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Formative Research on Hygiene Behaviors and Geophagy among Infants and Young Children and Implications of Exposure to Fecal Bacteria

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Abstract. We conducted direct observation of 23 caregiver–infant pairs for 130 hours and recorded wash-related behaviors to identify pathways of fecal–oral transmission of bacteria among infants. In addition to testing fingers, food, and drinking water of infants, three infants actively ingested 11.3 ± 9.2 (mean ± SD) handfuls of soil and two ingested chicken feces 2 ± 1.4 times in 6 hours. Hand washing with soap was not common and drinking water was contaminated with *Escherichia coli* in half (12 of 22) of the households. A one-year-old infant ingesting 1 gram of chicken feces in a day and 20 grams of soil from a laundry area of the kitchen yard would consume 4,700,000–23,000,000 and 440–4,240 *E. coli*, respectively, from these sources. Besides standard wash and nutrition interventions, infants in low-income communities should be protected from exploratory ingestion of chicken feces, soil, and geophagia for optimal child health and growth.

INTRODUCTION

Poor growth during infancy and early childhood remains an important risk factor for childhood morbidity and mortality and a major public health challenge in low- and middle-income countries. Childhood stunting is a risk factor for diminished survival, short adult height, impaired intellectual development, reduced economic productivity, and low offspring birthweight.[1] Globally, approximately 178 million children less than five years of age are stunted, with an estimated 35% of child deaths attributed to sub-optimal nutrition.[2] In Africa and Asia height-for-age Z scores decline from near zero at birth to –2.0 by 18 months of life. Rigorous dietary interventions have improved stunting by 0.7 Z scores at best,[3] only one-third of the average deficit.

Environmental enteropathy, a chronic subclinical intestinal pathology, is common among infants in low-income countries and has been proposed as a major pathway to childhood stunting.[4] Although diarrhea, the second leading cause of child mortality wordwide,[5] causes malnutrition, prevalent diarrhea is not always associated with poor growth in the long term because of catch-up growth between episodes.[6] Environmental enteropathy, which is characterized by reduced intestinal barrier function and chronic systemic inflammation, may be a more important cause of poor growth in children than diarrhea.[7] Research in the Gambia showed that 43% of linear growth failure could be explained by increased intestinal permeability, a measure of environmental enteropathy; in contrast, the prevalence of diarrhea was not associated with growth failure.[8] Although the precise cause of environmental enteropathy is unknown, it has been linked to unsanitary and unhygienic living conditions, and is likely to arise from chronic, subclinical exposure to fecal pathogens.[7]

The primary pathways of fecal–oral transmission have been described for decades using the F Diagram (food, flies, fingers, field, and fluids),[9] yet the relative importance of each pathway is not known. Furthermore, the primary routes of transmission may be different for infants and young children, because their primary food and fluid is breast milk and who regularly mouth objects as part of normal development. In developing countries, young children crawl and play in areas where they may come into contact with soil that is contaminated with human and animal feces. Human or animal feet that tread in feces deposited in the open bring pathogens into the domestic environment-home and immediate vicinity to infants and young children.[10] In rural Zimbabwe, a nosocomial pathogenic bacteria, *Clostridium difficile*, was isolated in 37% of soil, 17% of chicken feces, and 6% of water samples collected from 146 households.[11] More than half of these isolates in soil and chicken feces were toxigenic strains.

In Lima, Peru, an in-depth behavioral observation study of 21 children less than five years of age reported a mean (SD) of 3.9 (4.6) episodes of ingestion of chicken feces during a 12-hour period.[12] A recent comprehensive review reported that human geophagy (intentional consumption of earth) is common among children and pregnant women in low-income countries, where pathogen densities are highest.[13] Therefore, exposure to fecal bacteria among children in low-income countries might be greater than has been reported in studies that focused only on food and drinking water contamination.

During formative research to inform the design of interventions to improve infant growth, we conducted a study to identify the major pathways of fecal–oral microbial transmission among infants and young children in rural Zimbabwe. The study was executed in two phases. The first phase was observation of infants and their caregivers to identify the frequency of all objects put into childrens' mouths (whether swallowed) and presence of visible dirt over a six-hour period per household. The second phase was sampling and microbiologic analysis of the objects that were mouthed most frequently and/or were most visibly dirty.

METHODS

Study site and sample frame. The study was conducted in rural Shurugwi District, Midlands Province, Zimbabwe. Most households in this district subsist through small-scale farming, and poultry and cattle keeping. Twenty one households with seven infants in each age group (3–6 months, 6–12 months, and 12–18 months of age) were purposefully selected from village

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health workers' lists. An additional two households with infants less than three months of age were also included. The households consisted of ≥2 traditional mud or brick-walled houses. The immediate kitchen compounds were open yards with bare, loose sandy soil and no fence, referred to as a kitchen yard or yard in this report. Infants were free to crawl on bare soil where poultry and other animals were free to roam.

