Effect of water, sanitation, and hygiene interventions on enteropathogen detection in the environment: an individual-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 in the environment can help investigate if limited health effects occur because WASH interventions do not sufficiently reduce environment contamination.

**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 sanitation interventions. Most individual studies found no effects on pathogens or MST markers in environmental samples, including drinking water, hands, 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 trials have measured pathogens or host-specific fecal markers in the environment. The small effect of sanitation interventions on pathogens in the environment in this study is consistent with the lack of health impact in sanitation trials.

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## Research in context

**Evidence before this study.** Children in areas with poor drinking water, sanitation, and hygiene conditions (WASH) 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 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. We conducted a systematic review and individual participant data meta-analysis of WASH intervention studies that measured in the environment specific enteropathogens or microbial source tracking (MST) markers, which indicate the human or animal host of fecal microorganisms. We tested if interventions reduced the prevalence and abundance of these targets in drinking water, hand rinse, soil, and fly samples.

**Added value of this study.** We obtained data from 5 out of 6 eligible intervention studies identified in our systematic review that measured enteropathogens and MST markers in environmental samples. Most individual studies indicated a protective effect of interventions on the prevalence of individual 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 individual participant data meta-analysis design of our study 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 recently used 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.

**Implications of all the available science.** The environmental sampling in the studies in our review were mostly focused on sanitation interventions. The small reduction we observed in pathogen prevalence in the environment when pooled across all studies may explain the small effect the interventions had on child health. These findings also support previous findings of no effect from sanitation interventions on fecal indicator bacteria in the environment, further demonstrating the insufficiency of non-piped sanitation solutions in reducing fecal contamination in the environment and the importance of animal sources of feces. Possibly, high coverage of more intensive WASH interventions like safely managed water and sanitation 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 only a subset of households were environmentally sampled in each study. Pathogen targets and diagnostic methods varied by study, limiting comparability. Future research would benefit from environmental sampling following implementation of a more diverse set of WASH interventions using a standardized set of laboratory methods to enumerate a common range of pathogen targets.

## Introduction

Water, sanitation and hygiene (WASH) improvements are often assumed to reduce childhood enteric infections, subsequent 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 in human biological specimens. Without measuring these causal intermediates, trials are limited to a “black box” understanding, where underlying mechanisms of interventions are unknown and investigators can only speculate about reasons 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 recent 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 indicator organisms such as *E. coli* in the environment as a proxy for pathogens, and while *E. coli* has been shown to correlate with risk of diarrhea,10 fecal indicator bacteria (FIB) like *E. coli* correlate poorly with pathogens in the environment11 because both humans and animals shed FIB and FIB can originate from non-fecal sources.10,12 Recent applications of in nucleic-acid molecular methods to environmental sampling in low-income settings now allow for detection of a range of enteropathogens in human biological specimens and environmental samples,13,14 as well as distinction between human vs. animal fecal sources through microbial source tracking (MST).15 We conducted a systematic review and individual participant data (IPD) meta-analysis, where we combined sample-level observations of environmental sampling, allowing us to standardize the statistical approach, covariate adjustment, and subgroup analyses. We aimed to assess the effect of WASH interventions on detection of specific enteropathogens and human- and animal-specific MST markers in the domestic environment.

## Methods

### Search methods

We conducted a systematic literature search to identify WASH intervention studies in low-income countries that have measured pathogens and/or MST markers in environmental samples as well as at least one of the following health outcomes in children: caregiver-reported diarrhea, growth or pathogen detection in stool. We focused on studies 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 measures of environmental contamination and child health outcomes, and (3) examine the hypothesized causal pathway from WASH intervention 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. Search terms are listed in Supplementary Table S1 and our PubMed search string is listed in Supplementary Table S2. We only included studies published after 2000 to capture more recently developed advanced pathogen and MST detection methods. We limited our search to studies published 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 [Mertens et al. 2021 in prep]. We excluded studies that only measured fecal indicator bacteria such as coliforms or *E. coli*. One reviewer (AM) screened the abstracts of studies 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 study eligible for inclusion in the IPD meta-analysis, we contacted the corresponding authors to request individual data on the presence and abundance of pathogen and MST markers in environmental samples, child health outcomes, and potentially confounding baseline characteristics, including socioeconomic and demographic indicators. Unlike conventional meta-analyses that statistically pool reported summary estimates from different studies, IPD approach pools raw data from individual studies and then estimates the effect of interest.19 While effect estimates are often reported differently across studies (e.g., odds ratio vs. relative risk), individual participant data allow direct derivation of the effect estimate of interest, regardless of how it was reported in the original studies, with a standardized analysis approach, adjustment covariates, and subgroup analyses.

