Title

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Journal

Environmental Science and Technology, 55(12)

ISSN

0013-936X

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Publication Date

2021-06-15

DOI

10.1021/acs.est.1c01114

Peer reviewed

Environmental Science & Technology

pubs.acs.org/est

Article

Longitudinal Effects of a Sanitation Intervention on Environmental Fecal Contamination in a Cluster-Randomized Controlled Trial in Rural Bangladesh

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Cite This: *Environ. Sci. Technol.* 2021, 55, 8169–8179

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ABSTRACT: Household latrine access generally is not associated with reduced fecal contamination in the environment, but its long-term effectiveness has not been measured. We conducted an environmental assessment nested within the WASH Benefits Bangladesh randomized controlled trial (NCT01590095). We quantified *E. coli* and fecal coliforms in samples of stored drinking water, child hands, mother hands, soil, and food among a random sample of households from the sanitation and control arms of the trial. Samples were collected during eight quarterly visits approximately 1−3.5 years after intervention initiation. Overall, there were no substantial differences in environmental fecal contamination between households enrolled in the sanitation and control arms. Statistically significant reductions were found in stored water and child hands after pooling across sampling rounds, but the effects were small and not consistent across rounds. In addition, we assessed potential effect modification of intervention effects by follow-up time, season, wealth, community-level latrine density and coverage, population density, and domestic animal ownership. While the intervention had statistically significant effects within some subgroups, there were no consistent patterns of effect modification. Our findings support a growing consensus that on-site latrines are insufficient to prevent fecal contamination in the rural household environment.

KEYWORDS: *latrine, potty, child feces management, WASH, fecal indicator bacteria, E. coli, disease transmission pathways, environmental sampling*

INTRODUCTION

Acute and chronic enteric infections are associated with a high burden of childhood morbidity and mortality from diarrheal disease, enteric dysfunction, malnutrition, and growth faltering in low-income countries.[1−4] Enteric pathogens are transmitted from infected individuals to children through numerous environmentally mediated, fecal-oral pathways. Safely managed sanitation facilities are designed to reduce environmental contamination and prevent infections. However, historically, most latrine interventions have not led to improved health outcomes in children,[5] including two out of three recent large-scale trials that achieved high on-site latrine access among trial participants but did not target community-level coverage.[6−8] Observational and experimental research also has generally found no association between latrine access and measures of household fecal contamination.[9−13]

Factors that could explain the lack of effects on child health and environmental contamination of on-site latrine interventions include low community coverage, insufficient adherence, limited behavioral change, and inability to disrupt alternative pathways of fecal contamination, such as domestic animals.[14,15] Additionally, most sanitation intervention studies have measured environmental contamination at only one time point after intervention initiation,[10,16−18] and two of those were conducted shortly after intervention implementation.[10,16] Fecal contamination in the environment is temporally and seasonally variable, and latrine use and maintenance patterns among intervention recipients can change over time.[19,20] These

Received: February 16, 2021
Revised: May 18, 2021
Accepted: May 20, 2021
Published: June 4, 2021

https://doi.org/10.1021/acs.est.1c01114
Environ. Sci. Technol. 2021, 55, 8169−8179

factors may modify the observable impact of sanitation interventions over time, and long-term follow-up may reveal emergent effects that do not appear in the short term.[21] However, little data are available on the long-term effects of sanitation interventions on environmental contamination and how seasonal and temporal patterns might modify their effectiveness. Only one randomized controlled trial (RCT) has examined the impact of a sanitation intervention on environmental fecal contamination over time; it found no effect in source water, stored water, or hand rinses over two years.[22] However, the intervention in that trial focused on increasing demand for sanitation, and only 38% of intervention households had a functional latrine after implementation.[22] Thus, the long-term effects of effectively improving on-site sanitation facilities on environmental fecal contamination have not been directly assessed.

We conducted an environmental study nested within an RCT in rural Bangladesh (WASH Benefits, ClinicalTrials.gov NCT01590095) that implemented a latrine and child feces management intervention and achieved high intervention uptake. We collected samples from the home environment (stored drinking water, child hand rinses, mother hand rinses, soil, and food) to assess the effect of the intervention on the presence of fecal indicator bacteria (FIB) over a period of 2.5 years (1−3.5 years after intervention implementation) and investigated whether duration of follow-up, season, wealth, community-level latrine access, population density, or animal presence modify intervention effects.

MATERIALS AND METHODS

Study Design. The WASH Benefits trial was an RCT designed to measure the effect of water, sanitation, hygiene (WASH) and nutrition interventions on child growth and diarrhea over the first two years of life in rural Kenya and Bangladesh.[6,7,23] Households with a pregnant woman in her first or second trimester at enrollment were eligible. In Bangladesh, six to eight spatially contiguous enrolled households formed a cluster. A buffer zone of at least 15 min walking distance (one kilometer) was enforced between clusters to reduce spillover effects. Eight adjacent clusters were grouped into a study block. Clusters within each block were randomized to one of six intervention arms or into a double-sized control arm (twice the size of intervention arms), resulting in geographically matched clusters within blocks. The sanitation intervention comprised three products (a double-pit pour flush improved latrine, a sani-scoop for the removal of child and animal feces, and a children's potty) and behavioral messaging delivered by trained promoters on product use and maintenance. Households in rural Bangladesh are clustered in multifamily compounds; interventions were delivered to all households within the target household's compound. Each latrine in the compound that did not have slab or a functional water seal, or that failed to prevent surface runoff of feces, was replaced with a new latrine. The index household was provided a new latrine if the household did not already own one. All households in the compound received sani-scoops, children's potties (if they had children under three), and behavioral messaging. Behavioral messaging was continued over the duration of data collection. Households in the control arm were not visited by promoters.

