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Case Study/

Contrasting Influence of Geology on *E. coli* and Arsenic in Aquifers of Bangladesh

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Abstract

Arsenic in groundwater has been a concern in South and Southeast Asia for more than a decade. We explore here the possibility that hydrogeologic factors recently shown to influence the distribution of arsenic might also affect the level of contamination of shallow (<20 m) wells with microbial pathogens. A total of 96 shallow tube wells in two nearby villages of Bangladesh were surveyed during the wet and dry seasons, along with 55 deeper wells in neighboring villages. One of the two villages is located in a particularly sandy environment where recharge is rapid and shallow wells contain little arsenic. Shallow aquifers in the other village are capped with an impermeable clay layer, recharge is an order of magnitude slower, and arsenic levels are high. The fecal indicator *E. coli* was detected in 43% of shallow wells, compared with 12% of deeper wells. More shallow wells contained *E. coli* during the wet season (61%) than during the dry season (9%). In the wet season, a higher proportion of shallow wells in the village with low arsenic levels (72%) contained *E. coli* compared with the village having high arsenic levels (43%). Differences in arsenic and *E. coli* distributions between the two sites are likely due to the differences in permeability of near-surface sediments although differences in average well-depth between the two villages (9 ± 4 vs. 15 ± 3 m) may play a role as well. Hydrogeologic conditions that favor high levels of fecal contamination but low levels of arsenic in shallow groundwater should be taken into account during arsenic mitigation throughout South and Southeast Asia.

Introduction

Untreated groundwater is often the safest available drinking water source in developing countries that lack public sanitation and water infrastructure (Howard et al. 2006). In rural Bangladesh, for instance, at least 10 million relatively inexpensive tube wells have been installed since the 1970s for this reason, mostly privately by individual households (BGS and DPHE 2001; van Geen et al. 2003). Although switching from surface water to groundwater may have led to substantial reductions in infant mortality, diarrheal disease persists, and still accounts for 5% of all deaths in Bangladesh (Levine et al. 1976; D'Souza 1985; Pruss-Ustun et al. 2008). Aboveground, a number of factors may influence the risk of diarrheal disease in densely populated rural areas, including the availability and usage of latrines; the distance separating a latrine from a tube well, and its effect on hygiene; socioeconomic status, and the extent of seasonal flooding (Rahman et al. 1985; Emch 1999; Hoque et al. 1999; Luby et al. 2008). Below ground, much less attention has been paid to factors that might affect the microbial quality of groundwater tapped by shallow tube wells in Bangladesh, let alone other arsenic-affected countries in South and Southeast Asia. The extent to which ponds and

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streams highly contaminated with microbial pathogens might contaminate the underlying aquifers, in particular, has rarely been examined systematically (Hoque 1998; Islam et al. 2001; McArthur et al. 2004; Hoque et al. 2006; Luby et al. 2008).

The vulnerability of shallow sedimentary aquifers to microbial contamination is likely to vary over time and space in South and Southeast Asia as a result of the combination of a monsoonal climate and the highly heterogeneous distribution of sediment grain-size. Seasonal variations in groundwater level could play an important role given the strong dependence of bacterial transport on the degree of water saturation of the porous media (Crane and Moore 1984; Schäfer et al. 1998). However, microbial removal rates in groundwater also depend on other biological, chemical, and physical properties of both microorganisms and sediments, in addition to groundwater flow (Fontes et al. 1991; Dong et al. 2002; Ginn et al. 2002; Mailloux and Fuller 2003; Taylor et al. 2004; John and Rose 2005; Foppen and Schijven 2006). The implication is that microbial transport is difficult to predict in a complex fluvio-deltaic setting based on theoretical considerations alone (Ginn et al. 2002; Mailloux et al. 2003; McCarthy and McKay 2004). The present study takes an observational approach by building on the previously documented contrast in hydrogeological conditions between two densely populated villages of Araihazar, Bangladesh, to help identify factors that influence the vulnerability of shallow aquifers to microbial contamination.