Our two primary outcomes were the detection of any enteropathogen and any MST markers in any type of environmental sample. We analyzed prevalences separately for each sample type (e.g., water, hands, soil, flies) and also as a composite measure indicating detection of a given target in any sample type collected from the same compound (set of households with common courtyards, water sources, or latrines) during the same sampling round. 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 (general, human, animal), and the prevalence and abundance of individual enteropathogens and MST markers.

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 in 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 for samples below the limit of quantification (LOQ) and the limit of detection (LOD). For samples below the LOD, we used half the LOD and for samples below the LOQ, we used the midpoint between the LOD and LOQ for the imputation. To avoid analyzing targets where most of the data were imputed, we only included targets where at least 50% of samples were within the quantifiable range in our analysis. We did not pool abundance estimates because of issues in standardizing qPCR methods across sites.22,23

All analyses were adjusted for potential confounders. While estimated intervention effects from randomized controlled trials should be unconfounded, covariate adjustment may increase statistical efficiency, and improve exchangeability with matched cohorts and non-randomized trials.24 Covariates were prescreened using likelihood ratio tests, and only variables associated with the outcome with a p-value < 0.2 were included in the model for each outcome. We included the following variables in the prescreening set if they were measured within an included study: number of people in the household, age and education of primary caregiver in the household, 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. 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 any targets with fewer than 10 positive or negative values for a given sample type, or with fewer than two positive or negative values per study arm for a given sample type. Targets with <10 negatives were also excluded from the composite “any pathogen” and “any MST marker” variables to avoid generating sparse cells; targets with <10 positives were included in the composite variables.

Given the heterogeneity in study settings (e.g., local WASH conditions, climate, urbanization, population density, region-specific infectious disease patterns, intervention designs), we reported individual study-specific estimates for all analyses. For targets where data were available from four or more studies, we tested for heterogeneity in estimates using Cochran’s Q-test.25 If there was no significant heterogeneity (p-value>0.2), we pooled estimates using fixed-effects models. If there was evidence for heterogeneity but there was qualitative support for combining studies, we pooled estimates using random-effects models.

We conducted subgroup analyses by season (dry vs. wet), animal ownership (at least one vs. no animal owned) and pathogens with any zoonotic vs. no zoonotic transmission. The wet season for each study was defined as the 6 months of highest average rainfall, obtained from <https://www.weather-atlas.com/>.26 The pathogens we considered zoonotic were *Campylobacter, Salmonella, Yersinia enterocolitica, C. difficile, Cryptosporidium, Giardia* and *Ascaris*.27 Studies included detected virulence genes associated with specific *E. coli* pathotypes (EAEC, EPEC/EHEC, STEC, EIEC, ETEC). Among these, we classified STEC and EPEC (due to atypical EPEC) as zoonotic.27. We assessed effect modification by examining the p-values on the interaction terms between the treatment and the indicator variable for the subgroup in the regression models; a p-value <0.2 was considered evidence of effect modification. There was no heterogeneity in study setting within any individual study as each study was conducted either in a primarily rural or primarily urban setting. We therefore explored heterogeneity by study setting by pooling estimates separately for rural vs. urban studies and comparing the pooled estimates with Wald tests. We also separately pooled estimates from randomized and quasi-experimental studies and from 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>). The systematic review search strategies and the analysis plan were pre-registered on Open Science Framework (<https://osf.io/8sgzn/>). Our PRISMA checklist can be found 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 the 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,28 the Maputo Sanitation (MapSan) study in Mozambique,29 the Gram Vikas study in India,30 the Odisha Total Sanitation Campaign trial in India,5 and the CHoBI7 trial in Bangladesh (Table 1).31 Data were obtained from all studies except the CHoBI7 trial.

