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Animal Feces Contribute to Domestic Fecal Contamination: Evidence from *E. coli* Measured in Water, Hands, Food, Flies, and Soil in Bangladesh

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ABSTRACT: Fecal-oral pathogens are transmitted through complex, environmentally mediated pathways. Sanitation interventions that isolate human feces from the environment may reduce transmission but have shown limited impact on environmental contamination. We conducted a study in rural Bangladesh to (1) quantify domestic fecal contamination in settings with high on-site sanitation coverage; (2) determine how domestic animals affect fecal contamination; and (3) assess how each environmental pathway affects others. We collected water, hand rinse, food, soil, and fly samples from 608 households. We analyzed samples with IDEXX Quantitray for the most probable number (MPN) of *E. coli*. We detected *E. coli* in source water (25%), stored water (77%), child hands (43%), food (58%), flies (50%), ponds (97%), and soil (95%). Soil had >120 000 mean MPN *E. coli* per gram. In compounds with vs without animals, *E. coli* was higher by 0.54 $\log_{10}$ in soil, 0.40 $\log_{10}$ in stored water and 0.61 $\log_{10}$ in food ($p < 0.05$). *E. coli* in stored water and food increased with increasing *E. coli* in soil, ponds, source water and hands. We provide empirical evidence of fecal transmission in the domestic environment despite on-site sanitation. Animal feces contribute to fecal contamination, and fecal indicator bacteria do not strictly indicate human fecal contamination when animals are present.

INTRODUCTION

Fecal-oral pathogens are transmitted from feces to new hosts through complex, environmentally mediated pathways. The complexity arises from a multitude of transmission pathways, a broad diversity of pathogens, the influence of environmental conditions and interactions between the environment and human behavior. In the absence of effective sanitation and sewerage facilities that isolate human feces from the environment, human fecal organisms can spread into fields and ambient waters. These are subsequently transported by fomites and vectors (e.g., hands, flies) into drinking water and food as well as ingested through mouth contact with contaminated hands and objects or geophagia (deliberate ingestion of soil) by young children.[1,2]

Contamination of drinking water, a direct ingestion pathway, has been studied extensively, and water treatment has been shown to improve microbiological water quality as measured by fecal indicator bacteria (FIB) and reduce self-reported diarrhea.[3,4] Other transmission pathways remain understudied even though these could present major sources of fecal exposure. For example, complementary foods for young children contain FIB in low-income country settings[5] and child diarrhea has been linked to food contamination.[6] High FIB levels are found on hands in low-income countries[7] and handwashing interventions reduce self-reported diarrhea.[8] Flies are known to carry human pathogens[9,10] and fly control programs have successfully reduced diarrheal disease.[11] FIB and pathogens have been detected in soil[12] and geophagia has been associated with diarrhea, markers of environmental enteric dysfunction, and stunting in young children.[13,14] However, it has not been documented how soil contamination affects

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subsequent contamination of ambient and drinking waters, hands and food.

Sanitation interventions are a primary barrier to disease transmission and should block enteric pathogens both by stopping feces from spreading into the environment as well as eliminating fly breeding sites. However, recent sanitation trials have shown limited health impact. Two trials in India found no effect of sanitation improvements on child diarrhea, parasite infections, and growth,[15,16] while a trial in Mali demonstrated improved child growth but no diarrhea reduction.[17] These trials also found no reduction in FIB measured in source and stored water, and on hands and fomites.[15−17] A systematic review identified no overall reductions in environmental contamination in response to sanitation improvements.[18] Possible explanations include low latrine uptake and continuing open defecation; Clasen et al. (2014) and Patil et al. (2014) reported that <50% of households in intervention villages had a functional or improved latrine, respectively, and Patil et al. found that >70% of adults in intervention villages reported daily open defecation.[15,16]

Another potential explanation for the failure of sanitation improvements to reduce domestic fecal contamination and diarrhea is residual contamination from animal feces. Sanitation programs focus on isolating human feces from the environment, typically with no measures to reduce exposure to animal feces. Many households in low-income countries keep livestock in close proximity to living quarters.[19] Microbial source tracking studies in rural India and Bangladesh suggest that fecal contamination from animals is more prevalent than human contamination in the domestic environment, including source and stored drinking water, hands, and soil.[20−22] Courtyard soil, household floors, and child hands have been shown to contain animal fecal molecular markers.[20,23] Presence of animal feces in the compound has been associated with visible dirtiness of caregivers' and children's hands and faces.[24] There is also increasing evidence that exposure to domestic animals is associated with increased diarrhea.[19] However, the contribution of animal feces to fecal contamination along different transmission pathways in settings with on-site sanitation has not been assessed.

The objectives of our study were to (1) characterize levels of fecal contamination along multiple environmental transmission pathways (source, stored and ambient waters, child hands, complementary food, courtyard soil, and flies caught in the compound) in rural Bangladeshi households, (2) determine how the presence of domestic animals, household sanitary infrastructure and ambient climate conditions affect contamination levels, and (3) assess how different environmental pathways affect each other.

