Associations between detection of enteropathogens and microbial source tracking markers in the environment and child enteric infections and growth: an individual participant data meta-analysis

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## Abstract

**Background:** Fecal contamination is typically measured using fecal indicator bacteria (FIB). FIB in environmental samples have been associated with increased risk of diarrhea and reduced linear growth in children. However, FIB are imperfect predictors of enteropathogens, and few studies have assessed associations between detection of enteropathogens and microbial source tracking (MST) markers in the environment and child health outcomes.

**Methods:** We conducted an individual participant data (IPD) meta-analysis to pool data from WASH trials to assess associations between the presence of pathogens and/or MST markers in the environment and enteric infections with specific pathogens, caregiver-reported diarrhea and height-for-age Z-scores (HAZ) in children. We used covariate-adjusted regression models with robust standard errors to estimate associations and pooled results across studies. For the infection and diarrhea outcomes, we used environmental data from up to four months prior to the measurement of health endpoints; we used all available environmental data prior to the HAZ measurement.

**Findings:**

We identified and received data from nine studies. Detection of a specific pathogen in environmental samples was consistently associated with increased risk of subsequent child infection with the same pathogen. However, there was no consistent association between detection of enteropathogens in the environment and subsequent caregiver-reported diarrhea, except during wet seasons. Soil and child hands were predominant pathways associated with diarrhea. Detection of any pathogen in any sample type was associated with slightly lower HAZ (adjusted pooled mean difference: -0.08 (95% CI: -0.15, 0.00)). There was no consistent association between MST markers and diarrhea or HAZ; however, avian fecal markers were associated with increased risk of diarrhea and reduced child growth.

**Interpretation:** Detection of enteropathogens in the environment was associated with increased risk of pathogen-specific infections and lower HAZ but not with caregiver-reported diarrhea, highlighting the limitation of reported diarrheal symptoms as an outcome measure. Overall, conclusions were similar to studies that used FIB in the environment to predict child health outcomes. Measuring enteropathogens in environmental matrices can be useful for understanding transmission pathways for a specific pathogen and MST markers can be useful for assessing the disease burden associated with human vs. zoonotic pathogens, though most have limited associations with child health outcomes.

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## Research in context

**Evidence before this study.** Children in areas with poor drinking water, sanitation, and hygiene conditions (WASH) have reduced growth and a higher incidence of enteropathogen infections and diarrhea disease. Fecal contamination of the household environment, including in drinking water and soil and on flies and the hands of family members, is a primary route through which poor WASH leads to child pathogen ingestion which leads to poor health outcomes. Fecal contamination is usually measured using fecal indicator bacteria (FIB), and FIB presence in environmental samples have been associated with increased risk of diarrhea and reduced linear growth in children. However, FIB does not show the source of the fecal contamination or whether the contamination contains any or specific pathogens. As recent large WASH intervention studies have had limited effects on children’s health as well as on FIB in the environment, 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. MST markers indicate the human or animal host of fecal microorganisms, and the use of pathogen-specific and MST measures in environmental samples allowed us to examine if child morbidity was most related to exposure to specific pathogens through specific routes in the environment. We tested if the prevalence and abundance of enteropathogens and MST markers in drinking water, hand rinse, soil, and fly samples increased diarrhea disease or reduced growth in children under 5 years old.

**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 and child health outcomes. Several pathogens in the environment were strongly associated with infections of the same enteropathogen in children. There was no association between pathogens or MST markers and diarrheal disease, though there was a small association between pathogens and diarrheal disease during the wet season. There was a small overall association between the presence of any pathogen (but not any MST marker) in any sample type on child linear growth when data from all studies were combined, and the effect was larger in boys. Individual pathogens or MST markers did not have consistent associations with diarrhea or growth outcomes, and many estimates were imprecise due to small sample sizes, rare detection of some of the targets, rare diarrheal disease or measures of growth failure in some studies. By utilizing recently used methods to enumerate enteropathogens and host-specific fecal markers in a range of environmental samples and studies, we show that there is not a a clear pathway or pathogen through which environmental contamination effects child health.

