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Associations between floor material and *E. coli* contamination in rural Bangladeshi households

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ABSTRACT

Background: Soil floors are common in low-income countries and can harbor contamination from fecal waste. Soil/dust ingestion from floors or indirectly via hands, water and food can contribute to children's ingestion of fecal organisms. We assessed if finished (e.g., concrete) floors are associated with lower *E. coli* contamination in the domestic environment in rural Bangladesh.

Methods: We collected samples from 1864 households over 3.5 years, including stored drinking water, child and caregiver hand rinses, courtyard soil, food, and flies (n = 24,118 samples), and enumerated *E. coli* using IDEXX Quanti-Tray/2000.

Results: Controlling for socio-demographics, water/sanitation status, and animal ownership, households with finished floors had slightly lower log10-transformed *E. coli* counts (Δlog10 = −0.10 (−0.20, 0.00)) and prevalence (prevalence ratio [PR] = 0.90 (0.83, 0.98)) on child hands than households with soil floors; floor material was not associated with contamination levels in other sample types. In subgroup analyses, finished floors were associated with lower *E. coli* on child hands following heavy rainfall (Δlog10 = −0.23 (−0.39, −0.07)), above-median temperature (Δlog10 = −0.18 (−0.30, −0.06)), and in households with more domestic animals (Δlog10 = −0.16 (−0.32, −0.01)). Finished floors were also associated with slightly lower contamination of stored water following heavy rainfall (PR = 0.89 (0.81, 0.99)) and above-median temperature (PR = 0.91 (0.84, 0.98)), and lower contamination of stored food following higher rainfall and temperature but the associations for food were not statistically significant.

Discussion: Measures to control enteric infections in low-income countries should test flooring improvements to reduce exposure to fecal contamination.

1. Introduction

In settings where fecal waste is not well contained, children are frequently exposed to pathogens and experience a high burden of enteric infections and growth faltering (Petri et al., 2008). Enteropathogens are transmitted from the feces of infected humans or animals to the gastrointestinal tract of new hosts through various environmental pathways (Kawata, 1978). In particular, fecally contaminated soil in the

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domestic environment is increasingly recognized as a major source of pathogen exposure for young children in low-resource settings (Shivoga and Moturi, 2009). Children have frequent hand and object contact with soil and can accidently or deliberately ingest soil and/or dust (Kwong et al., 2016; Ngure et al., 2013), contributing substantially to their ingestion of fecal organisms (Kwong et al., 2020). Soil ingestion has been associated with increased risk of diarrhea, environmental enteric dysfunction and stunting in children (Shivoga and Moturi, 2009; Morita et al., 2017; George et al., 2015; Bauza et al., 2017, 2018).

Many homes in low-income countries have floors made of soil (Colston et al., 2024), and children may play, eat and sleep on soil floors, presenting a potentially substantial soilborne exposure pathway. Soil floors in low-income countries can harbor soil-transmitted helminths and other parasites, including hookworm (Halliday et al., 2019), *Ascaris lumbricoides* (Krause et al., 2015; Basualdo et al., 2007; Benjamin-Chung et al., 2015), *Strongyloides stercoralis* (Hall et al., 1994), and *Giardia duodenalis* (Basualdo et al., 2007; Benjamin-Chung et al., 2015; Hall et al., 1994; Cociancic et al., 2020). The eggs of some soil-transmitted helminths (e.g., *A. lumbricoides*) can remain infectious in soil for several months under favorable conditions, perpetuating infections (Brooker et al., 2006). Studies have also detected high levels of fecal indicator bacteria on interior and exterior soil floors (Exum et al., 2016; Navab-Daneshmand et al., 2018; Pickering et al., 2012; Montealegre et al., 2018), and fecal contamination of outdoor courtyard soil has been associated with increased contamination along other fecal-oral transmission pathways, including child hands, stored drinking water and food (Ercumen et al., 2017).

We hypothesize that a finished (e.g., concrete) floor may reduce hand and object contact with indoor soil and accidental and/or deliberate soil ingestion by young children, as well as provide a durable cleanable surface that does not support pathogen survival. Interventions to improve housing conditions have been associated with improved health outcomes, including respiratory and mental health (Thomson et al., 2009). Specifically, having finished floor materials as opposed to soil floors has been associated with a range of health benefits in children. A 2023 meta-analysis concluded that soil household floors are a potential contributor to enteric and parasitic infections in low- and middle-income countries (Legge et al., 2023). Replacing soil floors with finished floors in a large-scale Mexican program reduced diarrhea by 13 % and parasite infections by 20 % and improved child cognitive development (Cattaneo et al., 2009). In Zimbabwe, improved household flooring reduced infant diarrheal illness irrespective of the quality of the household's water or sanitation (Koyuncu et al., 2020). Few studies have linked indoor floor material to environmental contamination across multiple exposure pathways to substantiate the observed health effects associated with improved floors and investigate the environmental mechanisms behind them. Filling this gap in the mechanistic understanding of why floor material might affect microbial transmission in children's environments can inform the development of targeted interventions in homes with soil floors.

Here, we use data from a randomized controlled trial in Bangladesh (WASH Benefits) to assess associations between floor material and fecal indicator bacteria measured along several pathways in the household environment. The WASH Benefits trials assessed the effects of individual and combined water quality, sanitation, handwashing (WASH) and nutrition interventions on child health in rural Kenya and Bangladesh. In Bangladesh, the WASH interventions reduced child diarrhea and enteric infections by 31–40 % (Luby et al., 2018; Lin et al., 2018; Ercumen et al., 2019; Contreras et al., 2022), but did not improve child growth (Luby et al., 2018). In Kenya, the WASH intervention reduced *A. lumbricoides* infections by 22 % (Pickering et al., 2019a), but had no effect on *Giardia* infections, diarrhea, and child growth (Pickering et al., 2019a; Null et al., 2018). In both countries, the WASH interventions achieved limited reductions in environmental contamination with fecal indicators and pathogens, and the domestic environment remained heavily contaminated despite the interventions (Ercumen et al., 2018a, 2018b; Fuhrmeister et al., 2020; Contreras et al., 2021; Swarthout et al., 2024). These findings prompted calls for "transformative" interventions that more effectively interrupt infectious disease transmission (Cumming et al., 2019; Pickering et al., 2019b).

An observational analysis of child health data from both the WASH Benefits Bangladesh and Kenya trials showed that children in homes with finished floors had 38–67 % lower prevalence of soil-transmitted helminths and 18–22 % lower prevalence of *Giardia duodenalis* infections compared to children in homes with soil floors (Benjamin-Chung et al., 2021). To examine the environmental mechanisms behind these associations, here we use data from a subset of households enrolled in the WASH Benefits Bangladesh trial to assess associations between finished vs. soil floors and fecal contamination along various fecal-oral transmission pathways, including child and caregiver hands, stored drinking water, food, courtyard soil and flies.