MATERIALS AND METHODS

Data Collection. Our study was nested within a randomized controlled trial in rural central Bangladesh (WASH Benefits).[25] We randomly enrolled households from the trial's control arm between July 2013 and March 2014. During household visits, field workers conducted spot check observations on the presence of human and animal feces in the courtyard; human vs specific animal (cow, goat/sheep, chicken) feces were distinguished based on their visual characteristics. Field workers also administered a structured questionnaire on animal husbandry. Additionally, they observed water, sanitation, and hygiene indicators, including the cover status of the storage containers from which the drinking water and food samples were collected, presence of a handwashing station with soap and water (tubewell, pond, or container with water) within 10 m of the latrine, and presence and number of latrines in the compound and within 10 m of tubewells and ponds. They differentiated improved latrines based on Joint Monitoring Programme (JMP) categories[26] and observed whether the latrine drained into a septic tank, pit, or the environment (pond, ditch, etc.). When collecting soil samples, field staff observed whether the sampled area was visibly wet and in the sun or shade.

Sample Collection. Field workers collected samples from the compound, including tubewell water, drinking water stored in the home, pond water, child hand rinses, complementary foods given to young children, flies caught in the food preparation area, and courtyard soil from young children's outdoor play area. Samples were collected in sterile Whirlpak bags (Nasco Modesto, Salida, CA). To collect source water (tubewell) samples, field staff removed fabric or other materials attached to the tubewell mouth and flushed the tubewell by pumping five times before collecting 250 mL of water. To collect stored water, field workers asked the respondent to provide a glass of water from their primary drinking water storage container as if giving it to their children <5 years and pour 250 mL from the glass into a Whirlpak. Pond samples were collected by dipping a Whirlpak into the pond and collecting 250 mL of water from the area where the household reported most commonly accessing the pond. To sample child hands, field workers asked the respondent to place both hands of the youngest child <5 years, one at a time, into a Whirlpak prefilled with 250 mL of distilled water. Each hand was massaged from outside the bag for 15 s, followed by 15 s of shaking, and the rinsewater was preserved in the Whirlpak.[27] To collect soil samples, the respondent was asked to identify the outdoor area where the youngest child <5 years had most recently spent time. Field workers marked a 30 × 30 cm^2 area with a sterile stencil, and scraped the top layer of soil within the stencil into a Whirlpak using a sterile scoop to collect approximately 50 g of soil. To sample complementary food, field workers identified stored food to be served to children <5 years and asked the respondent to provide a small amount of food in the same manner they feed their children. Food was scooped to fill a 50 mL sterile plastic tube using a sterile spoon. Finally, field workers identified a suitable location in the food preparation area (away from the stove and smoke, under a roof or protected from rain if possible) and hung three horizontal 1.5-foot strips of nonbaited sticky fly tape. The tape was left in place for 3−6 h to capture flies. Field workers removed one fly from the center of the strip with the most flies using sterile tweezers and placed it into a Whirlpak. Clean gloves were worn to collect pond, hand rinse, soil, and food samples.

Sample Processing. Samples were preserved on ice and processed on the same day, typically within 12 h of collection. Tubewell and stored water samples were analyzed without dilution. Pond samples were diluted 1:100 and hand rinses 1:2 with distilled water. Food and soil were homogenized with distilled water using a sterile blending bag (BagFilter P, 400 mL, Interscience, Saint Nom, France) and a laboratory-scale food processor (BagMixer C, Interscience, Saint Nom, France) for 1 min at a specified mixing speed. A 10 g aliquot of food was homogenized with 100 mL of distilled water and then diluted 1:10. A 20 g aliquot of soil was homogenized with 200 mL of distilled water and then diluted $1:10^4$. An additional 5 g food and soil aliquot was oven-dried overnight to determine the

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moisture content and dry weight. Flies were homogenized with a pestle from outside the Whirlpak and mixed with 100 mL of distilled water; this slurry was diluted 1:100.

One field blank per sample collector per week, one laboratory blank per laboratory assistant per day, 10% field duplicates (two samples from one household), and 5% laboratory replicates (two aliquots from the same sample) were processed for quality control. Field workers collected two types of field blanks (1) by asking the respondent to pour distilled water from a sterile bottle into a Whirlpak and (2) by opening and shaking a prefilled Whirlpak in the field as if collecting a hand rinse. Samples were analyzed using IDEXX Quantitray with Colilert-18 media (IDEXX Laboratories, Maine, U.S.A.) and incubated at 44.5 °C for 18 h to enumerate *E. coli* with the most probable number (MPN) method. The Quantitray-2000 system with a wide detection range of 1−2419 MPN per tray was selected to accommodate variability within sample types.

Statistical Methods. We tabulated the presence/absence, $\log_{10}$-transformed counts, and geometric means of *E. coli*; we substituted the value of 0.5 MPN for samples below and 2420 MPN for samples above the detection limit to calculate the logarithm. We assessed the association between $\log_{10}$-transformed *E. coli* counts and ambient climate factors (e.g., season, sunlight and visible moisture in soil sampling area, measured soil moisture content), presence of animals, observed human/animal feces, and household sanitary infrastructure. Season was defined as wet vs dry as Bangladesh receives >80% of its rain during the monsoon season from June through October and is typically dry otherwise.[28] We also assessed the relationships between different transmission pathways by separately estimating the association of *E. coli* levels in different sample types (e.g., $\log_{10}$ increase in *E. coli* on hands for every $\log_{10}$ increase in *E. coli* in soil). We used generalized linear models with robust standard errors to account for the clustered design of the WASH Benefits trial. We assessed whether housing materials, reported income, land ownership, presence of electricity, and female education (≥1 year of formal schooling) as socioeconomic proxies were associated with the presence of animals and animal feces using chi-square tests; all models controlled for these potential confounders.