**Implications of all the available science.**

This is the first synthesis of evidence of the association of these important targets and child health outcomes, and it showed the utility of enteropathogen measurements to identify environmental routes most associated with pathogen-specific infections in children, and how MST markers can identify the human or animal source of contamination. Due to the expense of these measures and the limited overall associations with child health outcomes, FIB may be more useful to measure to assess the impact of WASH interventions as more samples can be collected across time and space, while enteropathogen and MST marker measurements should be to understand the specific pathogen transmission pathways or estimate the disease burden associated with human vs. zoonotic sources of fecal contamination. The associations between specific pathogens in the environment and in child stool samples, combined with the lack of associations between enteropathogens and MST markers and diarrheal disease highlights the limitations of caregiver-recall of diarrheal disease as an outcome to assess WASH enterventions or the effects of fecal contamination in the environment. Only a small number of trials met our inclusion criteria and only a subset of households were environmentally sampled in each study, leading to data sparsity, and studies had varied pathogen targets, diagnostic methods, and time between environmental sampling and health assessment. Future research should use standardized sets of laboratory methods to enumerate a common range of pathogen and MST targets, they should use objective and specific health outcomes like pathogen detection in stool, and continue to include less studied pathways such as child hands, flies, and soil in addition to sampling water.

## Introduction

Undernutrition is a leading contributor to child mortality and morbidity in low and middle income countries, and growth failure from undernutrition is associated with reduced cognitive development and adult income.1 Growth failure leaves children vulnerable to disease, as children with less tissue mass have weakened immune systems, and conversely, infections can lead to growth failure as children use nutrients for their immune systems instead of growth.2,3 Enteric infections may particularly cause growth failure, as both subclinical changes to the gut and symptomatic diarrhea lead to nutrient loss. Diarrhea also is a leading cause of death of children younger than 5 years, causing an estimated 534,000 deaths in 2017.4 Poor water, sanitation, and hygiene (WASH) conditions lead to the contamination of the local environment with fecal pathogens, with an estimated 62% of deaths from diarrhea and 16% of growth failure among children under 5 years attributed to fecal exposure from poor WASH.5

However, several large, recent trials of WASH interventions found small or null effects on child diarrhea and growth, which may be because the interventions failed to reduce environmental fecal contamination, or because environmental fecal contamination from inadequate WASH was not the primary cause of child diarrhea or growth failure in those populations.6–8 Fecal contamination in the environment is usually assessed by enumerating fecal indicator bacteria (FIB) such as *E. coli*, which have been associated with increased risk of diarrhea and reduced linear growth in children.9 However, FIB are imperfect markers as they can originate from non-fecal sources,10 cannot differentiate between human vs. animal fecal sources,11, and cannot tell if fecal contamination detected in a household’s environment contains the enteropathogens that cause diarrheal disease in children in the household.

Detection of specific enteropathogens or host-specific microbial source tracking (MST) markers in the environment using molecular methods addresses shortcomings of FIB. Few studies have assessed associations between DNA-based detection of enteropathogens and MST markers in the household environment and child health outcomes. We conducted a systematic review and individual participant data (IPD) meta-analysis to assess associations between detection of enteropathogens and MST markers in the environment and pathogen-specific enteric infections, diarrheal disease and growth failure in children. We investigated different types of household samples (source and stored drinking water, mothers’ and children’s hands, soil and flies) to investigate the specific pathways through which environmental contamination influences child health. Understanding whether and to what extent specific enteropathogens and fecal markers in the environment are associated with child health outcomes can help illuminate the mechanisms behind the modest or null effects in recent WASH intervention trials and guide the development and implementation of future WASH interventions.

## Methods

We conducted a systematic literature search to identify WASH intervention studies 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 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), 2) measured pathogens and/or MST markers in environmental samples, and 3) measured child anthropometry, diarrheal disease, or pathogen-specific infections.12 We excluded studies that only measured FIB such as coliforms or *E. coli*. We only included studies published after 2000 to capture more recently developed advanced pathogen detection methods. Details on the search strategy have been described elsewhere.

