Effect of water, sanitation, and hygiene interventions on detection of enteropathogens and host-specific fecal markers in the environment: an individual-participant data meta-analysis

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## Abstract

**Background:** Drinking water , sanitation, and hygiene (WASH) improvements are considered cornerstones to reduce diarrheal disease in low-income countries. However, recent trials have found no or mixed effects of household- and community-level WASH interventions on child health. Measuring pathogens and host-specific fecal markers in the environment can help investigate if limited health effects occur because WASH interventions do not sufficiently reduce environmental contamination or do not address animal fecal sources.

**Methods:** We conducted a systematic review and individual participant data meta-analysis to assess the effects of WASH interventions on enteropathogens and microbial source tracking (MST) markers in environmental samples. We used covariate-adjusted regression models with robust standard errors to estimate intervention effects and pooled results across studies.

**Findings:** We identified and received data from five randomized or quasi-experimental studies. Environmental sampling was primarily focused on onsite sanitation interventions. Most individual studies found no effects on pathogens or MST markers in environmental samples, including drinking water, hand rinses, soil and flies. We found a small but significant reduction in the prevalence of any pathogen in any sample type, with a pooled prevalence ratio of 0.94 (95% CI: 0.90, 0.99), 0.97 (95% CI: 0.91, 1.03). There was no overall effect on MST markers, and no consistent differences in intervention effects by season, animal presence, urbanicity, study design, or intervention uptake.

**Interpretation:** Few WASH trials to date have measured pathogens or host-specific fecal markers in the environment. The consistently small effect of onsite sanitation interventions on pathogens in the environment in these studies supports the broader evidence on lack of health impact in sanitation trials.

**Funding:** The Bill & Melinda Gates Foundation.

## Research in context

**Evidence before this study.** Children in areas with poor drinking water, sanitation, and hygiene (WASH) conditions experience increased diarrheal disease and reduced growth. Recent rigorous intervention studies on WASH improvements have shown mixed effects on reducing diarrheal disease in children and no improvements in child growth. Quantifying the effect of WASH improvements on enteric pathogens in environmental samples and on contamination originating from human vs. animal sources may help elucidate if interventions successfully interrupt the causal pathway between poor WASH, environmental exposure to fecal pathogens, and child health. Most previous studies and meta-analyses on the effect of WASH interventions on fecal contamination in the environment have focused on fecal indicator bacteria (FIB). Studies have shown FIB reductions in water and on hands due to water treatment and handwashing, respectively, but no effects from sanitation. However, limitations of FIB as predictors of enteropathogens and disease risk limit the interpretation of these findings. FIB also cannot distinguish between fecal contamination from humans and animals; this information can help illuminate whether lack of health effects from sanitation interventions are due to zoonotic disease transmission from unaddressed animal fecal sources. Recent applications of advanced analytic techniques to environmental sampling in low-income countries allow detection and enumeration of a range of enteropathogens, as well as fecal markers associated with specific hosts for microbial source tracking (MST). We conducted a systematic review and individual participant data (IPD) meta-analysis of WASH intervention studies to assess if interventions reduced the prevalence and abundance of enteropathogens or MST markers in the domestic environment.

**Added value of this study.** We identified 6 eligible intervention studies that measured enteropathogens and MST markers in environmental samples and obtained data from 5 studies. Most individual studies indicated a protective effect of interventions on the prevalence of pathogens and MST markers, but most estimates were not statistically significant due to small sample sizes and rare detection of some of the targets. The IPD meta-analysis approach allowed us to detect a small but significant reduction in the prevalence of any type of pathogen in any type of sample by pooling across all studies. There was no overall intervention effect on the prevalence of MST markers. This study takes advantage of advanced methods to enumerate enteropathogens and host-specific fecal markers in a range of environmental samples, including understudied reservoirs such as soil. We provide the first synthesis of evidence of the effect of WASH interventions on these important targets in the domestic environment to advance our understanding of the environmental mechanisms of interventions beyond the available evidence on effects on FIB.

