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

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## Abstract

**Background:** Quantifying the contribution of environmental faecal contamination to child diarrhoea and growth faltering can illuminate causal mechanisms behind the small/null effects on child health in recent water, sanitation and hygiene (WASH) trials. Fecal indicator bacteria (FIB) typically measured in the environment are imperfect proxies for enteropathogens and can come from human or animal faeces, which carry different levels of health risk. Detecting pathogens and host-specific microbial source tracking (MST) markers in the environment may better predict health outcomes. Methods: We conducted a systematic review and individual participant data meta-analysis of WASH intervention studies that measured enteropathogens and/or MST markers in environmental samples and subsequently measured child enteric infections, diarrhoea and height-for-age Z-scores (HAZ). We assessed associations between environmental measurements and child health outcomes using covariate-adjusted regressions with robust standard errors and pooled estimates across studies. Findings: We identified and received data from nine eligible publications within five intervention studies. Pathogen detection in environmental samples was associated with increased prevalence of infection with the same pathogen and lower HAZ (???HAZ=-0.09 (95% CI: -0.17, -0.01)) but not with diarrhoea (prevalence ratio [PR]: 1.17 (95% CI: 0.94, 1.46)), but during wet seasons pathogen detection was associated with higher diarrhoea prevalence (adjusted pooled prevalence difference [PD]= 0.05 (95% CI: 0.01, 0.09)). Detection of any pathogen in environmental samples was 1.17 (95% CI: 0.94, 1.46)). Detection of MST markers was not associated with diarrhoea (human markers: PR=1.01 (95% CI: 0.83, 1.24); animal markers: PR=1.21 (95% CI: 0.53, 2.77)) or HAZ (human markers?????????HAZ=-0.02 (95% CI: -0.15, 0.11); animal markers: ???HAZ=-0.06 (95% CI: -0.29, 0.18)). **Interpretation:** Our findings support a causal chain from faecal contamination to infection to growth faltering. Lack of health associations with most human and animal MST markers suggest a need for better faecal markers. Future studies should incorporate longitudinal and spatial environmental sampling using a combination of FIB, enteropathogens and well-performing MST markers and test for pathogens in stool to examine the theories of change between WASH interventions, faecal contamination and child health. **Funding:** The Bill & Melinda Gates Foundation.