RESULTS AND DISCUSSION

Household Characteristics. Of the 699 households randomly selected from the control arm of the parent trial, we successfully enrolled 608 (87%) households with 13% lost to follow-up (7% stillbirth, miscarriage, abortion, or death of children in the target age range, 5% relocation, and 1% refusal). Among 608 enrolled households, 97% had a latrine and 68% had an improved latrine as per the JMP definition;[26] 29% of latrines drained into the environment (Table 1). The presence and number of latrines were positively associated with all proxies of higher socioeconomic status (finished walls, electricity access, above-median reported income, land ownership, ≥1 years of female education, all *p*-values <0.05) while the presence of an improved latrine was not associated with any of these proxies. Human feces were observed in 4% of compounds. Half (47%) of households had water in the latrine area while 7% had soap. Fewer than 20% of drinking water storage containers were covered in contrast to 85% of food storage containers. At least one fly was caught in the food preparation area in 32% of households.

Table 1. Characteristics of Enrolled Households (N = 608)

household characteristics	%
household water, sanitation, hygiene conditions	
latrine in compound	97
improved latrine in compound (JMP definition[a])	68
latrine flushes to environment	29
household owns child potty	17
human feces observed in courtyard	4
stored water covered	17
water present in latrine	47
soap present in latrine	7
food container covered	85
flies captured in food preparation area	32
presence of domestic animals and animal feces	
compound has animals	94
chickens	91
cows	69
goats/sheep	39
animals roam free in compound	56
animal feces observed in courtyard	89
chicken feces	87
cow feces	30
goat/sheep feces	19

[a]JMP: Joint Monitoring Programme.

Animals and Animal Feces. Almost all compounds (94%) had domestic animals and the most common animal was chickens, while 89% of compounds had observed animal feces in the courtyard and chicken feces were the most common type of feces observed. Whether or not a household had animals was not associated with socioeconomic proxies; however, households that owned land were more likely to have >1 cow or >10 chickens ($p < 0.05$). Compounds were more likely to have animal feces in the courtyard if they had unfinished (e.g., bamboo, mud) walls or no electricity ($p < 0.05$).

Fecal Contamination. We tested 3254 samples and detected *E. coli* in every sample type, including 25% of source water, 77% of stored water, 43% of child hands, 58% of complementary foods, 50% of flies, 97% of ponds, and 95% of soil (Table 2). Geometric mean *E. coli* was <10 MPN per reporting unit in drinking water, food and on hands. The geometric mean *E. coli* for flies was 663 MPN per fly. Ponds and soil had extremely high contamination; geometric mean *E. coli* was >5000 MPN per 100 mL for ponds and >120 000 MPN per dry gram for soil. Across all samples types, 5% of samples exceeded the detection limit.

Ambient Climate Conditions Vs Fecal Contamination. During the rainy season, *E. coli* was detected significantly more frequently and at higher concentrations along all pathways compared to the dry season (all $p < 0.05$ except for $\log_{10}$ *E. coli* in soil) (Figure 1). Soil *E. coli* counts were not affected by whether the soil was visibly wet at the time of sampling. However, soil samples with above-median moisture content (median = 7%, range = 0−34%) had 0.70 $\log_{10}$ MPN higher *E. coli* per dry gram ($p < 0.005$). Soil from areas sunlit at the time of collection had 0.48 $\log_{10}$ MPN fewer *E. coli* per dry gram than soil from shaded areas ($p < 0.005$).

Presence of Animals and Animal Feces Vs Fecal Contamination. Animal presence was associated with higher levels of fecal contamination along multiple pathways; soil contamination in particular was independently associated with the presence of individual animal species (chickens, goats/

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Table 2. *E. coli* Detection among Environmental Pathways

type of sample	*N*	unit	lower detection limit (MPN[a])	upper detection limit (MPN[a])	geometric mean (MPN[a])	% positive
soil	591	1 dry gram	1000−1515[b]	2.4×10^6 to 3.7×10^6[b]	125 530	95
ponds	277[c]	100 mL	100	241 900	5918	97
tubewells	563	100 mL	1	2419	1	25
flies	193[d]	1 fly	100	241 900	663	50
child hands	584	2 hands	5	12 095	7	43
stored water	497	100 mL	1	2419	9	77
food	549	1 dry gram	1−8[e]	2426−20 158[e]	2	58

[a]MPN: Most probable number. [b]Corresponds to lower limit of 1000 MPN and upper limit of 2 419 000 MPN per wet gram given soil moisture content range of 0−34%. [c]Approximately half of households reported accessing a pond (typically to wash dishes and clothes). [d]A fly was captured in one-third of households. [e]Corresponds to lower limit of 1 MPN and upper limit of 2419 MPN per wet gram given food moisture content range of 3−88%.