**Implications of all the available science.** The environmental sampling in the studies in our review were mostly focused on onsite sanitation interventions that provided or promoted improved latrines with pits or septic tanks. The small reduction in pathogen prevalence in the environment when pooled across all studies may explain the small effect the interventions had on child health in the parent studies. These findings also support previous findings of no effect from sanitation interventions on FIB in the environment, further demonstrating the insufficiency of onsite sanitation solutions in reducing fecal contamination in the environment and the importance of animal sources of feces. Possibly, more intensive WASH interventions like safely managed water and sanitation, including safe disposal and/or treatment of excreta from both human and animal sources, are needed to reduce environmental contamination enough to improve child health. We note that only a small number of intervention studies measured pathogens and MST markers in the environment and environmental samples were collected from only a subset of households. Pathogen targets and analytic methods varied by study, limiting comparability. Future research would benefit from environmental sampling following implementation of a more diverse set of WASH interventions. Such studies should enumerate a common range of pathogen targets and use standardized laboratory methods for a given target and environmental matrix.

## Introduction

Water, sanitation and hygiene (WASH) improvements are assumed to reduce childhood enteric infections, diarrheal disease and growth faltering by reducing exposure to fecal-orally transmitted pathogens in the environment. Until recently, trials of WASH interventions have primarily focused on documenting health outcomes such as caregiver-reported diarrhea without quantifying intermediate outcomes along the causal chain, such as detection of pathogens in environmental samples and human biological specimens. Measuring these causal intermediates can illuminate underlying mechanisms of interventions and offer explanations for intervention success or failure. Inspecting the causal chain is especially important given the small or null effects on child diarrhea and growth reported in well-conducted trials of WASH interventions.1–5

Pathogens are transmitted from the feces of infected individuals to new hosts through interconnected environmental pathways. Studies assessing the effect of WASH interventions on environmental contamination have primarily focused on drinking water (and, to a smaller extent, hands and food) while other pathways such as soil and surfaces in the domestic environment have received less attention.6 Household water treatment and handwashing have been associated with reduced fecal contamination of drinking water7 and hands,8,9 respectively while sanitation interventions have had limited impact on environmental fecal contamination, including drinking water, hands, objects, surfaces, soil and flies.6

These studies have mostly relied on measuring fecal indicator bacteria (FIB) such as *E. coli* in the environment as a proxy for pathogens. While *E. coli* has been shown to correlate with risk of diarrhea,10 FIB correlate poorly with pathogens in the environment11 and can originate from non-fecal as well as both human and animal fecal sources.10,12 Recent applications of advanced analytic methods to environmental sampling in low-income settings allow for detection of a range of enteropathogens,13,14 as well as distinction between human vs. animal fecal sources through microbial source tracking (MST).15 We assessed the effect of WASH interventions in low-income countries on enteropathogens and human- and animal-specific MST markers in the domestic environment with a systematic review and individual participant data (IPD) meta-analysis, which allows combining observation-level data from studies with standardized statistical methods.

## Methods

### Search methods

We conducted a systematic literature search to identify WASH intervention studies in low-income countries with environmental as well as child health measurements in order to assess (1) the impact of WASH interventions on environmental contamination with human enteropathogens and fecal markers, (2) associations between these measures of environmental contamination and child health outcomes, and (3) the hypothesized causal pathway from WASH interventions to child health mediated through reduced environmental contamination. In this paper, we present findings for the first aim. The analyses for the latter two aims are presented in a companion paper.16

We searched the PubMed, Embase, CAB Direct Global Health, Agricultural & Environmental Science Database, Web Of Science, and Scopus databases. Our search terms and PubMed search string are listed in Supplementary Tables S1 and S2. We only included studies published after 2000 to capture more recently developed pathogen detection and MST methods. We limited our search to studies in English. We included studies meeting the following inclusion criteria: 1) prospective studies with a water, sanitation, or hygiene intervention and concurrent control (i.e., randomized controlled trial, matched cohort, controlled before-and-after study), consistent with prior WHO burden of disease reviews,17,18 2) measured pathogens and/or MST markers in environmental samples, and 3) measured child anthropometry, diarrheal disease, or pathogen-specific infections for use in a companion manuscript. We excluded studies that only measured FIB. One reviewer (AM) screened abstracts according to our inclusion/exclusion criteria, and two independent reviewers (AM, RT) examined the full texts of short-listed articles.