Ethics. On the first visit to each household, the study was introduced by the field supervisor and written informed consent to participate in the study was obtained from mothers. The written informed consent was first read to the mother in English. The field supervisor tested the mothers understanding of the consent by asking her to explain what she understood from the consent. If the mother did not understand the information in English, the supervisor read the consent in Shona. The Shona version of the consent form was translated and back-translated by the field research staff under the supervision of the field supervisor before the study. Ethical approval to conduct this study was granted by the Medical Research Council of Zimbabwe and the Institutional Review Boards of Johns Hopkins University Bloomberg School of Public Health and the Research Institute of McGill University Health Centers.

Caregiver–infant observation. To ensure uniform understanding among the research staff during data collection, prior training was conducted, and the same team was involved in pre-testing the tools and developing detailed field guides. During the observation phase in February 2011, two trained researchers conducted a six-hour in-depth observation between 8:30 AM and 2:30 PM in each of the 21 households with 6–18-month-old children. (Observation was stopped after two hours in the two household with children less than three months of age because these infants were mostly protected and inactive).

One researcher used a pre-tested semi-structured data collection tool to record every object that was either touched or mouthed by an infant, whether the object was visibly dirty, and the frequency of object–mouth episodes. Mouthing was defined as putting any item or fingers into a child's mouth, regardless of whether it was ingested. Researchers also observed and recorded the mother's hand-washing behavior and any infant diaper change and hand washing. The potential triggering events considered for hand washing were defined as after toilet use, after contact with animal stool, before feeding the baby, before handling food, before eating, and after sweeping the floor or yard.

After briefly interviewing the mother upon arrival at the household, the second researcher conducted, at two-hour intervals, spot checks and recorded the number of corralled and roaming animals and the presence of fecal material in the kitchen yard by using a pre-tested structured questionnaire. The spot checks also involved inspecting and recording the cleanliness of the mother's and infant's hands. The second researcher also determined whether the household had a hand washing station and functional latrine, and whether there was evidence of their recent use. To maintain the quality of data, debriefing sessions were held daily after every household visit. Researchers re-checked and cross-checked questionnaires and recording of key events and behaviors to maintain consistency in data collection.

After all infant observations, data were analyzed to identify the key potential vectors, defined as 1) objects mouthed most frequently and 2) objects that were ever mouthed and were most visibly dirty. The objects identified during phase I by the frequency definition were infant, mother, and sibling hands; food; water; toys/play objects (e.g., plastic cans and dolls); and food-serving utensils. Objects identified by the visibly dirty definition were soil and chicken feces.

Microbiologic analyses (May–June 2011). Two subsequent visits were made at 10-day intervals to the households visited in phase 1 to collect samples or swabs of the key potential vectors identified. A total of 22 households were sampled because one mother was away during the sampling phase. Three research staff with experience in aseptic sampling techniques and microbiologic analysis collected 14 samples from each household during mid-morning hours.

Soil samples were collected from three parts of the yard where children were most likely to play and likely point sources of fecal-contaminated soil where crawling children had access: areas used for laundry, diaper changing, and bathing; the kitchen door step; and the edge of the rubbish pit or path to the rubbish pit (if the pit was distant). On the second sampling visit, an additional soil sample was also collected from the field cultivated by the mother. Chicken feces were identified and collected within the yard from the closest spots to the kitchen door. We collected any chicken feces observed during the time of sample collection, whether fresh or dry. Most of the feces were dry and we avoided smaller droppings that could not be identified as chicken feces. The sample collectors were familiar with the local context and could easily identify chicken feces. Except for the initial sampling visit to the first four households, mothers and village health workers were not aware of the day and time of visits for sample collection to minimize social desirability bias.

Environmental samples (infant cup, infant toys and spoon, and kitchen floor area) and hand swabs (mother's and sibling's dominant hand) were taken by using commercial environmental sponge sampling kits (Bacti Sponge Kit; Hardy Diagnostics, Santa Maria, CA).[14] Four swabs were also taken from the child's left palm, right palm, right fingers, and left fingers at each visit. Approximately 20 grams of food, water, soil, and chicken feces samples were collected into sterile screw-cap bottles by using an aseptic technique. The mother was requested to scoop food during infant feeding into a sterile screw-cap bottle. If no infant feeding occurred during the sampling visit, the mother was requested to scoop any leftover food that was fed to the child by using the same spoon used for feeding. The procedure at the second visit replicated the first visit. A fixed area was sampled from the kitchen floor (equivalent to four sponge kits sizes [128 cm^2]) and from the infant cup and spoon (equivalent to six sponge sizes [192 cm^2]). Infant toys were of different sizes and shapes. Therefore, swabs were taken from a fixed area of surfaces that could easily fit into an infant's mouth (equivalent to two sponge kit sizes [64 cm^2]).

After sampling, swabs were immediately placed in cooler boxes, covered with ice packs, and transported to the field laboratory for microbiologic analysis within 12 hours. All samples were analyzed for total aerobic counts, *Escherichia coli*/coliforms, and *Enterobacteriaceae* by using 3M Petrifilm™ prepared plates (3M Microbiology, St. Paul, MN) according to the manufacturer's instructions.[15] Sterile Butterfield's phosphate buffer (99 mL) was added to 11 grams of food, soil, or chicken feces samples in sterile bags with filters and homogenized by

using a hand roller for three minutes to prepare a 1:10 dilution. Subsequent dilutions were prepared from the filtrate. Food and water samples were diluted (1:10–1:100,000) with sterile Butterfield's phosphate buffer. One milliliter of each sample dilution was plated in duplicate onto each of the 3 Petrifilm products and incubated at 35° ± 1°C for either 24 ± 2 hours (for coliforms and *Enterobacteriaceae*) or 48 ± 3 hours (for *E. coli* and total aerobic counts).