We examined associations between enteropathogens and MST markers in the environment and child health outcomes, including enteropathogen-specific infections, caregiver-reported diarrheal disease and growth . We classified enteropathogens and MST markers in the environment into multiple exposure variables. Our primary exposure variables were the prevalence of any enteropathogen or any MST markers in any type of environmental sample. We also tabulated prevalence separately for each sample type (source or stored drinking water, mothers’ and children’s hands, household and latrine soil, flies). Secondary exposure variables 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 or 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. The primary outcomes were height-for-age Z-scores (HAZ) and 7-day prevalence of caregiver-reported diarrheal disease. For specific enteropathogens detected in the environment, primary outcomes also included child infection with the same pathogen ascertained by stool testing. Secondary outcomes included Z-scores for weight-for-age (WAZ) and weight-for-length (WLZ) and the prevalence of stunting, underweight and wasting, defined as a Z-score below -2 for HAZ, WAZ and WHZ, respectively.13 For the growth outcomes, we used data from all environmental samples collected over the child’s lifetime prior to the anthropometry measurement; if there were repeated growth measurements after environmental sampling, we used the closest growth measurement taken to environmental sampling. For diarrheal disease and enteropathogen-specific infections, we only used environmental samples collected up to four months before the sampling of the child; we selected this window empirically to allow us to retain the highest number of time-matched pairs of environmental and health measurements from the available data while maintaining a time ordering window consistent with previous studies assessing associations between environmental contamination and diarrhea.14

For binary outcomes (prevalence of pathogen-specific infection, diarrhea, stunting, underweight, and wasting), we estimated prevalence ratios associated with the different exposure variables using modified Poisson regression.15 For continuous outcomes (child anthropometry Z-scores), we used linear regression to estimate mean differences. Because of repeated sampling or clustered designs in some studies, we used the Huber Sandwich Estimator to calculate robust standard errors.16 All analyses were adjusted for potential confounders. We included child age and asset-based household wealth as adjustment covariates for all adjusted estimates. Other 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: study arm, child sex, maternal age, household food security status, number of people in the household, age and education of primary caregiver in the household, number of rooms, construction materials (walls, floor, roof), access to electricity, land ownership and if anyone in the household works in agriculture. Within each study, we only estimated associations when there were at least 5 cases of the binary outcome in the rarest stratum of the exposure.

Given the heterogeneity in study settings (e.g., local WASH and nutrition conditions, climate, urbanization, population density, region-specific infectious disease patterns), we reported individual study-specific estimates for all analyses. For outcomes where data were available from four or more studies, we tested for heterogeneity in estimates using Cochran’s Q-test,17 and if there was no significant heterogeneity (p-value>0.2), we pooled estimates using fixed-effects models, otherwise we pooled estimates using random-effects models.

We conducted subgroup analyses by child age (immobile vs. crawling vs. walking pre-school-age vs. school-age) and sex, animal ownership in the household, season (dry vs. wet), and study setting (rural vs. urban). The wet season for each study was defined as the 6 months of highest average rainfall, obtained from <https://www.weather-atlas.com/>.18. For age, sex, animal presence and season, we included interaction terms between the exposure and the indicator variable for the subgroup in the regression models; a p-value <0.2 on the interaction term was considered evidence of effect modification. There was no variation in urbanicity within individual studies; we separately pooled estimates from urban vs. rural studies to assess effect modification.

As sensitivity analyses, we compared covariate-adjusted estimates with unadjusted estimates. We also compared adjusted estimates from parametric regression models with adjusted estimates from flexible machine-learning based targeted maximum likelihood estimation models.19 Additionally, to assess the impact of our chosen time window between environmental and health outcomes, we re-estimated associations using environmental data collected within a month prior to the diarrhea measurement, as well as using all environmental data collected at any time with respect to the diarrhea measurement.

All analyses were conducted in R 4.1.4, 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 S1.

## Results

### Included studies

The systematic review was conducted on 1/19/2021 and returned 3,376 publications, of which nine were included in the IPD analysis as they both met the inclusion criteria and the authors agreed to share data. The nine publications reported findings from five unique intervention studies: WASH Benefits Bangladesh and Kenya trials,20 the Maputo Sanitation (MapSan) study in Mozambique,21 the Gram Vikas study in India,22 and the Odisha Total Sanitation Campaign trial in India23. For the Odisha Total Sanitation Campaign trial, only village-level source water quality data were shared. Because individual studies within a given trial collected environmental measurements from different subsets of trial participants at different times, we report results stratified by publication rather than trial.

