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Human fecal and pathogen exposure pathways in rural Indian villages and the effect of increased latrine coverage

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ABSTRACT

Efforts to eradicate open defecation and improve sanitation access are unlikely to achieve health benefits unless interventions reduce microbial exposures. This study assessed human fecal contamination and pathogen exposures in rural India, and the effect of increased sanitation coverage on contamination and exposure rates. In a cross-sectional study of 60 villages of a cluster-randomized controlled sanitation trial in Odisha, India, human and domestic animal fecal contamination was measured in community tubewells and ponds (n = 301) and via exposure pathways in homes (n = 354), using *Bacteroidales* microbial source tracking fecal markers validated in India. Community water sources were further tested for diarrheal pathogens (rotavirus, adenovirus and *Vibrio cholerae* by quantitative PCR; pathogenic *Escherichia coli* by multiplex PCR; *Cryptosporidium* and *Giardia* by immunomagnetic separation and direct fluorescent antibody microscopy). Exposure pathways in intervention and control villages were compared and relationships with child diarrhea examined. Human fecal markers were rarely detected in tubewells (2.4%, 95%CI: 0.3–4.5%) and ponds (5.6%, 95%CI: 0.8–10.3%), compared to homes (35.4%, 95%CI: 30.4–40.4%). In tubewells, *V. cholerae* was the most frequently detected pathogen (19.8%, 95%CI: 14.4–25.2%), followed by *Giardia* (14.8%, 95%CI: 10.0–19.7%). In ponds, *Giardia* was most often detected (74.5%, 95%CI: 65.7–83.3%), followed by pathogenic *E. coli* (48.1%, 95%CI: 34.8–61.5%) and rotavirus (44.4%, 95% CI: 34.2–54.7%). At village-level, prevalence of fecal pathogen detection in community drinking water sources was associated with elevated prevalence of child diarrhea within 6 weeks of testing (RR 2.13, 95%CI: 1.25–3.63) while within homes, higher levels of human and animal fecal marker detection were associated with increased risks of subsequent child diarrhea ($P = 0.044$ and 0.013, respectively). There was no evidence that the intervention, which increased functional latrine coverage and use by 27 percentage points, reduced human fecal contamination in any tested pathway, nor the prevalence of pathogens in water sources. In conclusion, the study demonstrates that (1) improved sanitation alone may be insufficient and further interventions needed in the domestic domain to reduce widespread human and animal fecal contamination observed in homes, (2) pathogens detected in tubewells indicate these sources are microbiologically unsafe for drinking and were associated with child diarrhea, (3) domestic use of ponds heavily contaminated with multiple pathogens presents an under-recognized health risk, and (4) a 27 percentage point increase in improved sanitation access at village-level did not reduce detectable human fecal and pathogen contamination in this setting.

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1. Introduction

Despite reductions in global child mortality, an estimated 6.6 million children under age five still died in 2012, of which 22% were in India (UNICEF, 2013). Diarrheal diseases associated with poor sanitation are a leading cause of child deaths in developing countries, accounting for an estimated 13% of the deaths in India (Liu et al., 2012). While proper excreta disposal through good sanitation is necessary to reduce this disease burden, the impact of a specific sanitation intervention on health within a given setting is not always guaranteed as demonstrated by two recent evaluations of the Indian government's rural sanitation program (Clasen et al., 2014; Patil et al., 2014). Lack of measurable health impact may occur when the sanitation intervention does not adequately disrupt fecal-oral pathogen transmission pathways and/or address critical sources operating in the intervention setting. Pathways and sources have generally been difficult to assess using traditional fecal indicator bacteria (FIB) of microbial exposure such as total coliforms, thermotolerant coliforms, *Escherichia coli*, and members of the genus *Enterococcus* (the enterococci). FIB can originate from both humans and animals, and have been found in environments where fecal contamination is improbable, due to the presence of naturalized FIB and the ability to grow outside their hosts (Ishii et al., 2006; Leclerc et al., 2001; Power and Nagy, 1999). To move understanding forward, efforts using new research tools are needed to shed light on gastro-intestinal pathogen transmission pathways, pathogen sources, and the mechanisms by which different water, sanitation and hygiene interventions interrupt key pathways in diarrheal disease burden settings.