Any attempt to improve the quality of drinking water in Bangladesh is complicated by the presence in groundwater of geogenic arsenic across approximately half the country at concentrations often many times beyond the World Health Organization guideline value (10 μg/L) (BGS and DPHE 2001). The distribution of arsenic is highly patchy and can vary by orders of magnitudes over lateral distances of as little as 10 to 100 m, particularly in a geological transition area such as Araihazar (van Geen et al. 2003). While the causes and mechanisms of arsenic release are still debated, recent studies have shown that the distribution of arsenic in shallow (<20 m deep) aquifers is probably controlled at least in part by the local hydrogeology (Harvey et al. 2006; van Geen et al. 2008; Polizzotto et al. 2008). In our study area of Araihazar, for instance, arsenic concentrations in shallow groundwater are high (>50 μg/L) where fine-grained sediments cap the aquifer and local groundwater recharge is slow, whereas low (<10 μg/L) arsenic concentrations prevail in areas where permeable aquifer sands extend to the surface and local recharge is fast (Stute et al. 2007; Weinman et al. 2008; Aziz et al. 2008). A relation between surface permeability, local recharge, and arsenic concentrations in shallow aquifers was recently also reported for the Mekong delta in Cambodia (Robinson et al. 2009).

As a result of well-testing for arsenic, many villagers of Araihazar have switched their consumption away from a well that is elevated in arsenic to a nearby well that is low in arsenic (Chen et al. 2007; Opar et al. 2007). If this low-arsenic well happens to be shallow, this raises the possibility of increased exposure to microbial pathogens because such wells are typically located in sandy formations that are not capped with a relatively impermeable silt or clay layer (Stute et al. 2007; Aziz et al. 2008). In such a setting, local vertical recharge is relatively fast and filtration, die-off, adsorption, and inactivation of microorganisms is likely to be less effective, particularly during the wet season (Crane and Moore 1984; Taylor et al. 2004; John and Rose 2005; Foppen and Schijven 2006). A household that switches its consumption to a shallow well that is low in arsenic could, therefore, potentially increase its exposure to microbial pathogens. This unintended consequence of arsenic mitigation has, to our knowledge, never been evaluated and could be a concern for rural populations throughout arsenic-affected regions of South and Southeast Asia.

Methods

Site Description

The densely populated villages of Bangladesh are typically raised by local fill to avoid flooding during the monsoon and are surrounded by irrigated rice fields. A single village can contain more than 100 hand-pumped wells. Man-made, seasonally filled ponds, anywhere from several square meters to several hundred square meters in area, are distributed throughout the villages. Two households on average share a hand-pumped well and a latrine that discharges into either a shallow pit or a nearby pond. Latrines are frequently located within 10 m of wells and at times right next to them. Livestock, such as cattle and chickens, are another potential source of fecal contamination as these are kept within the villages.

To install a tube well, an approximately 20-cm diameter hole is first cleared to a depth of up to 100 m using a string of metal and PVC pipe that is lifted and lowered rhythmically to recirculate sediment-laden water, using a hand placed at the upper end of the pipe as a check-valve (Horneman et al. 2004). A string of glued 6-m (20 feet) sections of 4-cm (1.5″) PVC pipe, with a 0.9 to 1.8 m (3 to 6 feet) section of finely slotted PVC pipe at the end, is lowered into the hole to construct the actual well. Sand is then poured in the space between the hole and the well to cover the length of the filter, followed by finer sediment accumulated while drilling the hole. No special precaution is taken to seal the hole by grouting but the sediment gradually collapses back onto the pipe. Many villages also contain a few deeper wells extending to up to 250-m depth that have been installed using heavier metal pipe, a larger rig and a pump, typically under government direction.