The studies collected a range of sample types (source and stored drinking water, child and mother hands, soil from the courtyard, household and latrine areas, food, and flies caught in the compound’s latrine and kitchen areas). They measured bacterial, viral, protozoan and helminthic pathogens in environmental and child stool samples, including pathogenic *E. coli, V. cholerae, Shigella, Campylobacter, Salmonella, Yersinia, C. difficile*, rotavirus, norovirus, sapovirus, adenovirus, astrovirus, pan-enterovirus, *Cryptosporidium, Giardia, Entamoeba histolytica, Ascaris lumbricoides and Trichuris trichiura*. The MST markers included human (HumM2, HF183, BacHum, *M. smithii*), animal (BacCan, BacCow), ruminant (BacR) and avian (GFD) fecal markers. The most commonly used method was qPCR. Additional details on study designs, environmental sample collection, and laboratory methods are available elsewhere.12

The number of child diarrhea observations with time-matched environmental samples ranged from 210-2034 observations and diarrhea prevalence ranged from 6.1-25.9% across studies (Table 1). The number of HAZ observations with time-matched environmental samples ranged from 202 to 1800 observations and mean HAZ ranged from -1.90 to -1.33 (Table 1).

### Associations between environmental contamination and health

#### Associations with pathogen-specific infections

Detection of a specific enteropathogen in the compound environment was associated with higher prevalence of subsequent infection with the same pathogen in children living in the compound; trends were consistent across different enteropathogens and sample types (Figure 1). *Clostridium difficile*, *Ascaris* and *Trichuris* detected in courtyard soil were associated with 2-4 fold higher prevalence of infection the same pathogens, and *Shigella* and *Trichuris* detected in flies were associated with higher prevalence of *Shigella* and *Trichuris* infections, respectively. Pathogenic *E. coli* and *Giardia* detected in soil and/or flies and *Shigella* in soil also had borderline associations with higher infection prevalence. Few studies had time-matched data on water/hand samples and child infections; these studies found no association between pathogens measured in water or on hands and child infections with the same pathogen (Figure 1).

#### Associations with diarrhea

The presence of any enteropathogen in any type of environmental sample was not associated with diarrheal disease, except for significantly increased diarrhea prevalence associated with any enteropathogen detection on child hands in WASH Benefits Bangladesh (Figure 2); we note that the pathogen investigated in this study was rotavirus.24 When broken down by groups of pathogens, bacteria on child hands25 and protozoa in soil26 were also borderline associated with increasing risk of diarrhea but most other associationswere null (Figure S1). Similarly, most associations between specific pathogens in the environment and diarrhea were null, but rotavirus on child hands24 and *Giardia* in latrine soil26 were both significantly associated with an approximately two-fold increase in diarrhea risk (Figure S2). Detection of *Ascaris*, astrovirus and *Clostridium difficile* in soil26,27 and pathogenic *E. coli* on child hands25 was also borderline associated with increased risk (Figure S2). Increasing abundance of *Ascaris* and rotavirus in soil24,27 and rotavirus on child hands24 was associated with increasing risk of diarrhea (Figure S3).

There was no significant associations between the presence of any MST marker or groups of MST markers (human or animal) and child diarrheal disease in any sample type (Figure 2, Figure S1). Among individual markers, detection of the avian marker GFD in any sample was significantly associated with an over two-fold increase in diarrhea risk,28 and the same marker in stored water and on child hands was also borderline associated with increased diarrhea (Figure S4).24 Most other human and animal markers were not associated with diarrhea.

#### Associations with child growth

The presence of any enteropathogen in any environmental sample was significantly associated with lower HAZ when pooled across studies (adjusted mean difference: -0.08 (95% CI: -0.15, 0.00), Figure 3). This was driven primarily by the number of slightly harmful but insignificant effects rather than by any strong effect in specific studies, with the exception of water samples with any enteropathogen presence being significantly associated with lower mean HAZ.24 There was also a borderline association between detection of any pathogen in household soil and lower HAZ when pooled across studies (adjusted mean difference: -0.07 (95% CI: -0.15, 0.02), Figure 3).