Sponges from the hand and environmental swabs were transferred aseptically to homogenizer bags into which 20 mL of Butterfields phosphate buffer was added and squeezed for one minute by using a hand roller homogenizer. A sample of the resulting solution (1 mL) was plated in duplicate directly onto *Enterobacteriaceae* and *Escherichia coli*/coliforms Petrifilms and incubated as described above. Additional 1:10 dilutions for hand swabs and environmental surfaces swabs were undertaken for total aerobic count enumeration.

During sample collection period, we weighed the amount of dry soil that a one-year-old child held without spilling by folding its fingers onto the palm, and found this amount to weigh 2.5 grams. Half of this amount of soil was taken as the estimate for the soil that could be easily ingested because spills were observed for the three children who actively ingested dry soil. We also estimated the amount of soil that made a child's 2–3 fingers visibly dirty from crawling, by rubbing a known amount of dry soil on a baby's fingers and weighing the soil that was not captured on and between the fingers. We estimated 0.25 grams as the amount of soil that made 2–3 fingers visibly dirty and likely to be sucked into the mouth as commonly observed during the infant observation phase. These estimates were conservative because only little amounts of dry soil could stick on dry fingers. We also estimated the amount of chicken feces consumed in six hours during crawling on soil with chicken feces and exploratory ingestion of chicken feces. On average, a semi-dry chicken dropping weighed 1 g ram and a bite of chicken feces was estimated to weigh 0.25 grams by the two observers who saw the ingestion. An additional 0.5 grams was estimated from ingesting particles of chicken feces in soil assuming that 0.02 grams for every time visibly dirty hands went into the mouth ($0.02 \times 38 \times 0.75 = 0.56$ grams). These estimates were based on observational data during infant observation.

Data analysis. The frequencies of all objects put in the infant's mouths were calculated, and the 10 most frequently mouthed and the dirtiest objects were taken as the potential key vectors for phase II bacteriologic analyses. Geometric means for bacterial populations in each potential vector were calculated as colony-forming units per gram (CFU/g) of food, soil or chicken feces, CFU/mL of water, or CFU/swab for hands and environmental surface samples. All summary statistics and two sample *t*-test comparisons were carried out by using STATA statistical software version 10 (StataCorp LP, College Station, TX).

RESULTS

Household characteristics. We observed 23 caregiver–infant pairs for 130 hours during the infant observation phase. Baseline characteristics of households are shown in Table 1. All except one mother were married and had attained at least some level of primary education. Most houses had roofs made of thatch, and half had earthen or cow dung–smeared floors.

Table 1

Maternal and household characteristics (n = 23), Shurugwi District, Midlands Province, Zimbabwe

Characteristic	No. (%) or mean ± SD
Maternal	
Caretaker	
Mother	22 (95.7)
Grandmother	1 (4.3)
Mother's age, years	25.66 ± 6.5
Marital status	
Married	22 (96)
Single	1 (4)
Education level	
Primary	5 (22)
Secondary	18 (78)
No. children	
> 18	3.74 ± 2.0
> 5	1.56 ± 0.8
Household	
Latrine ownership	
Own	10 (43)
Neighbor's	5 (22)
Do not use/do not have	8 (35)
Hand washing	
Hand washing station	13 (56)
Soap at hand washing station	10 (44)
Wet ground at hand washing station	10 (44)
Primary water sources	
Borehole	13 (57)
Protected well	8 (35)
Unprotected well	1 (4)
River	1 (4)
Scooping container at point of use	
Specific scooping cup	9 (39)
Any cup	13 (56)

One-third of the households did not have a latrine. Hand washing stations and soap were found in approximately half of the households. More than half (56%) of the households had a borehole as their primary water source. Other households used either protected or unprotected wells.

General hygiene. At the beginning of the infant observation phase, one-third of the mothers had visibly dirty hands. Seventeen percent of the infants had visibly dirty hands (Table 2).

Table 2

General hygiene characteristics of infant's environment during observation, Shurugwi District, Midlands Province, Zimbabwe

Characteristics	On arrival, no. (%)
Caregiver's hands visibly dirty	7 (30)
Baby's hands visibly dirty	4 (17)
Diapers or child's bottom not clean	1 (4)
Stagnant water within infant's reach*	7 (30)
State of kitchen	
Unwashed utensils	9 (39)
Uncovered utensils	0 (0)
Uncovered food	1 (4)
Smooth concrete floor	12 (52)
Dirt or cow dung floor	11 (48)
Spill on the floor (food or drink)	5 (23)
Poultry feces visible on kitchen floor	8 (35)
Animals in kitchen	8 (35)
State of yard	
Kitchen yard swept	16 (70)
Area where child plays is swept	16 (70)
Poultry feces visible	20 (87)
Human feces visible	0 (0)
Animals feces visible	7 (30)

*Any stagnant water within infant's reaches in kitchen or outside yard.