2. Methods

2.1. Trial design

WASH Benefits included two cluster-randomized trials that provided WASH and child nutrition interventions -alone and in combination-to rural households in Kenya and Bangladesh (Arnold et al., 2013). In Bangladesh, participants were selected from the rural villages of Gazipur, Kishoreganj, Mymensingh, and Tangail districts in central Bangladesh. Households where pregnant women in their first or second trimester lived ($N = 5551$) were selected for enrollment. Groups of 6–8 proximate enrolled households were organized into clusters, and eight adjacent clusters were further grouped into geographic blocks. Within each study block, clusters were randomized into intervention vs. control groups. The trial design and specifics of the interventions have been previously reported (Arnold et al., 2013).

2.2. Assessment of floor material

Household characteristics, including floor material, were recorded at enrollment and at one and two years after intervention initiation when the trial visited households to record child health outcomes. Field staff observed the main floor material of the room where the enrolled pregnant woman or the index child (child who was in utero at enrollment) slept. Floors were considered finished if the most common floor material was wood, tile, or concrete, and unfinished if the floor material was entirely or mostly made of soil.

2.3. Environmental sub-studies

Environmental contamination measures were pre-specified as intermediate outcomes of the WASH Benefits trial. We used data from two sub-studies that measured environmental contamination among a subset of households enrolled in the trial (Ercumen et al., 2018b; Contreras et al., 2021). The first sub-study was conducted on average 4 months after the initiation of interventions and enrolled all 1840 households from the combined WASH, sanitation and control arms of the trial for a cross-sectional assessment (Ercumen et al., 2018b). Samples were collected from stored drinking water, food given to young children, hand rinses from index children, courtyard soil, flies captured near the kitchen, groundwater from tubewells, and ponds adjacent to enrolled households. The second sub-study was conducted between 1 and 3.5 years after the initiation of interventions and enrolled a random subset of 720 households from the sanitation and control arms of the trial for eight longitudinal assessments (Contreras et al., 2021). Samples were collected approximately quarterly from stored drinking water, food given to young children, hand rinses from index children and their caregivers, and courtyard soil. We combined data from these two sub-studies, resulting in nine rounds of environmental measurements from 1864 unique households over approximately 3.5 years. The present analysis is focused on samples of stored drinking water, food, index child and caregiver hand rinses, courtyard soil and flies. We excluded tubewell and pond samples from our analysis because we did not expect indoor floor materials to affect contamination in these sample types. Across the nine rounds of environmental measurements, stored drinking water and child hand rinses were collected at all rounds, caregiver hand rinses were collected at rounds 2–9, food and soil were collected at rounds 1, 4 and 5, and flies were collected at round 1 only (Table S1).

Sample collection: All samples other than food were collected using sterile Whirlpak bags (Nasco, Modesto, CA). Food samples were collected in a locally procured sterile plastic tube that contained a sterile spoon inside. A fresh bag or tube was used for each sample. We used sterile technique when collecting samples and followed previously published sampling methods (Ercumen et al., 2015; Pickering et al., 2010) when available.

Stored drinking water. Field staff asked participants to supply a glass of drinking water from their storage container the same way they would give it to a child <5 years old. Participants were instructed to pour the water into a sterile Whirlpak bag (Nasco, Modesto, CA) to collect approximately 150 mL of sample. If respondents reported using chlorine to treat their water, sodium thiosulfate was utilized to neutralize any residual chlorine.

Stored food. Food samples were collected by asking participants to provide a small portion of solid stored food in the same manner they feed their children, with a preference for rice. The food was scooped to fill a 50 mL sterile plastic tube using a sterile spoon.

Child and caregiver hand rinses. Hand rinses were collected from index children and their caregivers. A hand rinse was collected from the youngest child <5 years if the index child was unavailable. Sampled individuals placed one hand at a time into a sterile Whirlpak bag prefilled with 250 mL of distilled water. The hand was massaged from outside the bag for 15 s and shaken for 15 s. The same procedure was repeated for the other hand within the same bag, and the rinse water was preserved.

Courtyard soil. Soil samples were obtained from a 30 × 30 cm area in the courtyard at the entrance of the enrolled household. Field workers designated the area using an alcohol-sterilized metal stencil and used a sterile plastic scoop to scrape the top 1–2 mm of soil within the stencil once vertically and once horizontally into a sterile Whirlpak bag to obtain approximately 50 g of soil. They avoided collecting large debris (leaves, twigs); the samples were not sieved or homogenized prior to transport. To prevent cross-contamination, each tube containing a soil sample was placed in an individually sealed plastic bag, and the bags were placed in a different cooler than other sample types.

Captured flies. Field workers horizontally hung three 1.5-foot strips of non-baited sticky fly tape at suitable location (away from stove smoke, protected from rain, typically under a roof) in the kitchen and latrine areas. After 3–6 h, field workers returned to the household and removed one fly from the center of the strip with the highest number of flies using alcohol-sterilized tweezers and placed it into a sterile Whirlpak bag.

Quality control. Field workers collected 10 % field blanks for stored drinking water and hand rinse samples. For water samples, blanks were collected by transferring 100 mL of sterile water into the sterile Whirlpak in the same manner the water samples were collected. For hand rinse samples, blanks were collected by opening the pre-filled Whirlpak and performing the massaging and shaking steps without immersing a child or caregiver hand in the Whirlpak. No field blanks were collected for food, soil, and fly samples because sterile versions of these sample types to be used as blanks could not be generated. However, we expect that the water and hand blanks captured contamination risks from sample collection and transport procedures.

All samples were placed in coolers with ice packs and transported at 2–10 °C to the field laboratory of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) without preservatives or stabilizers.

Sample processing: Samples were processed within 12 h of collection. We used IDEXX Quanti-Tray/2000 to enumerate the most probable number (MPN) of *E. coli*. For water samples, we followed EPA Standard Methods 9223 B. For other sample types, we used modifications on this method based on pre-piloting. Specifically, for each sample type, an optimal dilution factor was determined by pilot testing to achieve counts in the quantifiable range of Quanti-Tray/2000 (1–2419.6 MPN) to avoid samples that are non-detect or exceed the upper limit of quantification. The range of quantification differed between sample types because different sample amounts and dilutions were used (Table S2). For solid samples, we also optimized homogenization steps based on pre-piloting.

Liquid samples (stored water, hand rinses). 100 mL aliquots of stored water samples were analyzed without dilution. For hand rinse samples, 50 mL of sample was diluted with 50 mL of distilled water.

Solid samples (food, soil). Food and soil samples were shaken in the Whirlpak to mix the sample, and then an aliquot was removed and homogenized with distilled water in a sterile blending bag for 1 min using a laboratory-scale food processor (Interscience, Saint Nom, France). For food samples, a 10 g aliquot was homogenized with 100 mL of water. From the resulting homogenate, 10 mL was mixed with 90 mL of distilled water to produce a 100 mL aliquot. For soil samples, a 20 g aliquot was homogenized with 200 mL of water. Then, 1 mL of the resulting homogenate was mixed with 99 mL of distilled water, and 1 mL of this solution was further diluted with 99 mL of distilled water. The dry weight of food and soil samples was determined by oven-drying 5 g wet aliquots at 110 °C for 24 h. The ratio of dry to wet weight was used to normalize bacterial counts to MPN per dry gram of sample.