Eight unique studies on pathogens or MST markers in the environment were nested within the five intervention studies with available data. Environmental results are reported in three separate publications from the WASH Benefits Bangladesh study, and two publications and one manuscript in preparation from the Mapsan study.32,33 These publications focused on samples collected from different subsets of trial participants at different times; therefore, we report results from these six studies separately. For the Odisha Total Sanitation Campaign trial, only village-level source water quality data were shared.

### Characteristics of included studies

All studies included a sanitation intervention. The WASH Benefits trials were cluster-randomized, multi-armed factorial designed 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 included promoting the construction of a pour-flush latrine with a single pit and Y-joint for a future second pit, which was subsidized post hoc at the household level by government funding. The Gram Vikas study was a matched cohort evaluating 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. No other study evaluated water interventions alongside sanitation intervention on enteropathogens or MST markers in the environment, and there were no studies examining the impact of hygiene or water interventions alone. Intervention uptake was high in most studies; 97% of intervention compounds in WASH Benefits Bangladesh had a latrine with a functional water seal compared to 31% of controls, 78% percent of intervention compounds in WASH Benefits Kenya had improved latrines compared to 20% of controls, 85% of intervention compounds in Gram Vikas had improved latrines compared to 18% of controls, and 86% percent of intervention compounds in MapSan had clean latrines compared to 45% of controls. Odisha had the lowest uptake, with 38% percent of intervention compounds having functional latrines at endline compared to 10% of controls.

### Sample types and targets in included studies

Samples were collected from 4 months34 to 6-10 years30 after intervention delivery, with most studies collecting samples at 1-2 years post intervention (Table 1). Types of samples collected 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 compound’s latrine and kitchen areas. The number of environmental samples in individual studies varied from 6035 to 210730. 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*.36 One study detected *Cryptosporidium* oocysts and *Giardia* cysts using direct fluorescent antibody (DFA) microscopy.35 Two studies used microscopy to enumerate STH eggs.37,38

Many targets had low or no variation, with 18/267 unique combinations of study, sample type, and target having no variation in target prevalence (all samples negative), and 43/267 of combinations having too little variation to estimate a prevalence ratio (<10 positive or negative samples). Among these sparse combinations, most (95.3%) had too few positive samples, and 4.7% had too few negative samples. Overall, 206/267 sample-target combinations had sufficient variability to be individually included in our meta-analysis. Among these, the prevalence of pathogens ranged from 1.4% for *Giardia* in mothers’ hand rinses39 to 62.3% for *Ascaris* in soil37. The prevalence of MST markers ranged from 2.4% for HumM2 on child hands34 to 97.5% for BacCow on mothers’ hand rinses.39

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

The interventions generally decreased the prevalence of pathogens and of 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, and among individual sample types there was only a significant pathogen prevalence reduction in flies (adjusted PR: 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, with an adjusted pooled PR of 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, with an adjusted pooled PR of 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 the presence of viruses or protozoa in any sample type or within specific sample types, though point estimates from individual studies were protective except for viruses on child and mother’s hands and household soil (Figure 1). Among specific pathogens, interventions reduced the prevalence of adenovirus (adjusted PR: 0.21 (95% CI: 0.06, 0.68)) and *Shigella* (adjusted PR: 0.28 (95% CI: 0.10, 0.78)) in any sample type in Capone et al. 2021, driven by significant reductions in latrine soil samples (Figure S1).

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

Among specific types of MST markers (human or animal), effects of interventions were inconsistent and largely null (Figure 2). The only significant estimates were observed for any animal MST marker in stored water in Boehm et al. 2016 (adjusted PR=0.69 (95% CI: 0.50, 0.95)), driven primarily by a significant effect of the sanitation intervention on the ruminant (BacR) marker (adjusted PR: 0.62 (95% CI: 0.43, 0.90), Figure S2). There were no other intervention effects on individual MST markers (Figure S2).