TABLE 3
Mother's hand washing practices, Shurugwi District, Midlands Province, Zimbabwe*

Key events	Opportunities	Any hand washing	HWWS†	Running water	Air drying‡
Possible contact with feces					
After adult toilet	10	4 (40.0)	0 (0)	1 (25)	4 (100)
Contact with animal feces	2	0 (0)	0 (0)	0 (0)	0 (0)
After diaper change§	41	13 (32)	4 (10)	2 (15)	13 (100)
Subtotal	53	17 (32)	4 (8)	3 (18)	17 (100)
After sweeping floor or yard	36	11 (31)¶	0 (0)	1 (9)	10 (91)
Before feeding the baby	32	14 (44)	0 (0)	1 (7)	10 (71)
Before handling food	51	23 (45)	1 (2)	3 (13)	22 (96)
Before eating	23	14 (61)	0 (0)	3 (21)	14 (100)
Before breastfeeding	44	19 (43)	0 (0)	0 (0)	16 (84)
Others#	11	11 (100)	2 (2)	0 (0)	11 (100)
Total	250	109 (44)	7 (3)	11 (10)	100 (92)

*Values are no. (%).
†HWWS = hand washing with soap. The denominator for HWWS and subsequent columns is the number of times of any hand washing.
‡Dried using a visibly dirty towel four times and a not visibly dirty towel five times.
§Twenty-five diaper changes were needed because of defecation and only four times were feces or diaper wash waste water disposed in a latrine. The four events of latrine disposal of feces were one for each of the age categories < 3 and 3–6 years and two for children 12–18 months of age. The two feces disposal in latrine for children 12–18 months of age were done by the same caregiver.
¶Hand washing with sand was done once.
#Include before milking the cow, after milking, after tethering goats, after feeding the child, after eating, after putting the baby to sleep, and after changing clothes.

The area where the child played was not swept in 16 (70%) of 23 households. The kitchen floor was made of cow dung or dirt in half of the households, and animals (mostly poultry) were observed in the kitchens of one-third of households on arrival. One-third of households had chicken feces on the kitchen floor and most (87%) had chicken feces in the kitchen yard. Other animal feces were also observed in 7 (30%) households. No human feces were observed in any yard. Chickens (median = 10, range = 1–31) were found freely roaming in the kitchen yards of 15–21 households. Similarly, guinea fowl (median = 8, range = 2–18) were freely roaming in yards of 4–7 households.

Hand washing and diaper change practices. In this study, hand washing was considered as the use of water with soap and/or sand as scrubbing agent. Wiping of hands with a towel or a piece of cloth without water and soap was described as wiping. Wiping was not considered hand washing. Overall, mothers washed hands 109 times during 250 triggering events (44%) but used soap only seven times (6%). Hand washing with sand was done once after sweeping the floor and yard. Mothers washed their hands after toilet use, contact with animal stool, or diaper changing on 17 (32%) of 53 occasions, but used soap during only 4 (24%) of these 17 hand washings. Mothers also washed their hands before 19 (43%) of 44 breastfeeding episodes, but did not use soap during any of these hand washings. Air-drying of hands was common but use of running water during hand washing was not (Table 3).

During 130 hours of observation, infant hands were washed 21 times: 8 of these were as part of a bath, 6 of which included soap (Table 4). Soap was not used in any of the 13 (62%) of 21 cases of infant hand washing that were not part of a bath; most (8 of 13) of these events occurred just before feeding **(**Table 4**).** Hand washing was more common for 12–18-month-old children, and in this age group none of the hand-washing events was during a bath. Among 3–12-month-old infants, hand washing occurred during a bath.

Mothers washed their hands after 13 (32%) of 41 diaper changes observed and used soap on only four occasions (Table 3). Of the 25 diaper changes involving feces, diaper water was either emptied into a latrine (4 times), yard (5 times) or a rubbish pit (12 times) (Table 5). The four times diaper waste water disposal in latrine was performed by caregivers with their own latrine, and none was reported for caregivers who shared a neighbor's latrine. The two latrine disposals for 12–18-month-old children were performed by the same caregiver. Two of the 25 times feces were buried in the garden. In two other instances, the observer was not able to see the method of disposal. Most commonly, mothers did diaper washing at the same spot/area (usually in the shade) where they did laundry.

Frequency of infant-mouth contact of potential fecal-oral vectors. Objects identified as major fecal–oral vectors by the frequency definition were infant, mother, and sibling hands; food; water; toys/play objects; and food service utensils. The objects identified as major fecal–oral vectors by most visibly dirty definition were soil and chicken feces (Table 6). Infant hands were put into the mouth a mean (SD) of 38 (38.9) times for the 20 infants and were visibly dirty during most (75%) of these episodes. Mothers' hand was put into infants' mouth less

TABLE 4
Infants hand washing practices, Shurugwi District, Midlands Province, Zimbabwe

Characteristic	Age category, months				
	< 3*	3–6	6–12	12–18	All
No. children	2	7	7	7	23
No. hand washing events, median (range)†	0.5 (0–1)	0 (0–1)	1 (0–1)	1 (0–4)	1 (0–4)
Total no. hand washing events	1	3	4	13	21
No. hand washing events as part of bath	1	3	4	0	8
Hand washing with soap	0	2	4	0	6
No. hand wiping and washing events, median (range)†	0.5 (0–1)	1 (1–1)	1 (0–2)	1 (0–5)	1 (0–5)
Total no. hand wiping and washing events	1	7	7	15	30

*Age category < 3 months had two infants observed for 2 hours each and only one hand washing event during bath.
†No. hand washing, wiped, or washed is median (range).

