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

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## Abstract

**Background:** Drinking water, sanitation, and hygiene (WASH) improvements are promoted as cornerstones to reduce diarrhoeal disease in low-income countries. However, recent trials have found mixed effects of household- and community-level WASH interventions on child health. Measuring pathogens and host-specific faecal markers in the environment can help investigate causal pathways between WASH and health by quantifying if and how much interventions reduce environmental exposure to enteric pathogens and faecal contamination from human and different animal 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). 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.

Few WASH trials to date have measured pathogens/MST markers in the environment. We extracted individual participant data from five eligible trials. Environmental sampling was primarily focused on onsite sanitation interventions at the household or community level and included drinking water, hand rinses, soil and flies. Most studies indicated a consistent protective effect of interventions on the detection of pathogens but effect estimates in individual studies could not be distinguished from chance. When pooled across studies, we found a small but significant reduction in the prevalence of any pathogen in any sample type (prevalence ratio [PR]: 0.94 (95% CI: 0.90, 0.99)) . There was no overall effect on MST markers from humans (pooled PR: 0.99 (95% CI: 0.88, 1.12)) or animals (pooled PR: 1.00 (95% CI: 0.97, 1.03)).

**Interpretation:** The small effect on pathogens and lack of effect on human/animal faecal markers in the studies included in our review are consistent with the limited health impact previously reported in these trials. These findings suggest that the sanitation interventions implemented in these studies did not fully isolate faecal waste from the environment. More comprehensive sanitation solutions are needed to reduce environmental faecal exposure.

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## Research in context

**Evidence before this study.** Children in areas with poor drinking water, sanitation, and hygiene (WASH) conditions experience increased diarrhoeal disease and reduced growth. Recent rigorous trials of WASH interventions have shown mixed effects on reducing diarrhoeal 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 faecal pathogens, and child health. Most previous studies and meta-analyses on the effect of WASH interventions on faecal contamination in the environment have focused on faecal 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 faecal contamination, enteropathogen presence, and disease risk limit the interpretation of these findings. Naturalized FIB can be present in the environment without faecal contamination, and FIB also poorly correlate with actual pathogen presence. FIB also cannot distinguish between faecal 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 unsafely managed animal feces. 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 faecal 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 studies indicated a consistent protective effect of interventions on the detection of pathogens, and some pathogens (e.g., adenovirus, Shigella) showed large significant reductions but most effect estimates in individual studies were not statistically significant due to small sample sizes and infrequent detection of some of the targets. The IPD meta-analysis approach allowed us to increase statistical power and 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 human or animal MST markers. This study takes advantage of advanced methods to enumerate enteropathogens and host-specific faecal 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 at the household or compound level. 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. Taken together, this evidence suggests that the sanitation interventions implemented in the studies included in our review achieved a small reduction in faecal contamination in the environment. More comprehensive WASH interventions, such as safely managed water and sanitation, including safe disposal and/or treatment of excreta from both humans and animals, are potentially needed to reduce environmental contamination enough to improve child health. We note that only a small number of intervention studies measured our targets of interest, and we identified no studies that assessed the effect of water supply/treatment, hygiene or safely managed and/or sewered sanitation interventions on pathogens and MST markers in the domestic environment. Also, pathogen targets and analytic methods varied by study, limiting comparability. Future research would benefit from environmental sampling following implementation of a more diverse and comprehensive 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

Every year, diarrhoeal disease kills an estimated 525,000 children under five.**troegerEstimatesGlobalRegional2018?** Enteropathogens (pathogens causing infections of the intestinal track) are transmitted from the feces of infected individuals to new hosts through a diverse set of interconnected environmental pathways. Water, sanitation and hygiene (WASH) improvements have been promoted to reduce childhood enteric infections and diarrhoeal disease by reducing environmental exposure to faecal-orally transmitted pathogens. Until recently, trials of WASH interventions have primarily focused on documenting health outcomes such as caregiver-reported diarrhoea without measuring intermediate outcomes along the causal chain, such as presence and abundance of pathogens in different types of environmental samples to characterize exposure. Such measurements 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 diarrhoea and growth reported in recent well-conducted trials of WASH interventions.1–5