## Research in context

**Evidence before this study.** Children in areas with poor drinking water, sanitation, and hygiene conditions (WASH) are exposed to enteric pathogens from faecal waste via environmentally mediated pathways such as drinking water, hands, food, soil and flies. These exposures can result in gut colonization with pathogens, which can lead to subclinical infections or diarrhoeal illness, which in turn can contribute to growth faltering. Recent large household- and community-level WASH intervention studies that aimed to interrupt environmental pathogen transmission have had limited effects on children’s health and on the detection of faecal indicator bacteria (FIB) in the environment. These findings have generated substantial debate about whether basic WASH interventions do not sufficiently reduce environmental pathogen exposure to prevent disease in high-burden settings, or whether environmental faecal contamination from inadequate WASH was not the primary cause of child diarrhoea or growth failure in these populations. Sensitive molecular methods allow simultaneous detection of multiple enteropathogens in environmental samples, and microbial source tracking (MST) methods can ideally help distinguish between human vs. animal faeces which carry different levels of health risk. Assessments using these methods can help illuminate the hypothesized causal chain between WASH improvements, environmental contamination and child health. We conducted a systematic review and individual participant data meta-analysis of WASH intervention studies that measured enteropathogens or MST markers along with child health outcomes. A previous analysis reported that WASH interventions led to a small reduction in enteropathogen detection in the environment and had no effect on MST markers. Here, we examine to what extent enteropathogens and MST markers along different pathogen transmission routes in the domestic environment are associated with pathogen-specific infections, diarrhoea and growth in children under 5 years old. **Added value of this study.** We obtained data from nine eligible publications reporting findings from five WASH intervention studies. Several pathogens in the environment were strongly associated with subsequently measured infection with the same pathogen in children. There was no overall association between pathogen detection in the environment and subsequent diarrhoea. Pooled across studies, pathogen detection in environmental samples was associated with slightly lower linear growth. Most human or animal MST markers were not associated with diarrhoea or child growth, except for avian markers. Previous meta-analyses have linked FIB presence in environmental samples to increased risk of diarrhoea and reduced linear growth in children. Data on health associations with enteropathogens and MST markers in the environment are scarce and mostly limited to high-income countries. This work is the first synthesis of evidence of the association between advanced environmental measurements and health outcomes in low-income countries to examine causal pathways between WASH interventions and health. **Implications of all the available science.** Enteropathogen detection in the environment was associated with increased risk of infection with the same pathogen and reduced child growth but not with caregiver-reported diarrhoea. These findings support the causal chain leading from environmental faecal exposure to infection to growth faltering. Our results also highlight the discordance between pathogen detection in the gut and symptomatic illness in settings where pathogen exposure is common, indicating that studies should augment self-reported diarrhoea outcomes with pathogen detection in stool. The reduction in HAZ associated with enteropathogens in the environment in our analysis was small and similar in magnitude to what has been reported for FIB. These findings indicate that environmental faecal contamination measurements with current methods only partially explain growth faltering in children, regardless of choice of analytical target. This could be because cross-sectional grab samples do not adequately characterize environmental contamination or capture the frequency and duration of exposure, which determine the internal dose ultimately ingested by children. Future studies should incorporate longitudinal and spatial environmental sampling using a combination of FIB, enteropathogens and well-performing MST markers. FIB may remain a useful tool as samples across time and space can be inexpensively analyzed to capture variability and predict health risks. Enteropathogen and well-performing MST marker measurements, respectively, can augment FIB measurements to examine transmission pathways for specific pathogens or identify zoonotic risk factors. We note that a small number of studies met our inclusion criteria and only a subset of households were environmentally sampled in each study, leading to data sparsity. Meta-analyses with additional data from future studies may detect associations we missed.

## Introduction

In settings with poor water, sanitation and hygiene (WASH) conditions, children are exposed to enteric pathogens through multiple environmentally mediated pathways, such drinking water, food, hands, flies, soil, surfaces and objects. These exposures can lead to gut colonization with pathogens, resulting in asymptomatic carriage, subclinical infection or symptomatic diarrhoeal disease1 Both subclinical changes to the gut and symptomatic diarrhoea can lead to nutrient loss and growth failure,1 and malnutrition leaves children further vulnerable to diarrhoeal disease through weakened immunity.2,3 Diarrhoea caused an estimated 534,000 deaths among children under 5 years in 2017,4and undernutrition is a leading contributor to child mortality and morbidity globally.5 An estimated 62% of diarrhoea deaths and 16% of growth failure among children under 5 years are attributed to faecal exposure from poor WASH.6 However, several large, recent trials of household- and community-level WASH interventions found small or null effects on child diarrhoea and growth, which may be because the interventions failed to reduce environmental faecal contamination, or because environmental faecal contamination from inadequate WASH was not the primary cause of child diarrhoea or growth failure in those populations.7–9 Faecal contamination in the environment is usually assessed by enumerating faecal indicator bacteria (FIB) such as *E. coli*, which have been associated with increased risk of diarrhoea and reduced growth in children.10 However, FIB are imperfect proxies of health risk as they can originate from non-faecal sources,11 and cannot confirm pathogen presence12 or differentiate between human and animal faeces which carry different levels of health risk13 Directly measuring enteropathogens in environmental matrices may better capture child exposures to disease-causing organisms and predict health outcomes, and detection of human vs. animal-specific microbial source tracking (MST) markers may indicate health risk of different magnitudes.14Understanding whether and to what extent specific enteropathogens and host-specific MST 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 of future interventions. We conducted a systematic review and individual participant data (IPD) meta-analysis to assess associations between enteropathogens and MST markers in different types of household samples and subsequently measured pathogen-specific enteric infections, diarrhoea and growth failure in children.

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