TABLE 5

Infant feces (diaper wash waste water) disposal, Shurugwi District, Midlands Province, Zimbabwe

Method of disposal	Age group, years				
Fecal disposal	< 3	3–6	6–12	12–18	Total no. (%)
Garbage/pit	1	4	4	3	12 (48)
Tossed in yard	0	1	3	1	5 (20)
Latrine	1	1	0	2*	4 (16)
Buried in garden	0	0	0	2	2 (8)
Not seen	1	1	0	0	2 (8)
Total, no. (%)	3 (12)	7 (28)	7 (28)	8 (32)	25 (100)

*The two feces disposed in the latrine for 12–18-month-old children were by the same caregiver.

frequently (mean [SD] = 11.3 [11.1] times in only three households), but were always visibly dirty (Table 6). Three infants ingested soil a mean (SD) of 11.3 (9.2) times, and two other infants ingested chicken feces a mean (SD) of 2 (1.4) times (Table 6). Two of the three infants who mouthed soil also put stones into their mouths.

Bacterial contamination of key vectors. As expected, all chicken feces samples were positive for *E. coli*. Compared with other potential vectors, chicken feces had far higher counts of *E. coli* and total coliforms (Tables 7 and 8). All soil samples from the kitchen yard and within reach of a crawling infant were also commonly and highly contaminated with *E. coli* (Table 7). Water samples were positive for *E. coli* in more than half of the households. However, on a per gram basis, *E. coli* counts were 3–35-fold higher in soil than in water. Total coliforms, *Enterobacteriaceae*, and total aerobic counts were 15–104-, 24–110-, and 88–947-fold higher in soil than in water based on 95% confidence intervals of the geometric means of bacterial counts. None of the food samples were positive for *E. coli*, and counts of the other bacteria were lower in food than in all other potential vectors. Mother's and infant's hand were contaminated with *E. coli* in 50% and 13% of households, respectively. The infant's cup and spoon cultures yielded *E. coli* in 5 (23%) households. Kitchen floor swabs were positive for *E. coli* in 18 (82%) households. Mean *E. coli* counts/swab for kitchen floors did not differ from those for cow dung (6,030 ± 22,286) compared with cement (5,705 ± 21,371; P ($|T| > |t|$) = 0.961).

TABLE 6

Key potential vectors, Shurugwi District, Midlands Province, Zimbabwe*

Potential vector	No. (%)	Vector-mouth episodes (mean ± SD)	% Visibly dirty†
Food‡	19 (83)	90.7 ± 70.5	32 (6/19)
Baby's hands	20 (87)	38.0 ± 38.9	75 (15/20)
Baby's cup and spoon	20 (87)	33.6 ± 20.2	25 (5/20)
Fresh fruits§	12 (52)	13.3 ± 10.3	58 (7/12)
Toys	13 (57)	13.3 ± 8.0	54 (7/13)
Mother's hands	3 (13)	11.3 ± 11.1	100 (3/3)
Soil	3 (13)	11.3 ± 9.2	100 (3/3)
Breasts	18 (78)	7.9 ± 4.5	0 (0/18)
Sibling's hands	3 (13)	7.3 ± 5.5	100 (3/3)
Water	10 (44)	3.2 ± 2.0	30 (3/10)
Stone	4 (17)	3.0 ± 2.7	100 (4/4)
Chicken feces	2 (9)	2.0 ± 1.4	100 (2/2)

*Total number of households = 23.
†Values in parentheses are no. episodes/no. infants or households where episodes occurred.
‡Refers to home-cooked food: porridge, *sadza*, bread, milk, green maize, beans, fat cook, sweet potato, pumpkin, eggs, nuts, crisps, beef, fish, and green peas.
§Guava, mangoes, and sweet reeds, although sweet reeds is not a fruit *per se*.

Daily exposure to fecal bacteria. The amount of soil ingested by a one-year-old child was estimated as the sum of soil ingested from crawling and active ingestion {[i.e., 0.25 grams (soil stuck in three fingers) × 38 (mean hand-mouth episodes) × 0.75 (frequency of visibly dirty hands)] + 11.3 (mean soil–mouth episodes) × 1.25 grams (estimate of soil ingested in an episode)} = 21.25 grams. A one-year-old child was used as a reference because the three infants who actively ingested soil had an average age of one year. We estimated chicken ingestion as mean vector–mouth episodes × amount per episode (i.e., 2 × 0.25 grams = 0.5 grams). An additional 0.5 grams was estimated from ingestion of soil with chicken feces. Similarly, average quantity of water intake per day was estimated by using a 375-mL metallic cup common among the households. A one-year-old child took at least more than a full cup of water in six hours. Our observation data was consistent with self-reports of five mothers of children more than 12 months of age. Therefore, we estimated average water intake for a one-year-old child to be 400 mL.