### Data Collection and Analysis

For each eligible study, we contacted the corresponding authors to request individual data on the presence and abundance of pathogens and MST markers in environmental samples, child health outcomes, and potentially confounding baseline characteristics, including socioeconomic and demographic indicators. If the corresponding author was unwilling to share individual data, the study was excluded from our analysis. Prior to sharing data, all personal identifiers such as GPS locations were removed, and indirect identifiers such as sampling dates were coarsened to a monthly resolution. Unlike conventional meta-analyses that statistically pool reported effect estimates from different studies, the IPD approach pools raw data from individual studies and then estimates the effect of interest.19 While effects are often reported differently across studies (e.g., odds ratio, relative risk), IPD allow direct derivation of effect estimates, regardless of how they were reported in the original studies, with a standardized analysis approach, including consistent adjustment for covariates. The IPD approach also allows subgroup analyses beyond those reported in the original studies, by pooling effect estimates stratified by subgroups of interest.

Our two primary outcomes were the detection of any enteropathogen and any MST markers in any type of environmental sample. We generated these two composite measures to indicate detection of any pathogenic target or any MST target in any sample type collected during the same sampling round from the same compound, where a compound was defined by the original studies as a set of households with common courtyards, water sources, or latrines. Given that many targets were infrequently detected in individual studies, these composite outcomes allowed us to pool information from studies that focused on different targets and sample types, leveraging the IPD approach for increased statistical precision. We also analyzed the prevalence of any pathogen and any MST marker separately for each sample type (e.g., water, hand rinses, soil, flies). Secondary outcomes included the prevalence of specific pathogen types (any viruses, any bacteria, any protozoa, any helminths), the prevalence of MST markers from specific host types (human, animal), and the prevalence and abundance of individual enteropathogens and MST markers. We did not include general MST markers in our analysis as they are not host-specific.

We compared each outcome between the WASH intervention and control arms of the included studies. We estimated prevalence ratios using modified Poisson regressions.20 For abundance outcomes, we used linear regressions to estimate differences in log-transformed gene copies and negative binomial regressions to estimate ratios of soil-transmitted helminth (STH) egg counts. Because of repeated sampling or clustered designs in some studies, we used the Huber Sandwich Estimator to calculate robust standard errors.21 For abundance measures, we imputed values below the limit of detection (LOD) with half the LOD and values below the limit of quantification (LOQ) with the midpoint between the LOD and LOQ. To avoid analyzing targets where most data were imputed, we only included targets where >50% of samples were within the range of quantification (ROQ) in our analysis of abundance measures.

All analyses were adjusted for potential confounders. While estimated intervention effects from randomized trials should be unconfounded, covariate adjustment may increase statistical efficiency and improve exchangeability with matched cohorts and non-randomized trials.22 Covariates were prescreened using likelihood ratio tests, and those associated with the outcome with a p-value <0.2 were included in the model for each outcome. We prescreened the following variables if they were measured within an included study: number of people in the household, age and education of primary caregiver, asset-based household wealth, number of rooms, construction materials (walls, floor, roof), access to electricity, land ownership and if anyone in the household works in agriculture. When analyzing binary outcomes, we only included one independent variable (including the treatment arm) per 10 positive samples, or per 10 negative samples if <50% of samples were negative. We therefore did not estimate prevalence ratios for targets with <10 positive/negative values for a given sample type, or <2 positive/negative values per study arm for a given sample type. Given the heterogeneity across studies (e.g., local WASH conditions, climate, urbanization, population density, regional infectious disease patterns, intervention designs), we reported individual study-specific estimates for all analyses. For targets where data were available from ???4 studies, we tested for heterogeneity using Cochran’s Q-test.23 If there was no significant heterogeneity (p-value>0.2), we pooled estimates using fixed-effects models. If there was evidence for heterogeneity but qualitative support for combining studies, we pooled estimates using random-effects models. We did not pool abundance estimates because of issues in standardizing qPCR methods across sites and the small number of available abundance estimates.24,25

