zymo RNA/DNA shield.

Table 1. Comparison of control assays between Samburu and Turkana counties, ct = cycle threshold, which is inversely proportional to concentration of the target.

Target	County	n	Fraction Positive	Mean Ct	sd Ct
18s	Samburu	66	1.00	16.80	2.72
	Turkana	98	1.00	17.58	3.83
Total *E. coli uidA*	Samburu	66	1.00	26.18	4.42
	Turkana	98	1.00	22.60	4.73

TAC Validation

To determine the validity of PCR performed by TAC, a subset of soil and stool samples were analyzed for soil transmitted helminths via traditional qPCR at KEMRI. The same samples were analyzed by TAC at Berkeley. Within both human stool and soil samples, all samples were negative for all soil transmitted helminths by both methods (Table 2).

Table 2. Comparison of PCR and TAC results for soil transmitted helminths. Number of samples positive by assay.

		A. duodenale		*A. lumbricoides*		*N. americanus*		*S. stercoralis*		*T. trichiura*	
	n	PCR	TAC	PCR	TAC	PCR	TAC	PCR	TAC	PCR	TAC
Soil	20	0	0	0	0	0	0	0	0	0	0
Human stool	20	0	0	0	0	0	0	0	0	0	0

Exposure Assessment

Dose Equations

Drinking water

Dose of *E. coli* in drinking water is calculated as:

$$D_{DW} = C_{DW}\ x\ V_{dw} \qquad \textit{Eq. 1}$$

where C_{DW} is the concentration of *E. coli* in drinking water (MPN / 100 mL) and V_{dw} is the volume of water consumed per day (mL). The concentration of *E. coli* in drinking water was obtained from the field testing results from this study. Child water consumption per day was obtained from EPA estimates of child water consumption in the United States, as no local estimates of drinking water were available and estimates from other settings such as Bangladesh reported greater drinking water consumption than measured in the United States.[22] We selected the most conservative drinking water consumption estimates available to us because the study sites have poor water availability. We plan to do sensitivity analyses comparing dose when using US EPA estimates *versus* other data sources to see how this impacts our results.

Food

Dose of *E. coli* in food is calculated as:

$$D_{food} = C_{food}\ x\ V_{food} \qquad \textit{Eq. 2}$$

where C_{food} is the concentration of *E. coli* in food (MPN / dry gram) and V_{food} is the quantity of food consumed per day (grams). The concentration of *E. coli* in food was obtained from this study. Food consumption estimates were obtained from a 2016 study in Turkana county that measured the dry weight of food consumed by children daily.[34] In total, food consumption was measured for 3,331 child-days, with 2,434 observations in the 12-23 month age group and 897 in the 6-11 month age group. We estimated food consumption separately for these two age groups. No food consumption was reported for children younger than 6 months; based on the Turkana food consumption results and our own survey, where no caregiver with a child under 6 months reported feeding them solid food, we assumed food consumption for children under 6 months was zero.

Hands

Dose of *E. coli* from child hands is calculated as:

$$D_{hands} = C_{hands}\ x\ F_{h,m}\ x\ P_{h,m}\ x\ TE_{h,m}\ x\ awake \qquad \textit{Eq. 3}$$

where C_{hands} is the concentration of *E. coli* on the hands (MPN / 2 hands), $F_{h,m}$ is the frequency of hand-to-mouth contact (contacts / hour), $P_{h,m}$ is the proportion of hand area in contact with the mouth (unitless), $TE_{h,m}$ is the transfer efficiency from hands to mouth (unitless), and $awake$ is the number of hours the child is awake per day (hours). The concentration of *E. coli* on child hands was obtained from the field measurements of this study; the frequency of hand-to-mouth contacts are estimated separately for each age group based on the results of our structured observations. The proportion of hand area in contact with the mouth was obtained from the literature, and was estimated based on child observations.[20] The transfer efficiency from hands to mouth was obtained from the literature, and was estimated with laboratory experiments that seeded viruses and bacteria on surfaces and hands and measured the transfer efficiency.[35] The hours awake per day is estimated separately for each age group and is obtained from the US EPA based on child structured observations.[20]

Soil

Dose of *E. coli* from soil is calculated as:

$$D_{soil} = C_{soil}\ x\ M_{soil,event} x\ F_{s,m}\ x\ awake \qquad \textit{Eq. 4}$$

where C_{soil} is the concentration of *E. coli* in soil (MPN / dry gram), $M_{soil,event}$ is the quantity of soil ingested per soil-to-mouth contact (grams / contact), $F_{s,m}$ is the frequency of soil-to-mouth contacts (contacts / hour), and $awake$ is the number of hours the child is awake per day (hours). The concentration of *E. coli* in soil was obtained from the field measurements of this study. The mass of soil consumed per contact event was obtained from the literature, and was determined from structured observations of children in Bangladesh.[36] The frequency of soil-to-mouth contact was measured during the structured observations of this study. As mentioned above, the hours awake per day is estimated separately for each age group and is obtained from the US EPA based on child structured observations.[20]

Fomites

$$D_{fomite} = C_{fomite}\ x\ F_{f,m}\ x\ FSA\ x\ TE_{f,m}\ x\ awake \qquad \textit{Eq. 5}$$

where C_{fomite} is the concentration of E. coli on the object, $F_{f,m}$ is the frequency of fomite-to-mouth contact, FSA is the fraction of the surface area of the fomite mouthed, $TE_{f,m}$ is the transfer efficiency from fomites to mouth, and $awake$ is the number of hours the child is awake per day. The concentration of E. coli on the objects were obtained from this study, as was the frequency of fomite-to-mouth contact. Because we did not have any information on the fraction of the fomite surface area mouthed and observed children mouthing a wide variety of object sizes in the field, we used a uniform distribution ranging from 0 to 1 to estimate the fraction of surface area mouthed. For the transfer efficiency from the fomite to the mouth, we obtained distributions from the literature based on laboratory experiments.[35] As mentioned above, the hours awake per day is estimated separately for each age group and is obtained from the US EPA based on child structured observations.[20]

Total dose

Total dose was estimated by summing the doses along each pathway (Eq. 7).

$$D_{total} = D_{drinking\ water} + D_{food} + D_{hands} + D_{soil} + D_{fomites} \qquad \textit{Eq. 6}$$

For full details of the parameters used in our models and their sources, see Appendix A.

Fitting Distributions to Sample Data

IDEXX E. coli values by sample type

The limit of detection (LOD) for the IDEXX Colilert MPN method is 1 colony/100 mL. LODs for each sample type were estimated by converting the method LOD of 1 colony/100mL to minimum colonies per unit of sample (per dry g of soil, per 2 hands, or per fomite) based on input volumes into the IDEXX trays.

Fitting distributions

We used the R package fitdistrplus to fit distributions to the *E. coli* concentration and child behavior data. Distribution form was selected based on theory and confirmed by checking data fit to various distributions. To account for the nature of our left-censored data, distributions were fit using the fitdistcens equation, which uses maximum likelihood estimation to fit a distribution of a specified form to both the quantifiable and non-detect results.

Model Details

The Monte Carlo simulations estimated infection risk for 1,000 children per age group and included n=10,000 iterations. For each iteration, parameter values were randomly sampled from their distribution. The dose along each pathway were estimated and summed to estimate total daily dose. We also conducted analyses

stratified by sub county to estimate daily doses and dominant pathways separately for Turkana South and Samburu North. All analyses were conducted using R 4.1.1.

For the QMRA, we made the decision to end the model at daily dose and not proceed to calculating infection risk and DALYs because of limitations in the infection risk models and the number of assumptions needed to estimate infection risk. The infectious dose for pathogenic *E. coli* is reported for adults, and we expect the true infectious dose for children under 2 may differ significantly from this estimate due to the children's developing immune systems and microbiomes.

Focus Group Discussions

We conducted focus group discussions (FGDs) with study participants separately in Turkana and Samburu. The purpose of these FGDs was to get input from caregivers on best ways to prevent young children from getting sick by stopping them from being exposed to disease-causing organisms. Our goal was to get feedback on the best ways to prevent children from getting sick, and what barriers households face when keeping households healthy. Another key goal was to get caregiver input on which products or behaviors will be easiest and most feasible to implement and prevent child exposure to enteric pathogens.

We invited research participants about one week before the actual date of the FGD held on the 28th of November 2022 in Turkana, and the 5th of December 2022 in Samburu. Sixteen research participants were randomly selected from the original list of respondents who participated in the study between March- April 2022 in Turkana south sub county. Out of the 16 invited 15 participants attended the meeting at RCEA health center located at Lokichar Centre. In Samburu, 13 research participants were randomly selected from the list of respondents, and all 13 participants attended the meeting. All participants were female caregivers of children under two years old.

The same methods were used at both FGDs. The meeting started with introductions by the IPA team of three. Thereafter we read and explained the consents, the purpose of the meeting to the participants and the voluntary participation. Each participant was requested to sign the consent forms to show they are willing to participate in the FGD and the recordings. We requested permission to allow for audio recordings.

After all the participants had signed the consents and accepted to be recorded, the participants were assigned name tags from P1-P15 to conceal their identity during the discussions and recordings.

The facilitator started by discussing the following key themes:

1. What can families do to keep children under 2 healthy and free from disease in the home?
2. How can drinking water be handled in the home to ensure it is free from contamination?
3. How can food for children under 2 be handled to minimize contamination?
4. How could you make it easier to wash your hands?
5. How could you keep a child's mouthing toys/objects free from contamination?
6. What can families in this community do to keep young children under 2 years from animal feces?
7. What can be done to ensure young children do not come into contact with human feces in the home (both from the latrine and from child potty)?

