



## Impact of population and latrines on fecal contamination of ponds in rural Bangladesh

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### ABSTRACT

A majority of households in Bangladesh rely on pond water for hygiene. Exposure to pond water fecal contamination could therefore still contribute to diarrheal disease despite the installation of numerous tubewells for drinking. The objectives of this study are to determine the predominant sources (human or livestock) of fecal pollution in ponds and examine the association between local population, latrine density, latrine quality and concentrations of fecal bacteria and pathogens in pond water. Forty-three ponds were analyzed for *E. coli* using culture-based methods and *E. coli*, Bacteroidales and adenovirus using quantitative PCR. Population and sanitation spatial data were collected and measured against pond fecal contamination. Humans were the dominant source of fecal contamination in 79% of the ponds according to Bacteroidales measurements. Ponds directly receiving latrine effluent had the highest concentrations of fecal indicator bacteria (up to 10<sup>6</sup> Most Probable Number (MPN) of culturable *E. coli* per 100 mL). Concentrations of fecal indicator bacteria correlated with population surveyed within a distance of 30–70 m ( $p < 0.05$ ) and total latrines surveyed within 50–70 m ( $p < 0.05$ ). Unsanitary latrines (visible effluent or open pits) within the pond drainage basin were also significantly correlated to fecal indicator concentrations ( $p < 0.05$ ). Water in the vast majority of the surveyed ponds contained unsafe levels of fecal contamination attributable primarily to unsanitary latrines, and to lesser extent, to sanitary latrines and cattle. Since the majority of fecal pollution is derived from human waste, continued use of pond water could help explain the persistence of diarrheal disease in rural South Asia.

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### 1. Introduction

Over the past few decades the incidence of diarrheal disease has declined throughout the developing world. Despite this decline, 1.5 million children under five die of diarrhea annually (UNICEF/WHO, 2009). The decline in diarrheal disease has generally been attributed to improved medical treatment and interventions focused on safe drinking water, as well as improved sanitation and hygiene (Esrey, 1996; Pruss et al., 2002). The inability to reduce diarrheal disease levels further suggests that transmission pathways in the

developing world are complex and that environmental conditions may play a larger role than previously thought. Substantial amounts of surface water are contained in ponds scattered throughout villages in rural Bangladesh. Due to poor sanitation, ponds receive fecal contamination from numerous latrines and could be a source of pathogens overlooked by intervention programs focused exclusively on drinking water quality. Contact with pond water has previously been identified as a driver of diarrheal disease in Bangladesh when people drink, bathe in, or live near a pond (Emch et al., 2008; Ali et al., 2002). Although surface water from ponds is generally not used for drinking and cooking, it is used for bathing and brushing teeth (Aziz et al., 1990).

In recent decades, the number of ponds excavated in Bangladesh has outpaced population growth (see Supp. Material in Neumann et al., 2010). While many ponds are excavated to raise the ground to

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protect dwellings from flooding, they often fulfill alternate purposes, including aquaculture, bathing, irrigation, or holding latrine effluent. In the area surrounding the ponds, latrines, the primary mode of handling human feces in rural Bangladesh, are often deliberately placed, effectively creating sewage lagoons. Global studies have revealed that substantial decreases in diarrheal disease morbidity by a quarter to over a third (Fewtrell et al., 2005) and improvements in childhood nutritional indexes (Esrey, 1996) have accompanied a gradual switch from open pit latrines to more sanitary latrines consisting of concrete foundation rings to prevent leakage of human feces onto the open ground. In rural Bangladesh specifically, epidemiologic studies have reported reductions in diarrheal disease morbidity due to improved sanitation (Aziz et al., 1990; Emch, 1999; Emch et al., 2008; Hoque et al., 1996) where only half the population currently has access to sanitary latrines (WHO/UNICEF, 2008).

Although several diarrheal disease pathogens are transmitted through human feces including, *Shigella* and rotavirus, livestock feces can carry diarrheal pathogens including *Campylobacter*, *Salmonella* and certain types of pathogenic *E. coli* (Nicholson et al., 2005), all of which are routinely identified in the stools of diarrhea patients at the International Center for Diarrheal Disease Research Bangladesh (ICDDR) hospital (Albert et al., 1999). Villagers live very close to their livestock on a daily basis and the livestock are frequently kept near ponds where latrines and tubewells are also located, resulting in opportunities for both types of fecal contamination to impact pond water quality. Therefore, understanding the relative contributions of fecal sources, as well as the impact of waste containment on surface water fecal contamination, could lead to better public health interventions.