We conducted subgroup analyses by season (dry vs. wet), animal ownership (at least one vs. no animal owned) and pathogens with vs. without zoonotic transmission. The wet season for each study was defined as the 6 months of highest average rainfall.26 The pathogens we considered zoonotic were *Campylobacter, Salmonella, Yersinia enterocolitica, C. difficile, Cryptosporidium, Giardia and Ascaris*.26 We included Ascaris as a zoonotic pathogen because the microscopy methods in some of the included studies do not differentiate Ascaris lumbricoides from the morphologically identical Ascaris suum which can infect humans.26–28 Studies included detected virulence genes associated with specific E. coli pathotypes (EAEC, EPEC/EHEC, STEC, EIEC, ETEC). We classified STEC and EPEC (due to atypical EPEC) as zoonotic.26 We assessed effect modification by examining the p-values on the interaction terms between the treatment and the indicator variable for the subgroup in regression models; a p-value <0.2 was considered evidence of effect modification. We also assessed heterogeneity by study setting, study design and intervention uptake. There was limited heterogeneity in urbanicity within any individual study. We therefore pooled estimates separately for rural vs. urban studies and compared the pooled estimates with Wald tests. We also separately pooled estimates from randomized and quasi-experimental studies and studies with high vs. low intervention uptake and compared pooled estimates with Wald tests.

All analyses were conducted in R 4.0, and analysis scripts are publicly available (<https://github.com/amertens/wash-ipd>). Our systematic review search strategy and analysis plan were pre-registered on Open Science Framework (<https://osf.io/8sgzn/>). Our PRISMA checklist is available in Supplementary Table S3.

## Results

### Search results and data acquisition

The systematic review was conducted on 1/19/2021 and returned 3,376 results after removing duplicates. Of these, 3,253 were excluded by abstract screening, and of 125 short-listed studies, eight met the inclusion criteria after full-text screening. The eight publications reported findings from six unique intervention studies: WASH Benefits Bangladesh and Kenya trials,29 the Maputo Sanitation (MapSan) study in Mozambique,30 the Gram Vikas study in India,31 the Odisha Total Sanitation Campaign trial in India,5 and the CHoBI7 trial in Bangladesh32 (Table 1). Data were obtained from all studies except the CHoBI7 trial.

Environmental results were reported in three separate publications from the WASH Benefits Bangladesh study, and two publications and one manuscript in preparation from the Mapsan study.33,34 Multiple publications within a trial collected samples from different subsets of trial participants at different times; therefore, we report their results separately rather than combined by trial. For the Odisha Total Sanitation Campaign trial, only village-level source water quality data were shared.

### Characteristics of included studies

All but one of the eligible studies focused on onsite interventions, and one31 evaluated a combined water supply and onsite sanitation intervention. No studies examined the impact of hygiene or water interventions alone on pathogens and MST markers in the environment. The WASH Benefits trials were cluster-randomized, multi-armed factorial trials of water, hygiene, sanitation, and nutrition interventions in rural Kenya and Bangladesh, but pathogens and MST markers in environmental samples were only measured in the control and sanitation arms. The sanitation intervention included the construction of a double-pit pour-flush improved latrine and provision of a child potty and sani-scoop for feces removal. The MapSan study was a controlled before-and-after study of a decentralized sanitation intervention in urban Mozambique. The intervention entailed the construction of pour-flush toilets that drain to septic tanks, shared by a minimum of 12 people. The intervention delivery was not randomized, but control sites were matched to intervention sites on compound size and time of enrollment. The Total Sanitation Campaign study in Odisha, India, was a cluster-randomized trial assessing the Government of India’s Total Sanitation Campaign, which promoted the construction of a pour-flush latrine with a single pit and Y-joint for a future second pit, subsidized post hoc at the household level by government funding. The Gram Vikas matched cohort study evaluated the effect of a piped water and sanitation intervention provided by the Gram Vikas non-governmental organization in rural India. Gram Vikas built a water tank and piped distribution system, and provided materials for the construction of pour-flush toilets in each household. After each household in the village completed latrine construction, the water system was turned on for the whole village. The control villages were matched to intervention villages on pre-intervention characteristics.