The discussion took about one hour after which we shared the preliminary findings of the *E. coli* contamination and exposure assessment with the participants to get their input. This discussion took another 30 minutes for participants giving their reaction on the findings. After the facilitator had presented the findings, the moderator posed questions to the participants to obtain their perceptions of the results.

RESULTS

Household characteristics

Household Enrollment

In total, we enrolled 100 households into the study, 61 of which were sampled longitudinally. Households with children in the youngest age groups (0-2 months, 3-5 months, and 6-11 months) were enrolled into the longitudinal cohort in order to study early exposures. In each sub county, fifty households total were enrolled, of which 30 households were enrolled as longitudinal. Of the 100 households enrolled, approximately 25 were enrolled in each target age group: 0-2 months, 3-5 months, 6-11 months, and 12-23 months. Fifty-one children enrolled were male, and 49 female (Table 3).

Table 3. Household enrollment by age group and subcounty, including number of households enrolled in longitudinal data collection and structured observations.

				Turkana South		Samburu North	
Age Group	**Total**	**Structured Observations**	**Longitudinal**	**Total**	**Longitudinal**	**Total**	**Longitudinal**
All	*100*	*56*	*61*	*50*	*30*	*50*	*31*
0-2 months	24	14	24	12	12	12	12
3-5 months	22	15	21	11	10	11	11
6-11 months	30	16	16	15	8	15	8
12-23 months	24	11	0	12	0	12	0

Using the Kenya Poverty Probability Index, we found that 64% of households were below the Kenya poverty line and 66% below the Kenya food poverty line. Improved flooring was rare (n=31 households), as were improved wall materials (n=32 households). Among respondents, 71 caregivers either did not attend school or did not finish primary school and only 11 completed secondary school; 47 reported being illiterate. Further, 93 households lacked electricity access.

WASH access

Access to water, sanitation, and hygiene (WASH) was limited in the study regions. Fifty-nine percent of households lacked access to any type of latrine, and only 4% reported having a handwashing station available. The primary drinking water sources were piped water (n=50) and boreholes (36), with the remaining households relying on springs (5), dug wells (5), surface water (2), and vendor water (2; Fig 3). Sixty-six households reported that their drinking water source was not available when needed at least once in the last two weeks; 50% of these cases were due to the source running out of water, 8% due to lack of electricity or fuel, and the remaining cases for various reasons. Reported one-way walk times to the primary water source ranged from 0 to 300 minutes, with a median time of 10 minutes and a standard deviation of 46 minutes. Notably, no respondents reported treating drinking water. When households had stored water on premises at the time of our visit (n=98), the median water storage time was 6 hours. Notably, the median water storage time varied by sub county; the median storage time in Turkana was 5 hours *versus* 8 hours in Samburu, and the difference was statistically significant (student's t-test, $p = 0.003$). On average, Turkana households lived further from their water sources than Samburu households (median walk time = 17.5 minutes in Turkana *versus* 10 minutes in Samburu; student's t-test, $p = 0.02$).

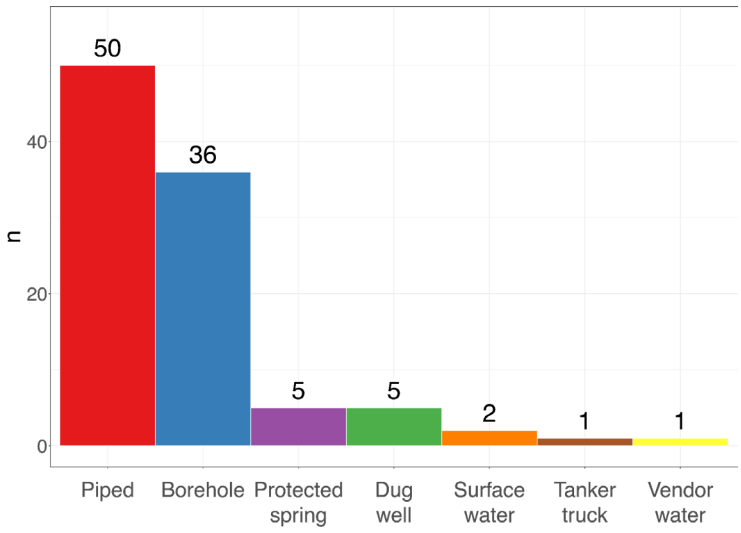

Figure 3. Reported drinking water source.

Child food consumption

Caregiver-reported child food consumption varied by age group (Fig 4). All children (100%) under 6 months of age were breastfed, with limited supplemental other liquids. Breastfeeding was still very common in the 6-11 month age group (>90%) but decreased to less than 60% by 12-23 months. Consumption of other liquids increased in the 6-11 and 12-23 month age groups, with limited introduction of supplemental foods, mostly soft foods (Fig 4).

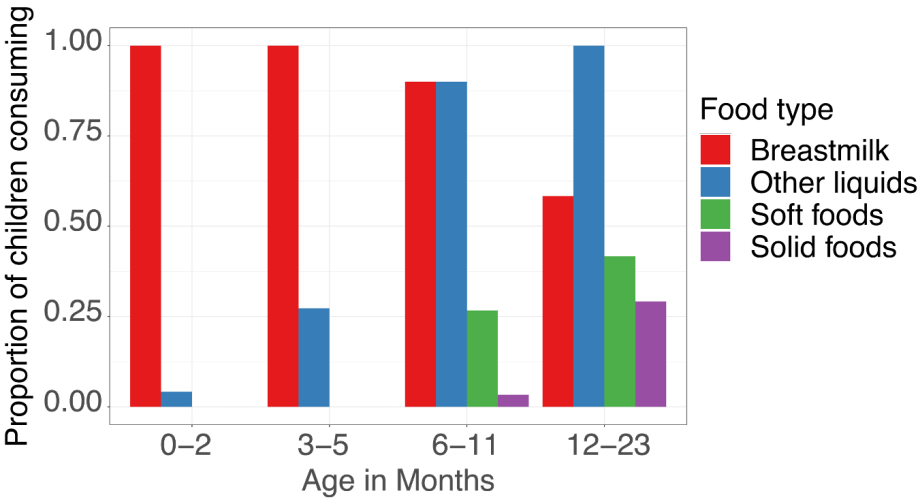

Figure 4. Child food consumption by age group.

Child Health

Overall, caregivers reported diarrhea in the last 7 days for over ⅓ of children (Table 4). Diarrheal prevalence peaked in the 3-5 and 6-11 month age groups. Further, diarrheal prevalence was greater in Samburu than Turkana. In our surveys we asked about diarrhea using two different definitions: 1) caregiver-defined diarrhea, 2) watery/soft stool and 3+ bowel movements in 24 hours. Presented in Table 2 are results of caregiver-defined diarrhea; diarrheal prevalence was similar between the two definitions. The overall prevalence of diarrhea in the last 7 days using each definition were 1) 36% and 2) 28%.

Table 4. Prevalence of child diarrhea in the last 7 days, overall and by sub county.

			Overall			Turkana South			Samburu North	
Age group	n	Diarrhea last 7 days (Caregiver defined)	Proportion diarrhea (caregiver defined)	Proportion diarrhea (WHO defined)	n	Diarrhea last 7 days (Caregiver defined)	Proportion diarrhea (caregiver defined)	n	Diarrhea last 7 days (Caregiver defined)	Proportion diarrhea (caregiver defined)
ALL	100	36	0.36	0.28	50	12	0.24	50	24	0.48
0-2 months	24	7	0.29	0.17	12	3	0.25	12	4	0.33
3-5 months	22	10	0.45	0.41	11	3	0.27	11	7	0.64
6-11 months	30	11	0.37	0.27	15	2	0.13	15	9	0.6
12-23 months	24	8	0.33	0.33	12	4	0.33	12	4	0.33

Caregiver Risk Perceptions

Household surveys included questions to understand which pathways caregivers perceive as contributing to child diarrhea. Caregivers were asked, "How likely is your child to develop diarrhea after contacting" chicken feces, soil, human stool, and caregiver hand. Answer options ranged from very likely to very unlikely (Fig 5). Most caregivers perceive contact with soil and human stool as risky; however, fewer caregivers perceive chicken feces as contributing to diarrhea.

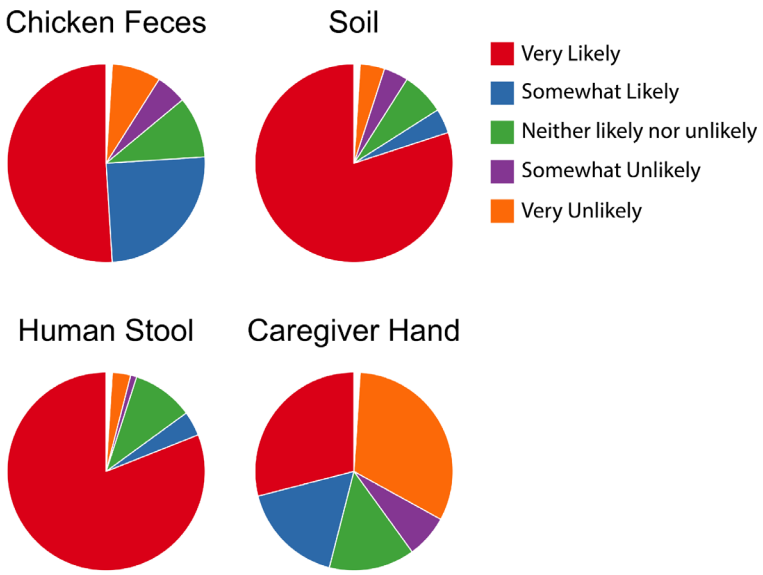

Figure 5. Caregiver risk perceptions. Respondents were asked "How likely is your child to develop diarrhea from contacting" chicken feces, soil, human stool, and their own hand.