To date, WASH intervention studies that have measured environmental contamination have mostly relied on faecal indicator bacteria (FIB) such as E. coli as a proxy for a wide range of enteropathogens, including bacteria, viruses, protozoa, and helminths. Studies have also primarily focused on enumerating contamination in 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 faecal contamination of drinking water7 and hands,8,9 respectively, while sanitation interventions have had limited impact on environmental faecal contamination as measured by FIB, including drinking water, hands, objects, surfaces, soil and flies.6 However, FIB are imperfect predictors of faecal contamination, pathogen presence and ultimate health risk. While E. coli in drinking water has been shown to correlate with increased risk of diarrhoea,10 FIB can originate from non-faecal sources**devaneFecalIndicatorBacteria2020?** and generally correlate poorly with pathogens in the environment.11 Also, FIB are found in both human and animal feces, and their detection in the environment therefore cannot differentiate the source of the contamination.10,12,**devaneFecalIndicatorBacteria2020?**

Recent applications of advanced molecular methods to environmental sampling in low-income settings can offer advantages over FIB measurements in characterizing environmental contamination. These methods can be used to detect a range of enteropathogens,13,14 or distinguish between human vs. animal faecal 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 that measured human enteropathogens and human/animal MST markers in environmental samples. 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 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,16,17 2) measured pathogens and/or MST markers in environmental samples, and 3) measured child anthropometry, diarrhoeal disease, or pathogen-specific infections. We restricted the search to studies measuring child health outcomes to estimate associations between environmental contamination and child health in a companion paper.18 We included studies published after 2000 to reflect recent advances in pathogen detection and MST methods but we did not limit our search to any specific laboratory method (e.g., molecular, culture-based, microscopy). We excluded studies that only measured FIB. We limited our search to studies in English. One reviewer (AM) screened abstracts, and two independent reviewers (AM, RT) examined the full texts of short-listed articles with differences resolved with a third reviewer (AE). We followed PRISMA reporting guidelines (Table S3), and we used an adapted version of the Newcastle-Ottawa scale to evaluate bias risk in individual studies (Table S4).**wellsg.sheab.oconnelld.petersonj.welchv.lososm.tugwellpNewcastleOttawaScaleNOS?**

### Data Collection and Analysis

For each eligible study, we contacted 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 could not be included in 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.

Our two primary outcomes were the detection of any enteropathogen and any MST markers in any type of environmental sample. We did not pre-specify specific pathogens or markers as outcomes as each study measured a different set of targets. We used the pathogens and MST markers measured in the included studies to generate two composite measures: (i) detection of any pathogenic target or (ii) 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. Because many targets were infrequently detected in individual studies, 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 class (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 that 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.19 For abundance outcomes, we used linear regressions to estimate differences in log10 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.20 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, our analysis of abundance measures only included targets where >50% of samples were within the range of quantification (ROQ).

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.21 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. These variables reflect socio-demographic conditions that are commonly considered potential confounders in WASH studies. 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 type), we reported individual study-specific estimates for all analyses. For outcomes where data were available from 4 or more studies, we pooled using random-effects models fit using restricted-maximum likelihood with the metafor package.[@ viechtbauerConductingMetaAnalysesMetafor2010]. We did not pool abundance estimates because of issues in standardizing qPCR methods across sites and the small number of available abundance estimates.22,23

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 six months of highest country-level average rainfall.24 We classified the pathogens measured in the included studies as potentially zoonotic or not zoonotic. The pathogens we considered as potentially zoonotic were *Campylobacter, Salmonella, Yersinia enterocolitica, Clostridium difficile, Cryptosporidium, Giardia* and *Ascaris*.25 We included Ascaris as a potentially zoonotic pathogen because Ascaris lumbricoides and Ascaris suum cross-infect humans and pigs, and microscopy methods do not distinguish them as they are morphologically identical.25–27 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.25 We assessed additive interaction by examining the p-values on the interaction terms between the treatment and the indicator variable for the subgroup in linear regression models estimating prevalence differences because additive interactions have been argued to be more mechanistic and of public health importance.**vanderweeleTutorialInteraction2014?** A p-value <0.2 was considered evidence of effect modification given the lower power of interaction analyses.**selvinStatisticalAnalysisEpidemiologic2004?**

We also assessed heterogeneity by study-level characteristics, including setting, study design, intervention uptake and time between intervention onset and environmental sampling. 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 vs. quasi-experimental studies and studies with high vs. low intervention uptake and compared pooled estimates with Wald tests. We did not have data on the dates of intervention delivery for the individual studies so we pooled estimates separately for studies with shorter follow-up ($$1 year) and longer follow-up (>1 year) between intervention onset and environmental sampling as reported by the individual studies.

All analyses were conducted in R 4.0.4, 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/>).