In this substudy, environmental contamination was measured over time in a random subset of households from the sanitation and control arms of the trial. Four households per cluster were randomly selected from each sanitation cluster and from one of two control clusters in the same block, maintaining the pair-matched design of the parent trial. In total, 360 households from the sanitation arm and 360 households from the control arm were sampled. Participating households were visited approximately quarterly eight times over 2.5 years, starting approximately one year after the intervention was initiated.

Procedures. *Sample Collection.* At each visit, samples were collected from various sources within the home environment that represent potential pathways of environmental contamination from fecal sources. Stored drinking water, children's hands, and mothers' hands were sampled during all eight sampling rounds. During the third and fourth rounds, soil from the courtyard and stored food for young children were also sampled from a random subset of households.

Trained field staff asked participants to provide a glass of water from their storage container that they would give to their children under five years old to drink. Participants were asked to pour the glass of water into a sterile Whirlpak bag (Nasco Modesto, Salida, CA) to collect approximately 150 mL of water. Hand rinses were collected from index children (the child born to women pregnant at enrollment) and their mother. If the index child was not available, a hand rinse was collected from the youngest child available under five years old. Samples were collected by placing hands, one 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 process was then repeated for the other hand in the same bag.[24] Soil samples were collected from a 30 cm by 30 cm area of the courtyard at the entrance of the study household. Field workers scraped the top layer of soil from the area into a sterile Whirlpak bag using a sterile plastic scoop. The sample area was scraped once vertically and once horizontally to collect 50 g of soil. Food samples were collected by asking participants to provide a small amount of stored solid food in the same manner they feed their children, with a preference for rice. Food was scooped to fill a 50 mL sterile plastic tube using a sterile spoon.

Sample Processing. Samples were preserved on ice and transported to the field laboratory of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). Samples were typically processed within 7 h of collection with the IDEXX Quanti-Tray/2000 method. Stored water was analyzed undiluted in 100 mL aliquots, and 50 mL of hand rinse sample was diluted with 50 mL distilled water. Soil and food samples were homogenized with distilled water in a sterile blending bag using a laboratory-scale food processor. A 20 g soil aliquot was homogenized with 200 mL water; 1 mL of homogenate was then mixed with 99 mL of distilled water, and 1 mL of the resulting mixture was mixed with 99 mL of distilled water to generate a final 100 mL aliquot. A 10 g food aliquot was homogenized with 100 mL water; 10 mL of homogenate was then mixed with 90 mL of distilled water. Additional 5 g aliquots of soil and food were oven-dried at 110 °C for 24 h to determine moisture content. Colilert-18 media was added to samples, followed by incubation at 44.5 °C for 18 h to enumerate the most probable number (MPN) of *E. coli* and fecal coliforms (per 100 mL of stored water, per two hands, or per one dry gram of soil/food). Quality control procedures including blanks and replicates were followed (SI Text S1).

https://doi.org/10.1021/acs.est.1c01114
Environ. Sci. Technol. 2021, 55, 8169−8179

On-Site and Community-Level Sanitation Conditions. At each visit, field staff administered a structured survey on household sanitation behaviors (e.g., use of the intervention hardware, disposal of child and animal feces, etc.) and presence and number of domestic animals. Field staff also completed spot check observations of environmental sanitary conditions. During the second round, an additional survey was completed to quantify population density and sanitation coverage within a 100 m radius of study households. This radius was chosen to reflect the upper range of the lateral distance pathogens travel through groundwater from a fecal source.[25] Field workers identified all compounds within 100 m of the participating study compound by walking 300 steps in each direction away from the compound. At each compound within that radius, they recorded the global positioning system (GPS) coordinates and total number of residents and latrines in the compound as reported by respondents and observed the type and hygienic condition of all latrines in the compound.

Ethics. Participants provided written informed consent in the local language (Bengali). The study protocol was approved by human subjects committees at the icddr,b (PR-11063), University of California, Berkeley (2011−09−3652), and Stanford University (25863).

Statistical Methods. *Outcomes and Parameters of Interest.* Data analysis was blind to participants' intervention arm and followed a preregistered analysis plan.[26] The analysis plan, deidentified data sets, and analysis scripts are available at OSF (https://osf.io/6u7cn/). Our outcomes were the presence and counts of *E. coli* and fecal coliforms. We estimated intervention effects using prevalence ratios (based on presence/absence of each indicator) and log10 differences in counts (based on log-transformed MPN of each indicator after replacing nondetects with half the detection limit) for each sample type separately.