Livestock ownership & animal hygiene practices

In total, 86% of households reported owning livestock. Of those, the most common animals owned were goats (83%), sheep (71%), chickens (57%), dogs (49%), cattle (42%), and cats (29%). Respondents also reported limited ownership of camels (10%), donkeys (8%), ducks (1%), and other poultry (1%). Respondents commonly reported that animals entered their home (77%) and the sleeping area (35%). When asked how they dispose of animal feces in their compound, respondents reported collecting feces for domestic purposes (28%), tossing into the bush/field (26%), tossing into the garbage (19%), left in place (17%), and never present (10%).

However, during the structured observations, enumerators observed animal feces present in 98% of households observed, and multiple types of feces present in 21% of households.

Food hygiene

For all 28 food samples collected, field staff recorded information on food storage and serving practices. Overall, 57% of food was cooled without a lid, and 64% was not reheated before serving. Food was frequently stored uncovered (46%) and on the ground (43%). Field staff observed the food preparation areas and found flies (54%), trash (43%), and animal feces (21%).

Interpretation

Our observed household characteristics broadly agree with prior surveys undertaken in these sub counties. These results support findings that these study regions have high poverty rates, limited WASH access, and high levels of child illness. We also find high animal ownership, and widespread animal feces present in household compounds. The child diarrhea rate is high, higher than what has been reported in the region previously, and much higher than recorded in other study settings. The WASH benefits study in Kenya found diarrheal prevalence around 27% among children in western Kenya, compared to the 36% found in this study,[37] indicating that children in the study region have an elevated burden of gastrointestinal illness compared to other regions of Kenya. Moreover, none of the households we surveyed reported consuming treated drinking water; there is a clear need in these sub counties for improvements to safe drinking water. Further, we found that the water situation may differ between Samburu North and Turkana South sub counties; Samburu North households reported shorter walk times to collect water but longer water storage times than Turkana households. Interventions to improve water access will need to consider the unique situation of each sub county.

Sample Collection and *E. coli* measurement

In total, 1454 samples were collected and we enumerated *E. coli* in all soil, food, hand rinse, drinking water, source water, and fomite rinse samples collected at the first visit for a household (n=1008 samples). Feces and stool samples were not analyzed for *E. coli* counts due to budget limitations. Breakdown by sample type is displayed in Table 5.

Table 5. Samples collected by type.

Sample Type	Total	Samburu	Turkana	*E. coli* Measured
Child Stool	191	109	81	0
Mother/caregiver stool	192	99	75	0
Animal feces	358	252	72	0
Soil	240	128	106	155
Food	68	50	10	41
Hand rinse	172	112	105	120
Drinking water (stored)	177	112	96	125
Source water	56	27	46	92
Fomite rinse	78	40	38	99

Stored water was the sample type most likely to contain *E. coli*, and we measured higher *E. coli* levels in stored *versus* source waters. Further, samples in Samburu were more likely to contain *E. coli*, and when contaminated, contained higher levels of *E. coli* than observed in Turkana. We also find that food contained approximately 1 log10 lower (1/10) as much *E. coli* as soil (Fig 6).

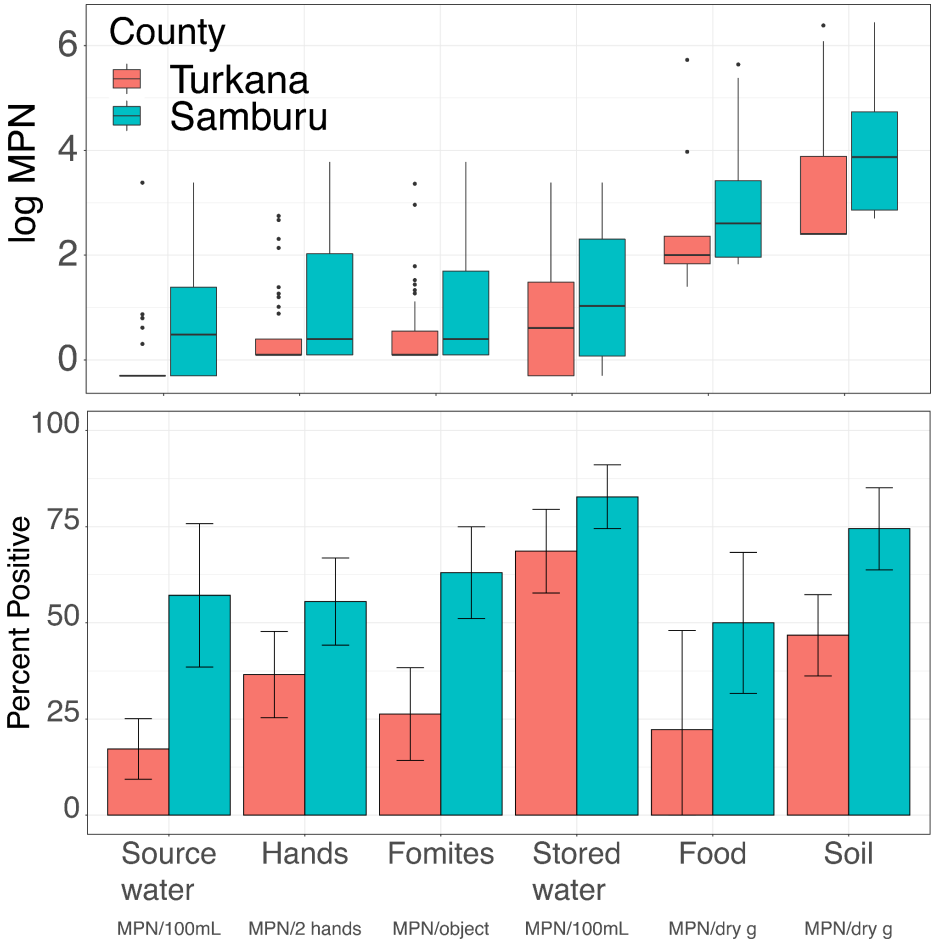

Figure 6. E. coli levels (log10 most probable number [MPN]) in environmental samples (upper) and percent of samples positive for E. coli (lower). Results are disaggregated by study site, Turkana versus Samburu counties. Error bars represent the 95% confidence interval around the mean.

Higher *E. coli* levels in stored *versus* source water indicates contamination after the point of collection, during transport or storage in the household. Contamination can come from dirty jerricans, when dirty hands contact drinking water, or from contact with contaminated scoops or cups. Prior studies have found that longer storage times in the household are associated with increased *E. coli* contamination in stored water, both because the chances of contamination increase with water age and due to bacterial growth in stored water.[38] In this study, the average drinking water storage time in Samburu is greater than in Turkana by 3 hours, a 60% increase in storage time, and this longer storage is one possible explanation for the higher *E. coli* levels in Samburu stored water. However, Samburu source waters are more often contaminated, and the increase in *E. coli* levels between source water and stored water is significantly greater in Turkana. These results indicate that post-collection contamination is greater in Turkana than in Samburu, and that higher contamination in source waters likely drives the greater *E. coli* levels in Samburu stored water.

Samburu may have more *E. coli* contamination than Turkana because the higher temperatures in Turkana may result in pathogen die-off and degradation.[39] We also may find lower *E. coli* levels in Turkana soils because the Turkana soil was very sandy and dry, a matrix that is not conducive to bacterial growth.

Child Behaviors

We observed 56 children for a total of 204 hours during structured observations. The locations children spent their time varied by age (Fig 7). Children in the 0-2 month age group predominantly spend their time in the house, while children in the 3-5 and 6-11 month age groups spend approximately equal amounts of time in the house and courtyard. For the 12-23 month age group, more time was spent in the courtyard than the house.

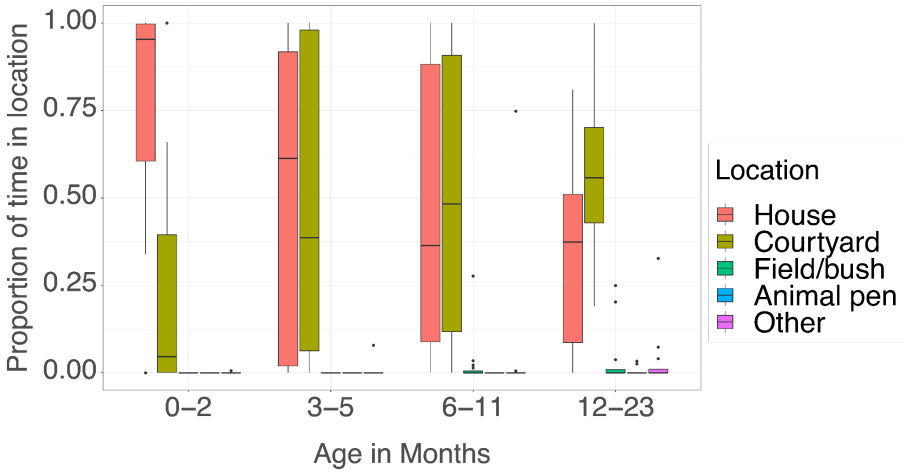

Figure 7. Time spent in various locations by child age, as recorded during structured observations.

Child behavior, such as hand or mouth contacts per hour, also varied by age group (Fig 9). The frequency of child hand contacts increases with age. The most frequent hand contacts were with caregivers, fomites, and soil. Across the age groups, we observed a transition from the children contacting primarily caregivers to more contacts with fomites and soil.

The overall frequency of mouth contacts peaked in the 3-5 and 6-11 month age groups. Children most commonly have mouth contact with their own hands, caregivers, fomites, water, and food, although we did observe occasional mouth contacts with animals and animal feces.