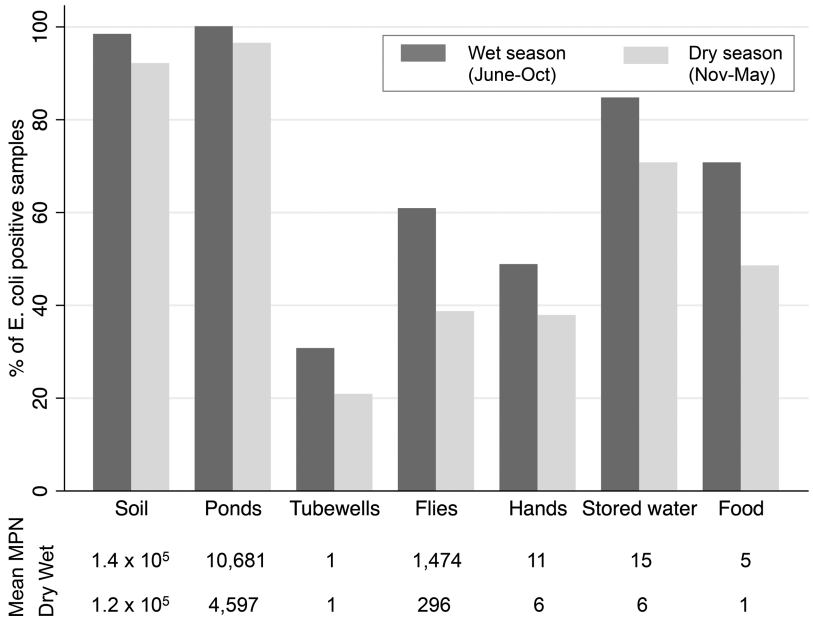

Figure 1. *E. coli* detection during wet season (Jun−Oct) vs dry season (Nov−May). The *y*-axis shows the percentage of *E. coli* positive samples. Geometric mean *E. coli* counts are displayed beneath the bars.

sheep, cows) as well as the presence of any animal in the compound (Figure 2). Compounds with animals had 0.54 $\log_{10}$ MPN higher *E. coli* in soil, 0.40 $\log_{10}$ MPN higher *E. coli* in stored water, and 0.61 $\log_{10}$ MPN higher *E. coli* in food (all $p < 0.05$). This was primarily driven by the presence of chickens; compounds with chickens had 0.70 $\log_{10}$ MPN higher *E. coli* in soil, 0.49 $\log_{10}$ MPN higher *E. coli* in stored water, and 0.40 $\log_{10}$ MPN higher *E. coli* in food (all $p < 0.05$). Compounds where animals roamed freely had 0.22 $\log_{10}$ MPN higher *E. coli* in soil and 0.27 $\log_{10}$ MPN higher *E. coli* in ponds (all $p < 0.05$) than compounds with no animals at all or no free-roaming animals. Food had 0.32 $\log_{10}$ MPN higher *E. coli* in compounds where ≥1 fly was captured in the food preparation area ($p = 0.02$).

Similarly, the presence of animal feces in the courtyard was significantly associated with increased contamination in the domestic environment; especially soil *E. coli* was independently associated with the presence of feces from individual animal species as well as the presence of any animal feces in the compound (Figure 2). Compounds with animal feces had 0.55 $\log_{10}$ MPN higher soil *E. coli*; the increase was 0.51 $\log_{10}$ for chicken feces, 0.33 $\log_{10}$ for goat/sheep feces and 0.25 $\log_{10}$ for cow feces (all $p < 0.05$). Animal feces were associated with higher levels of *E. coli* in ponds and food as well. Surprisingly, the presence of animal feces was associated with lower *E. coli* levels in tubewells and not associated with *E. coli* levels on flies. Because human feces were observed very infrequently (4% of households), we did not have sufficient statistical power to assess associations with this variable.

Sanitary Infrastructure vs Fecal Contamination. The presence of a latrine was associated with significantly lower *E. coli* in soil and ponds, while the presence of an improved latrine was associated with reduced contamination of ponds, and a higher number of latrines in the compound was associated with reduced contamination of soil, child hands and stored drinking water (Figure 2). In contrast, ponds had increased *E. coli* if there was a latrine within 10 m ($\Delta\log_{10}$=0.21, 0.02−0.41) or if the latrine was observed to drain into the environment ($\Delta\log_{10}$ = 0.22, 0.00−0.45) or directly into the pond ($\Delta\log_{10}$ = 0.30, 0.13−0.47). The presence, number, improved vs unimproved status, proximity or drainage location of latrines in the compound was not associated with tubewell water quality or *E. coli* on flies.