Effect of the Intervention. The effect of the intervention was estimated through intention-to-treat analysis comparing the sanitation arm to the control arm using generalized linear models. Robust sandwich standard errors were estimated using study block to account for cluster-randomization and repeated measures of households. An indicator variable for study block was included in regression models to account for geographical matching. The overall effect of the intervention on each outcome was estimated by pooling all samples of a given type across sampling rounds. Estimates were also made for each sampling round separately to observe temporal trends. Possible confounding of effect estimates was assessed through adjusted secondary analyses. Potential confounders were screened for an association with each outcome in bivariate analysis ($p < 0.2$) and associated variables were included as covariates in adjusted models. Variables with <5% or >95% prevalence in the study population were excluded. Potential confounders included baseline characteristics and covariates measured at the time of sample collection (e.g., water treatment, time of sample collection) but not expected to be on the causal pathway. The list of potential confounders is available in our prespecified analysis plan (https://osf.io/6u7cn/).[26] To assess the impact of missing data, we (i) compared the rate of loss to follow-up between the two study arms, (ii) compared the enrollment characteristics of households that did not participate in all sampling rounds vs households that did, and (iii) compared intervention effects among all households vs households that participated in all sampling rounds.

Effect Modification. We assessed modification of intervention effects by prespecified variables, including duration of follow-up in years, season (dry vs wet), household wealth, community-level sanitation coverage, population density, and presence and number of domestic animals. Effect modification for each variable was assessed by estimating intervention effects within its subgroups and by including its interaction with intervention arm as a model covariate. Effect modification was assessed after pooling samples by type across all rounds. We excluded soil and food samples due to their smaller sample size. To reduce the number of model comparisons made, we used log10 *E. coli* counts as the only outcome in effect modification analyses.

We defined the wet season as June through October, when Bangladesh receives 80% of its annual rain.[27] We created a wealth index using principal component analysis (PCA) from 21 household assets and divided households into quartiles of wealth. Community-level sanitation coverage was quantified in two ways. First, we estimated per-capita sanitation coverage by dividing the number of hygienic latrines within 100 m of each study compound (including public latrines) by the number of people living within the same area. Second, we estimated the proportion of households within 100 m of each study compound that had at least one hygienic latrine. A hygienic latrine was defined as an improved facility (according to the Joint Monitoring Programme definition) that was observed to safely contain feces.[28] Population density was defined as the number of people living within 100 m of each study compound. Sanitation coverage and population density variables were divided into tertiles. As a sensitivity analysis on the definition of community-level sanitation coverage, we repeated analyses using a radius of 50 m around each study compound. We assessed effect modification for domestic animals by type: cattle, poultry, goats and sheep, and other animals (donkeys, pigs, dogs, and cats). Counts for each animal type were categorized into four groups: no animals of the given type and tertiles of the number owned.

RESULTS AND DISCUSSION

Enrollment. A total of 720 households were enrolled from the sanitation and control arms of the intervention trial. Data collection occurred between June 2014 and December 2016. Most households (80%) completed all eight sampling rounds (Table S1), while 10% of households completed seven rounds, 4% completed six rounds, and 6% completed between one and five rounds. Households that participated in all eight rounds provided 87% of samples in the intervention arm and 82% of samples in the control arm. Covariates and effect modifiers were balanced between intervention arms and between households that participated in all eight rounds and households that did not (Table 1, Tables S2, S3).

Fecal Contamination. We analyzed a total of 4727 stored water samples, 5324 child hand rinses, 5397 mother hand rinses, 749 soil samples, and 535 stored food samples. *E. coli* was detected in 81% of stored water samples, 74% of child hand rinses, 75% of mother hand rinses, 95% of soil samples, and 68% of stored food samples (Figure 1, Table S4). The geometric mean MPN for *E.* coli was 9.8 per 100 mL of drinking water, 29.2 per two child hands, 29.5 per two mother hands, 144,050.7 per dry gram of soil, and 51.7 per dry gram of food (Figure 1, Table S4). Fecal coliforms were detected in over 90% of samples of each type (Figure S1, Table S5).

https://doi.org/10.1021/acs.est.1c01114
Environ. Sci. Technol. 2021, 55, 8169−8179