Interventions led to high latrine access among intervention recipients in most studies. Individual studies used different definitions of latrine access, including improved latrines, clean latrines, functional latrines or latrines with a functional water seal, as observed by field staff. In four studies, 78-97% of intervention recipients had access to these types of facilities, compared to 18-45% of controls.2,3,31,**bickUsingPathAnalysis2021?** The Odisha trial had the lowest effect on latrine access, with 38% of intervention compounds having functional latrines at endline compared to 10% of controls.5 Usage of latrines in intervention households was variable and especially low among children. In WASH Benefits Bangladesh, 94% of adults were observed to defecate in a hygienic latrine in structured observations but only 54% of children were observed using the latrine or potty and only 15% of animal feces were observed to be removed with the provided sani-scoop.35 In WASH Benefits Kenya, reported safe disposal of child feces dropped from 77% one year after intervention to 37% after two years.3 In Odisha, 50% of households reported children using a latrine,5 and in Gram Vikas, 35% of intervention villages reporting disposing of child feces in improved latrines.31 However, access to and usage of improved latrines were higher in intervention households than control households in all studies.

### Sample types and targets in included studies

Samples were collected from 4 months36 to 6-10 years31 after intervention delivery, with most studies collecting samples at 1-2 years post intervention (Table 1). Types of samples included source and stored drinking water, child and mother hand rinses, soil from the courtyard, household and latrine areas, food, and flies caught in the latrine and kitchen areas. The number of samples in individual studies varied from 6037 to 210731. The pooled dataset across all studies included 12,184 samples, with a total of 40,150 observations for pathogen/MST marker prevalence.

The studies measured a range of bacterial, viral, protozoan and helminthic pathogens, including pathogenic *E. coli, V. cholerae, Shigella, Campylobacter, Salmonella, Yersinia, C. difficile*, rotavirus, norovirus, sapovirus, adenovirus, astrovirus, enterovirus, *Cryptosporidium, Giardia, Entamoeba histolytica, Ascaris lumbricoides and Trichuris trichiura* (Tables S4-S7). The MST markers included human (HumM2, HF183, BacHum, *M. smithii*), animal (BacCan, BacCow), ruminant (BacR) and avian (GFD) fecal markers (Tables S4-S7). Most studies used quantitative polymerase chain reaction (qPCR) or reverse-transcriptase (RT)-qPCR to quantify these targets (Table 1). One study used slide agglutination serotyping to detect *V. cholerae* and *Shigella*.38 One study detected *Cryptosporidium* oocysts and *Giardia* cysts using direct fluorescent antibody microscopy.37 Two studies enumerated STH eggs by microscopy.39,40

Many targets had low or no variation. Out of 267 unique combinations of study, sample type, and target, 18 had no positive values, 41 had <10 positive values and two had <10 negative values. Therefore, 206/267 sample-target combinations had sufficient variability in target prevalence to estimate a prevalence ratio and be individually included in our meta-analysis. Among these, the prevalence of pathogens ranged from 1.4% for *Giardia* in mothers’ hand rinses41 to 62.3% for *Ascaris* in soil39. The prevalence of MST markers ranged from 2.4% for HumM2 in child hand rinses36 to 97.5% for BacCow in mothers’ hand rinses.41