Fly samples. Our goal was to assess potential transmission of fecal organisms from flies both mechanically (via their legs, wings, or body surface), and by regurgitation from their gastrointestinal tract; therefore, we homogenized the whole body of captured flies. Flies were homogenized with a pestle from outside the Whirlpak and mixed with 100 mL of distilled water to make a slurry. 1 mL of this slurry was then mixed with 99 mL of distilled water.

Quality control. Laboratory staff processed 5 % of samples in replicate and performed 10 % laboratory blanks. Our criterion for blanks was to drop the samples collected or processed on the same day as the contaminated blanks if any field and lab blanks showed contamination. We calculated the intraclass correlation coefficient between samples processed in replicate to assess agreement. While we did not set a threshold of acceptable agreement between replicates, 90 % agreement is typically considered adequate.

Colilert-18 media was added to the final 100-mL sample aliquots. Trays were incubated at 44.5 °C for 18 h (Yakub et al., 2002). Bacterial counts were normalized to MPN per 100 mL for water samples, per 2 hands for child and caregiver hand rinses, per 1 dry gram for food and soil samples, and per 1 fly for flies using a sample type-specific conversion factor that accounted for the amount of sample material contained in the 100 mL aliquot used in the IDEXX tray, the dilution factor and (for food and soil) the moisture content of the original sample.

2.4. Ethics

The primary caregiver of the index child provided written informed consent in the local language (Bengali). Because children were young (<3 years old at enrollment), we did not obtain assent; caregivers consented on behalf of minors for hand rinse sampling. The study protocol was reviewed and approved by human subjects committees at the icddr,b (PR-11063), University of California, Berkeley (2011-09-3652), and Stanford University (25863).

2.5. Statistical methods

We conducted a prospective, observational analysis of associations between household floor material and subsequently measured environmental contamination. We used the floor material recorded at baseline for our primary analysis as this assessment preceded all environmental measurements. To account for changes in floor material over time, we conducted a sensitivity analysis where we only used data from households that had the same floor material at both enrollment and the two-year follow-up. Our outcomes of interest were the abundance and prevalence of *E. coli* in the environmental samples; we considered both outcomes to assess whether floor material influences both the levels and binary presence of *E. coli*. We quantified abundance using log10-transformed MPN counts after replacing non-detects with half the lower detection limit (0.5 MPN) (Wendelberger and Campbell, 1994) and values above the upper detection limit of 2419.6 MPN with 2420 MPN before normalizing MPN values to the sample type-specific reporting units.

Estimation strategy: We used generalized linear models to estimate log10-transformed *E. coli* count differences (Δlog10) and prevalence ratios (PR) comparing households with finished vs. unfinished floors, separately for each sample type and pooling data across all trial arms and data collection rounds. Models used robust standard errors at the study block level (i.e. the unit within which randomization was performed) to account for cluster-randomization and repeated measurements; we used the block as the clustering unit as it represented the independent unit in the study. Unadjusted models included floor material as the only independent variable. Adjusted models controlled for potential confounders, which were measured either at the trial's baseline (caregiver's age and education, number of children <18 years in the household, number of individuals living in the compound, food security assessed by the Household Food Insecurity Access Scale (HFIAS) index (Coates et al., 2007), asset-based wealth index, wall materials, drinking water source, minutes to primary drinking water source) or at the time of sample collection (sex and age of index child, number of cows, goats, and chickens owned) (see Table S3 for full description of adjustment covariates). We controlled for baseline values of most covariates to avoid potential bias associated with controlling for post-exposure covariates (Groenwold et al., 2021); for the few covariates where we used values measured at the time of sample collection, we assumed that floor material does not influence the covariates. We controlled for animal ownership rather than whether animals entered the indoor living space because another study in Bangladesh by our team found that households with finished floors are less likely to allow their domestic animals indoors (Ercumen et al., 2025); therefore, this variable is a potential mediator on the causal pathway between floor material and contamination outcomes. Models for food and stored water samples included additional variables measured at the time of sample collection. For water samples, we controlled for whether the storage container was covered and had a narrow mouth and the number of hours the water had been stored. For food samples, we controlled for the number of hours since the food was prepared. All models also controlled for study arm (binary variable for intervention vs. control). Observations with missing covariate data were excluded from adjusted models.

Effect modification: We assessed potential modification of the association between floor material and *E. coli* contamination by rainfall, temperature, and animal ownership. We used publicly available daily observations from GloH2O's Multi-Source Weighted-Ensemble Precipitation (MSWEP) dataset and the National Aeronautics and Space Administration's Famine and Early Warning Systems Network (FEWS NET) Land Data Assimilation System for Central Asia (FLDAS-Central Asia) dataset and matched daily rainfall and temperature values to our study data by date and GPS coordinate (Contreras et al., 2021). We generated four binary effect modification variables for weather: two indicators for whether heavy rain and elevated temperature occurred within 2 days prior to sample collection, and two indicators for whether the 2-day rolling-average rainfall or temperature prior to sample collection were above median. Heavy rainfall and elevated temperature were defined as >80th percentile of daily values for the study region over the 3.5-year data collection period; this cut-off was selected because it is commonly used in studies of weather parameters and was associated with increased *E. coli* levels in environmental samples in our prior analysis (Mertens et al., 2019; Niven et al., 2023). Because this definition can lead to sparse data in the heavy rainfall and elevated temperature strata (i.e., only 20 % of observations exceed this cut-off), we used above-median rainfall and temperature as additional indicators that split the data into evenly sized strata. For animal ownership, we generated a categorical effect modification variable based on tertiles of the total number of animals owned by the household. We estimated associations between floor material and *E. coli* outcomes within each rainfall, temperature and animal ownership subgroup and included interaction terms between floor material and the effect modification variables. A p-value <0.20 on the interaction term was interpreted as significant effect modification. The effect modification models controlled for the same covariates as the primary models.

3. Results

3.1. Enrollment

The first environmental sub-study visited households once between July 2013 to March 2014, and the second sub-study visited households eight times between June 2014 and December 2016 to collect a total of 6350 stored drinking water samples, 2181 stored food samples, 7092 child hand rinse samples, 5397 caregiver hand rinse samples, 2538 courtyard soil samples, and 610 fly samples (Table S1).

3.2. Floor material

Among 1864 unique households with available environmental samples, 11.8 % (219) had finished floors and 88.3 % (1645) had unfinished floors, as measured at the baseline of the trial. Of 1864 households, all had available floor material data at baseline, and 91.3 % (1703) had available floor material data at the two-year follow-up. Among these 1703 households, 93.8 % (1597) retained the same floor material at the two-year follow-up, while 4.3 % (74) transitioned from soil to finished or concrete floors and 1.9 % (32) from finished to soil floors. Of the 1597 households that retained the same floor material, 10.8 % (173) had finished floors and 89.2 % (1424) had unfinished floors at both measurement timepoints.