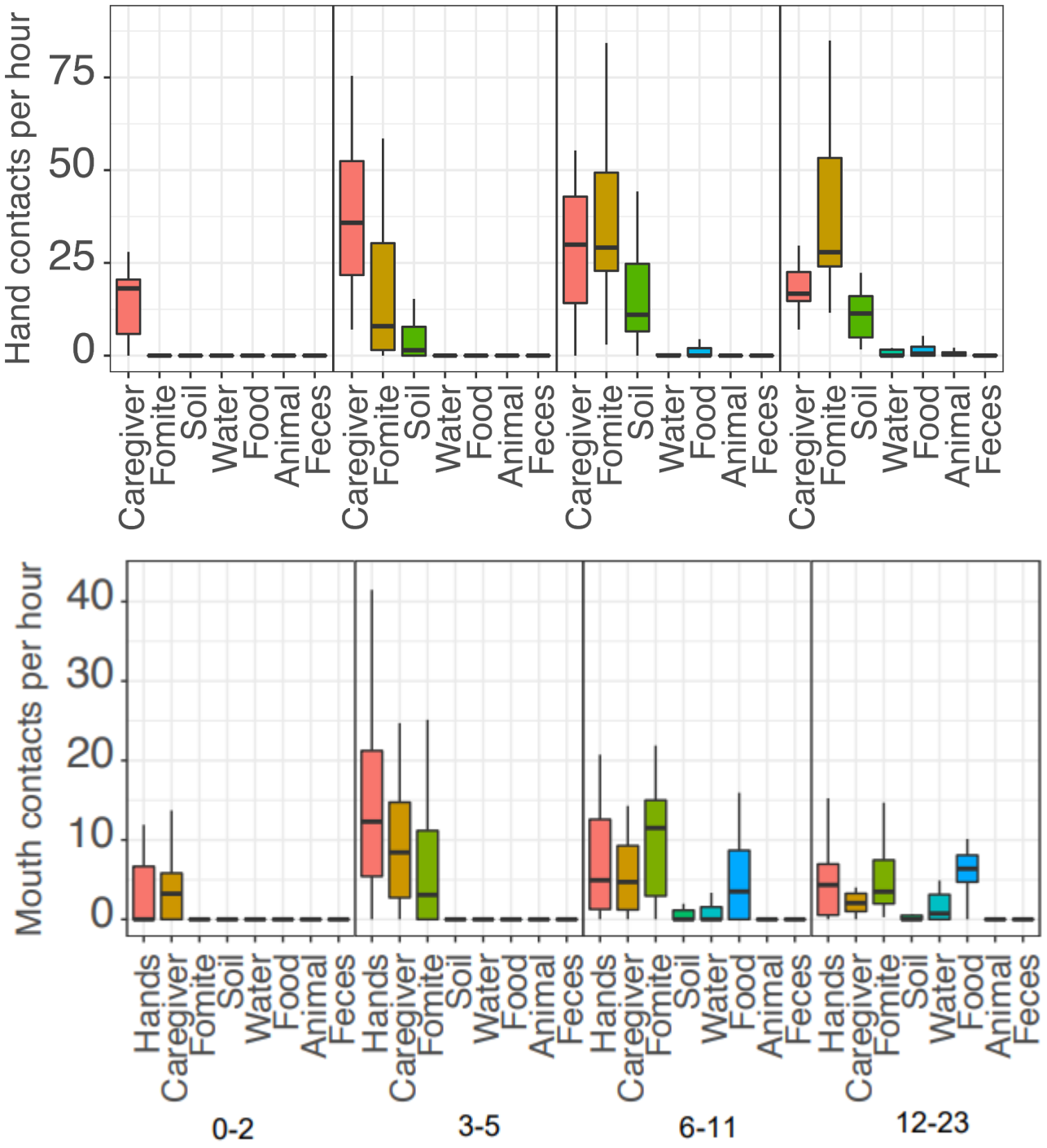

Figure 8. Observed hourly hand contacts and mouth contacts by age group (in months).

Our structured observations demonstrate the importance of including young infants (<6 months) in structured observations; the rates of hand contacts with caregivers, fomites, and soil, and of mouth contacts with their own hands, caregivers, and fomites are similar to those of the older age groups. We also demonstrate that child behavior changes with child age, and that the dominant ways children physically interact with the world evolve throughout development stages. Our results also highlight the importance of collecting site-specific data on child behavior; we find that child behaviors varied significantly between our study site and the site of Kwong et al. in Bangladesh, and the patterns used by Mattioli et al. for a QMRA in Tanzania (Table 6).[20,22]

Table 6. Comparison of child behavior in this study to other settings; reported are median contact rates used in QMRA models in each study.

	Child behavior (3-6 months) *		
	Hand-to-mouth contacts	**Fomite-to-mouth contacts**	**Soil-to-mouth contacts**
This study	16.5 times/hour	6.9 times/hour	0.1 times/day
Kwong et al, Bangladesh[22]	17.6 times/hour	17.75 times/hour	1.03 times/day*
Mattioli et al, Tanzania[20]	1.3 times/hour		
	Child behavior (6-11 months)		
	Hand-to-mouth contacts	**Fomite-to-mouth contacts**	**Soil-to-mouth contacts**
This study	7.3 times/hour	11 times/hour	5.9 times/day
Kwong et al, Bangladesh[22]	13.04 times/hour	19.01 times/hour	6.76 times/day
Mattioli et al, Tanzania[20]	0.99 times/hour		
	Child behavior (12-24 months)		
	Hand-to-mouth contacts	**Fomite-to-mouth contacts**	**Soil-to-mouth contacts**
This study	4.1 times/hour	6.4 times/hour	12.2 times/day
Kwong et al, Bangladesh[22]	10.58 times/hour	17.40 times/hour	5.08 times/day
Mattioli et al, Tanzania[20]	0.88 times/hour		
* The 0 - 2 month age group is excluded here because no other study conducted structured observations with children under 3 months			

E. coli Exposure Assessment

Modeled *E. coli* doses were low for children under 6 months (<30 MPN/day) but increased to >10^3 MPN/day in the 6-11 and 12-23 month age groups (Fig. 9). Food is the dominant exposure pathway for *E.coli* based on the model, accounting for >40% of the average daily dose for children over 6 months. Drinking water is also an important pathway for these age groups, accounting for approximately 25% of the average daily dose. For the younger age groups, which exclusively consume breastmilk and other liquids, the dominant pathways are through water and child hands.

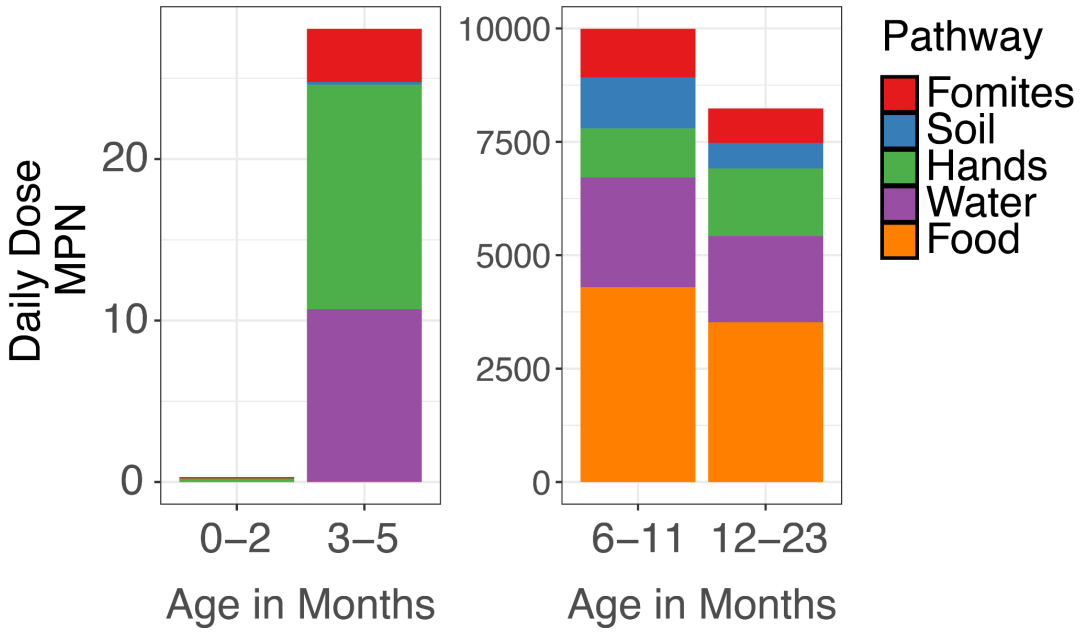

Figure 9. E. coli QMRA results: modeled median daily E. coli dose by child age group for the 0-2 and 3-5 month age groups, ranging from 0 - 30 MPN (left) and the 6-11 and 12-23 month age group, ranging from 0 to 10,000 MPN (right).

The estimated daily dose differs between sub counties; estimated doses in Samburu are approximately twice the doses in Turkana, driven by higher *E. coli* levels within the pathways (Fig 10). However, while the specific doses vary by sub county, the relative importance of each pathway is similar. In both locations, food and water are the most important pathways among older children (>6 months), while water and hands are the dominant pathways for children under 6 months.