Table 1. Baseline Characteristics of Participating Households by Study Arm

	Sanitation Arm n = 360	Control Arm n = 360	Total n = 720
WASH Characteristics			
Shallow tubewell is primary water source, % (n)	74 (266)	73 (264)	74 (530)
Had stored water at home, % (n)	48 (174)	48 (171)	48 (345)
Reported treating water yesterday, % (n)	0 (0)	<1 (1)	<1 (1)
Reported defecating in the open daily			
Adult men, % (n)	7 (25)	5 (19)	6 (44)
Adult women, % (n)	5 (18)	4 (15)	5 (33)
Children aged 8 to <15 years, % (n)	8 (13)	8 (11)	8 (24)
Children aged 3 to <8 years, % (n)	40 (75)	34 (62)	37 (137)
Children aged 0 to <3 years, % (n)	81 (60)	78 (54)	80 (114)
Latrine			
Owned, % (n)	52 (189)	56 (201)	54 (390)
Concrete slab, % (n)	94 (323)	96 (332)	95 (655)
Functional water seal, % (n)	34 (105)	30 (95)	32 (200)
Visible stool on slab or floor, % (n)	51 (171)	47 (163)	49 (334)
Owned a child potty, % (n)	3 (10)	4 (15)	3 (25)
Human feces observed in the			
House, % (n)	8 (30)	10 (35)	9 (65)
Child's play area, % (n)	1 (3)	1 (4)	1 (7)
Handwashing location within six steps of latrine, % (n)	13 (46)	17 (61)	15 (107)
Has water, % (n)	98 (45)	95 (58)	96 (103)
Has soap, % (n)	54 (25)	46 (28)	50 (53)
Handwashing location within six steps of kitchen, % (n)	11 (38)	11 (41)	11 (79)
Has water, % (n)	84 (32)	88 (36)	86 (68)
Has soap, % (n)	24 (9)	27 (11)	25 (20)
Household Characteristics			
Mother's age (years), median (range)	23 (15, 41)	23 (15, 41)	23 (15, 41)
Mother's education level			
Secondary or higher, % (n)	56 (201)	56 (200)	56 (401)
Primary, % (n)	32 (114)	29 (106)	31 (220)
No education, % (n)	12 (45)	15 (54)	14 (99)
Food security (HFIAS, Coates 2007)			
Food secure, % (n)	68 (244)	69 (249)	68 (493)
Mildly food insecure, % (n)	9 (32)	8 (29)	8 (61)
Moderately food insecure, % (n)	19 (69)	20 (71)	19 (140)
Severely food insecure, % (n)	4 (15)	3 (11)	4 (26)
Number of children under 18 years old in household, median (range)	1 (0, 8)	1 (0, 6)	1 (0, 8)
Total number of individuals living in compound, median (range)	10 (2, 45)	9 (2, 40)	9 (2, 45)
Distance to primary drinking water source (minutes), median (range)	0 (0, 62)	0 (0, 10)	0 (0, 62)

There was no statistically significant difference in the prevalence of *E. coli* between sanitation and control arms for any sample type, with all sample collection rounds pooled or during any individual round (Figure S2, Table S6). The intervention resulted in statistically significant overall reductions in mean log10 counts of *E. coli* in stored drinking water (Δlog10 = −0.08, 95% CI −0.15, 0.00) and child hand rinses (Δlog10 = −0.08 (−0.15, 0.00)), but the effect sizes were modest (Figure 2, Table S7). The intervention resulted in sporadic reductions in *E. coli* counts during individual rounds for these two sample types and a marginally significant reduction in food during one round, but there were no consistent trends (Figure 2). There was no effect on *E. coli* counts in mother hand rinse or soil samples. Intervention effects on fecal coliforms were similar to those on *E. coli* (Tables S8, S9). Effects on both indicators changed little after adjusting for potential confounders or excluding samples from households that did not participate in all eight sampling rounds (Tables S6−S10).

Effect Modification. Overall, effects on *E. coli* counts were not modified by season, duration of follow-up, or population density (Figures 3−4; Tables S11−S13). Wealth was not an important modifier overall, although there were modest reductions in *E. coli* counts on children's hands in the top two wealth quartiles (Δlog10 = −0.13 and −0.17). Overall, 63% of compounds within 100 m of study households had at least one hygienic latrine and coverage tertiles were 0%−60%, 61%−82%, and 83%−100%. There were an average of 0.11 hygienic latrines per capita (9 people per hygienic latrine) within 100 m and latrine density tertiles were 0.00−0.08, 0.08−0.13, and 0.13−0.43. Using either definition of community-level sanitation coverage, intervention effects were mostly null among study households surrounded by high latrine coverage; there were marginally significant reductions on the order of 0.10-log10 among households surrounded by low latrine coverage (Figure 3). Results did not change substantially using a 50 m radius. In total, 68% of households had cattle (median n = 2), 92% had poultry (median n = 14), and 37% had goats or sheep (median n = 0). Intervention effects were mostly null among households with no animals of each group. There were statistically significant reductions ranging from 0.14-log10 to 0.26-log10 between the sanitation and control arms among households that owned different numbers of different animals but no clear trends with increasing tertiles of animals owned.

DISCUSSION

The sanitation intervention in the WASH Benefits Bangladesh trial did not meaningfully reduce indicators of fecal contamination in the household environment in stored water, children's hands, mothers' hands, soil, and food. There were modest reductions of *E. coli* counts in stored water and on children's hands. Our results are consistent with those of previous trials on sanitation and indicators of fecal contamination.[16−18,22] The longer-term results in this study (1−3.5 years after intervention implementation) are consistent with an earlier evaluation of the WASH Benefits Bangladesh trial that found no reduction of *E. coli* in the environment four months after intervention implementation.[10]

In contrast with previous trials with low uptake, most households in the sanitation arm (94%) maintained functional, hygienic sanitation facilities throughout the duration of the study.[29] Only two households from the sanitation arm reported adults practicing open defecation during our final visit. However, it remains possible that behavior change achieved by the intervention was insufficient to impact contamination, as 89% of households with children <3 years old and 40% of households with children aged 3−8 reported that those children practiced open defecation at our final visit. Among index children who did not use a latrine the last time they defecated, 63% of households reported at the final visit that they disposed of the feces in a latrine, 19% in a drain or ditch, 12% in a bush, forest, or field, 3% left the feces on the ground in or outside of the compound courtyard, and the remaining

https://doi.org/10.1021/acs.est.1c01114
Environ. Sci. Technol. 2021, 55, 8169−8179