Households with finished floors generally had higher levels of education and were wealthier and more food secure. On average, 83.1 % of caregivers in households with finished floors at baseline had secondary or higher education compared to 49.8 % in households with unfinished floors (Table 1). Among households with finished floors, 93.6 % reported being food secure compared to 64.9 % of households with unfinished floors (Table 1). Households with finished floors were more likely to have electricity, improved wall materials and assets (e.g., television, mobile phone, bicycle, motorcycle) while households with unfinished floors appeared to own slightly more animals (Table 1). Of 1864 households, 3.2 % (60) households had at least one covariate missing and were excluded from adjusted analyses.

3.3. E. coli counts and prevalence

Overall, courtyard soil samples had the highest mean log10 MPN of *E. coli* (5.10) and prevalence (94.5 % positive) (Table S4). In households with unfinished floors, log-10 transformed counts of *E. coli* ranged from 0.7 in food samples to 5.1 in courtyard soil samples (Fig. 1, Table S5), while in households with finished floors, log-10 transformed counts ranged from 0.6 in food samples to 5.0 in courtyard soil samples (Fig. 1, Table S5). In households with unfinished floors, *E. coli* prevalence ranged from 54 % in fly samples to 95 % in courtyard soil samples (Fig. S1, Table S6), and in households with finished floors, *E. coli* prevalence ranged from 52 % in fly samples to 93 % in courtyard soil samples (Fig. S1, Table S6). Replicate samples had an intraclass correlation of 86 %. We detected *E. coli* in 1 % of blanks, with a geometric

Table 1
Characteristics of enrolled households by floor material.

	Finished floor	Unfinished floor
	N = 219	N = 1645
Demographics		
Caregiver's age (years), mean (range)	24.1 (16.0, 43.0)	24.0 (15.0, 44.0)
Caregiver's education, % (n)		
None	3.7 (8)	16.2 (267)
Primary	13.2 (29)	34.0 (559)
Secondary or higher	83.1 (182)	49.8 (819)
Age of index child (years), mean (range)	1.90 (0.01, 4.8)	1.73 (0.01, 4.8)
Female index children, % (n)	53.6 (118)	49.4 (824)
Number of individuals ≤18 years living in household, median (range)	1 (0, 9)	1 (0, 8)
Number of total individuals living in compound[a], median (range)	8 (2, 31)	10 (2, 52)
Socioeconomic indicators		
Food security, % (n)[b]		
Secure	93.6 (205)	64.9 (1068)
Mildly insecure	2.3 (5)	9.5 (157)
Moderately insecure	3.7 (8)	21.6 (355)
Severely insecure	0.5 (1)	4.0 (65)
Household characteristics, % (n)		
Has electricity	85.4 (187)	56.0 (921)
Has improved wall material[c]	90.4 (198)	68.5 (1126)
Has improved roof material[d]	100 (219)	98.5 (1620)
Household assets, % (n)		
Owns ≥1 television	65.3 (143)	25.5 (419)
Owns ≥1 bicycle	34.5 (75)	29.9 (492)
Owns ≥1 motorcycle	28.3 (62)	5.0 (82)
Owns ≥1 mobile telephone	97.7 (214)	84.3 (1387)
Animal ownership		
Owns at least one domestic animal, % (n)	92.2 (202)	96.2 (1583)
Number of animals owned, mean (range)		
Cows	2.0 (1.0, 13.0)	2.7 (1.0, 37.0)
Poultry	10.9 (1.0, 69.0)	17.2 (1.0, 2700.0)
Goats/sheep	0.6 (1.0, 12.0)	1.2 (1.0, 16.0)

[a] **Compound** is a group of households around a central courtyard shared by extended families.
[b] Food security was assessed using the Household Food Insecurity Access Scale (HFIAS) index, which records the self-reported frequency of how often the household experienced uncertainty of food supply, and insufficient quality or quantity of food. The total score ranges from 0 to 27 and households are classified based on their score as f**ood secure (0–1), mildly food insecure** (2–8), m**oderately food insecure** (9–16), and **severely food insecure** (17–27).
[c] Improved walls included concrete and corrugated steel, and unimproved walls included soil/clay, leaves or thatch, and bamboo, based on local norms.
[d] Improved roofs included concrete and corrugated steel, and unimproved roofs included thatch, bamboo, and plastic sheets, based on local norms.

mean *E. coli* count of 6 MPN among positive blanks.

3.4. Associations between floor material and E. coli counts and prevalence

For all sample types, households with finished floors had slightly lower *E. coli* counts and prevalence than those with unfinished floors (Fig 1, Fig S1).

Unadjusted analyses: In unadjusted analyses, households with a finished floor had lower mean log10-transformed *E. coli* counts in stored drinking water (Δlog10 = −0.21, (−0.31, −0.11), child hand rinses (Δlog10 = −0.17 (−0.26, −0.09)), and caregiver hand rinses (Δlog10 = −0.13 (−0.23, −0.04)) (Fig. 2, Table S5). Findings were similar for *E. coli* prevalence; households with a finished floor had 6–13 % lower *E. coli* prevalence in stored drinking water, and child and caregiver hand rinses (Fig. S2, Table S6).

Adjusted analyses: In adjusted analyses, having a finished floor was associated with slightly lower log10-transformed *E. coli* counts (Δlog10 = −0.10 (−0.20, 0.00)) and slightly (10 %) lower *E. coli* prevalence on child hands only (Fig. 2, Fig. S2, Tables S5 and S6). Having a finished floor was not associated with *E. coli* counts or prevalence in other sample types in adjusted models.

Sensitivity analysis: In the sensitivity analysis limited to households who had the same floor material at baseline and the two-year follow-up, point estimates for associations between floor material and *E. coli* counts and prevalence on child hands remained similar to the primary analysis but associations were no longer statistically significant, and there was no statistically significant association between floor material and *E. coli* counts or prevalence for any other sample type (Tables S7 and S8).

3.5. Effect modification by rainfall and temperature

Following heavy rain, above-median rain and above-median temperature, finished floors were associated with 0.14–0.23 log lower *E. coli* counts on child hands (interaction p-values<0.20 compared to periods of no heavy rain, below-median rain and below-median temperature, Fig. 3, Table S9). Following heavy or above-median rain and elevated or above-median temperature, finished floors also appeared to be associated with 0.11–0.32 log lower *E. coli* counts in food (interaction p-values<0.20 compared to periods of no heavy rain, below-median rain, no elevated temperature and below-median temperature, Fig. 3, Table S9). However, the associations between floor material and *E. coli* counts in food were not statistically significant in any rainfall or temperature stratum (Fig. 3, Table S9). Following periods of above-median temperature, finished floors were associated with 0.60 log lower *E. coli* counts in/on flies (Fig. 3, Table S9). There was limited evidence of effect modification by rainfall or temperature on *E. coli* counts in stored water, on caregiver hands and in courtyard soil (Fig. 3, Table S9).