Among specific types of MST markers (human or animal), effects of interventions were inconsistent and largely null (Figure 2). There wasa reduction in any MST marker in any sample type in Boehm et al. 2016 (adjusted PR=0.99 (95% CI: 0.93, 1.06)) but and increase in Holcomb et al. 2022 (in prep., adjusted PR=1.16 (95% CI: 1.02, 1.32)). There was also a reduction any animal MST marker in stored water in Boehm et al. 2016 (adjusted PR=0.69 (95% CI: 0.50, 0.95)), driven primarily by a significant effect of the sanitation intervention on the ruminant (BacR) marker (adjusted PR: 0.62 (95% CI: 0.43, 0.90), Figure S2).In addition, there was a reduction in the human marker (HF183) in any sample type (adjusted PR=0.67 (95% CI: 0.48, 0.95)), 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, 20119/78539 (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. (in prep.) Of these, 18% were below the specific study reported limit of detection, 24% were below the study-reported limit of quantification, and 58% were in the study-reported range of quantification. Of targets enumerated within specific sample types, only 18% had >50% of samples within the range of quantification and were therefore included in our analysis. Table 2 shows the mean log10-transformed abundances stratified by arm as well as covariate-adjusted differences in abundances between the intervention and control arms for these combinations. The interventions had significant effects on the abundance of one MST target, but not on STH egg counts. 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). There were not sufficient studies with abundance data to pool estimates.

### 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. Interventions had significantly different effects by season in several study-specific sample types, but the direction of the effects was inconsistent (Figure S3). Interventions decreased the prevalence of any MST marker on child hands and in soil in Boehm et al. 2016, the prevalence of any MST marker in stored water in Furhmeister et al. 2020, and the prevalence of any pathogen in soil in Kwong et al. 2021 in soil, but only during the wet season. All three of these studies were from rural Bangladesh. Conversely, interventions increased the prevalence of any pathogen in stored water in Reese et al. 2017 and in stored water (marginally significant) and courtyard soil in Fuhrmeister et al. 2020 in the wet season but had no effect during the dry season.

There was no significant effect of any interventions on any pathogen or any MST marker prevalence when households were stratified by animal presence, but there was one significant interaction in Holcomb et al. 2020, the only urban study, with a non-significant protective effect of the intervention in homes without animals, and a non-significant harmful effect of the intervention in homes with animals (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 (28.5%). There were also no significant differences in pooled estimates between the one urban study (Holcomb et al. 2020) and the four rural studies (Wald-test p-value for any MST in any sample type: 0.22), between randomized trials and quasi-experimental studies (Wald-test p-value: 0.43), or between the four studies with higher intervention uptake compared to the Odisha Total Sanitation Campaign trial (Wald-test p-value: 0.59). Unadjusted estimates did not vary greatly from adjusted estimates (Figures S6-S8).

## Discussion

We obtained individual participant data from five different WASH intervention studies for an IPD analysis reported in 7 individual papers and one unpublished study.30,34,34,35,37–39 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. There were, however, limitations arising from heterogeneity in included studies. Covariates were measured differently across studies (Table S8), though it is unlikely there is substantial residual confounding biasing the results because of the randomized or quasi-experimental nature of included studies and the similarity between unadjusted and adjusted estimates, (Tables S6-S7). We did not correct for multiple comparisons, and so some study-specific intervention effects may be type-1 errors. 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. For example, we defined the wet season as the six rainiest months and found a significant reduction in the prevalence of any STH eggs in soil in the wet season while Kwong et al. 2021 defined the wet season as the five rainiest months in the original publication and found no evidence of effect modification. The included studies were also only able to sample a relatively small quantity from single locations at single points in time; it is possible reductions in pathogen concentrations would be more apparent with larger quantities of environmental samples combined before testing. Environmental sampling in general is not necessarily reflective of a children’s exposure, as the cross-sectional presence or concentration of pathogens in on location is an indirect proxy that may be poorly correlated with actual ingestion of pathogens over time.40 Lastly, there was also issues with small sample sizes and low target prevalence in the primary analysis, especially as the intervention studies were designed and powered to test for effects on child health and not pathogen or MST markers in the environment, leading to many un-estimated intervention effects and low power in estimated effects.