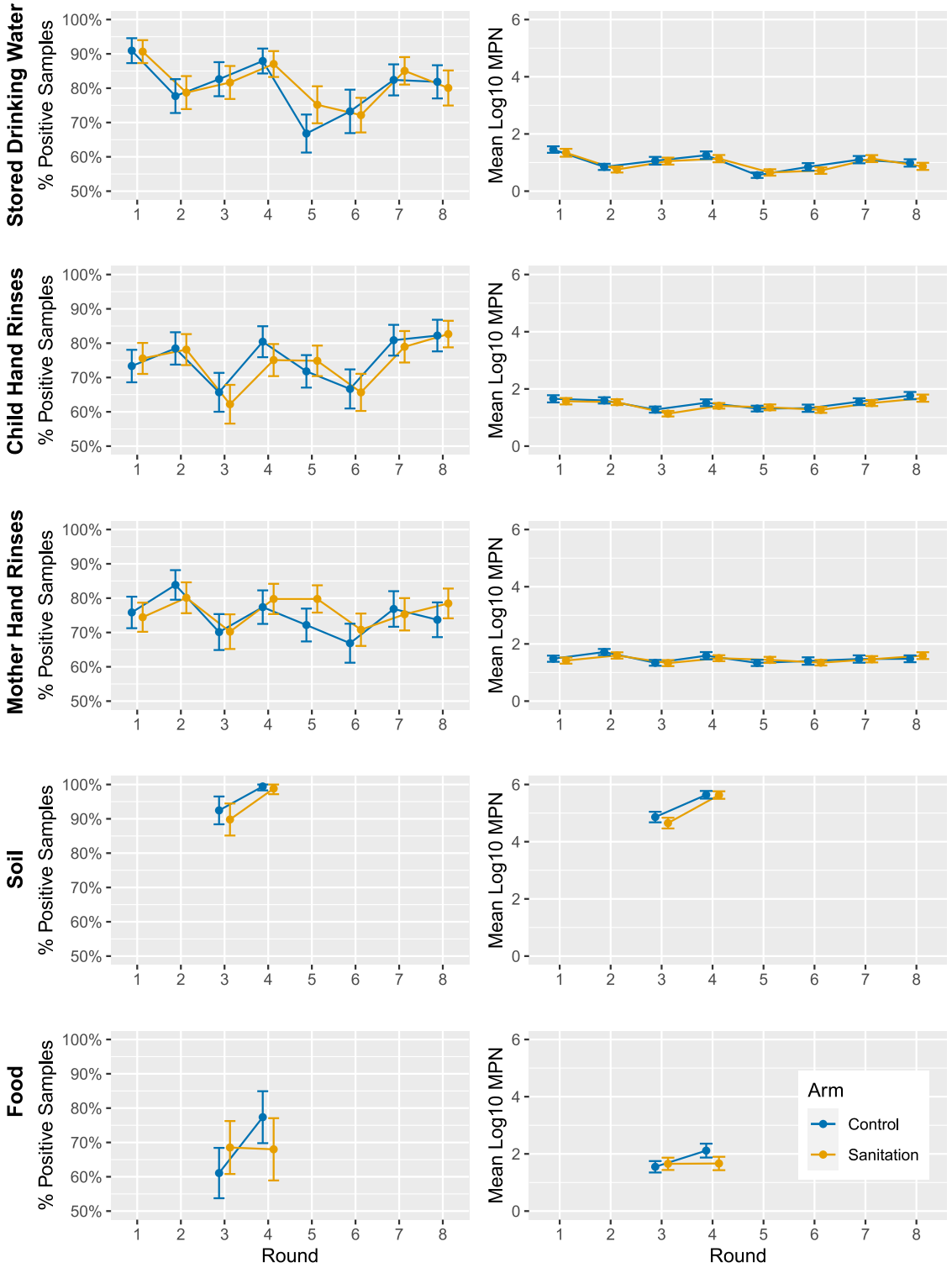

Figure 1. Prevalence (left column) and mean log10 most probable number (MPN) (right column) of *E. coli* with 95% confidence intervals by sample type, study round, and intervention arm. Each row contains a separate sample type. Units by sample type are 100 mL of stored water, two child/mother hands, and one dry gram of soil/food. Each study round took place approximately three months after the preceding round.

households left the feces in the potty or in a specific pit for children's feces. Among the same households, 39% reported using the provided sani-scoop to handle the feces.

There is mixed evidence on the impact of community-level latrine coverage on health,[30−33] but one theoretical model suggests that the level of coverage in one's community is more important for health than their own personal access.[34] The WASH Benefits trial employed a compound-based sanitation intervention that delivered interventions to all households within each extended-family compound but reached <10% of compounds in the villages where it was implemented. If fecal contaminants primarily enter study households from outside sources, the compound-based intervention would not prevent most contamination. Households in this study were well