## Results

### Search results and data acquisition

The systematic review was conducted on 19 January 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 articles reported findings from six unique intervention studies: the WASH Benefits Bangladesh (WBB) and Kenya (WBK) trials,28 the Maputo Sanitation (MapSan) study in Mozambique,29 the Gram Vikas study in India,30 the Total Sanitation Campaign (TSC) trial in India,5 and the CHoBI7 trial in Bangladesh31 (Table 1). Data were obtained from all studies except the CHoBI7 trial where individual participant data was not shared; this trial was not included in our IPD analysis. For the TSC trial, only village-level source water quality data were available. For WBB and Mapsan, multiple substudies within the trials collected samples from different subsets of trial participants at different times; therefore, we report their results separately rather than combined by trial.

### Characteristics of included studies

Three studies were cluster-randomized controlled trials (WBB, WBK, TSC). The MapSan study was a controlled before-and-after study where control sites were matched to intervention sites on compound size and time of enrollment. Gram Vikas was a matched cohort study where control villages were matched to intervention villages on 12 pre-intervention WASH and socio-economic characteristics. Based on the Newcastle-Ottawa scale, studies had low risk of bias due to blinded outcome assessments, with the Gram Vikas and MapSan studies having a lower rating due to higher loss to follow-up and lack of randomization (Table S4). WBB, WBK, TSC and Gram Vikas were conducted in rural settings while the MapSan study was urban. All included studies evaluated sanitation interventions (Table 1). The TCS and MapSan studies focused on sanitation alone. The WBB and WBK trials included individual and combined water, hygiene, sanitation, and nutrition interventions but pathogens and MST markers in environmental samples were only measured in the sanitation and control arms. The Gram Vikas study evaluated a combined piped drinking water and sanitation intervention. No included studies evaluated drinking water supply, drinking water treatment or hygiene interventions alone.

All sanitation interventions evaluated were onsite (i.e. non-sewered) technologies delivered at the household or community level. None of the interventions met the Sustainable Development Goal standard of “safely managed sanitation” and would be classified as “basic” or “limited” sanitation. In the WASH Benefits studies, the sanitation intervention included a new or upgraded improved latrine for each household in enrolled compounds and provision of a child potty and sani-scoop for feces removal. WBB latrines were dual pit latrines with a water seal and in WBK plastic latrine slabs were used to improve existing latrines. MapSan provided pour-flush latrines draining to septic tanks, shared by multiple households. TSC promoted construction of a pour-flush latrine with a single pit and Y-joint for a future second pit, subsidized post hoc by government funding. In the Gram Vikas study, a non-governmental organization provided materials for the construction of pour-flush latrines in each household in selected villages and built community water tanks and piped distribution systems providing household connections. When every household in the village completed latrine construction, the water system was turned on for the whole village.

Access to and use of improved latrines were higher in intervention households than control households in all 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.2,3,30,32 The TSC trial had the lowest effect on latrine access, with 38% of intervention compounds having functional latrines at endline compared to 10% of controls.5 Use of latrines in intervention households was variable and especially low among children, and safe management of child and animal feces was uncommon. In WBB, 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.33 In WBK, reported safe disposal of child feces dropped from 77% one year after intervention to 37% after two years.3 In TSC, 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.30

### Environmental sample types and targets in included studies

Environmental 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 environmental samples included source and stored drinking water, child and mother hand rinses, soil from the courtyard, household and latrine areas, and flies caught in the latrine and kitchen areas. Food samples were collected in one study**holcombImpactsUrbanSanitation2021?** but were not included in our IPD analysis because only 9 samples were positive for MST targets. The number of samples in individual studies varied from 6035 to 210730. The pooled dataset across all studies included 12,184 samples, with a total of 40,156 observations for pathogen or 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 S5-S8). The MST markers included human (HumM2, HF183, BacHum, *M. smithii*), animal (BacCan, BacCow), ruminant (BacR) and avian (GFD) fecal markers (Tables S5-S8). 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 microscopy.35 Two studies enumerated STH eggs by microscopy.37,38

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 rinses39 to 62.1% for *Ascaris* in soil37 and the prevalence of MST markers ranged from 2.4% for HumM2 in child hand rinses34 to 97.5% for BacCow in mothers’ hand rinses.39

### Effects on the prevalence of pathogens and any MST markers

Interventions decreased the prevalence of any pathogen in any sample type in most individual studies but the confidence intervals for prevalence ratios often crossed the null (Figure 1 ). Among individual sample types, pathogen prevalence was significantly reduced 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. When pooled across 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 (pooled aPR= 1.01 (95% CI: 0.98, 1.04)) or within specific sample types (Figure 2).

### Effects on the prevalence of pathogen classes and specific 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 prevalence in any sample type (pooled aPR= 0.90 (95% CI: 0.62, 1.33) or within specific sample types (Figure 1). Intervention effects in individual studies were generally in the protective direction for protozoa and helminths but we did not have sufficient studies to generate pooled estimates. 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 soil around latrines (Figure S1).