### Intervention effects on the prevalence of any enteropathogen and any MST marker

The interventions generally decreased the prevalence of pathogens and MST markers but the confidence intervals for prevalence ratios often crossed the null (Figure 1-2). Interventions decreased the prevalence of any pathogen in any sample type in all specific studies except for Boehm et al. 2016. Among individual sample types, pathogen prevalence was significantly reduced only in flies (adjusted prevalence ratio [aPR]=0.37 (95% CI: 0.16, 0.85), Figure 1). Overall, study-specific estimates were largely homogeneous, with no significant Cochran’s Q-tests of homogeneity. Therefore, we pooled estimates using fixed-effects models. When pooled across all studies, there was a small reduction in the prevalence of any pathogen detected in any sample type (pooled aPR= 0.94 (95% CI: 0.90, 0.99), Figure 1). Interventions had no effects on the prevalence of any MST marker in any sample type or within specific sample types.

### Intervention effects on the prevalence of pathogen types and individual pathogens

Interventions reduced the prevalence of any bacterial pathogens in any sample type (pooled aPR=0.92 (95% CI: 0.85, 0.99)), though intervention effects were not significant in any individual study (Figure 1). Interventions did not significantly reduce virus or protozoa prevalence in any sample type or within specific sample types, though point estimates from individual studies were protective except for viruses in child and mother hand rinses and household soil (Figure 1). Among specific pathogens, interventions reduced the prevalence of adenovirus (aPR=0.21 (95% CI: 0.06, 0.68)) and *Shigella* (aPR=0.28 (95% CI: 0.10, 0.78)) in any sample type in Capone et al. 2021, driven by significant reductions in latrine soil (Figure S1).

### Intervention effects on the prevalence of MST marker types and individual markers

Among specific types of MST markers (human or animal), interventions effects were inconsistent and largely null (Figure 2). There wasa reduction in any MST marker in any sample type in Boehm et al. 2016 (aPR=0.99 (95% CI: 0.93, 1.06)) but an increase in Capone et al. 2022 (in prep.) (aPR= (95% CI: , )). There was also a reduction any animal MST marker in stored water in Boehm et al. 2016 (aPR=0.69 (95% CI: 0.50, 0.95)), driven primarily by a significant effect of the sanitation intervention on the ruminant BacR marker (aPR= 0.62 (95% CI: 0.43, 0.90), Figure S2). There was a reduction in the human marker HF183 in any sample type (aPR=0.67 (95% CI: 0.48, 0.95)) in Holcomb et al 2020, but not individual sample types. There were no other intervention effects on individual MST markers (Figure S2).

### Intervention effects on the abundance of individual pathogens and MST markers

Of all observations, 20% had abundances quantified, including STH egg counts in Steinbaum et al. 2019 and Kwong et al. 2021 and gene copies of enteropathogens and MST targets in Boehm et al. 2016, Fuhrmeister et al. 2020, and Capone et al. 2022 (in prep.)(Figure S8). Of these, 18% were below the specific study-reported LOD, 24% below the study-reported LOQ, and 58% within the study-reported ROQ. Of targets enumerated within specific sample types, only 18% had >50% of samples within the ROQ and were therefore included in our analysis. The interventions had significant effects on the abundance of one MST target, but not others, and not on STH egg counts (Table 2). The abundance of the BacCow animal marker was lower in mothers’ hand rinses in the intervention arm in Fuhrmeister et al. 2020, with an adjusted log10-transformed difference of -0.28 (95% CI: -0.49, -0.07).