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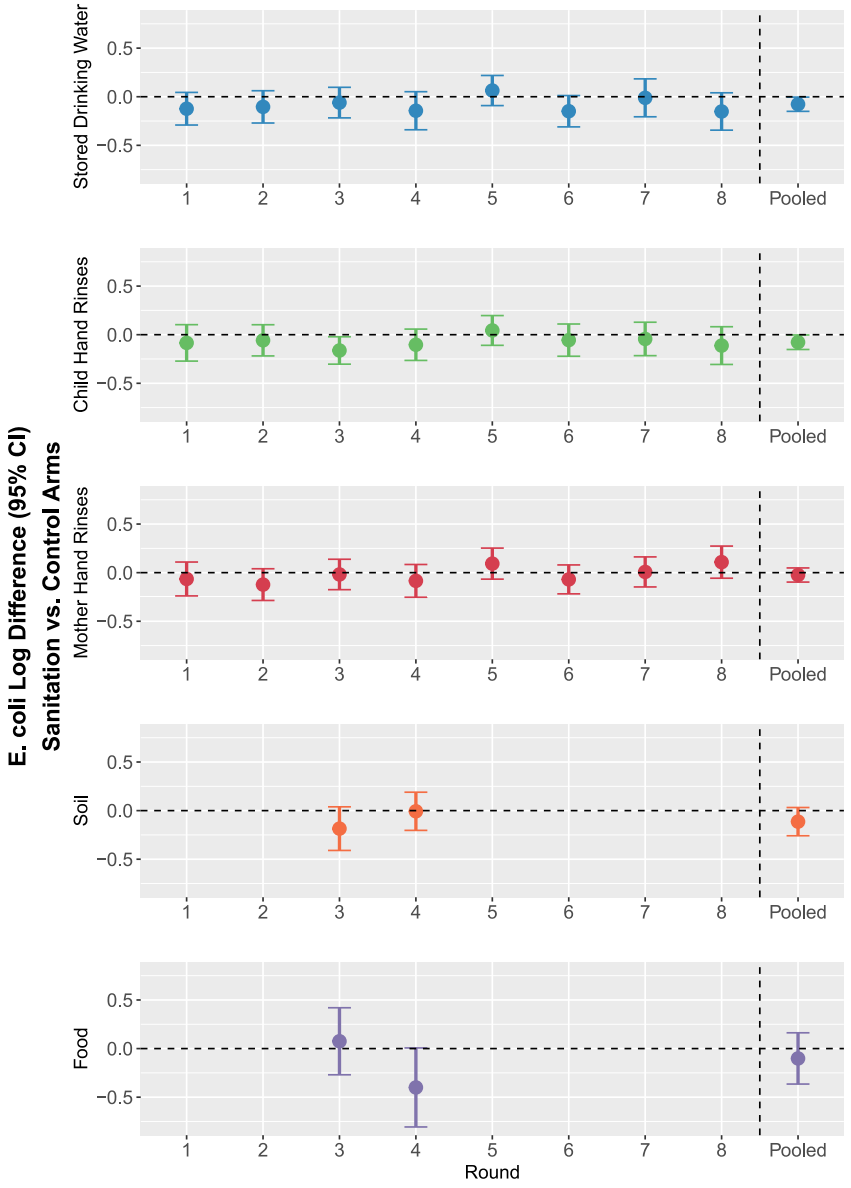

Figure 2. Effect of the intervention on log-transformed *E. coli* counts in environmental samples by sample type and round. Unadjusted log10 differences for sanitation vs control arms and 95% confidence intervals are shown for each sampling round and for all rounds pooled. Each row contains results for a separate sample type. Units by sample type are 100 mL of stored water, two child/mother hands, and one dry gram of soil/food. Each study round took place approximately three months after the preceding round.

distributed across the range of community-level coverage (with tertiles of 0−60%, 61−82%, and 83−100%), which allowed for a robust assessment of effect modification across relevant coverage thresholds.[5,35] However, community-level coverage of hygienic latrines did not modify the effect of the intervention. Field workers observed that hygienic latrines in neighborhood compounds appeared to safely isolate feces (i.e., not draining into the environment, no feces overflowing from pit), but it is possible that those latrines were not of high enough quality to prevent contamination through other pathways (e.g., subsurface infiltration, unhygienic pit emptying into waterbodies). In addition, the 100 m radius around study compounds that we used to measure community-level coverage may have missed pathogen transmission across longer distances, such as through tracking by people or animals.

Improved latrines are designed to separate human feces from the environment, but they do not prevent contamination from animals. Domestic animals have been implicated as a major source of fecal contamination and associated with diarrhea and other health outcomes.[36,37] An observational analysis within the WASH Benefits Bangladesh control arm found that domestic animal presence was associated with increased fecal contamination in the environment.[38] Another substudy within the sanitation and control arms found more animal-associated fecal genetic markers in the household environment than human-associated markers.[39] In this study, we found 0.14 to 0.26-log10 reductions in stored water and hand *E. coli* counts in some subgroups of animal ownership but no consistent patterns. Contamination from animal sources may have been mitigated through use of the sani-scoop to handle animal feces, which varied by animal type. Among intervention households at the end of our study that reported disposing of animal feces, 68% reported using the sani-scoop to dispose of cattle feces, 75% for poultry feces, and 91% for dog feces. Most animal