When broken down by groups of enteropathogens, presence of viruses in stored water and protozoa on child hands was significantly associated with a reduction in HAZ (by approximately z= -0.5) in individual studies24,25 while presence of bacteria in source or stored water and STH in soil were borderline associated with with small reductions in HAZ (Figure S1).24,29 Individual pathogens whose detection was significantly associated with reduced HAZ were *Ascaris* in soil and flies, *E. histolytica* in soil, *Giardia* on child hands and rotavirus in water (Figure S2). However, many associations between individual pathogens and HAZ were null, and multiple pathogens in different sample types were associated with higher HAZ (Figure S2). Similarly, there were inconsistent associations between the abundance of specific enteropathogens and HAZ (Figure S3). For other measures of growth, associations between the presence/abundance of enteropathogens and WAZ, WHZ, stunting and wasting were mostly inconsistent but both the positive presence and increasing abundance of rotavirus in stored water was consistently associated with reduced HAZ (positive z=-0.37 (95% CI: -0.66, -0.07)) WAZ (positive z=-0.97 (95% CI: -1.28, -0.66)) and WHZ (positive z=-1.13 (95% CI: -1.43, -0.83)), and many pathogens showed some degree of association with increased risk of being underweight (Figures S1-S3).

The presence of any MST in any environmental sample was not associated with HAZ when pooled across studies, and individual studies showed significant associations both in the harmful and protective direction (Figure 3). The associations between HAZ and the presence of groups of MST markers and individual MST markers were inconsistent, with most estimates having null effects (Figure S1, Figure S4). Of the statistically significant associations with individual MST markers, half of the sample-specific estimates were associated with increased HAZ and half were associated with decreased HAZ. Associations between the abundance of specific MST markers and HAZ were similarly inconsistent (Figure S5). For other measures of growth, there were inconsistent associations between the presence or abundance of any MST marker and WAZ, WHZ, stunting, underweight and wasting across studies, with most estimates having null effects, and with significant effects occurring in both harmful and protective directions (Figures S1, S4, S5). However, some markers were consistently associated with reduced growth across multiple metrics within individual studies, such as the animal marker BacCow in multiple sample types (z from -0.27 to -0.45),25,26 and the avian marker GFD (z from -0.28 to -0.40) and ruminant marker BacR in stored water (z from -0.25 to -0.30; Figures S4, S5).24 The abundance of MST markers generally had similar association with health outcomes as the corresponding prevalences, though the abundance but not presence of BacCow in household soil, stored water, and flies was associated with lower HAZ and higher prevalence of stunting, and the abundance but not presence of HumM2 in household soil was associated with more stunting and wasting.

#### Subgroup analyses

There were no consistent differences in associations between enteropathogens or MST markers and diarrhea or HAZ when analyses were stratified by child age (Figures S6, S7). However, most studies did not have children measured in all age categories. There was also no significant effect of child sex on associations between environmental pathogens or MST markers and diarrheal disease (Figure S8). Pooled across studies, pathogen presence was associated with twice the reduction in HAZ in boys (adjusted mean difference: -0.18 (95% CI: -0.30, -0.06)) than in girls (adjusted mean difference: -0.18 (95% CI: -0.30, -0.06), Figure S9). The decrease in HAZ associated with the presence of any pathogen in any environmental sample was also higher among boys than girls in most individual studies, though the difference was only significant in one study (Figure S9).27 There was no effect modification by sex on associations between MST markers and HAZ (Figure S9).

Diarrheal disease was too sparse to estimate differences in associations between households with and without animals. When pooled across studies, there was a significant decrease in child HAZ in compounds with any sample with any enteropathogen detected when the child lived in a compound with no animals, but not in compounds that had animals (Figure S10). There was no interaction by animal presence for associations between MST and HAZ (Figure S10).

Also when pooled across studies, there was a significant increase in child diarrheal disease risk in compounds with any sample with any enteropathogen detected when the child diarrheal disease occurred during the wet season (Figure S11). There was no association with between MST markers and diarrhea in either season. There were also no significant differences in pooled estimates between the one urban study28 and the four rural studies for any combination of exposures and outcomes.