Associations between Pathways. Contamination levels along different environmental pathways were associated with each other (Figure 3). Pond *E. coli* increased for each $\log_{10}$ *E. coli* increase in soil ($\Delta\log_{10}$ = 0.13, 0.03−0.23). *E. coli* on child hands increased for each $\log_{10}$ *E. coli* in soil ($\Delta\log_{10}$ = 0.07,

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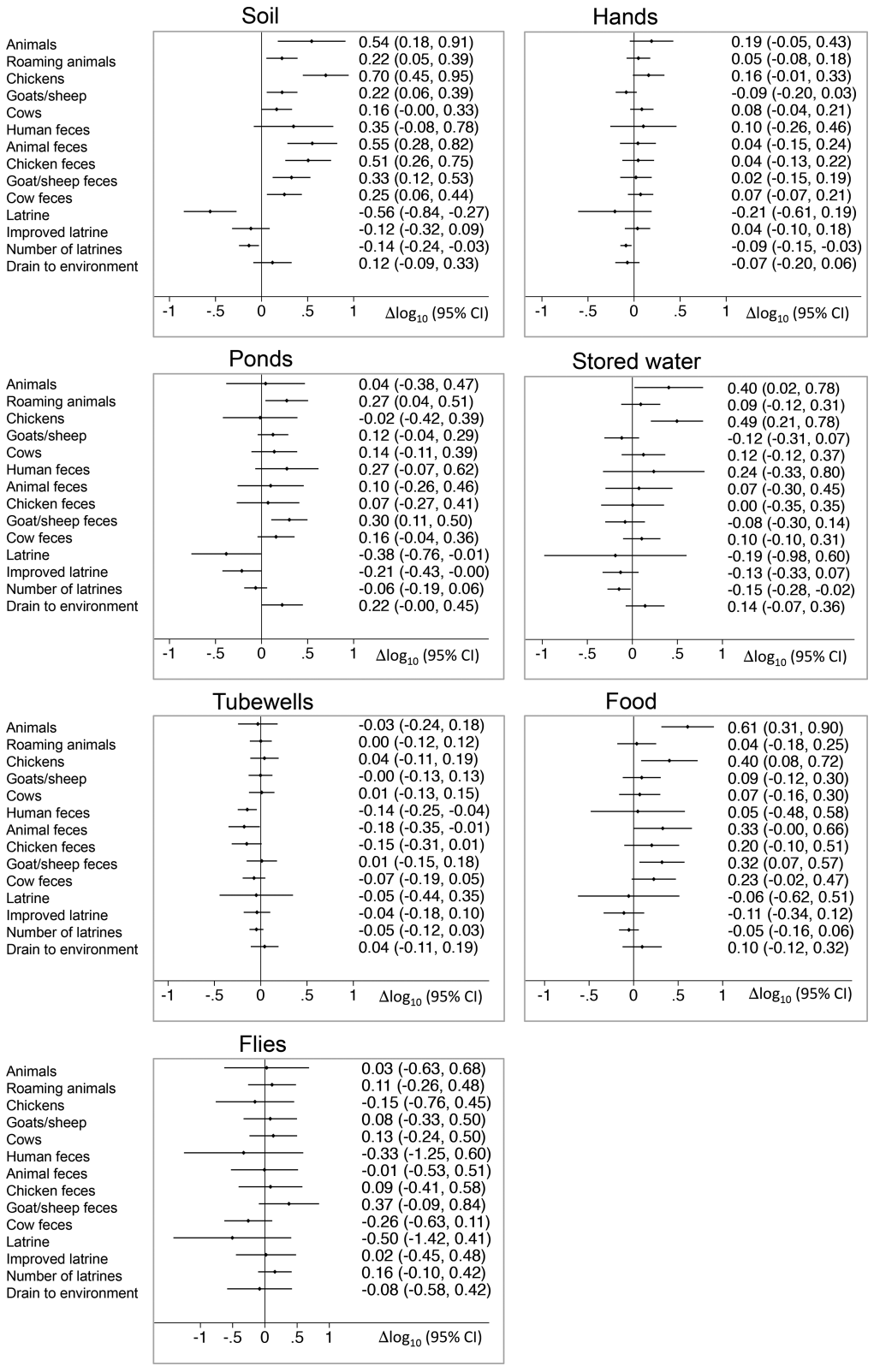

Figure 2. Increase in $\log_{10}$ *E. coli* associated with the presence of animals, animal and human feces, and sanitary infrastructure in compound.

0.01−0.12) and ponds ($\Delta\log_{10}$ = 0.15, 0.05−0.25). *E. coli* in stored water increased for each $\log_{10}$ *E. coli* in soil ($\Delta\log_{10}$ = 0.15, 0.06−0.24), ponds ($\Delta\log_{10}$ = 0.27, 0.09−0.44), hands ($\Delta\log_{10}$ = 0.21, 0.08−0.33), and source water ($\Delta\log_{10}$ = 0.39, 0.23−0.55). Finally, *E. coli* in food increased with each $\log_{10}$ *E. coli* in all other pathways, including soil ($\Delta\log_{10}$ = 0.12, 0.02−0.22), ponds ($\Delta\log_{10}$ = 0.28, 0.08−0.48), hands ($\Delta\log_{10}$ = 0.18, 0.04−0.32), source water ($\Delta\log_{10}$ = 0.21, 0.05−0.37), stored water ($\Delta\log_{10}$ = 0.28, 0.17−0.39), and flies caught in the food preparation area ($\Delta\log_{10}$ = 0.21, 0.08−0.34).