Heavy precipitation is generally limited to the months of May through August in Bangladesh, although there are significant year-to-year variations. The rainy season effectively started 2 months earlier in 2003 (Figure 1a) and was delayed by several months in 2009,

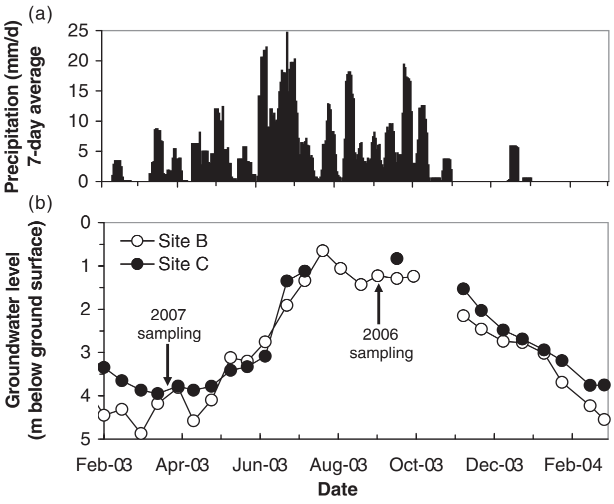

Figure 1. (a) Daily precipitation in Dhaka averaged with a 7-d running from February 2003 through February 2004, as presented by Stute et al. (2007). (b) Variations in groundwater levels at Sites B and C relative to the ground surface over the same period, modified from Stute et al. (2007). Also shown is when wells were sampled for microbial testing in 2006 and 2007 (no groundwater level data are available for 2006 through 2007).

for instance. The shallow aquifers of the region are recharged both vertically by precipitation and laterally by rising local streams (Harvey et al. 2006; Stute et al. 2007). Groundwater levels in Araihazar respond by varying between 1 and 5 m below the ground surface during the wet and dry season, respectively (Figure 1b). Occasionally, most recently in 2003, widespread flooding causes some villages to be temporarily abandoned. Groundwater flow reverses as water levels drop in local streams during the dry season and is also influenced at least locally by mechanized irrigation wells (Harvey et al. 2006; Aziz et al. 2008).

Analysis of the hydrogeologic and biogeochemical factors controlling the highly variable distribution of arsenic in groundwater of Araihazar began in 2000 with a survey of arsenic levels in groundwater pumped from 6500 private tube wells distributed across a 25 km^2 area (van Geen et al. 2003). Groundwater from the majority of wells in Araihazar contain >10 µg/L arsenic, but there are also a few villages or portions of villages where arsenic concentrations in shallow wells are consistently low (Figure 2).

Two villages were selected to span the full range of hydrogeologic conditions (and therefore arsenic levels) observed in shallow aquifers of Araihazar. Coring and a geophysical survey of the surrounding fields has shown that Baylakandi, (23.780°N, 90.640°E, hereon and in previous publications referred to as Site B) is underlain by a 7-m thick layer of fine-grained sediments which extends to the surface (Zheng et al. 2005). Sediments at Site B are composed of anywhere from 10 to 60% clay in the top 1 m. Nearly all shallow wells at Site B contain dissolved arsenic concentrations higher than 50 µg/L and often considerably higher levels (van Geen et al. 2003; Figure 2). Dating of groundwater using the ^{3}H/^{3}He technique indicates an age of 23 years at 19 m depth at Site B and relatively slow (0.05 m/year) recharge from the surface (Stute et al. 2007). This is probably the result of the low hydraulic conductivity of fine-grained surface sediments in the area (Aziz et al. 2008).

Satyabhadi (23.790°N, 90.611°E, hereon and in Dhar et al. (2008) referred to as Site C is located 3 km west of Site B (Figure 2). Site C is underlain by coarse aquifer sands that extend to or near to the surface (Aziz et al. 2008). In contrast to Site B, shallow wells of Site C typically contain less than 10 µg/L arsenic (van Geen et al. 2003) and meet the World Health Organization guideline for arsenic in drinking water. Tritium-helium age dating of the shallow aquifer at Site C indicates that groundwater recharge from the surface is more than an order of magnitude faster than at Site B (averaging 1.1 m/year), with an age of only 3 years measured at 14 m depth (Stute et al. 2007). Temporal variations in groundwater composition at Site C were consistent with rapid penetration of flood water to a depth of 11 m in 2003 (Dhar et al. 2008).