### Effects on the prevalence of MST marker types and individual markers

Across specific types of MST markers, interventions effects were inconsistent and largely null for both human markers (pooled aPR: 0.99 (95% CI: 0.88, 1.12)) and animal markers (pooled aPR: 1.00 (95% CI: 0.97, 1.03)) (Figure 2). Among significant effects, there was a reduction in any MST marker in water samples in Boehm et al. 2016 (0.69 (95% CI: 0.50, 0.95)) but an increase in any sample type in Capone et al. 2022 (aPR=1.16 (95% CI: 1.02, 1.32)). There was also a reduction in 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).

### Effects on the abundance of specific 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 (Figure S3). 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 abundance of the BacCow animal marker was lower in mothers’ hand rinses in the sanitation intervention arm in Fuhrmeister et al. 2020, with an adjusted log10-transformed difference of -0.28 (95% CI: -0.49, -0.07) per pair of hands. The interventions did not have significant effects on the abundance of any other MST target, nor STH egg counts (Table 2).

### 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 WBK. Intervention effects differed by season, but the direction of the effects was inconsistent (Figure S4). There was no significant effect of any interventions on any pathogen or any MST marker prevalence when households were stratified by animal presence (Figure S5). There were no differences in intervention effects on pathogens with possible zoonotic transmission versus pathogens with only human hosts (Figure S6). 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: 0.22), between randomized trials and quasi-experimental studies (p-value: 0.43), between studies with sampling closer in time to intervention delivery or later in time (p-value: 0.51) or between the four studies with high latrine access among intervention recipients compared to the TSC trial with lower access (p-value: 0.57). Adjustment covariates were measured differently across studies (Table S9). Unadjusted estimates did not vary greatly from adjusted estimates (Figures S7-S8).

## Discussion

Our IPD analysis of environmental samples within five intervention studies, mostly focused on household- and community-level onsite sanitation improvements, indicates a small overall reduction in pathogen prevalence in the environment associated with the interventions. While individual studies were underpowered to detect effects on pathogen prevalence with precision, point estimates of intervention effects were consistently in the protective direction across studies, despite differences in setting, sanitation intervention designs, and time between intervention delivery and sampling. There were no overall effects on human or animal faecal markers.

These findings add to a body of literature on the effectiveness of sanitation improvements in low-income countries in interrupting faecal-oral pathogen transmission. A previous systematic review found no effect of sanitation interventions on FIB in the environment.6 The small pooled effect on pathogens in the environment in our analysis indicates that any reductions in pathogen transmission through environmental pathways was likely small. This can help explain the null findings of the parent trials on child diarrhoea.2,3,5,29,30 Among the five included studies, only the WASH Benefits Bangladesh trial found a significant reduction in diarrhoea prevalence2 as well as a reduction in parasite infections**linEffectsWaterSanitation2018?**,**ercumenEffectsWaterSanitation2019?** in the sanitation arm compared to controls.**bauzaMicrobialSourceTracking2019?** Diarrhoea was reduced by 2.2 percentage points on the absolute scale, compatible with a small reduction in pathogen transmission. Taken together, these findings indicate that the sanitation interventions in the studies included in our review did not sufficiently isolate faecal waste from the environment, despite most of them achieving high levels of latrine access and latrine use by adults among intervention recipients. Young children’s feces are a dominant source of faecal contamination in the household environment**bauzaMicrobialSourceTracking2019?**, while animal feces make up the majority of global faecal waste,40,**berendesEstimationGlobalRecoverable2018?** and are associated with increased domestic contamination.40 Therefore, containment of adult human waste may be insufficient to reduce environmental contamination in settings with continued child open defecation and high exposure to animal waste.41 Only the WASH Benefits Bangladesh and Kenya trials included tools for child feces management (potties and scoops), and the scoops could also be used to dispose of animal feces but adoption of these tools was low.33,**islamAssessingSustainedUptake2022?**. Notably, we found reduced prevalence of ruminant (BacR) markers in stored water and reduced abundance of animal markers (BacCow) on mothers’ hands in two studies nested within the WASH Benefits Bangladesh trial. The reduction in animal faecal contamination can help explain the unique health impacts in this trial in contrast with the other four studies. In our analysis, only the MapSan study achieved a reduction in a human (HF183) marker. More comprehensive sanitation programs, such as safely managed sanitation services that include safe removal in addition to containment of faecal waste, and interventions targeting child and animal feces can potentially more effectively interrupt environmental pathogen transmission.**amebeluLancetCommissionWater2021?**