The goals of this study were to assess the levels and factors influencing concentrations of fecal bacteria and adenovirus in ponds within a representative village of Bangladesh. The following hypotheses were tested: 1) fecal bacteria in ponds of rural Bangladesh are primarily human in origin, rather than from domestic livestock; 2) concentrations of fecal bacteria and adenovirus are related to local population density; and 3) concentrations of fecal bacteria and adenovirus are related to the number of latrines around a pond, as well as the type of latrine. To test these hypotheses, this study integrates pond concentrations of fecal bacteria and viral pathogens with detailed spatial data from the same village. The novelty of this study is: 1) both *E. coli* and total Bacteroidales were compared as fecal indicators; 2) host animal specific Bacteroidales quantitative PCR assays were used to identify samples impacted by human or livestock fecal waste (Bernhard and Field, 2000; Layton et al., 2006; Lee et al., 2008); and 3) human adenovirus, representing both a human pathogen and a human fecal indicator, was analyzed (Castignolles et al., 1998; Wyn-Jones and Sellwood, 2001; Jiang, 2006).

## 2. Methods

### 2.1. Site description

The village of Char Para is located in Araihsar upazilla, about 25 km east of Dhaka. On-going public-health and earth-science studies focused on the groundwater arsenic problem were launched in the area in 2000 (van Geen et al., 2003). Char Para, also referred to as Site K (Radloff et al., 2007), is underlain by fine to medium grained deltaic sands, which form a shallow aquifer that is tapped by tubewells that are screened from 10 to 20 m and are the primary drinking water source in the village. The shallow aquifer below Char Para is bounded hydrologically on three sides by a former channel of the Old Brahmaputra River which floods to the edge of the village by the late wet season (van Geen et al., 2003; Weinman et al., 2008). Many ponds in Char Para are empty at the end of the dry season in April when the groundwater table falls below the bottom of the pond. The ponds that do not dry out are often the deepest ponds, or are

artificially maintained for fish farming by pumping from a deeper aquifer. Standing water can also be maintained year-round in latrine ponds that receive latrine effluent and runoff of wash water from surrounding wells. At the beginning of the monsoon in late May, some ponds may also fill and drain rapidly; fluctuations in pond water levels of up to 1 m were observed within 24 h in June 2008. As a result of the initially depressed water table and the flat topography between pond basins, little mixing between surface water bodies occurs until late in the monsoon (August) when some ponds may overtop their basins.

### 2.2. Field methods and pond classification

#### 2.2.1. Village-wide GPS survey

High accuracy (sub-meter) GPS coordinates were collected for all ponds, latrines and households throughout the village during June 2009 using a Trimble GeoXH receiver and Terrasync 2.4 software (Table A1). GPS data were post-processed using Pathfinder Office 3.0 (Trimble Navigation Ltd., Sunnyvale, CA). Latrines were classified as sanitary if the concrete platform and rings were intact with no visible sign of effluent discharging onto the ground. A latrine was classified as unsanitary if the ring was absent or cracked, or if visible effluent discharged directly into a pond via a PVC pipe. A population survey was conducted to determine pond ownership and the number of persons living in each household (cattle were not counted during the village-wide survey). Population and latrine totals within a given radius of each pond were determined using the buffer and intersect tools in ArcGIS software. The population and number of latrines (sanitary and unsanitary) surrounding ponds within a radius of 10 and 50 m was calculated at 5-m intervals and within a radius of 50 to 100 m at 10-m intervals. This method is referred to hereon as the spatial buffer method.

#### 2.2.2. Pond drainage basin survey

An alternative survey was carried out in June 2008 to identify latrines within a radius of ~20 m of a pond located within drainage basin that sloped downward toward the water edge. The rationale for this hydrological approach is that latrines within the basin could have a greater influence on microbial pond water quality than latrines at similar distances outside the drainage basin. Information collected for each pond drainage basin includes water depth, long and short axes of the pond water surface (measured in meters), designated purpose as identified by the owner, number and type of latrines (unsanitary or sanitary) and the number of cattle residing within the drainage basin (Table A1). Ponds receiving direct latrine effluent were classified as latrine ponds. Unless local households identified a specific use such as bathing or aquaculture, ponds not receiving direct latrine effluent were categorized as having no specific use. Specific conductivity, pH, dissolved oxygen and temperature were measured at each pond at the time of microbial sampling using a calibrated handheld multiprobe (556 Multiprobe System, YSI Inc., Yellow Springs, OH).

### 2.3. Microbiological assays

Water from 43 ponds was collected in sterile bottles in mid-June 2008, during the early monsoon when surface runoff was common but the ponds were not yet full. One hundred milliliters of pond water samples were collected in sterile containers to measure culturable *E. coli* using the MPN based Colilert™ test kit (IDEXX Laboratories, Inc., Westbrook, ME). Triplicate samples were taken from the first 30 ponds, and after the Coefficient of Variation (C.V.) between replicates was found to be low (24%), remaining ponds were sampled in duplicate (C.V. 31%). Based on the assumption that replicates contained the same true concentration of cultured *E. coli* cells, triplicate and duplicate samples were pooled to produce a single MPN with 95% confidence intervals (Hurley and Roscoe, 1983; Knappett et al., 2010). Pond water samples were diluted 1:100 or 1:1000 with commercial bottled drinking water

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