### Subgroup and adjusted analyses

The wet season was May-October in Bangladesh and India and November-April in Mozambique. Western Kenya has two distinct periods of high rain, so the wet season was defined as March-May and October-December for WASH Benefits Kenya. Intervention effects differed by season but the direction of the effects was inconsistent (Figure S3). There was no significant effect of any interventions on any pathogen or any MST marker prevalence when households were stratified by animal presence (Figure S4). There were no differences in intervention effects on pathogens with possible zoonotic transmission versus pathogens with only human hosts (Figure S5). Samples from compounds with animals did not have a significantly higher prevalence of zoonotic pathogens (36%) than households without animals (29%). In Wald tests, there were no significant differences in pooled estimates between the one urban intervention study (Mapsan) and the four rural studies (p-value for any MST in any sample type: 0.22), between randomized trials and quasi-experimental studies (p-value for any pathogen in any sample type: 0.43), or between the four studies with high latrine access among intervention recipients compared to the Odisha Total Sanitation Campaign trial with lower access (p-value for any pathogen in any sample type: 0.57). Adjustment covariates were measured differently across studies (Table S8). Unadjusted estimates did not vary greatly from adjusted estimates (Figures S6-S8).

## Discussion

We obtained individual participant data from five different WASH intervention studies reported in eight individual publications31,33,34,36,37,39–41 and one unpublished study (Capone et al. 2022, in prep). Despite differences in study settings and intervention designs across studies, results were relatively consistent, with no statistically significant heterogeneity in any of the pooled estimates.

While individual studies were likely underpowered to detect effects, their point estimates of intervention effects were consistently protective, and there was a small but significant reduction in the prevalence of any pathogen in any sample type when pooled across studies. Increased power from combining studies highlights a strength of the IPD meta-analysis approach, which allows pooling data with standardized estimation approaches across studies.42 Our findings indicate that there was a small effect of sanitation interventions on reducing the overall prevalence of pathogens but not MST markers in the environment, regardless of the study setting or the specifics of the sanitation improvements. These findings can help explain the null findings of the parent trials on child diarrhea,5,29–31 except for the WASH Benefits Bangladesh trial that found a significant reduction in diarrhea prevalence in the sanitation arm compared to controls.

Domestic animals can contribute to fecal contamination in the environment,43 and have been hypothesized to partly explain why sanitation interventions focused on isolating human fecal matter have achieved limited improvement in child health outcomes.44 MST markers allow differentiating the effect of sanitation improvements on fecal markers from human vs. animal sources. In our analysis, there was only a reduction in one of two human-specific MST markers in the MapSan study when aggregated across sample types, but not in individual sample types. We observed reduced prevalence of ruminant markers in stored water and reduced abundance of animal markers on mothers’ hands in two studies nested within the WASH Benefits Bangladesh trial; there was no effect on the prevalence of the latter (>95% in both arms), highlighting the utility of having quantitative data on high-prevalence targets. Notably, the sanitation intervention in this trial included a scoop for disposal of child and animal feces and may thus have reduced animal fecal contamination in environmental samples, while the sanitation interventions from other included studies would not be expected to reduce animal-specific MST markers.

Molecular methods to detect specific enteropathogens or MST markers are typically more expensive than measuring FIB and also require skilled staff and more advanced laboratory facilities. Therefore, a small number of studies have employed these methods in low-income countries and were eligible to be included in our meta-analysis, and environmental samples were only analyzed from a subset of participants in each study. In addition, MST markers have limitations with their specificity and sensitivity in low-income country settings.25,45 Our findings using these more advanced methods were not substantially different from previous evidence that sanitation interventions had no effect on FIB in the environment,6 suggesting null effects were not solely due to limitations of FIB. Therefore, FIB remain a useful tool to assess the impact of WASH interventions, as more locations and points in time can be sampled for the same cost to capture spatial and temporal variability. Enteropathogen-specific testing may be most useful to supplement FIB measurements to identify the specific etiologies through which WASH interventions may improve health or assess the effects of targeted interventions on a specific pathogen. Advances in technology that reduce the costs of molecular diagnostics or increased funding for environmental testing within WASH trials may allow broader use of these methods to more precisely estimate intervention effects on environmental contamination. Additionally, standardization of a broad panel of important enteropathogens, using methods sensitive enough to permit simultaneous detection in both environmental and clinical samples, as well as standardized reporting guidelines, would allow for better comparability for future IPD meta-analyses.13