https://doi.org/10.1021/acs.est.1c01114
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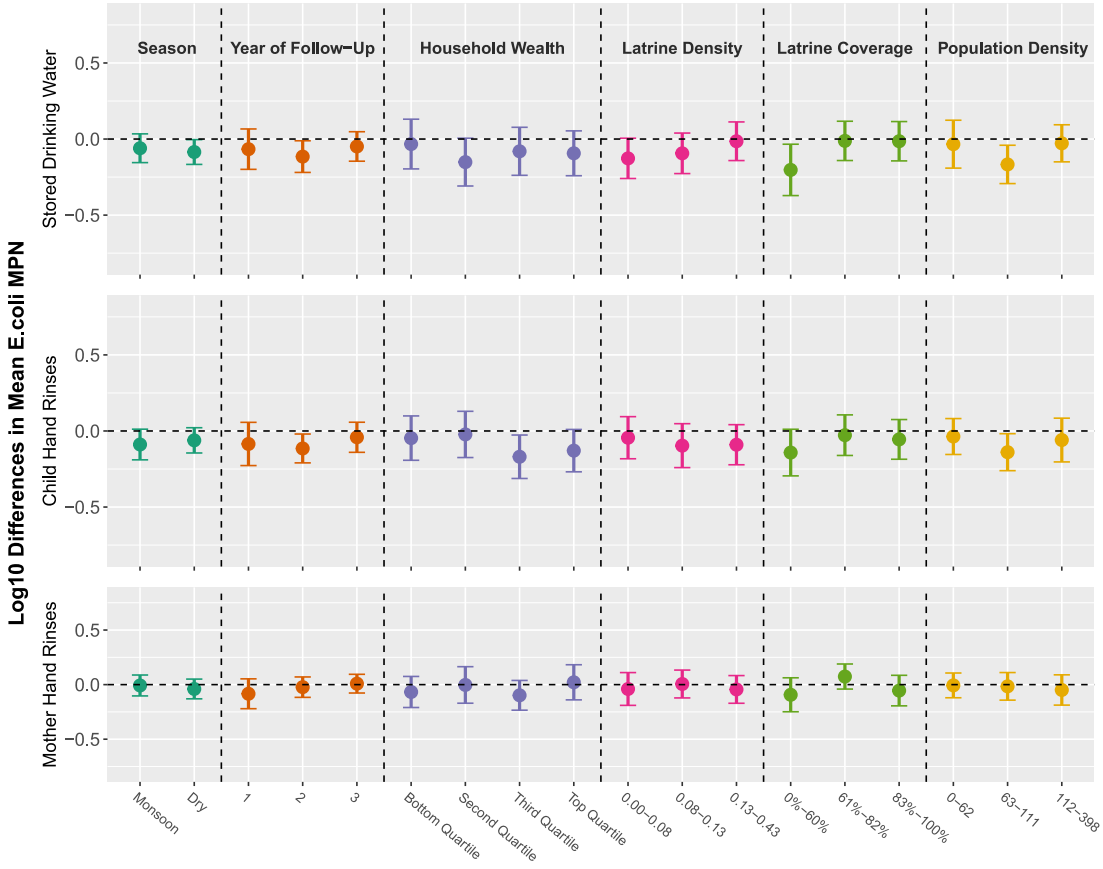

Figure 3. Effect modification on log-transformed *E. coli* counts in environmental samples by season, year, household wealth, and hygienic latrine density, hygienic latrine coverage, and population density within 100 m. Unadjusted log10 differences for sanitation vs control arms and 95% confidence intervals are shown for individual categories of each potential effect modifier. Latrine density was defined as the number of hygienic latrines per capita within 100 m, and latrine coverage as the percent of compounds within 100 m with at least one hygienic latrine. Latrine and population categories represent tertiles. Each row contains results for a separate sample type. Units are 100 mL of stored water and two child/mother hands.

feces were disposed of in an open pit. Some households stored animal feces for domestic use, including 22% of those with goat feces and 49% of those with cattle feces. An earlier evaluation also found reduced ruminant-specific fecal markers in stored water among sanitation-arm households, suggesting potentially improved animal feces management.[39] We did not account for animals owned by neighboring compounds, the feces of which could enter study households via roaming animals, compound residents' feet, or surface runoff and/or subsurface infiltration.

Despite having no overall effect on environmental *E. coli* contamination, the WASH Benefits Bangladesh sanitation intervention resulted in statistically significant reductions in enteric infections objectively assessed by stool testing and caregiver-reported child diarrhea.[6,40,41] It is possible that we missed true intervention effects on the environment due to limitations of *E. coli* as a fecal indicator.[42] Detecting *E. coli* in the environment does not necessarily indicate presence of human or animal fecal contamination, as *E. coli* can be naturalized in the environment,[43−45] and fecal-borne pathogens may be present even in the absence of detectable *E. coli*.[46,47] We also may have missed intervention effects due to sampling at random times rather than during key moments of contamination or transmission.[48] Other evaluations of the WASH Benefits Bangladesh sanitation intervention also found no reduction of pathogens in the environment, including pathogenic *E. coli*, rotavirus, norovirus, *Giardia lamblia* (enumerated by qPCR) and *Ascaris lumbricoides, and Trichuris trichiura* (enumerated by microscopy).[39,49,50] While low pathogen prevalence and smaller samples sizes in some of these studies limit inference, they consistently point to a lack of robust environmental impact from the sanitation intervention. The reductions in enteric infections among children receiving the intervention may have been achieved through alternate pathways, such as reduced contamination of the latrine facility, unidentified behavioral changes, or reduced person-to-person transmission.