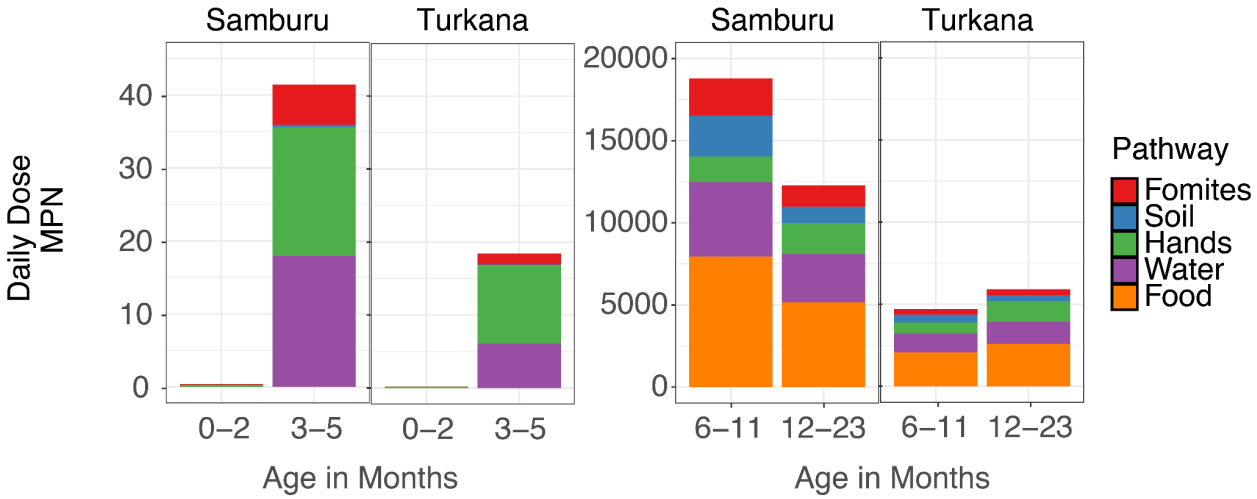

Figure 10. Modeled median dose along each pathway for Turkana versus Samburu sub counties.

These results demonstrate that multiple pathways are important for pathogen exposure, and that these pathways differ by child age. Even though modeled daily *E. coli* dose was low for children between 0-5 months old, these children still had high diarrhea prevalence (29% for 0-2mo; 45% for 3-5mo), indicating that even early, low exposures can affect child health. One explanation for high diarrhea among young children with an estimated low *E. coli* daily dose is that the immune systems of young children are still developing, which means they could be susceptible to pathogens at lower infectious doses.

Another explanation for the high diarrheal prevalence among children under 6 months is that they are exposed to *E. coli* via pathways not included in the model. We observed that the youngest children frequently had both hand and mouth contact with caregivers, a pathway that we were unable to evaluate because we did not sample caregiver hands. Previous studies in rural, low-income settings have documented high fecal contamination on mothers' hands, suggesting mothers' hands are a likely exposure route.[40,41] Further, all children under 6 months in our study were breastfed and we were unable to measure transmission during breastfeeding; however, breastfeeding is generally protective against diarrhea.[42]

A limitation of our exposure assessment model is the reliance on *E. coli*, an indicator organism for fecal contamination that is not necessarily pathogenic, to estimate transmission along various pathways. While *E. coli* is widely used for these and similar purposes, it is recognized that the transport and persistence of *E. coli* in the household environment differs from other enteric pathogens.[24] Relying only on *E. coli* to determine the key transmission pathways may result in an over- or under-estimation of the importance of the pathways, especially because viruses, helminths, and protozoa profiles likely differ the most from *E. coli* compared to other bacteria, and have been found to play a significant role in child health and diarrhea. Because of this limitation, we also conducted testing for 39 pathogens in a variety of sample types to understand how pathogen prevalence differs from *E. coli* and refine our intervention recommendations accordingly. The pathogen testing results are presented below.

Pathogen Results

In total, 292 samples have been analyzed by TAC. Human stool (child stool 59, mother stool 63) makes up approximately one third of all samples analyzed, soil another one third, while animal stool (camel, cattle, chicken, duck, dogs, goats, and sheep) make up the final third.

We detected pathogen genes in every sample type, with mean target detection >1 in all sample types. Across all sample types, we detected a median of 3 pathogens per sample and a mean of 4 pathogens per sample, with a standard deviation of 2.5 pathogens. The number of pathogens detected varied by sample type (one-way anova, $p < 0.01$). We found a lesser number of pathogens in child stool compared to: soil (Tukey's post-hoc test, $p < 0.01$), poultry feces ($p = 0.05$), and dog feces ($p < 0.01$). Child stool was not significantly different from goat, sheep, or cattle feces. Dogs carried a statistically significant greater number of pathogens in their stool than cattle, goats, sheep, or camels, and poultry carried a greater number of pathogens than cattle (Figure 11).

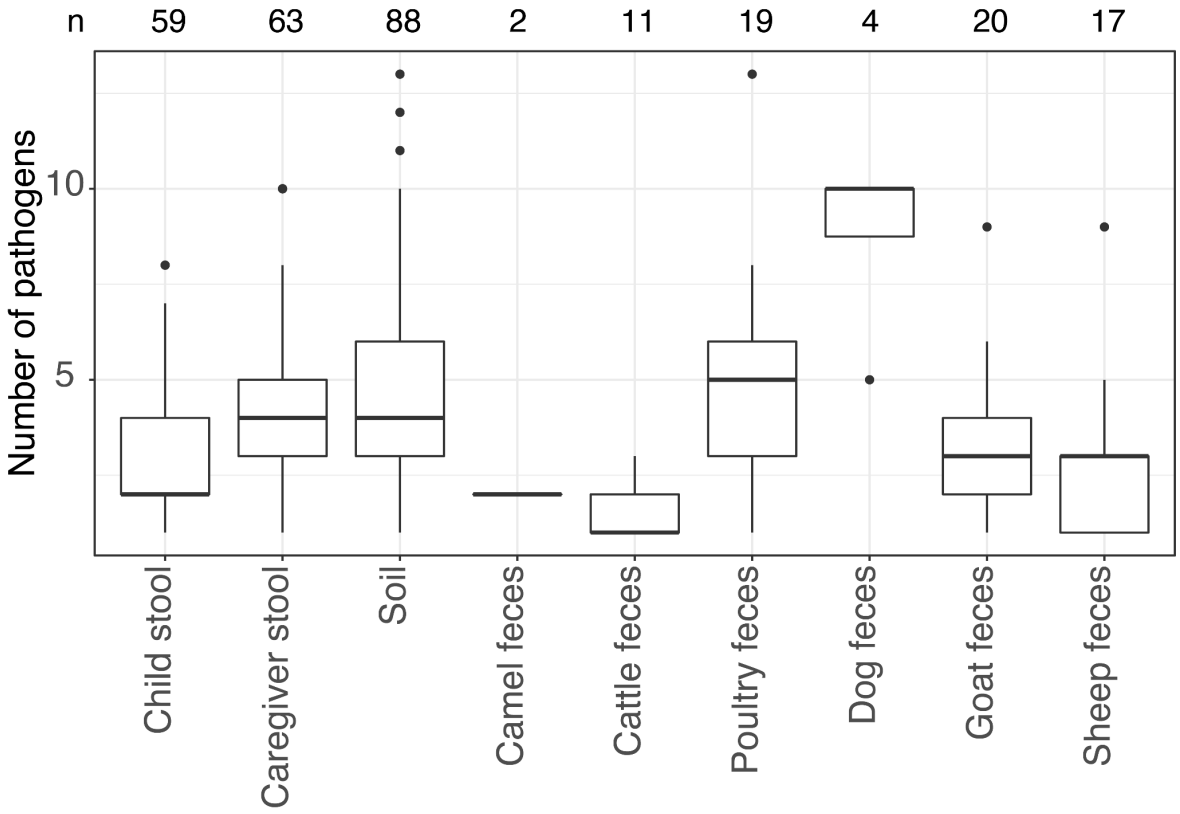

Figure 11. Number of pathogen gene targets present by sample type.

In child stool, the most commonly detected pathogens were enterovirus (48% prevalence), EAEC (48%), EPEC (33%), *Giardia duodenalis* (19%), and Adenovirus 40/41 (17%). In mother stool, the most commonly detected pathogens were EAEC (65%), EPEC (53%), ETEC (33%), EIEC*/Shigella* (27%), and Enterovirus (26%). Dogs were most likely to contain pathogen gene targets, primarily STEC (83%), *Giardia duodenalis* (83%), EPEC (83%), Cryptosporidium spp. (83%), and EAEC (67%). Goats and sheep primarily carried STEC. Chicken stool most frequently contained EPEC, EAEC, STEC, ETEC, and *Giardia duodenalis*. The most commonly detected pathogens in soil were *Cryptosporidium* spp. (66%), *Giardia duodenalis* (46%), EAEC (46%), EPEC (45%), and ETEC (41%; Fig 12). Profiles of child pathogen carriage were most similar to dogs, chickens, soil, and their caregivers.

Overall, the most common pathogens we detected across all sample types were EAEC (39%), EPEC (35%), *Giardia duodenalis* (24%), ETEC (23%), and enterovirus (22%). Over two-thirds of all samples (68%) were positive for one or more types of pathogenic *E. coli*, including 55% of animal feces, 69% of human stool, and 74% of soil samples.

Children in our study were typically infected with 2 enteric pathogens. This is comparable to but slightly less than the pathogen loads found in child stool in the RISE study in urban informal settlements in Fiji (mean 2.8 pathogens per sample), which used a custom TAC to detect a similar number of pathogens as this study.[43] While no data on diarrheal prevalence or household characteristics are available for the RISE study to provide the most relevant comparisons, it is not surprising the children in urban informal settlements would carry more pathogens than those in rural Kenya, given the close proximity of many households and animals that are typically present in informal settlements.

In the GEMS study, the pathogens associated with diarrhea for children under 12 months in Nyanza Province, Kenya were found to be rotavirus (22% of cases), *Cryptosporidium* spp. (9.9%), ETEC (8.8%), and adenovirus

40/41 (8.5%). For children between 12 and 24 months of age, the most important pathogens were EIEC/*Shigella* (15.4%), rotavirus (14.5%), ETEC (9.5%), and *Cryptosporidium* spp (6.3%). In this study, the prevalence of all of these pathogens in child stool are low, with the exception of adenovirus 40/41, while diarrheal prevalence is high, suggesting that the dominant causal agents for child diarrhea vary by setting and depend on the prevailing pathogen profiles of the study site.