Following heavy rain and above-median temperature, finished floors were associated with approximately 10 % lower *E. coli* prevalence in stored drinking water (interaction p-value<0.20, Fig. S3, Table S10). Finished floors appeared to be associated with lower *E. coli* prevalence in/on flies following periods of no heavy rain and below-median rain (interaction p-values <0.20, Fig. S3, Table S10) but associations were not statistically significant in any stratum. Finished floors were also associated with approximately 50 % lower *E. coli* prevalence in/on flies following periods of elevated temperature (Fig. S3, Table S10). There was limited evidence of effect modification by rainfall or temperature on *E. coli* prevalence in other sample types (Fig. S3, Table S10).

3.6. Effect modification by animal ownership

In the bottom tertile of animal ownership, finished floors were associated with approximately 0.20 log higher *E. coli* counts on stored water and caregiver hands, and there was a similar trend for food that was not statistically significant (Fig. 4, Table S11). In the middle and top tertiles of animal ownership, finished floors were associated with approximately 0.20 log lower *E. coli* counts on child hands (interaction p-values <0.20 compared to the bottom tertile) (Fig. 4, Table S11). Similarly, finished floors were associated with approximately 20 % lower *E. coli* prevalence in child hand rinses compared to unfinished floors in the top tertile of animal ownership (interaction p-value<0.20 compared to bottom tertile, Fig. S4, Table S12). There was insufficient evidence of effect modification by animal ownership categories on *E. coli* prevalence in other sample types (Fig. S4, Table S12).

4. Discussion

Among rural Bangladeshi households, having finished floors was associated with slightly lower log10-transformed *E. coli* counts (Δlog10 = −0.10 (−0.20, 0.00)) and prevalence (PR = 0.90 (0.83, 0.98)) on child hands compared to unfinished floors adjusting for potential confounders

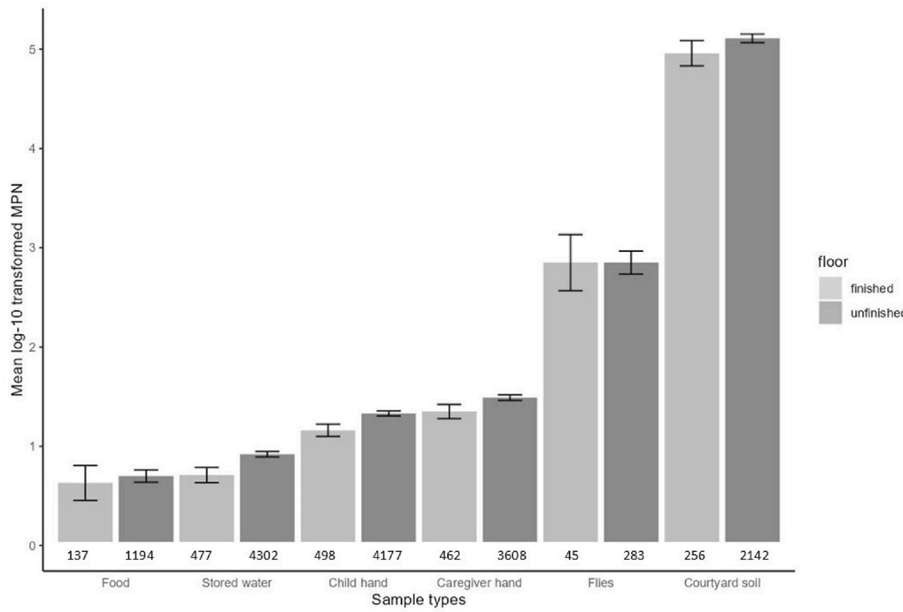

Fig. 1. Mean log10-transformed most probable number (MPN) of *E. coli* with 95 % confidence intervals (CI) by sample type and floor material. Each pair of columns represents a sample type. The number of samples is listed below the columns. Units by sample type are per 100 mL of stored water, 2 child and caregiver hands, 1 dry gram of soil and food, and 1 fly.