Discussion. We found ubiquitous fecal contamination along multiple environmentally mediated pathways in rural Banglade-

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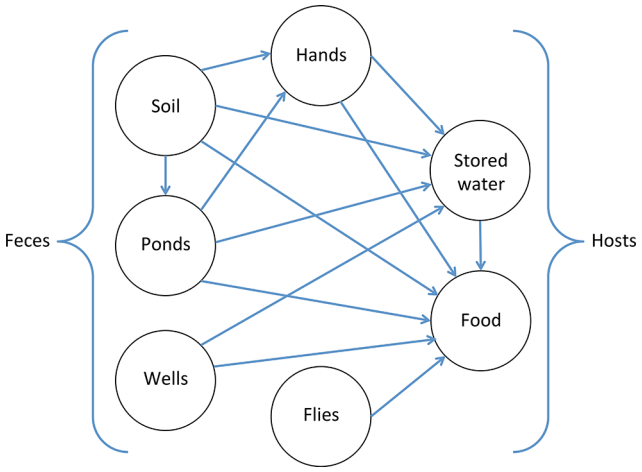

Figure 3. Associations between environmental transmission pathways, measured as increase in $\log_{10}$ *E. coli* on a pathway associated with each $\log_{10}$ increase in *E. coli* along another pathway. Arrows indicate associations that are significant at the $p < 0.05$ level; the lack of an arrow between two sample types indicates that we did not observe a significant association.

shi households with on-site sanitation access. We detected *E. coli* in 25% of tubewells, compared to 77% of stored drinking water, supporting prior evidence on tubewell water quality in Bangladesh[29−31] and subsequent contamination at the point-of-use.[32,33] We found *E. coli* on 43% of child hands. Hand contamination levels were similar to findings from Tanzania[34] and urban Bangladesh[23] but much higher than in high-income countries.[35] *E. coli* was detected in 58% of complementary food, consistent with previous studies in Bangladesh.[6,36,37] Among flies captured at the food preparation area, 50% had *E. coli*, which could have been present on the outside or in the gut of the fly. Soil and ponds had high levels of *E. coli*, suggesting these are major reservoirs for fecal organisms. Soil *E. coli* levels in our study were substantially higher than previously documented in Tanzania and Zimbabwe.[12,38] Our method may have had higher recovery efficiency since it did not require a settling step like the protocol used in the Tanzania study. Our results could also indicate heavier fecal input into the environment due to the high population density of Bangladesh and/or enhanced bacterial growth in soil due to the wet climate and high groundwater table in Bangladesh; saturated subsurface conditions favor the transport, survival and growth of microorganisms.[39] This is consistent with our finding that soil with higher moisture content had higher *E. coli* counts. However, soil in our study was also more contaminated than measured in a similar rural Bangladeshi setting by enumerating soil homogenates on Petrifilm without a settling step;[13] one reason for this could be our method's high upper detection limit (2 419 000 MPN per wet gram of soil). We also found associations between *E. coli* levels measured in soil, ponds, groundwater, hands, flies, stored water, and food. Previous evidence supports these findings; however, few studies have explored associations between several different pathways. Ambient (pond) water quality has been shown to affect groundwater quality.[40] Source water quality, in turn, is a known determinant of stored water quality.[41] A link between contamination of stored water and hands has also been demonstrated.[22,34,42]

Our findings suggest that animal feces contribute more substantially to domestic fecal contamination in rural Bangladeshi households than human feces; 4% of compounds had observed human feces in the courtyard vs almost 90% having animal feces. This is not surprising considering that 97% of enrolled households had a latrine. Open defecation is commonly practiced by young children in this setting;[43] nonetheless, our infrequent observation of human feces indicates that child feces are removed from the compound's living area. Indeed, other work in our study area indicated that, among households where child feces are not disposed of in a latrine, 64% reported disposing of them in the bushes surrounding the compound, 18% in open waste heaps and 13% in drains while only 11% left the feces on the ground.[44] However, human feces likely contribute to domestic fecal contamination through other routes, such as latrines draining into ponds/canals or pits leaking into the environment. Indeed, ponds with a latrine within 10 m and ponds receiving latrine effluent had higher *E. coli* levels in our study, consistent with previous evidence from rural Bangladesh.[40,45]

Chickens presented the most prevalent domestic animal exposure in our study. Roughly 90% of compounds had chickens, followed by cows (69%) and goats/sheep (39%). Similarly, 87% of courtyards had chicken feces, followed by cow feces (30%) and goat/sheep feces (19%). Chickens typically roam and deposit feces throughout the compound while scavenging for food,[46] and because their feces are small and relatively odorless, they are likely to be left in place, even though some households collect chicken feces to use as fertilizer.[47] Cow dung is often collected and used as cooking fuel and housing material in rural Bangladesh.[48] This could explain the relatively low prevalence of cow feces; while 69% of compounds had cows, only 30% had observed cow feces.