While individual studies were likely underpowered, there was generally consistency in protective point estimates of intervention effects (67% (138/206)), and there was a small but significant reduction in the prevalence of any pathogen in any sample type when pooled across studies. The increase in power when combining studies highlights a strength of the IPD meta-analysis approach, along with standardized estimation approaches and subgroup analyses, and also indicates that there was a small effect of sanitation interventions on reducing the prevalence of aggregated pathogens in the environment, regardless of the study setting or the specifics of the sanitation improvements.41 The pooled estimates should be interpreted in conjunction with the individual estimate, 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. This limits the comparability of different and increases the risk that the assumption of random-effects meta-analysis that intervention effects arise from normal distribution of true effects is violated.[@ borensteinBasicIntroductionFixedeffect2010] Despite that caveat, the small reduction in any pathogen prevalence in the environment and lack of effect on MST markers is consistent with the null findings of the parent sanitation studies on child diarrhea,[ref [ except for the WASH Benefits Bangladesh trial that found a significant reduction in diarrhea prevalence in the sanitation arm compared to controls.

However, effects on composite outcomes, like the pooled reduction of any pathogen presence, should be interpreted with caution. While composite outcomes increase statistical efficiency and allowed us to combine estimates from diverse trials, targets, and sample types, they obscure the specific pathways, pathogens, and sources of contamination which sanitation interventions affected, a primary advantage of using pathogen specific measures and MST markers over FIB.42 Unlike with FIB, though, aggregated pathogens still ensure a fecal source and pathogenicity, indicating WASH interventions have a small effect on some of the causes of diarrheal disease within the specific populations studied. This aggregate result is supported by the mostly protective but insignificant effects on individual pathogens in specific samples.

Nucleic-acid (NA)-based diagnostics allowing for the detection of specific enteropathogens or MST markers are typically more expensive and complex than measuring FIB, contributing to the small number of eligible studies to be included in our meta-analysis and included studies only environmentally sampling a subset of enrolled households. Therefore, FIB remain a useful tool to assess the impact of WASH interventions, as they can sample more locations and points in time for the same cost, while enteropathogen-specific testing may be most useful supplementing FIB by identifying the specific infection pathways and causes through which WASH interventions may improve health. Advances in technology that reduce the costs of NA-based diagnostics or increase funding for environmental testing of enteropathogens within WASH trials, may more precisely estimate the impact of WASH interventions on environmental contamination. Additionally, standardization of a broad panel of important enteropathogens, using molecular methods sensitive enough to permit simultaneous detection in both environmental and clinical samples, as well as standardized reporting guideline, would allow for better comparability for future IPD meta-analyses.13

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 were only a reduction in one of two human-specific MST markers in the MapSan study when aggregated across sample types, and not in individual sample types , while we observed reduced prevalence of ruminant fecal markers in stored water and reduced abundance of animal fecal markers on mothers’ hands in two studies nested within the WASH Benefits Bangladesh trial. There was a reduced abundance of the BacCow MST on mother’s hands and slightly reduced the abundance on children’s hands, though no change in the very high prevalence (>95% in both arms), highlighting the utility of quantifying high prevalence environmental 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 have reduced animal-specific MST markers. In general, MST markers have strong limitations in assessing WASH impacts, as they have poor specificity and sensitivity in low-income countries in addition to greater expense compared to FIB.[23; holcombMicrobialIndicatorsFecal2020]

The limited effects of sanitation interventions on enteropathogens and MST markers in this analysis and on diarrheal disease in the parent studies are consistent with previous studies that found no effect on fecal indicator bacteria in the environment.6 This suggests that null effects are not solely due to limitations of indicator bacteria but rather indicate the insufficiency of basic sanitation solutions in reducing fecal contamination in the environment. The lack of intervention impacts may be due to the design of lower-cost interventions studies here, or a lack of compliance, especially among children. In WASH Benefits Bangladesh, uptake of the improved intervention was high and 94% of adults were observed using the latrine during structured observations, but only 54% of children used the latrine or potty and only 15% of animal feces was removed with the provided sani-scoop [@ parvezAchievingOptimalTechnology2018] Public health programs should pursue “transformative WASH” that intervenes at the community-level to avoid spillover from neighboring households, focuses on the full chain of fecal contamination including safe removal rather than just containment in latrines, and addresses child and animal feces tomore effectively interrupt environmental pathogen transmission.45 Future WASH intervention studies should also assess the effect of water treatment and hygiene interventions across a range of pathogens on the understudies infection pathways of hand, flies, and food as well as in drinking water and soil.

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