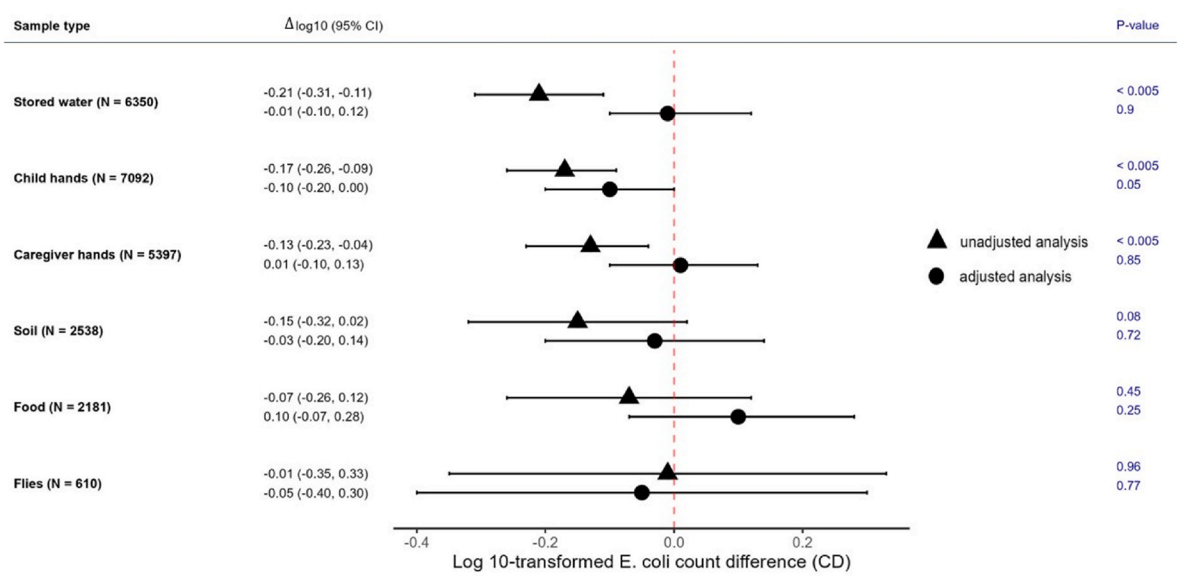

Fig. 2. Associations between floor material and log10-transformed *E. coli* counts in environmental samples by sample type. Unadjusted and adjusted log10-transformed count differences (Δlog10) for finished vs unfinished floors, 95 % confidence intervals (CIs) and associated p-values are shown for each sample. Effect estimates where the confidence intervals cross the null (red dashed line) are not statistically significant at the $p < 0.05$ level. Adjusted analyses controlled for study arm (intervention vs. control), sex and age of index child, caregiver's age and education, number of children <18 years in the household, number of individuals living in the compound, food security, asset-based wealth index, wall materials, drinking water source, minutes to the primary drinking water source, number of cows, chickens and sheep/goats, narrow mouth container (only for stored water samples), cover status of the container (only for stored water samples), hours since water has been stored (only for stored water samples), and hours since stored food was prepared (only for food samples). Units by sample type are per 100 mL of stored water, 2 child and caregiver hands, 1 dry gram of soil and food, and 1 fly. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(socio-demographics, water/sanitation status, animal ownership). While households with finished floors also appeared to have less *E. coli* contamination of stored drinking water and caregiver hands, only the association with child hands remained statistically significant after adjusting for potential confounders. Following periods of increased rainfall and temperature, finished floors were associated with up to 0.23-log lower *E. coli* counts on child hands, approximately 10 % lower *E. coli* prevalence in stored drinking water and up to 0.33-log lower *E. coli* counts in food but the associations for food were not statistically significant. Finished floors also appeared to be associated with lower *E. coli* counts and prevalence on flies following periods of less rain and increased temperature.

A growing body of observational literature reports child health benefits associated with improved floor materials in low-income

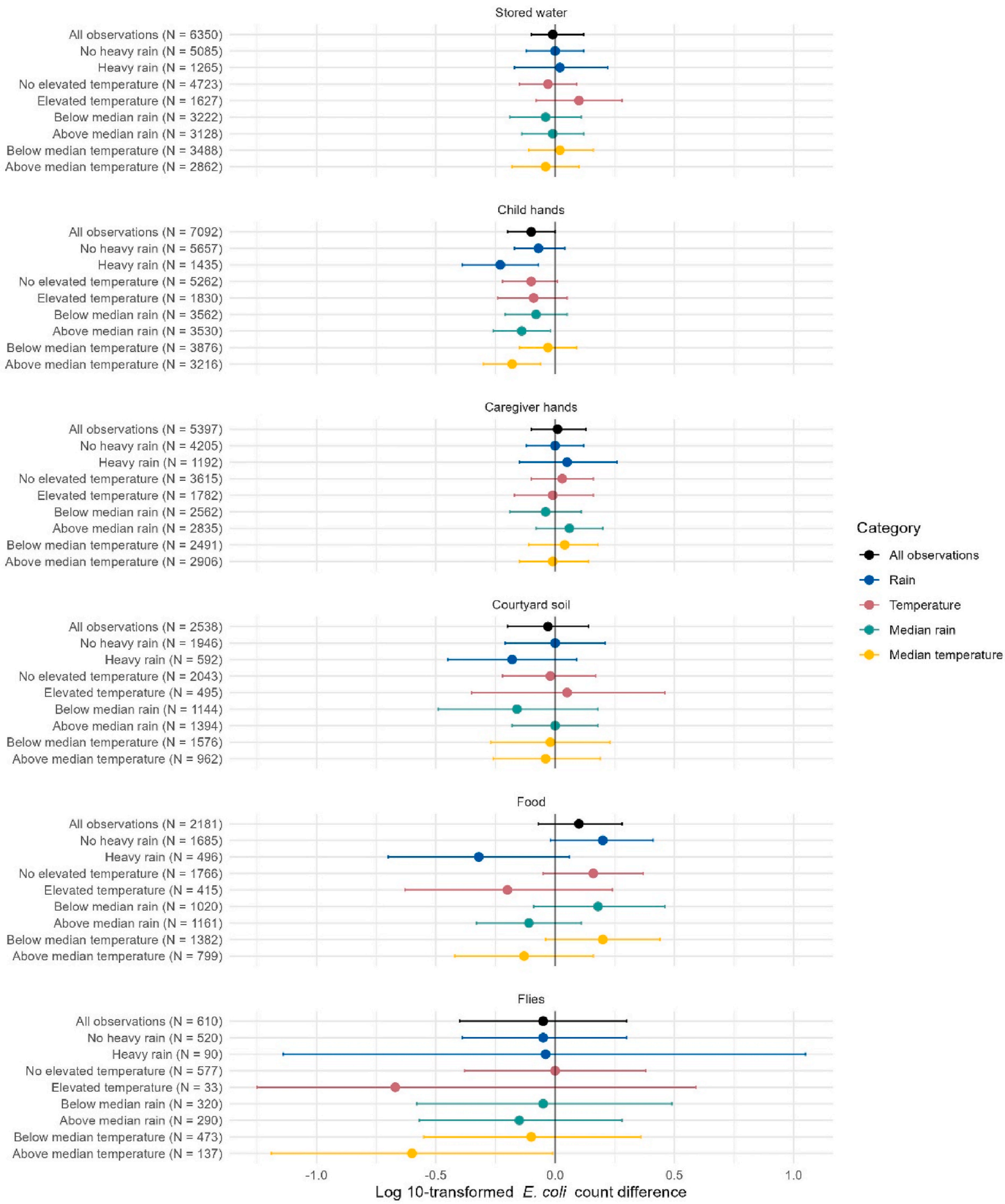

Fig. 3. Effect modification by heavy rain, elevated temperature, above-vs. below-median rain, and above-vs. below-median temperature within 2 days before sampling on adjusted associations between finished floors and log 10-transformed *E. coli* counts in environmental samples. Heavy rain and elevated temperature are each defined as >80th percentile of daily values during the study period. Above-median rain and above-median temperature are each defined as >50th percentile of 2-day rolling-average rainfall and temperature values during the study period. The circles denote differences in log10-transformed *E. coli* counts between households with finished vs. unfinished floors, and the horizontal lines denote 95 % confidence intervals. Confidence intervals for fly samples are truncated in the "elevated temperature" stratum because of low precision due to small sample size. Effect estimates where the confidence intervals cross the null (vertical line) are not statistically significant at the $p < 0.05$ level. The numerical estimates corresponding to this figure are provided in Table S9.

countries (Cattaneo et al., 2009; Koyuncu et al., 2020). Among children enrolled in the WASH Benefits trial in Kenya and Bangladesh, finished floors were associated with reduced diarrhea and parasite infections caused by STH and Giardia (Benjamin-Chung et al., 2021). Our findings indicate that these health benefits in the WASH Benefits Bangladesh trial are not mediated by lower fecal contamination of the domestic environment as measured by *E. coli,* except for child hands. In Bangladesh, children's mouthing of their own hands was the largest contributor to *E. coli* ingestion by children aged 6–35 months old (Kwong et al., 2020). A recent meta-analysis found that the odds of child diarrhea increased