Due to the infrequency of observed human feces, we had limited power to detect associations between this exposure and *E. coli*. Animal feces were associated with increased contamination of soil, ponds and complementary foods. The association with food contamination might indicate that, when preparing food, caregivers do not wash hands after handling animal feces. Previous work in Bangladesh found that, during food preparation, caregivers feed dung cakes to the fire with bare hands and resume food handling or feed children without washing hands.[49] However, while dung cakes are moist when handled to form them for subsequent use, they are sun-dried before being used as fuel, and desiccation should substantially reduce pathogen concentrations.[50] Surprisingly, the presence of animal (as well as human) feces was associated with lower tubewell contamination. Tubewells in rural Bangladesh are typically located on the periphery of the compound rather than in the central courtyard area. Animal and child feces are often disposed of in bushes or open waste heaps on the compound periphery as well. Observed feces in the courtyard area could indicate that feces have not been disposed of near the tubewell, where they could more easily infiltrate into the well. *E. coli* on flies was not associated with animal feces in the courtyard, potentially indicating that flies can acquire fecal contamination from distal sources beyond a given compound.

Evidence from microbial source tracking supports the contribution of animal feces to domestic fecal contamination in our study setting. A subset of 500 stored drinking water, child hand and soil samples from our study were analyzed by quantitative polymerase chain reaction (qPCR) for human, ruminant and avian molecular fecal markers.[20] Over 50% of soil and hands and 22% of stored water contained ruminant markers while the avian marker was detected in 33% of soil,

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16% of hand rinses and 9% of water samples.[20] Ruminant and avian markers were more commonly detected in compounds that had ruminants and birds, respectively,[20] consistent with our finding of higher *E. coli* levels in compounds with animals. In contrast, human fecal markers were detected in 9% of soil, 2% of hand rinse and none of the water samples.[20] Others have reported similar findings. A recent study in India detected animal fecal markers in 75% of ponds, 15% of tubewells, 52% of stored water, and 96% of hands in contrast to human markers detected in 8% of ponds, 2% of tubewells, 20% of stored water and 37% of hands.[22] A similar study found evidence of animal contamination in 70% of households in rural India compared to human contamination in 35%, based on testing stored drinking water and hands.[21] Ruminant fecal markers have also been detected on child hands and household floors in urban Bangladesh.[23]

The high prevalence of animals and animal feces in our study and their associations with fecal contamination in the domestic environment suggest that animals can be a source of fecal pathogen exposure. A previous study in Bangladesh found 8.5 $\log_{10}$ MPN *E. coli* and 7.8 $\log_{10}$ MPN *Enterococcus* per gram of chicken feces[23] and 6.8 $\log_{10}$ MPN *E. coli* and 3.8 $\log_{10}$ MPN *Enterococcus* per gram of cow feces.[23] Animal feces also carry pathogens that infect humans, such as pathogenic *E. coli*, *Salmonella* and *Campylobacter*.[50] A study in Ecuador found that 76% of chickens were positive for *Campylobacter*;[51] *Campylobacter* can persist in chicken feces for days after deposition.[38,52] Animal feces pose variable levels of human health risk, depending on the prevalence of human-infective pathogen strains in the host species.[53−55] A study in rural India found similar odds of diarrhea associated with animal and human fecal markers in the domestic environment.[21] Identical strains of *Campylobacter* were isolated from the feces of children and chickens in Ecuador, suggesting zoonotic transmission.[51] Pathogens can be transmitted from animal feces to human hosts through direct and indirect routes. Previous studies have observed children ingesting chicken feces.[38,52] Structured observations of 148 children in our study demonstrated that roughly 20% of young children touched animal feces but direct ingestion was rare (<3%).[56] However, up to 35% of children placed soil in their mouth or put their hands in their mouth without handwashing after touching soil.[56] Compounds with animals had higher levels of soil contamination in our study, as well as higher contamination of stored water and food. Taken together, these findings suggest that animal feces are a source of fecal exposure for children in this setting. Environmental pathways, including highly contaminated soil, potentially mediate transmission by direct and indirect ingestion.

Our findings are consistent with an emerging body of literature that exposure to domestic animals, especially chickens, is associated with increased risk of enteric infection and adverse child growth. A meta-analysis found associations between diarrheal infections and domestic animal exposure, with an almost 3-fold increase in *Campylobacter* infections associated with poultry exposure.[19] The presence of animal fecal markers in the household environment was associated with an over 4-fold increase in the odds of child diarrhea in rural India; the magnitude of the effect was similar to that observed for the presence of human markers.[21] The presence of animal feces was associated with lower height-for-age in children in Ethiopia and Bangladesh.[24] In rural Ethiopia, while poultry ownership was associated with improved child growth (presumably by providing nutrition-rich foods such as eggs), corralling poultry inside the home overnight was associated with growth faltering; indoor corralling of other domestic animals was not associated with adverse growth outcomes.[46] Similarly, keeping animals in the room where children sleep was associated with environmental enteric dysfunction scores and stunting among rural Bangladeshi children; chickens were the most common animal corralled in the sleeping area (61%) followed by cows (39%).[57] In Peruvian shantytowns, children living in households with chickens were at increased risk of *Campylobacter* infections;[58] an intervention to corral chickens in an attempt to reduce children's exposure to feces deposited by free-ranging chickens substantially increased rather than decreased the risk of *Campylobacter*-related diarrhea compared to letting the chickens free-range.[59] This could have been due to exposure to concentrated rather than dispersed fecal matter from chickens. In contrast, cow exposure was not associated with child diarrhea or growth in rural India.[60]