Our primary outcomes were composite measures of contamination to allow us to combine estimates from different targets and sample types across multiple studies to increase statistical efficiency. Effects on these composite outcomes should be interpreted with caution as they provide no information on specific transmission pathways and also obscure the specific pathogens and fecal sources the interventions affected, a primary advantage of pathogen-specific measures and MST markers over FIB.46 However, our results using these composite measures are supported by the mostly protective but insignificant effects on individual pathogens in specific sample types. Similarly, pooled estimates from multiple studies should be interpreted in conjunction with study-specific estimates, as studies in our review measured different targets in different environmental matrices using different methods, had different interventions, and were conducted in locations with different climates, built environments, and sociocultural settings. However, we found no statistical evidence of heterogeneity in intervention effects between studies, and low-prevision study-specific estimates and high-precision pooled estimates were qualitatively aligned.

One limitation of our study was that covariates were measured differently across studies (Table S8) and we were able to control for a small number of covariates in some cases due to sparse data. However, it is unlikely that there is substantial residual confounding biasing our results given the randomized or quasi-experimental nature of included studies and the similarity between our unadjusted and adjusted estimates, (Figures S6-S7, Tables S6-S7). Definitions of effect modifiers also varied by study, as seasonal rainfall patterns vary by location and studies measured different types of animals in the compound as well as different types and numbers of zoonotic and non-zoonotic pathogens. To limit the number of comparisons, we did not evaluate effect modification by different types and numbers of animals, or different wet season definitions. The effect modification analyses involve small sample sizes after stratification, so they may be sensitive to the definitions of the effect modifiers. We did not correct for multiple comparisons, and so some study-specific intervention effects may be type-1 errors. The intervention studies were designed and powered to test for effects on child health and not pathogen or MST markers in the environment, and due to small sample sizes and low target prevalence we were not able to estimate intervention effects for some targets and had low power in estimated effects for individual studies.

One limitation of our analysis was that covariates were measured differently across studies and we were able to control for a small number of covariates in some cases due to sparse data. However, substantial residual confounding is unlikely given the randomized or quasi-experimental design of included studies and the similarity between our unadjusted and adjusted estimates. Definitions of effect modifiers also varied by study, as seasonal rainfall patterns vary by location and studies measured different types of animals in the compound as well as different types and numbers of zoonotic and non-zoonotic pathogens. The effect modification analyses involve small sample sizes after stratification, so they may be sensitive to the definitions of the effect modifiers. We did not correct for multiple comparisons, and so some study-specific intervention effects may be type-1 errors. The intervention studies were designed and powered to detect effects on child health and not pathogens or MST markers in the environment, and due to small sample sizes and low target prevalence we were not able to estimate intervention effects for some targets and had low precision in estimated effects for individual studies. In addition, the included studies only collected a small quantity of samples from a single location at a single point in time; any reductions in pathogen presence could be more apparent with larger quantities of environmental samples (e.g., water, soil) combined before testing or with repeated testing. Environmental sampling in general is not necessarily reflective of children’s exposure, as cross-sectional detection of a pathogen in a given environmental sample is a poor proxy of actual ingestion of pathogens over time.47

The limited effects of sanitation interventions on enteropathogens and MST markers in this analysis, and on diarrheal disease in the parent studies indicate that onsite sanitation solutions are insufficient to reduce fecal contamination in the environment. This could be due to limited use of the latrines, especially among children, failure to address animal feces, poor containment of feces within onsite facilities or lack of safe disposal and treatment of fecal waste. Public health programs should pursue “transformative WASH” approaches that encompass the full chain of excreta management including safe removal rather than mere containment, and address child and animal feces to more effectively interrupt environmental pathogen transmission.48 Future WASH intervention studies should also assess the effect of water treatment and hygiene interventions across a range of pathogens in the household environment and include understudied pathogen transmission pathways such as child hands, soil and flies.

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