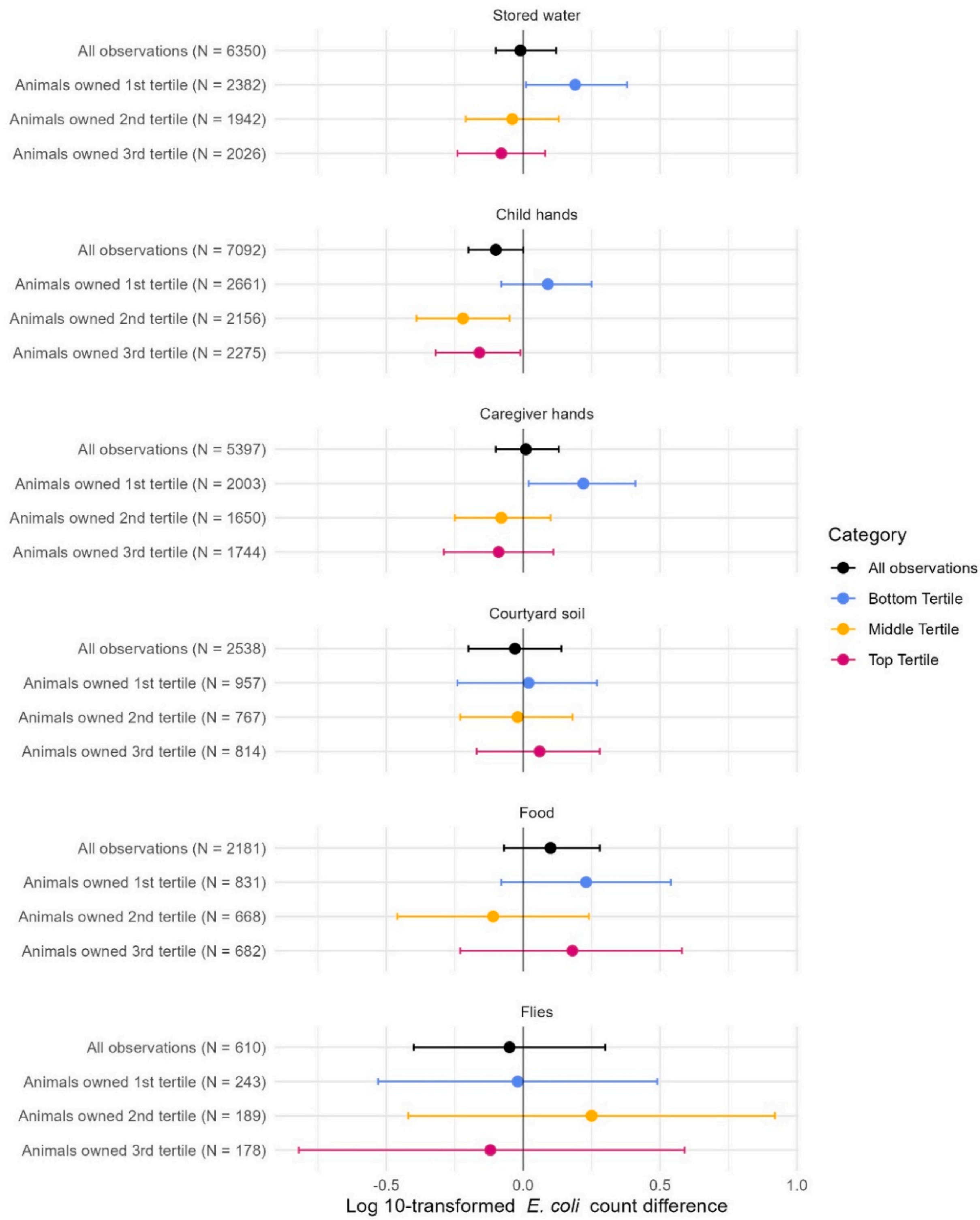

Fig. 4. Effect modification across tertiles of animals owned on adjusted associations between finished floors and log 10-transformed *E. coli* counts in environmental samples. Households owned the following number of animals, mean (range): 1st tertile: 4.6 (0–9), 2nd tertile: 14.4 (10–19), 3rd tertile: 57.6 (20–2700). The circles denote differences in log10-transformed *E. coli* counts between households with finished vs. unfinished floors, and the horizontal lines denote 95 % confidence intervals. Effect estimates where the confidence intervals cross the null (vertical line) are not statistically significant at the $p < 0.05$ level. The numerical estimates corresponding to this figure are provided in Table S11.

by approximately 10 % for each 1-log increase in *E. coli* on child hands (Goddard et al., 2020), while in Bangladesh the incidence of child diarrhea increased by 23 % for each 1-log increase in *E. coli* on child hands (Pickering et al., 2018). Therefore, we expect lower *E. coli* contamination of children's hands in homes with finished floors to result in reduced diarrheal illness. However, the associations we observed between floor material and *E. coli* on child hands were small in magnitude and unlikely to fully explain the previously reported protective associations between finished floors and child health outcomes in the WASH Benefits study (Pickering et al., 2018).

Following periods of increased rainfall and temperature, finished floors were associated with larger reductions in fecal contamination of child hands. There was also some evidence of larger reductions in contamination of food and stored drinking water following higher rainfall and temperature but these associations should be interpreted with caution given the large number of subgroup analyses (Lagakos, 2006; Burke et al., 2015). We have previously reported similar effect modification by rainfall and temperature for effects of WASH interventions on child health and fecal contamination in the WASH Benefits trial (Nguyen et al., 2024; Niven et al., 2024). Bangladesh experiences a pronounced monsoon season, and heavy rainfall and/or flooding events are common. Our findings indicate that finished floors can reduce exposure to fecal organisms via child hands, and potentially via food and stored water during these events. Rainfall and temperature extremes are expected to increase in the future (Myhre et al., 2019), and finished floors may yield increased health benefits under climate change.

Finished floors also appeared to be associated with larger reductions in *E. coli* contamination of child hands but not caregiver hands in households that kept more vs. fewer domestic animals. While cohabitation with domestic animals is associated with higher levels of *E. coli* on floors regardless of floor material, our previous work indicates that, among households that keep animals, finished floors harbor less *E. coli* than soil floors (Ercumen et al., 2025). Children have frequent hand contact with floors, and our current results indicate that improved floor materials may translate to reduced child hand contact with animal fecal contamination via floors. We did not observe a similar trend for caregiver hands. Women in rural Bangladesh often bear the primary responsibility for the daily upkeep and welfare of domestic animals (Mostari et al., 2021) and may have frequent direct hand contact with animal fecal waste; therefore, improved floor materials may not be sufficient to reduce *E. coli* contamination of caregiver hands. Caregiver hand contamination is also highly temporally variable (Ram et al., 2011) and increases after completing domestic tasks (e.g. handling child feces, sweeping, washing dishes, handling food) (Pickering et al., 2011); compared to these drivers, our findings indicate that soil floors are not a primary contributor to caregiver hand contamination.

Notably, all studies of health outcomes associated with floor material to date are observational, and confounding by socioeconomic status could have influenced their findings. In our analysis, households with finished floors were better educated, wealthier and more food secure than households with unfinished floors. While households with finished floors appeared to have lower *E. coli* contamination of stored drinking water, and caregiver and child hand rinses in unadjusted analyses, after adjusting for potential confounders, finished floors were only associated with lower *E. coli* counts and prevalence on child hands. While our analysis controlled for several sociodemographic indicators, it is possible that residual confounding remains. Future efforts to investigate the effects of finished floors on child health and environmental contamination should consider randomized study designs to minimize confounding (Rahman et al., 2024).