#### Sensitivity analyses

Most covariates were not strongly associated with enteropathogen or MST marker presence in the environment, suggesting they are not strong confounders of the relationship between these exposures and our child health outcomes (Figure S12). Measures of household wealth generally had the strongest association with environmental contamination, though the association varied by study and microbial target. Additionally, data sparsity allowed controlling for a small number of covariates in most analyses. On average, covariate adjustment had small effects on the results; adjusted estimates were slightly larger in magnitude than unadjusted estimates and the effect of adjustment was slightly more pronounced when a larger number of covariates was used for adjustment (Figures S13-S14). Comparison between associations estimated with generalized linear models (GLM) vs. machine-learning based targeted likelihood estimation models (TMLE) showed no major differences, indicating that the linear assumptions and lack of interactions in the GLMs used for the primary analysis did not lead to greater residual confounding compared to more flexible methods (Figures S15-S16). Lastly, results were similar when we used data from environmental samples up to four months prior, one month prior or at any time with respect to diarrhea measurements (Figure S17).

## Discussion

Detection of enteropathogens in the compound environment was associated with increased risk of subsequent infection with the same pathogen among children living in the same compound, as well as with lower HAZ (-0.08 (95% CI: -0.15, 0.00)) when pooled across studies, especially among boys (-0.18 (95% CI: -0.30, -0.06)). Enteropathogen detection in the environment overall was not associated with risk of subsequent diarrhea, except during the rainy season, but we observed associations between some individual pathogens and higher diarrhea risk. MST markers were generally were not associated with diarrhea, except for the avian GFD marker. Associations between MST markers and child growth outcomes were inconsistent across studies but detection of some animal markers, such as the avian GFD and animal BacCow markers in environmental samples was consistently associated with multiple reduced growth metrics within individual studies.

Strong positive associations between detection of pathogens in the environment and subsequent detection in child stool samples demonstrates environmental transmission and provides a link in the causal chain between environmental contamination and lower child HAZ, though few pathogens were measured in both environmental matrices and stool. However, the reduction in HAZ associated with enteropathogens in the environment was modest overall; small non-significant effects in individual studies became significant when pooled, highlighting the strength of IPD meta-analyses. The diarrhea outcome did not capture these risks, consistent with prior research showing self-reported all-cause diarrhea is a poor proxy for enteropathogen infections and highlighting its limitations as an outcome to assess the impact on environmental pathogen contamination.**regoComparisonTraditionalDiarrhoea2021?** This is also consistent with research on pathogens in recreational water in high-income countries, where specific enteropathogens had limited associations with all-cause diarrhea.30 In contrast, a previous study in India found that detection of any pathogen (*rotavirus*, *adenovirus*, *pathogenic E. coli*, *Cryptosporidium* or *Giardia*) in improved water sources was associated with increased risk of child diarrhea.31

Pathogens in the environment that had associations with increased risk of child diarrhea in our analysis included rotavirus, *Giardia* and, to a smaller extent, pathogenic E. coli, *Ascaris*, astrovirus and *Clostridium difficile*. Among these, rotavirus, pathogenic E. coli and astrovirus have been identified in multi-country case-control studies among the pathogens with the highest attributable burden of child diarrhea in low-income countries.32,33 Other dominant pathogens in these studies included Cryptosporidium, *Shigella*, Campylobacter and norovirus; we note that we did not have sufficient time-matched data to estimate associations between detection of these pathogens in the environment and child diarrhea. Rotavirus in drinking water was also significantly associated with lower HAZ, WAZ and WHZ (z ranging from -0.37 to -1.13) in one study;24 we note that this study was conducted among young children where half of the children were aged 0-5 months and 75% were aged <2 years, representing the critical window for growth faltering34.

The avian marker GFD was the only MST marker associated with increased risk of diarrhea, while GFD and the animal marker BacCow were associated with reduced child growth across multiple metrics. In a previous study in India, detection of both human and animal fecal markers in household samples was associated with >4-fold increase in child diarrhea, but only when the marker was detected in all vs. none of the sample types tested.31 Our findings support growing evidence that animals, specifically poultry, are a major source of diarrhea transmission in low-income countries.35 Close contact with domestic animals has been shown to be associated with diarrhea, markers of enteric dysfunction and reduced growth among children.36,37 Poultry have been specifically associated with increased risk of Campylobacter diarrhea,38,39 and *Campylobacter* infections have been linked to reduced child growth.40 A study in Ethiopia found lower HAZ among children in homes where chickens were corralled, though owning chickens was associated with higher HAZ overall.41