Our results add to an existing body of research by measuring the long-term effects of latrine-based sanitation interventions on fecal contamination. The consistent null results between this and similar trials, despite variation in study characteristics, duration and local contexts, suggests that household latrines are not a sufficient technology to disrupt fecal contamination from entering the home environment. More work is needed to understand the mechanisms through which the intervention reduced enteric infections despite no apparent reduction in fecal contamination in the domestic environment, as measured by fecal indicator bacteria as well as selected pathogens. Further research should explore the pathogen-specific environmental disease transmission pathways (e.g., through domestic animals or on crops), both at the household and community levels, to identify potential strategies to maximize the benefits of on-site sanitation. Ultimately, improved sanitation strategies

https://doi.org/10.1021/acs.est.1c01114
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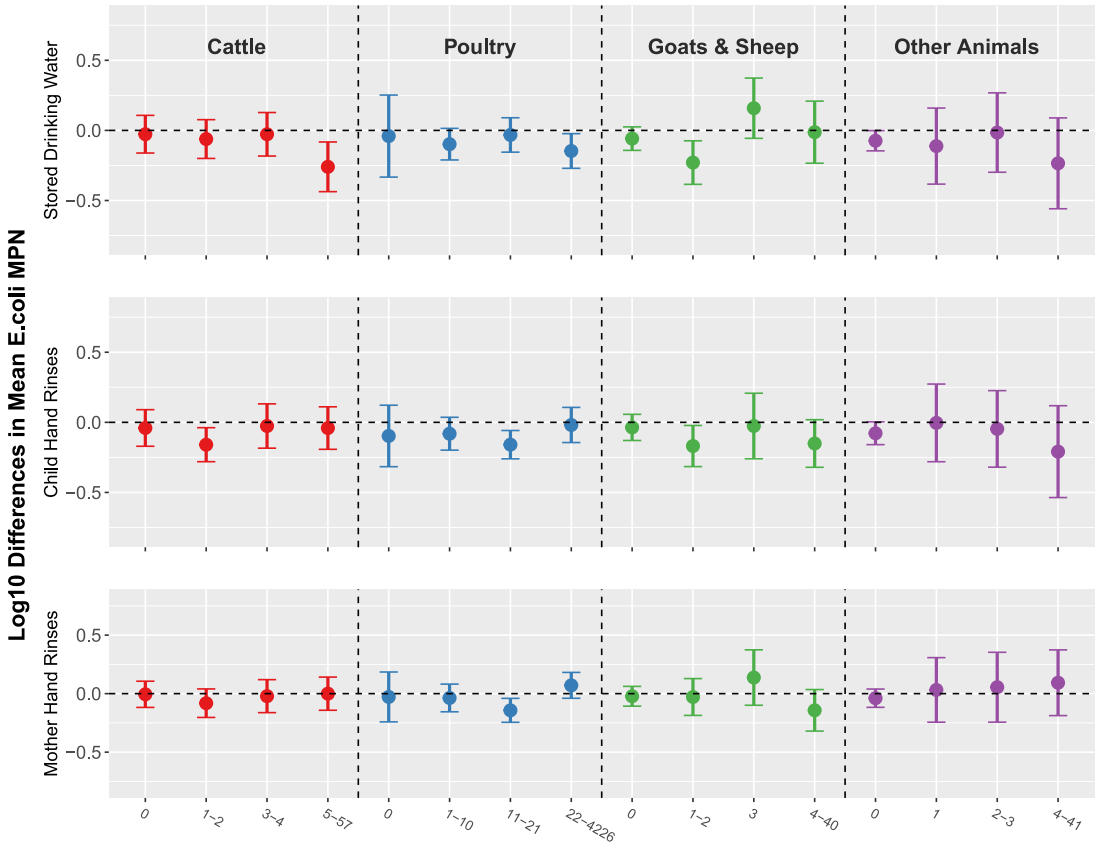

Figure 4. Effect modification on log-transformed *E. coli* counts in environmental samples by domestic animal ownership. Unadjusted log10 differences for sanitation vs control arms and 95% confidence intervals are shown for individual categories by animal type. "Other" includes donkeys, pigs, dogs, and cats. For each animal type, the categories represent those with no animals and tertiles of number of animals owned. Each row contains results for a separate sample type. Units are 100 mL of stored water and two child/mother hands.

and implementation programs are needed that fundamentally transform the hygienic profile of whole communities and manage excreta flows throughout their lifecycle. Those strategies must consider the complex system of factors that lead to environmental contamination in order to disrupt transmission and improve health.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c01114.

Quality Control; prevalence and mean log10 most probable number (MPN) (right column) of fecal coliforms with 95% confidence intervals; effects of the intervention on the presence of *E. coli* in environmental samples; flowchart of household participation and sample collection; distributions of potential effect modifiers; characteristics of environmental samples and sampling process; effect modification of intervention effects in various samples; covariates selected for adjusted models (PDF)

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https://doi.org/10.1021/acs.est.1c01114
Environ. Sci. Technol. 2021, 55, 8169−8179

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research was supported by Grant R01HD078912 from the NIH and in part by Grant 0PPGD759 from the Bill & Melinda Gates Foundation to the University of California, Berkeley. The authors declare no competing financial interest.

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Environ. Sci. Technol. 2021, 55, 8169−8179