A strength of this study was the large sample size, with >20,000 samples collected across several fecal-oral transmission pathways over 3.5 years. The large sample size indicates that lack of observed associations is unlikely to be due to lack of statistical precision. However, some sample types were collected in smaller numbers than others and only approximately 10 % of study households had finished floors, leading to small analysis strata. Food and soil samples were only collected during three out of nine measurement rounds, and flies were only collected during one round. The food and soil sample collection rounds intentionally targeted both rainy and dry seasons to capture weather variation but the smaller sample size for these sample types may have limited the statistical power of our effect modification analyses, and the one-time collection of fly samples may have captured limited weather variability. Another limitation is that our primary analysis used floor material measured at baseline while approximately 6 % (106) of households switched floor materials over the course of the study. Our sensitivity analysis only using data from households that retained the same floor type at the two-year follow-up yielded similar effect estimates but had lower statistical precision due to smaller sample size; therefore, the switching of floor materials at an unknown timepoint in the study did not affect our conclusions. Additionally, we did not collect data on whether households had any coverings over the primary floor material in key areas, such as the child's sleeping area; coverings may reduce the dissemination of fecal contamination from floors or alternatively provide an environment that supports bacterial growth. Given the large number of comparisons conducted, it is possible that some of the observed associations are chance findings. We did not correct for multiple hypothesis testing because Bonferroni or similar adjustments can lead to overcorrection, especially for correlated outcomes (Schulz and Grimes, 2005). However, our analyses were pre-planned, and our findings were consistent using different outcome specifications (e.g. continuous count vs. binary presence of *E. coli*) and between primary and sensitivity analyses; these trends are unlikely to be explained by chance.

Another weakness is that we relied on *E. coli* to measure fecal contamination across a range of environmental matrices. *E. coli* can originate from natural sources (Hardina and Fujioka, 1991). However, in a subset of our hand rinse and soil samples, biochemical assays and phylogrouping of *E. coli* isolates from positive Quanti-Trays showed a low false-positive rate and no genotypic or phenotypic differences compared to *E. coli* isolates from fecal samples, indicating contamination of fecal origin in the environmental samples (Julian et al., 2015). Additionally, *E. coli* does not correlate well with the presence of actual pathogens (Wu et al., 2011). Therefore, it is possible that the associations we observed between floor material and *E. coli* do not apply to pathogens, and especially non-bacterial pathogens such as viruses and protozoa. Given that child hand contamination with *E. coli* is associated with diarrhea (Pickering et al., 2018), we expect our findings to be predictive of health risks but future studies of floor materials should consider measuring specific pathogens in addition to *E. coli* in household environmental samples. Given the wide range of variation in contamination levels among sample types, we used different sample amounts and dilution factors for each sample type to achieve counts in the quantifiable range; differences in dilutions may have impacted our ability to detect associations between floor material and *E. coli* counts for some sample types. Further, while we used pre-piloting to optimize sample processing, we did not determine the recovery efficiency of our methods; it is likely that we did not fully capture all *E. coli* present in complex environmental matrices.

While we investigated several fecal-oral transmission pathways, we did not measure *E. coli* directly on floors. It is possible that the health benefits associated with finished floors in previous studies were mediated by reduced direct exposure to pathogens on floors. Prior studies have demonstrated lower *E. coli* contamination on finished vs. unfinished floors. A study of peri-urban households in Peru enumerated *E. coli* on floors at the household entrance and kitchen areas (Exum et al., 2016). In both locations, dirt/soil floors had higher mean *E. coli* counts compared to wood or cement floors. Other studies in Bangladesh have detected fecal indicator bacteria such as *E. coli* on 25 % of floors and enterococcus on 100 % of floors, animal-specific fecal markers (BacR) on 27 % of floors (Harris et al., 2016) and antimicrobial-resistant *E. coli* on 71 % of floors (Tabassum et al., 2024) but these studies did not compare contamination between finished and unfinished floors. A study in Ghana found that floor material (concrete vs. soil) in the bedroom did not influence the prevalence and frequency of soil ingestion and the amount of soil ingested by children (Bauza et al., 2018). We also note that even in houses with finished floors, children can still be frequently exposed to soil and dust when they spend time outdoors (e.g. in courtyards). In a different ongoing study in rural Bangladesh, we found that households with finished floors were less likely to allow their domestic animals inside the home while family members were more likely to remove their shoes when entering the home (Ercumen et al., 2025). Therefore, it is also possible that finished floors improve health via improved domestic hygiene.

In recent large trials, WASH interventions achieved limited child health improvements (Cumming et al., 2019; Pickering et al., 2019b) and reductions in environmental contamination with pathogens (Mertens et al., 2023). Public health policies are typically focused on WASH infrastructure and behaviors; broader housing improvements including floor materials have policy relevance as part of a more comprehensive "transformative WASH" agenda. Finished floor materials could be a promising, passive, infrastructure-based intervention to reduce child exposure to fecal contamination and improve child health in low-income countries. Finished floors are easier to keep clean than soil floors, do not provide a reservoir for pathogen growth and do not generate dust, which can carry pathogens as well as chemicals. In contrast to WASH interventions, finished or concrete floors, once installed, do not necessitate ongoing behavior change and are an aspirational product, which can potentially facilitate implementation by policymakers and NGOs. Our findings from rural Bangladesh indicate that finished floors may reduce exposure to fecal organisms via reduced contamination of child hands, and following periods of increased rainfall and temperature, via reduced contamination of child hands, food and stored drinking water. Measures to control enteric infections in low-income countries should test flooring improvements to reduce exposure to fecal contamination. Future studies of flooring interventions should augment fecal indicator measurements with pathogen-specific outcomes and use randomized study designs to eliminate the possibility of unmeasured confounding.

CRediT authorship contribution statement

Sumaiya Tazin: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Mahfuza Islam:** Writing – review & editing, Methodology. **Amy J. Pickering:** Methodology. **Laura H. Kwong:** Writing – review & editing, Methodology. **Andrew Mertens:** Methodology. **Caitlin Niven:** Methodology. **Benjamin F. Arnold:** Writing – review & editing, Methodology. **Alan E. Hubbard:** Methodology. **Mahfuja Alam:** Methodology. **Debashis Sen:** Methodology. **Sharmin Islam:** Methodology. **Mahbubur Rahman:** Writing – review & editing, Resources, Methodology. **Leanne Unicomb:** Writing – review & editing, Methodology. **Stephen P. Luby:** Writing – review & editing, Investigation, Funding acquisition. **John M. Colford:** Investigation, Funding acquisition. **Jade Benjamin-Chung:** Writing – review & editing, Methodology. **Ayse Ercumen:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2025.114641

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