In this research, we apply advanced microbial source tracking (MST) techniques to assess human-associated fecal exposure in the public and domestic domains of 30 intervention and 30 control villages of a large cluster-randomized controlled trial of impacts of improved household sanitation on health in rural India (the Sanitation Trial) (Clasen et al., 2012), and examine the effect of increased intervention latrine coverage on fecal exposure via different pathways in the community. We employ a set of MST host-associated fecal *Bacteroidales* assays recently validated to distinguish and quantify human versus non-human livestock and other domestic animal sources of fecal contamination in the study area (Odagiri et al., 2015).

The primary objectives were to (1) measure prevalence of human and animal fecal contamination of community water sources, and of household stored drinking water and mothers' and children's hands; (2) assess the microbiological safety of community water sources by measuring locally important child diarrhea-causing pathogens; and (3) examine the effect of increased latrine coverage in intervention villages on contamination and pathogen exposure rates. In a secondary analysis, we explore associations between detected human and animal fecal contamination in homes and household child diarrhea, and between observed pathogen contamination of village water sources and village-wide child diarrhea prevalence to shed light on potential diarrheal pathogen transmission pathways and sources operating in study communities.

2. Materials and methods

2.1. Study setting

The study was carried out in Puri District in Odisha, India in a subset of 30 intervention and 30 control villages of the Sanitation Trial. The Sanitation Trial design, pour-flush latrine construction intervention, study setting and population characteristics have been described elsewhere (Boisson et al., 2014; Clasen et al., 2012). Briefly, key characteristics of the study population include (1) low sanitation coverage prior to the intervention (10% of households); (2) high access to improved drinking water sources such as public (deep groundwater) and private (shallow groundwater) tubewells (82% of households); (3) daily usage of open ponds for non-drinking purposes including anal cleansing after defecation, bathing, brushing teeth and domestic hygiene activities (>50% of households); and (4) livestock ownership (59% of households), comprising cattle (most frequently owned), sheep, goat and buffalo.

The latrine promotion and construction program in intervention villages was undertaken between January 2011 and January 2012, yielding mean village-level coverage with functional latrines in the intervention and control villages of the present study of 37.5% and 10.2%, respectively.

2.2. Village and household selection

Each Sanitation Trial intervention village (n = 50) was uniquely paired with one nearby control village (n = 50), and 30 pairs randomly selected into this study. Testing occurred in each village of a pair on consecutive days to minimize spatial and temporal confounding over the monsoon season study period. Twelve pairs were sampled in 2012 from June 19th to July 26th; 18 pairs in 2013 from June 26th to August 22nd. In each village, households with a child under 5 enrolled in the Sanitation Trial health surveillance study were stratified based on drinking public or private tubewell water, and three households in each stratum randomly selected for testing. In total, 354 households were sampled.

2.3. Community water source selection

Two public tubewells (deep groundwater), two private tubewells (shallow groundwater) and two open ponds (surface water) in each village were tested, unless fewer existed. Further details are provided in Supplemental Material. Sources were each sampled once, during or just after household sampling between 8 and 11 a.m. In total, 111 public and 98 private tubewells, and 94 open ponds were sampled.

2.4. Sample collection

2.4.1. Community water sources

A 20-L sample was collected for molecular analysis as previously described (Schriewer et al., 2015). For thermotolerant coliform (TTC; also known as fecal coliform) measurement, an additional 100-mL sample was collected in a sterile 4-oz Whirl-Pak (NASCO Corp., Fort Atkinson, WI). Samples were placed on ice, transported to a laboratory in Bhubaneshwar, and processed within 8 h of collection.

2.4.2. Household stored drinking water and hand rinses

Approximately 500 mL of stored drinking water (SDW) was collected for molecular analysis and a further 100 mL separately collected for TTC measurement using sterile 69-oz and 4-oz Whirl-Paks, respectively. The collectable amount of SDW was limited by the small volumes of stored drinking water and unwillingness of households to give all of it for research purposes. Hand rinses (HR) were obtained from the mother and youngest child following a published protocol (Pickering et al., 2010). Because recent household activities can significantly affect the level of hand fecal contamination (Devamani et al., 2014; Pickering et al., 2011), we standardized HR collection times, resulting in 76% and 75%, respectively, of intervention and control villages' HR samples collected when mothers were preparing or eating foods.