Using these estimates from our structured observation, a one year old child in rural Zimbabwe may typically ingest up to 1 gram chicken feces in a day, 20 grams of laundry area soil and 400 mL of water from a contaminated source. As a result the child will consume 4,700,000–23,000,000; 440–4,240 and 400–1,200 *E. coli* counts, from these sources respectively, based on the 95% confidence intervals (Table 7).

DISCUSSION

In this study, we showed that infants in rural Zimbabwe are frequently exposed to fecal indicator bacteria through daily activities. Active exploratory ingestion of soil and chicken feces had the greatest risk of fecal bacteria exposure in terms of high microbial load. Crawling on bare highly contaminated soils and kitchen floors exposes infants and young children to low but frequent dosages of fecal bacteria for most part of their active developmental stage. These novel data on fecal bacteria from soil and chicken feces identify these two factors as the predominant pathways in this study. Fecal contamination is represented by the frequency of *E. coli,* the classical inhabitant of the intestinal tract of warm-blooded animals. Ingestion of fecal bacteria may cause environmental enteropathy, the major pathway associated with poor growth in early life.[7]

Optimal hand washing practices that could mitigate fecal–oral transmission of bacteria in children were uncommon among the caregivers and children observed (Tables 3 and 4). Hand washing for children was rare in this rural setting. Children were frequently exposed to fecal bacteria by crawling on cow dung–smeared kitchen floors, on bare soil, and on chicken feces in the yard. Hand washing for older infants was only performed during bathing. Although most (78%) caregivers had some level of secondary education, hand washing with soap was rarely observed after animal or human fecal contact. These findings suggest that other socioeconomic factors and accessibility or acceptability of soap may play a role in the use of soap and other hygiene practices. The observed rate of hand washing with soap after fecal contact (7%) was much lower than that reported in a study from Bangladesh, which was based on five hours of structured observation, and reported that hand washing with soap was performed after adult caregiver defecation (33%)[16] and after adult caregiver's defecation

TABLE 7

Overall mean and number of samples (%) in each category of *Escherichia coli* counts, Shurugwi District, Midlands Province, Zimbabwe

Vector	No. samples	*E. coli* positive, no. (%)		*E. coli*	No. samples under each category of counts (%)			
		Samples positive	Households positive	Mean (95% CI)*	< 100	100–10,000	10,000–1,000,000	> 1,000,000
Food (porridge)	15	0 (0)	0 (0)	0 (0–0)	0 (0)	0 (0.0)	0 (0)	0 (0)
Water	43	14 (33)	12 (55)	2 (1–3)	13 (30)	1 (2)	0 (0)	0 (0)
Breast	36	0 (0)	0 (0)	0 (0–0)	0 (0)	0 (0.0)	0 (0)	0 (0)
Hand swabs								
Index child's left fingers	37	4 (11)	3 (14)	1 (0–2)	4 (11)	0 (0.0)	0 (0)	0 (0)
Index child's right fingers	37	2 (5)	2 (9)	1 (0–2)	1 (3)	1 (3)	0 (0)	0 (0)
Siblings dominant hand	20	1 (5)	1 (5)	1 (0–2)	1 (5)	0 (0)	0 (0)	0 (0)
Caregiver's dominant hand	43	13 (30)	11 (50)	4 (2–8)	9 (21)	3 (7)	1 (2)	0 (0)
Environmental samples								
Index child's cup and spoon	40	7 (18)	5 (23)	2 (1–4)	4 (10)	3 (8)	0 (0)	0 (0)
Kitchen floor	42	25 (60)	18 (82)	42 (14–130)	6 (14)	14 (33)	5 (12)	0 (0)
Soil								
Field soil	22	1 (5)	1 (5)	1 (0–2)	1 (5)	0 (0)	0 (0)	0 (0)
Trodden path to pit	43	17 (40)	14 (64)	5 (3–8)	12 (28)	5 (12)	0 (0)	0 (0)
Kitchen door step	43	24 (56)	16 (73)	17 (7–43)	9 (21)	15 (34)	0 (0)	0 (0)
Laundry area	43	30 (70)	18 (82)	69 (22–212)	10 (23)	16 (37)	4 (9)	0 (0)
Chicken feces	42	22 (100)	22 (100)	10.3 (4.7–22.67) m	0 (0)	1 (2)	7 (17)	34 (81)

*CI = confidence interval. Mean counts are geometric means (95% CI) colony-forming units (CFU)/gram for food, soil, and chicken feces, CFU/mL for water, and CFU/swab for breast, hand swabs, and environmental samples. No. households = 22. m = million.

and cleaning a child's bottom (23%),[17] and less than half the rate reported (17%) in a comprehensive review of formative research studies carried out in 11 low-income countries.[18] A similar low rate of hand washing with soap after fecal contact was reported in several other low-income countries.[19]