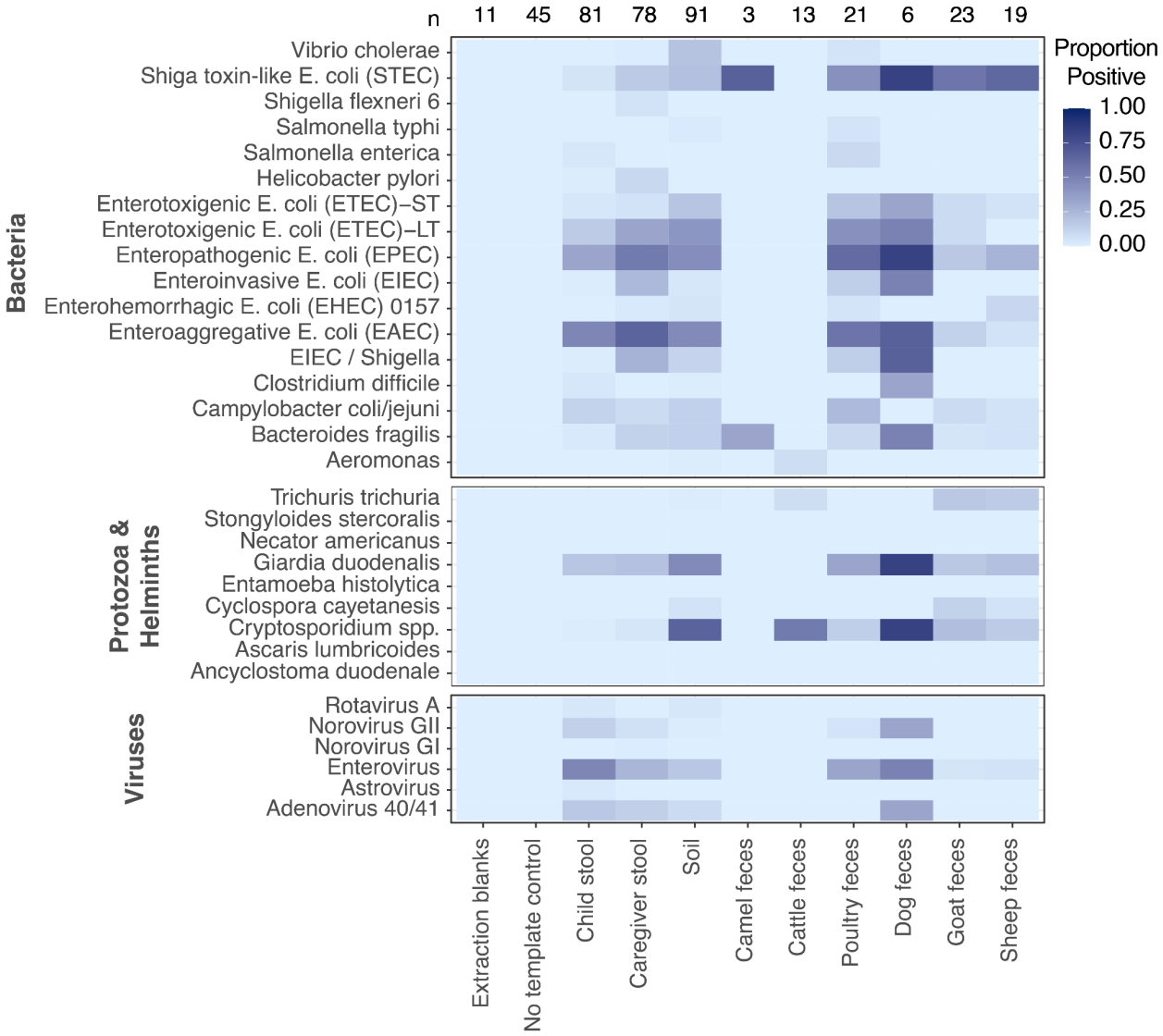

Figure 12. Pathogen detection by target and sample type.

A limitation of this study is that, due to budget constraints, we were unable to analyze pathogen profiles in other sample types, including food, child hand rinses, drinking water, and source water. We find in our exposure assessment model that food is a key exposure pathway for *E. coli* to children who consume complementary foods, but were unable to determine the prevalence of specific enteric pathogens. Given the importance of food in child exposure to *E. coli* in this setting, we plan to further pursue efforts to detect and quantify pathogen genes in the food samples collected. We note that budget constraints limited the number of households we could enroll, the number of longitudinal sampling visits we could conduct, and the number of samples we could analyze. However, a study in Kisumu, Kenya, found frequent contamination of infant weaning foods. Enteric pathogens were present in 62% of infant foods, and 2+ pathogens in 37%.[44] The most

common pathogens in infant foods were *A. hydrophila*, EPEC, EHEC O157, ETEC, adenovirus, and *Cryptosporidium* spp. While we may expect dominant pathogens to vary between Kisumu and rural, northern Kenya, these results demonstrate that infant weaning food can be frequently contaminated and agree with our finding of the importance of infant food in pathogen transmission.

Further, we find that child pathogen carriage is similar between Turkana and Samburu counties, and that pathogen carriage increases with age; the lowest pathogen carriage is seen in the 0-2 month age group (Fig 13). On average, child stool in Turkana contains more pathogens than in Samburu (median = 2 pathogens in Turkana *versus* 1 pathogen in Samburu); however, this difference is borderline statistically significant (student's t-test, $p = 0.06$). Young infants aged 0 - 2 months carry fewer pathogens in their stool than children aged 3 - 5 months ($p < 0.01$) and 6 - 11 months ($p = 0.03$), while there is no significant difference in pathogen carriage between children aged 3 - 5 and 6 - 11 months old ($p = 0.12$). Children aged 12 - 23 months were excluded from this analysis due to the small sample size ($n = 2$). When we compare between sub counties within each age group, we find no significant difference in pathogen carriage in the 0 - 2 month age group ($p = 0.34$), but a greater number of pathogens in Turkana children aged 3 - 5 months ($n = 16$) compared to Samburu children in the same age group ($n = 11$; $p < 0.01$). Older age groups were excluded from this subanalysis due to limited sample sizes.

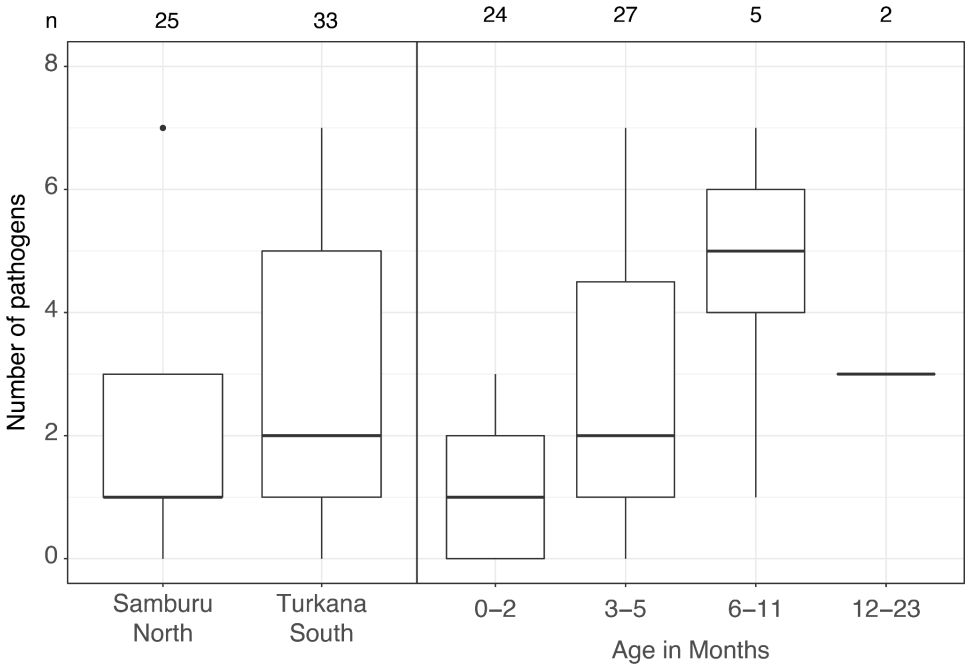

Figure 13. Pathogen carriage in child stool by county and age group.

Insights from Focus Groups and Workshops

From our *E. coli* exposure assessment modeling, we determined that key pathways for child fecal exposure in the study sites were food and drinking water (pathogen results were not yet available at the time of focus group discussions). To assess community opinions on enteric pathogen transmission along these and other pathways (including exposure to animal feces), and garner community input on perceived barriers to preventing child exposure to pathogens, we held focus group discussions (FGDs) with 13-15 caregivers from each study site. These results are summarized below and more details for next steps are outlined in Appendix B.

Turkana Focus Group

Preexisting risk perceptions. Prior to learning of the study results, caregivers perceived poor WASH access, animal feces, and inadequate diet as contributing to pathogen transmission to children. Pathways related to poor WASH mentioned by caregivers included: inadequate access to water for drinking and hygiene practices, lack of hand washing stations and products such as soapy water tippy taps, contaminated source and drinking water, open defecation and lack of pit latrines, and poor mother/caregiver hygiene when feeding infants. Animal-related pathways mentioned included: domestic animal feeding on child feces and animal feces contaminating child play areas. Food pathways mentioned included infant weaning before 5-6 months of age, improper cleaning of complementary feeding utensils, feeding children cold food, and inadequate balanced diet due to food insecurity. Respondents also mentioned that child ingestion of contaminated soil from play areas contributed to pathogen transmission.