It is also possible that current environmental measures are limited in their ability to detect intervention effects on pathogen presence in the environment. Faecal contamination in the domestic environment varies spatially and temporally**levyDriversWaterQuality2009?**,**ramVariabilityHandContamination2011?**, and pathogen presence in the environment is intermittent48, depending on the presence of infected individuals, shedding rates and pathogen fate and survival in environmental reservoirs**paymentPathogensWaterValue2011?**. Different pathogens have different predominant transmission pathways, and specific pathogens may cause illness through a particular pathway too infrequently to capture with cross-sectional grab samples. Additionally, pathogen prevalence and abundance in the environment is typically low**paymentPathogensWaterValue2011?**, leading to low statistical power to detect intervention effects. Any reductions in pathogen presence might be more apparent with larger sample sizes and/or repeated sampling with high temporal and spatial resolution, which is costly for currently available pathogen detection methods, or by analyzing larger quantities of composite samples (e.g., water, soil). In addition, human MST markers have low specificity and sensitivity in low-income country settings with high levels of faecal contamination in the environment.23,42 Also, molecular methods for pathogen detection do not provide information on viability, and the clinical implications of small amounts of pathogen DNA/RNA detected in a sample are not clear. While FIB have limitations in terms of low specificity to faecal sources and poor correlation with pathogens, culture-based FIB enumeration captures viable organisms, and large numbers of temporal/spatial samples can be analyzed at low cost. Therefore, studies evaluating the environmental impact of WASH interventions can benefit from combining molecular pathogen measurements with culture-based FIB measurements to leverage the respective strengths of these approaches. Pathogen-specific testing can supplement FIB data 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 pathogen detection methods to more precisely estimate intervention effects on environmental contamination.

Our analysis had some strengths and limitations. The IPD meta-analysis approach allowed use to pool data with standardized estimation approaches across studies,43 increasing our statistical power for rare outcomes and allowing sufficient precision to detect a small overall effect on pathogens that individual studies were underpowered to detect. Pooling data assumes that findings from individual studies are sufficiently homogeneous despite implementing different interventions in different climates, built environments, and sociocultural settings. Pooled estimates should therefore be interpreted in conjunction with estimates from individual studies. In our analysis, we detected no statistical heterogeneity between individual studies, and low-precision estimates from individual studies were qualitatively aligned with high-precision pooled estimates, suggesting that pooling data did not obscure any study-specific trends. Similarly, because the studies measured different targets in different environmental matrices and many targets were detected infrequently, we relied on composite measures such as detection of any pathogen and any MST marker in any sample type to compare and pool data across studies. This highlights both a strength and limitation of pathogen detection in the environment. While measuring pathogens directly provides high specificity and avoids the false positives associated with FIB, the low prevalence of a given pathogen along a given pathway results in low statistical power. Combining data on different pathogens along different pathways increases power and can provide a general understanding of intervention impacts but obscures nuances on which specific pathogens along which pathways are influenced by interventions. Therefore, effects on these composite outcomes should be interpreted in tandem with pathogen-specific estimates. Standardized measurement and reporting of a harmonized panel of enteropathogens in a consistent set of environmental matrices can allow better comparability of pathogen-specific data for future IPD meta-analyses.13

Additionally, only a small number of studies met our inclusion criteria, limiting the generalizability of our findings. Four of the included studies focused on onsite sanitation interventions and one evaluated a combined piped water and sanitation intervention. Therefore, we were unable to explore the effects of individual water supply/treatment and hygiene interventions, and other, more comprehensive sanitation modalities such safely managed sanitation services and sewer connections. Also, while the studies tested a diverse set of samples, including understudied reservoirs such as a soil, they have missed potentially important pathways. For example, contaminated food has been identified as a dominant pathogen transmission pathway[@ aminQuantitativeAssessmentFecal2019] but only one study in our review sampled food and we could not include these data in our analysis as the target was infrequently detected. Similarly, 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 as the original studies were designed and powered to detect effects on child health rather than pathogens or MST markers in the environment.

Our results show that the sanitation interventions in our review led to a small reduction in the presence of enteropathogens in the environment, consistent with the limited health effects in these trials and suggesting that the interventions insufficiently isolated faecal waste. Large-scale piped water and sewerage improvements in high-income countries that effectively separate feces from the environment have drastically improved community health.**mackenbachSanitationPragmatismWorks2007?** Public health programs in low-income countries should pursue more comprehensive “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. Future WASH intervention studies should assess the effect of such interventions on environmental contamination, using a combination of molecular pathogen measurements and culture-based FIB proxies and including understudied transmission pathways such as soil, food, and flies.

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