Half of the caregivers' dominant hands were positive for *E. coli* in a context where hand washing with soap after fecal contact was rarely practiced. Our findings further strengthen the need to effectively break the fecal–oral transmission route via hands through effective interventions such as hand washing with soap.[10] Similar fecal contamination frequency of mothers and children hands was also reported in Tanzania.[20] Fecal indicator bacteria contamination of mothers' and children's hands was associated with fecal indicator bacteria contamination of stored drinking water in Dar es Salaam, Tanzania, which was more contaminated than source water,[20] suggesting that water was re-contaminated by hands at the point of use. Such an association could explain contamination of drinking water with *E. coli* in half of the households in our study. Eleven of 12 households with *E. coli* in water at the point of use had improved water sources (borehole or protected well). Therefore, fecal contamination was unlikely to occur at the water sources. Fecal contamination of water increased between source and household storage in rural South Africa and Zimbabwe.[21]

TABLE 8

Overall mean and no. samples (%) in each category of other bacteria counts for selected vectors, Shurugwi District, Midlands Province, Zimbabwe

Vector	No. samples	Mean (95% CI) *	No. samples under each category of counts (%)			
			< 100	100–10,000	10,000–1,000,000	> 1,000,000
Coliforms						
Food (porridge)	15	2 (0–6)	14 (93)	1 (7)	0 (0)	0 (0)
Water	43	18 (10–33)	36 (84)	7 (16)	0 (0)	0 (0)
Kitchen floor	42	658 (324–1,340)	9 (21)	29 (69)	4 (10)	0 (0)
Trodden path to pit	43	274 (126–596)	12 (28)	28 (65)	3 (7)	0 (0)
Kitchen door step	43	639 (294–1,390)	9 (21)	29 (70)	6 (4)	0 (0)
Laundry area	43	1,880 (718–4,950)	5 (12)	22 (51)	16 (37)	0 (0)
Chicken feces	42	23.2 (10.1–53.4) m	0 (0)	0 (0)	5 (12)	37 (88)
Enterobacteriaceae						
Food (porridge)	15	4 (1–13)	14 (93)	1 (7)	0 (0)	0 (0)
Water	43	52 (27–100)	29 (67)	13 (30)	1 (2)	0 (0)
Kitchen floor	42	1,850 (1,030–3,340)	2 (5)	35 (83)	5 (12)	0 (0)
Trodden path to pit	43	1,290 (623–2, 690)	3 (7)	34 (79)	6 (14)	0 (0)
Kitchen door step	43	2,970 (1,390–6,350)	3 (7)	28 (64)	13 (30)	0 (0)
Laundry area	43	5,750 (2,560–12,900)	2 (5)	24 (56)	16 (37)	1 (2)
Chicken feces	42	29.5 (14.3–60.7) m	0 (0)	0 (0)	3 (7)	39 (93)
Aerobic counts						
Food (porridge)	15	1,420 (168–12,000)	3 (20)	8 (53)	2 (13)	2 (13)
Water	43	21,400 (10,200–45,100)	0 (0)	19 (44)	19 (44)	5 (12)
Kitchen floor	42	105,000 (94,600–117,000)	0 (0)	0 (0)	42 (100)	0 (0)
Trodden path to pit	43	1.89 (1.23–2.92) m	0 (0)	0 (0)	13 (30)	30 (70)
Kitchen door step	43	4.44 (2.49–7.93) m	0 (0)	0 (0)	8 (18)	35 (81)
Laundry area	43	20.3 (11.6–35.5) m	0 (0)	0 (0)	2 (5)	41 (95)
Chicken feces	42	1.30 b (474 m–3.58 b)	0 (0)	0 (0)	1 (2)	41 (98)

*CI = confidence interval. Mean counts are geometric means (95% confidence interval) colony-forming units (CFU)/gram for food, soil, and chicken feces, CFU/mL for water, and CFU/swab for breast, hand swabs, and environmental samples. No. households = 22. m = million; b = billion.

However, endemic dysentery among 12–24-month-old children was associated with only fecal contamination of source water and not during storage in the same study.

Although food did not appear to be a critical source of fecal indicator bacteria in this study, the infant feeding environment was frequently contaminated with fecal matter. The kitchen floor, where infant feeding commonly took place, was frequently contaminated with *E. coli* and in part chicken feces. Although hand washing with soap can be effective in breaking the fecal–oral pathway, it was an uncommon practice. Most mothers did not dry their infant's hands or restrain the baby after hand washing. It was common for infants to place their wet fingers on the kitchen floor or bare soil and then put their soiled fingers into their mouths soon afterwards. In addition, food, fruits, and objects were often picked straight up from the floor into an infant's mouth. Infant hand washing might increase the risk of fecal bacteria exposure if thorough drying of the hands and subsequent protection from dirt is not implemented.