Water safety practices. Respondents mentioned a range of water practices to reduce contamination in drinking water, including boiling water and keeping it covered, avoiding hand contact with water while collecting, using AquaTabs or PUR for treatment, sedimenting water, filtration, or using local herbs to clean the water. Respondents also mentioned using clean jerricans, avoiding rolling jerricans on the ground when collecting water, and avoiding sending children to collect water.

Food safety practices. Specific food safety measures mentioned by participants included hand washing before feeding, selecting clean foodstuff at markets, washing foods before cooking, cleaning food containers, limiting the time of food storage, and reheating food before feeding children.

Barriers to reducing transmission. Caregivers also reported a variety of barriers to reducing the risks of pathogen transmission to children, including a lack of caregiver education and knowledge on water treatment and food hygiene, and no/limited education by the government through community health volunteers and public health officers. Caregivers also reported a lack of enough food for the mother during breastfeeding, which leads to early weaning. Also mentioned were the challenges of mothers' time; mothers reported often being busy with other chores, leaving the child to crawl around the compound, and secondary caregivers are not as careful in taking action to reduce transmission risk during childcare. Other challenges mentioned included a lack of access to clean treated drinking water in proximity to the household and resources to purchase water treatment and sanitation products, and a lack of latrines within the compounds and in market areas and food kiosks, leading to open defecation and environmental contamination. Caregivers also discussed the support that would be needed to reduce pathogen transmission, and primarily mentioned increased education and support from CHVs and CHWs and access to WASH products and interventions. Caregivers mentioned a desire for soap for cleaning complementary feeding utensils, handwashing stations with adequate soap and water, improved cement floors, and adequate supply of water for drinking and cleaning. Caregivers requested the county government to construct toilets in market centers with handwashing stations nearby.

Samburu Focus Group

Preexisting risk perceptions. Prior to learning of the study results, FGD participants in Samburu discussed their current understanding of pathogen transmission to children under 2, and mentioned primarily WASH, feces management, and food hygiene. Participants stated that child consumption of contaminated food, drinking water, and soil contributed to transmission, as well as contact with contaminated play objects. Feces management was mentioned in the context of animals feeding on human feces and being infected with pathogens. Also mentioned were caregivers not washing hands before feeding children or after using the toilet.

Water safety practices. Participants believed that water safety could be improved by boiling drinking water or by treating water with chlorine. Safe water storage was also mentioned; participants suggested ensuring water storage containers had lids, and that drinking water be stored separate from water for other uses. Participants also believed water safety could be improved by preventing contamination of water sources, primarily by avoiding open defecation or by burying feces.

Food safety practices. Various types of food hygiene practices were mentioned by participants, including caregivers washing hands before feeding the child, cleaning of serving utensils and storage away from animals, and keeping food covered and reheating food before serving to the child. Participants also mentioned transmission via food could be reduced by regular breastfeeding and/or by feeding children vegetables and fruits. Finally, participants mentioned that keeping dogs separate from children and away from the food preparation area may reduce transmission.

Barriers to reducing transmission. Caregivers mentioned that a key challenge in reducing transmission via animals and animal feces would be keeping animals separate from children; in the Samburu culture, animal ownership is important and it may not be feasible to keep livestock separate from children. Respondents also reported a fear of banditry attacks and were concerned about potential theft of livestock. Caregivers also mentioned challenges around inadequate water sources and treatment supplies, and mentioned difficulties obtaining chlorine for water treatment. A lack of WASH infrastructure poses a challenge to caregivers as well; caregivers mentioned a lack of latrines and child potties, boreholes, and handwashing facilities in their communities.

Future focus group discussions. The enteric pathogen analysis results from this study were not available at the time of the focus group discussions described above. We recommend that additional focus group discussions be held to highlight the finding that chicken and dog feces have much higher pathogen burdens than ruminant feces, and to obtain community input on potential strategies for mitigating child exposure to poultry and dog feces in particular (see appendix B).

Implications of Study Findings and Community Preferences

We found that pathogen carriage and diarrheal prevalence were high in all child age groups, even those with low modeled daily *E. coli* dose by QMRA, demonstrating that Turkana and Samburu are both high disease burden settings for children under two years of age. Children in our study were typically infected with an average of two enteric pathogens, with pathogen burden increasing with age. We also find that multiple pathways are important in child exposure to fecal contamination, including food, drinking water, hands, and soil. These results demonstrate that multiple interventions are likely needed to be implemented to curb pathogen transmission. Focus group discussions with community members indicated a high level of knowledge of fecal-oral transmission pathways, indicating that physical resource constraints (e.g. access to piped water supplies, food insecurity) were the main barriers to preventing child exposure to enteric pathogens.

Food Hygiene. Contaminated food was identified as an important exposure pathway for children post-weaning. Food hygiene is a complex problem, particularly for households in the study setting lacking piped water access and electricity access.[45] For example, cleaning dishes and feeding utensils can be a time consuming process when caregivers have to fetch water from outside the home.[46] Reheating food can also be a burden if households don't have access to gas or electric stoves. Providing food storage containers with tight sealing lids could reduce the introduction of pathogens to food during storage, but would not prevent the growth and persistence of pathogens that may have already been introduced during feeding (as reheating would). Focus group participants noted that it may be possible to prepare food in such a way to limit leftovers that would need to be stored as a strategy to prevent contamination in food. Notably, community members

identified food insecurity as a barrier for mothers to continue exclusive breastfeeding, leading to early weaning and early child exposure to contaminated food. Food supplements for nursing mothers, enabling them to continue exclusive breastfeeding, could be an effective intervention to reduce child consumption of contaminated food, and could potentially have co-benefits to child development, microbiome, and immune system.[42]

Drinking water infrastructure. We also found drinking water to be an important exposure pathway for all age groups. Improvements to drinking water could be made on two fronts: proximity and quality. On-plot access to water sources would enable households to spend less time on each collection trip, and could result in shorter water storage times, reducing the chances for contamination of stored water; increased quantities of water collected, enabling more water for hygiene and cleaning purposes; and more time spent on income-generating activities, which could reduce food insecurity.[47,48] Further, community members expressed a desire for additional water access points (e.g. shortening water fetching walk times).

Water quality could be improved by the use or development of improved sources and implementing chlorine dosers at the community level. Chlorination is effective at inactivating the majority of pathogens that children were infected with in the study communities, with the exception of *Giardia* and *Cryptosporidium*.[49] A recent meta-analysis of water treatment interventions (of mostly chlorination interventions) estimated that the interventions reduced all-cause child mortality by 30%.[50] Point of use (POU) chlorination methods that require users to devote daily unpaid labor to use them consistently often see adherence quickly drop off after behavior promotion ends.[51] We would instead suggest chlorination methods that do not require users to change their water collection practices, such as passive (in-line) chlorination, to be explored.[52,53] Community members also highlighted that access to chlorine products in the study sites has been a problem in the past, and thus interventions in this space would need to ensure a consistent and adequate supply of chlorine for water treatment.

Most households in the study communities reported using boreholes or piped water, which can be compatible with passive chlorination technologies if connected to a storage tank.[54] Possible implementation and funding partners for improving water infrastructure are the government or Evidence Action, an NGO which has implemented point of collection chlorination technologies in Western Kenya. Installation of solar powered boreholes connected to overhead tanks would enable gravity-fed piped distribution systems to distribute piped water to household plots, and would also be compatible with tablet erosion in-line chlorine dosers. Conducting a demand assessment among study communities to understand willingness to pay for household piped water connections delivering chlorinated water could provide valuable information for government planning to upgrade water infrastructure in the study communities.

Animal feces management. A key finding of this study is the high levels and diversity of pathogens present in animal feces, and that many of the same pathogens are carried in animal feces and human stool. In particular, chicken and dog feces contained many zoonotic pathogens while ruminant feces had lower pathogen burdens. These results suggest that dog and poultry feces could be an important reservoir of pathogens in this setting, and highlight the importance of including animal waste management in discussion of interventions.[15] Community members identified animal feces as an exposure route of pathogens for young children even before learning of the study results, but mentioned that because animals play such an important role in their culture, interventions to keep animals separate from children may not be feasible. Our findings are useful in identifying which types of animal feces need to be managed: poultry and dog. Community members expressed a preference for interventions aimed at removing animal feces from child play areas. Thus, intervention co-design and piloting could focus on providing caregivers with scoops to remove feces, implementing improved flooring (e.g. easy to clean surfaces such as concrete) in child play areas, or enabling caregiver hand hygiene

after handling animal feces. Local stakeholders also suggested incorporating safe poultry and dog feces management into government Community-led Total Sanitation programs.

Recommendations for Co-Creating Solutions. We suggest using principles of human-centered design to co-create potential solutions in partnership with the study communities. The human-centered design process involves: 1) Needs assessment, 2) Defining the problem, 3) Ideation, 4) Prototyping, and 5) Testing. The process includes frequent cycling between stages and interaction with the end users during each phase. We have outlined some suggested potential activities in Appendix B focused on enabling food hygiene, facilitating safe chicken/canine feces management, and obtaining community input on water infrastructure planning.

CONCLUSIONS

Our study results emphasize that children get exposed to fecal contamination and enteric pathogens through multiple pathways (food, water, fomites, and hands), indicating that single interventions (e.g. a household food hygiene intervention) will be unlikely to lead to a substantial reduction in the burden of enteric disease for young children. Further, we identified animal feces, namely chicken and dog, as an important source of pathogens in the household environment. Sharing this knowledge and engaging with communities will be important to identify culturally appropriate and feasible interventions that address food hygiene and safe management of chicken and dog feces. However, we also acknowledge that the burden of food hygiene, water treatment, and animal feces management is predominantly borne by women caregivers, who are usually already constrained by other competing domestic socio-cultural demands. Considering the reality of how difficult it is to transform a highly contaminated environment with household-level interventions, stakeholders with available resources may want to consider partnering or supporting the government in investing in the services and infrastructure identified as the top priorities by the study communities and their leadership. While we would suggest further community engagement to identify these priorities, our initial results suggest there is strong demand for improved water infrastructure. Available resources may be best spent supporting government and/or non-profit entities to install or upgrade water infrastructure to improve access to safely managed drinking water as defined by the Sustainable Development Goals (i.e., on-plot access to water free from microbial contamination).