Limitations. One limitation of our study is that *E. coli* is an imperfect proxy for fecal contamination. It has been suggested that tropical soils and waters can harbor naturally present *E. coli*;[61,62] these are phenotypically identical to *E. coli* from fecal sources and can only be distinguished by genotypic comparison.[63,64] While soil collected from compounds with animals and observed animal feces consistently had higher levels of *E. coli* in our study, the level of contamination in compounds without animal feces was still high (4.7 $\log_{10}$ MPN). This could indicate that soil accumulates fecal indicator contamination beyond the immediate contribution of feces observed at the time of sampling; however, it could also point to the presence of naturally present *E. coli*. Soilborne *E. coli* can persist and multiply outside animal hosts, especially in warm and moist tropical conditions; when incubated at 30−37 °C in the laboratory, naturally present *E. coli* can grow in soil to concentrations of ∼5 $\log_{10}$ per gram (similar to the soil *E. coli* levels in our study).[65] However, testing of a subset of our soil samples with biochemical assays, phylogrouping and PCR detection of genes associated with enteric vs environmental origin showed no differences between *E. coli* isolates in soil vs those from fecal samples collected from cattle, chickens, and humans in the study area.[66] Additionally, qPCR testing of our soil samples for microbial source tracking markers revealed high prevalence of ruminant and avian fecal molecular markers, providing evidence for contamination of fecal origin from animal sources, while human fecal markers were rare.[20] This evidence suggests that, while *E. coli* can signal fecal contamination, its presence should not be interpreted as evidence of strictly human fecal contamination when animals are present.

E. coli is also imperfectly correlated with the presence of fecal pathogens.[50] The associations we observed between animal feces and *E. coli* therefore do not provide standalone evidence for pathogen transmission from animal feces to the domestic environment.[67] Multiplex PCR testing of a subset of *E. coli*-positive food and fly samples from our study found pathogenic *E. coli* genes in 14% of *E. coli*-positive food and 2% of *E. coli*-positive flies.[68] A previous study in rural Bangladesh found that among tubewells with 1−10 MPN/100 mL *E. coli* (similar to our tubewell *E. coli* levels), pathogenic *E. coli* was detected by qPCR in 21%, rotavirus in 57%, *Shigella* in 7% and *Vibrio cholerae* in 7% of wells.[69] Another study in a similar Bangladeshi setting found that, while 97% of soil samples were positive for *E. coli*, only 14% contained pathogenic *E. coli* detected by

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multiplex PCR.[13] However, despite its limitations as an indicator organism, *E. coli* is used globally to monitor microbiological contamination.[70−72] A systematic review has demonstrated that *E. coli* in drinking water is associated with diarrhea, supporting its use as an indicator for diarrhea-causing pathogens.[73]

Another limitation is that we collected all environmental samples simultaneously; we therefore cannot ascertain the causal direction of observed associations. For example, we can only hypothesize that soil contamination preceded the associated contamination of hands, stored water and food, and not vice versa. However, while we cannot directly compare levels of contamination along the different pathways due to different reporting units and detection limits, the environmental media we would expect to be more proximal to fecal sources (e.g., soil, ponds, flies) were more heavily contaminated than those further down the transmission pathway (e.g., hands, stored water, food). Similarly, all molecular fecal markers had higher prevalence in soil than on hands or in stored water,[20] although it is difficult to compare PCR results across sample types because of differential recovery efficiency. This pattern is consistent with the assumption that contamination would progressively decrease as we move further from fecal sources due to limited transfer efficiencies of transmitting vectors/fomites. One could therefore assume that heavily contaminated soils and ponds led to the lower levels of contamination observed on hands, stored water, and food, and not vice versa.

A related limitation is that we sampled each household once. Domestic contamination levels vary temporally, even within 1 day;[74] this variation is not captured by our one-time sampling. *E. coli* counts in duplicate samples collected simultaneously from the same household in 10% of households were correlated 69−79% depending on sample type; repeat samples collected at different times would likely exhibit greater variability.

Finally, our analysis was observational and therefore susceptible to confounding. A recent study in India found that animal ownership was associated with higher socioeconomic status.[60] Almost all compounds in our study owned domestic animals. Socioeconomic proxies were not associated with animal ownership but significantly associated with the presence of animal feces. We controlled for housing materials, reported income, land ownership, presence of electricity, and caregivers' education in all models; however, it is possible that residual confounding remained in our analysis.

CONCLUSIONS

We provide a novel assessment of fecal contamination along several different pathogen transmission pathways in the household environment in a setting with high on-site sanitation coverage. Our findings demonstrate widespread fecal contamination. The presence of animals and animal feces, especially chickens, was associated with domestic fecal contamination. It is likely that animal feces are a dominant source of fecal contamination in low-income country settings with high sanitation coverage and low rates of open defecation; under these circumstances, fecal indicator bacteria will be poor proxies for human fecal contamination. Intervention studies on hygienic removal of animal feces from children's environment can assess whether reducing exposure to fecal contamination from animal sources can reduce child enteric illness.

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Notes

The authors declare no competing financial interest.

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