A substantial minority of infants and young children actively ingested soil and chicken feces or licked stones from the bare yard soil. Caregivers did not stop babies from active soil ingestion. Two subsequent focus group discussions in rural Zimbabwe confirmed that babies eat soil and either fresh or dried chicken feces (Zvitambo qualitative research, unpublished data). Some mothers reported that in-laws or village elders advised soil eating because it was good for the baby's intestines or treated stomach illnesses. Ingestion of chicken feces (overall mean = 0.2 episodes per child) was less frequent than that reported among toddlers in Peru (3.9 episodes/child).[12] Many pathogenic bacteria have been isolated from chicken feces. In rural Zimbabwe, toxigenic *C. difficile* was isolated from soil and chicken feces.[11] In the study in Peru, viable *Campylobacter jejuni,* an important cause of dysenteric diarrhea, was isolated from infected chicken's feces up to 48 hours after deposition.[12] As expected, in our study, all chicken feces contained *E. coli* regardless of the time of deposition. Ingestion of chicken feces and soil containing chicken feces potentially represents a huge burden of pathogenic bacteria, which may cause diarrhea and may be important in the etiology of environmental enteropathy.[7]

This study suggests that existing wash interventions are failing to protect infants and young children from ingesting soil and feces at a critical growth and developmental stage. Interventions focusing on containing animals and prevention of exposure of children's hands from fecal bacteria from contaminated floors and yard soil are just as, if not more important than hand washing and water treatment, because these transmission pathways may lead to greater dosages of exposure. Whereas wash interventions to date have focused on hand washing, improved drinking water sources, point-of-use water treatment and improved sanitation, no attention has been given to exploratory ingestion of soil and chicken feces and geophagy. To our knowledge, no studies to date have quantified the burden of fecal bacterial ingestion by young children through geophagia, exploratory behavior or crawling on bare soil. These exposures place infants at risk of diarrheal diseases (*C. jejuni*, enteropathogenic strains of *E. coli*, and *Salmonella* species).[10,12]

Our study had several limitations. We only took one hand swab per caregiver or sibling per visit. The four swabs taken from parts of index child's hands were also taken once in one visit and were therefore not representative of a day's microbial exposure for the infant. A more extensive sampling strategy in future studies would enable variability in counts of fecal bacteria to be evaluated. In Tanzania, mother's hands were quickly re-contaminated with fecal bacteria during household activities, such as sweeping, after hand washing.[22]

Samples were taken during the dry hot season. Fresh food diversity that was common during infant observation was not observed during microbiology sampling. For example, fruits were available during the infant observation but were no longer in season during the microbiology sampling. The dry hot winter days and the nature of the soil (sandy with less than 20% silt) was a less conducive environment for survival of fecal bacteria. The bacterial counts reported in this study could therefore be much lower than would be typical during the wet season. Many of the poultry feces samples were dry and could have been exposed to the sun for hours. Therefore, the counts reported for chicken feces could be lower than those for fresh feces. However, these limitations do not negate the implications of this study.

Infants and young children in rural Zimbabwe are frequently exposed to highly contaminated surfaces such as kitchen floors and yard soils. Chicken and soil feces ingestion were identified as the predominant pathways of fecal–oral transmission of bacteria during this study.

Effective interventions should be carefully designed to break this prominent route of fecal–oral transmission. More attention should be devoted to interventions aimed at reducing animal fecal contamination of child's environment. Poultry containment in Peru was found to be intermittent even after extensive orientation and technical assistance.[23] Chicken corrals were not entirely successful at separating children from the poultry. Mothers who participated in aforementioned focus group discussions were strongly opposed to corralling chickens because of resources to feed chickens. The frequency of contamination of soil samples with *E. coli* suggests that sweeping may not be effective in reducing exposure to fecal bacteria in households where poultry are freely roaming the kitchen yard. Most of the floors and yards were swept by the time of sample collection and they were also frequently contaminated with *E. coli*. Our study showed no difference in fecal bacteria count between cemented and earthen/cow dung–smeared floors. Improvement in floor materials (such as cementing) is therefore not likely to reduce the microbial load.

This study demonstrates that existing evidence based wash interventions will not effectively eliminate fecal–oral transmission of bacteria among infants and young children. New interventions and programs are needed to address these environmental health risks that potentially diminish the benefits achievable for child hood health and growth from improved dietary interventions. A clear separation of the infant from the frequently contaminated soil without negating the child's physical and cognitive development, through restricting exploratory behavior, seems the most practical and feasible way to reduce the risk of exposure to fecal bacteria from these environmental sources. Educating mothers on personal and environmental hygiene and safe disposal of human and animal feces should complement efforts to provide a clean environment for young children.

Further research is recommended to analyze the presence of fecal pathogens, such as gastrointestinal viruses, diarrheagenic *E. coli,* and human-specific *Bacteriodales* species, by using highly specific molecular techniques. Microbial source tracking

to identify the source of fecal contamination is necessary in these settings to provide evidence of the relative importance of animal and human feces in contaminating domestic environment. Studying the distribution of fecal bacteria and pathogens within the household environment can guide future interventions on improving domestic hygiene. In addition, this distribution is critical for elucidating the causal association between poor sanitation and hygiene, exposure to fecal pathogen, and environmental enteropathy and further to poor child health and stunting. Furthermore, it is important to show evidence of the causal association between hygiene practices, diarrhea, and stunting. If diarrhea is associated with long-term linear growth faltering, the relative contribution of environmental enteropathy and diarrhea would require further clarification.

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