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Note: The content and material in this report will be published in a future peer-reviewed manuscript. Results, analysis, and interpretation included in this report are preliminary and may change in future analyses and publications of these study results

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APPENDIX A: QMRA PARAMETERS

Pathway	Parameter	Units	Age group	Distribution	Source
DRINKING WATER	Concentration of *E. coli* in drinking water	MPN / 100 mL	all	lnorm(2.03,3.09)	this study
	Volume of drinking water consumed daily	mL	0-2	ln N(0.18, 3.72)	[22]
	Volume of drinking water consumed daily	mL	3-5	ln N(5.18, 1.19)	[20]
	Volume of drinking water consumed daily	mL	6-11	ln N(5.43, 0.96)	[20]
	Volume of drinking water consumed daily	mL	12-23	ln N(5.19, 0.91)	[20]
FOOD	Concentration of *E. coli* in food	MPN / dry g	all	ln N(2.20, 4.71)	this study
	Volume of food consumed daily	dry g	0-2	0	this study
	Volume of food consumed daily	dry g	3-5	0	this study
	Volume of food consumed daily	dry g	6-11	lnorm(6.0, 0.64)	[34]
	Volume of food consumed daily	dry g	12-23	lnorm(6.9, 0.28)	[34]
HANDS	Concentration of *E. coli* on child hands	MPN / 2 hands	0-2	lnorm(-2.35, 3.98)	this study
	Concentration of *E. coli* on child hands	MPN / 2 hands	3-5	lnorm(0.62, 3.80)	this study
	Concentration of *E. coli* on child hands	MPN / 2 hands	6-11	lnorm(1.18, 2.83)	this study
	Concentration of *E. coli* on child hands	MPN / 2 hands	12-23	lnorm(1.56, 3.96)	this study
	Frequency of hand-to-mouth contact	contacts / hr	0-2	exp(0.17)	this study

Pathway	Parameter	Units	Age group	Distribution	Source
	Frequency of hand-to-mouth contact	contacts / hr	3-5	exp(0.062)	this study
	Frequency of hand-to-mouth contact	contacts / hr	6-11	exp(0.13)	this study
	Frequency of hand-to-mouth contact	contacts / hr	12-23	exp(0.21)	this study
	Proportion of hand area in contact with mouth	unitless	all	unif (0.06, 0.33)	[20]
	Transfer efficiency from hands to mouth	unitless	all	N (0.41, 0.25)	[20]
	Hours awake per day	hr	0-2	unif (7.15, 13.78)	[20]
	Hours awake per day	hr	3-5	unif (7.15, 13.78)	[20]
	Hours awake per day	hr	6-11	unif (7.15, 13.78)	[20]
	Hours awake per day	hr	12-23	unif (8.58, 15.55)	[20]
FOMITES	Concentration of *E. coli* on fomite	MPN / object	all	lnorm(0.47, 3.62)	this study
	Frequency of object-to-mouth contact	contacts / hr	0-2	exp(31.27)	this study
	Frequency of object-to-mouth contact	contacts / hr	3-5	exp(0.13)	this study
	Frequency of object-to-mouth contact	contacts / hr	6-11	exp(0.10)	this study
	Frequency of object-to-mouth contact	contacts / hr	12-23	exp (0.17)	this study
	Transfer efficiency from object to mouth	unitless	all	N (0.41, 0.25)	[20]
	Surface area mouthed (SAM)	unitless	all		[36]
	Fraction of surface area mouthed (FSA)	unitless	all	unif (0, 1)	assumption

Pathway	Parameter	Units	Age group	Distribution	Source
SOIL	Concentration of *E. coli* in soil	MPN / dry g	all	lnorm(7.44, 4.11)	this study
	Mass of soil per ingestion event	dry mg	all	beta(5.30, 158.6)	[36]
	frequency of soil-to-mouth contact	contacts / hr	0-2	empirical	this study
	frequency of soil-to-mouth contact	contacts / hr	3-5	empirical	this study
	frequency of soil-to-mouth contact	contacts / hr	6-11	empirical	this study
	frequency of soil-to-mouth contact	contacts / hr	12-23	empirical	this study

APPENDIX B. RECOMMENDED HUMAN CENTERED DESIGN PROCESS FOR CO-CREATING SOLUTIONS

In this appendix we outline suggested activities using principles of human-centered design for co-creating strategies for preventing child exposure to enteric pathogens in the study communities. The human centered design process includes five stages: 1) Needs assessment, 2) Defining the problem, 3) Ideation, 4) Prototyping, and 5) Testing. There is often frequent iteration between stages and interaction with the end users during each phase. We suggest the following human-centered design activities to co-create, test, and refine solutions with the study communities in Turkana and Samburu.

Problem Definition

We would suggest first having community members identify and rank their top three priorities for government or NGO investments in their communities. While the below activities may be helpful in developing interventions that respond to household needs and address child exposure to enteric pathogens, we note that household-level interventions (e.g. promoting a specific set of behaviors or usage of a specific product) are unlikely to address all exposure pathways for young children. Understanding community priorities could be helpful for determining how to support the government in upgrading services or infrastructure in the study area, which may be a more effective use of resources than developing household-level interventions.

Drawing on the study results, we would suggest starting with the following questions and validating them during focus group discussions with 10-15 community members in each subcounty to ensure they are the correct problem definitions:

Food Hygiene

1. How might we design a feeding utensil for young children that would be easy to clean?
2. How might we facilitate a way for caregivers to re-heat food quickly to be fed to young children?

3. How might we design a product that enables safe storage of food, including tight sealing containers?
4. How might we support caregivers in preparing quantities of food that minimizes the need for storage?
5. How might we support mothers in their efforts to maintain a sufficient breast milk supply for their children?
6. How might we facilitate hand hygiene for caregivers and children prior to feeding?

Animal Feces Management

1. How might we prevent dogs and/or chickens from defecating where children sleep or play?
2. How might we train dogs to defecate in specific locations to make feces management easier?
3. How might we create a product that facilitates easy removal of dog and/or chicken feces from areas where children sleep or play?
4. How might we safely dispose of animal feces?
5. How might we create an easily cleanable surface for children to play on?
6. How might we design a product to keep dogs and chickens out of food preparation areas and/or child play areas?

Water infrastructure

1. How might we use our resources to engage other stakeholders on improving water infrastructure in the study communities?
2. How might we use our resources to advocate for improved water infrastructure in the study communities?
3. How might we assess household willingness to pay for access to chlorinated piped water connections?
4. How might we obtain community input on water infrastructure and water treatment planning?

Ideation

Hold a brainstorming session internally and/or with community members to ideate potential solutions to the above problems. When brainstorming, it's helpful to follow these guidelines: 1) defer judgment, 2) encourage all types of ideas, 3) build off ideas, 4) go for quantity not quality, and 5) be visual. During idea generation, it's helpful to be positive and build off ideas rather than discount ideas. At the end of the session, you can rank ideas (vote on them) and come to a consensus on which ones to pursue further.

Based on study results and focus group discussions, some initial possible ideas to seed brainstorming efforts and obtain feedback on from community members are as follows:

- Moveable/adjustable fencing or netting to keep chickens separate from young child play areas
- Food preparation products that would enable cooking compartmentalized servings for young children that could remain sealed until ready for consumption
- Feeding utensils that are easy to clean and facilitate clean feeding practices
- Tools for removing chicken feces and/or dog feces
- Child bibs that prevent food from being dropped in soil during feeding events
- Concrete patios or concrete flooring

- Animal behavior training programs (e.g. to defecate in specific locations or stay out of child play areas)
- Nutrition supplements for breastfeeding mothers
- In-line chlorination devices at water points with storage tanks

Prototyping and Testing

For one or more potential solutions, it may be appropriate to first quickly create rough prototypes that convey the main idea of a product feature or concept to eventual end users. Rapid prototyping strategies can include drawing or sketching what a product will look like or how it will work (drawings can be shown to users or stakeholders to get feedback). Another strategy is building with cardboard or other easily obtainable materials to inform user-interface design features. For example, if you are creating a tool for caregivers to use to remove poultry feces from a compound, you could cut wooden sticks at different lengths and ask users to mimic usage to determine how long the handle of the tool will need to be. To test function, you could try cleaning up poultry feces with different features attached to the tool (e.g. brush, blade, scoop).

Once you have validated a concept, build functional prototypes and have households test them out for 48 hours. Obtain user feedback and incorporate feedback into the design. Consider what education or training will be needed to accompany a physical product or service. Once you have developed an intervention or service, conduct a longer term pilot (e.g. 3-6 months) at the scale of 50-100 households before investing in larger scale implementation.

Iterative Feedback

Throughout these phases, it's helpful to interact frequently with end users and other stakeholders to inform design decisions. It usually takes multiple field testing sessions with users to develop a product or service that sufficiently meets the needs of users.

Financial Viability

Once you have a viable product, assess both the capital cost and any operational costs. Assess effective demand for the product through actual sales to community members or by charging monthly fees for a service. Gain an understanding of what product or service features community members demand and/or would be willing to pay for. Explore diverse revenue streams, including subsidization by the government or NGOs, advertisements on physical products, or carbon credit financing (e.g. for chlorinating water supplies or food hygiene products that would reduce heating/energy requirements).

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