In our analysis, soil and child hands stood out as dominant pathways of environmental diarrhea transmission, and pathways associated with reduced child growth also included source and stored water. A recent meta-analysis showed increased risk of diarrhea associated with increasing levels of fecal indicator bacteria in drinking water and on child hands, and reduced HAZ associated with increasing FIB in drinking water.9 Child hands have been identified as a major source of children’s fecal exposure, in terms of frequency of mouth contacts,42 estimated *E. coli* ingestion43 and associations with diarrhea.44 Similarly, soil has been shown to account for a significant portion of estimated *E. coli*ingestion for children45 and ingestion of soil has been associated with markers of environmental enteric dysfunction and stunting in children.46,47 Our findings corroborate the role of child hands and soil in diarrhea transmission.

Our analysis adds to a body of research that assessed the relationship between child health outcomes and environmental fecal contamination measured by FIB. Meta-analyses have found that E. coli and thermotolerant coliforms in water are associated with increased risk of diarrhea.48,11 A recent IPD analysis found that the odds of diarrhea increased by 9% of each log10 increase in FIB in drinking water and by 11% for each log10 increase in FIB on child hands.9 In the same IPD analysis, a log10 increase in FIB in drinking water and on fomites was associated slightly reduced HAZ (z = -0.04 and -0.06, respectively).9 Our analysis using specific enteropathogens and MST markers in the environment did not yield major advantages over using FIB with respect to predicting child diarrhea and growth outcomes; we found no consistent association between these more advanced environmental measurements and diarrhea, and the reduction in HAZ associated with enteropathogens in the environment was similar in magnitude to what has been reported for FIB. Measuring enteropathogens and MST markers in environmental samples is subject to some of the same limitations as measuring FIB, such as temporal and spatial variability, exacerbated by smaller sample sizes for these more expensive measurements and the low prevalence of enteropathogens in the environment. Additional limitations for linking these measurements to health outcomes include inaccurate recall of self-reported diarrhea symptoms, multiple etiologies for diarrhea and a multitude of risk factors for impaired growth beyond environmental exposures. Nonetheless, measuring enteropathogens in the environment is useful for assessing transmission pathways for a specific pathogen, as evidenced by our findings of increased risk of infection with a pathogen following its detection in the environment. Similarly, MST markers are a useful tool for understanding the health burden from human vs. zoonotic pathogens, as evidenced by increased diarrhea and reduced child growth associated with avian fecal markers in our analysis.

Our analysis had several limitations. Due to the smaller sample size of the environmental samples within the eligible studies, the rare detection of many of the enteropathogens in environmental samples and the low prevalence of diarrheal disease in children in many individual studies, data sparsity limited the feasible analyses. Many exposure-outcome associations were not estimated due to sparse data and there was only a small number of pathogens measured in both the environment and subsequently in children’s stool. Additionally, we could only adjust for a small subset of potentially confounding covariates in some analyses due to the small number of available observations. However, most covariates were weakly associated with measures of environmental contamination, and our unadjusted and adjusted estimates were similar, even when controlling for a larger number of covariates. Flexible covariate adjustment through TMLE did not change the associations between environmental contamination and diarrheal disease or HAZ. Therefore, we believe our modeling approach adequately adjusted for measured confounding but unmeasured confounding may bias our results. We did not correct for multiple comparisons, and so some significant associations are likely type-1 errors, especially when results across sample types and individual studies were inconsistent. The differences in the time window between environmental and child health measurements across different studies may have also led to inconsistencies in associations between studies. However, shrinking or expanding the window we allowed between environmental and diarrhea measurements in our analyses did not change our findings.

Future studies investigating the associations between environmental fecal contamination and child health should continue to include less studied pathways such as child hands and soil in addition to waterborne pathways, and use objective and specific health outcomes, such as pathogen detection in stool, instead of self-reported all-cause diarrhea. Enteropathogen and MST marker measurements can augment or replace FIB measurements when the goal is to understand the transmission of a specific pathogen or estimate the disease burden associated with human vs. zoonotic pathogens.

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