Field Collections

Shallow wells (<20 m deep), deeper wells (up to 150 m) and ponds were sampled from August 28 to September 2, 2006, near the close of the wet season, and again from March 10 to 17, 2007 during the dry season. In 2006, villagers reported little flooding in months prior to sampling. Although most ponds in the study area were full in August 2006, no flooded wells were observed. In March 2007, most village ponds were either dry or water levels were extremely low.

During the wet season, a total of 65 shallow wells were randomly selected at Sites B and C to provide broad geographic coverage of the village. Based on the 2000 through 2001 arsenic survey (van Geen et al. 2003), approximately half the existing shallow wells were sampled in both villages in August, although some of the unsampled wells were probably no longer present (Opar et al. 2007). During the dry season, an effort was made to return to the same wells sampled in August. Fifty-seven wells were re-sampled, while eight had either been moved or broken in the interim or could not be located. An additional 31 new wells were sampled in March for a total of 88 wells. Nearly all shallow, accessible wells were sampled at both sites in March. Wells that required priming, a practice that could contaminate a well with surface water, were generally avoided, or, in a few cases, were primed with groundwater from a different well and then purged before sampling.

Samples were also collected in August 2006 from a set of 35 to 150 m (115 to 490 feet) deep community wells distributed across the entire study area of Araihazar, including Sites B and C (van Geen et al. 2007). Most villages have only one community well. In March 2007, groundwater was collected from another set of 12 deeper

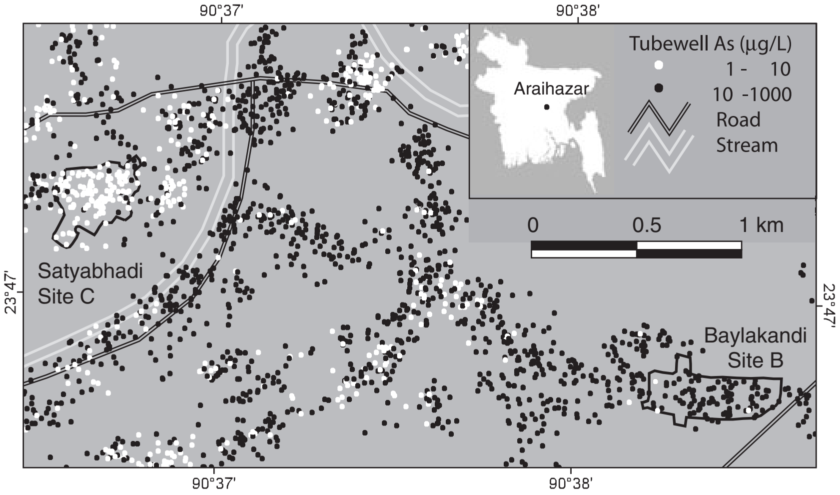

Figure 2. Map of portion of Columbia University's study area in Araihazar showing the distribution of tube wells and their arsenic content relative to the WHO guideline of 10 µg/L (data from van Geen et al. 2003). The perimeter of the two villages sampled in 2006 through 2007 and enlarged in Figure 5 is also shown.

(27 to 76 m; 90 to 250 feet) private wells in the vicinity of Site B.

Concrete platforms 4 m^2 in size are sometimes poured around new tube wells to facilitate water collection and to prevent surface water infiltration along the sides of the well. The presence of a platform at a well was noted and its condition was recorded as either "broken" if it was cracked or crumbling, or "good" if it was not. At each well, the sampler also recorded the GPS coordinates and the well identification number from the 2000 through 2001 survey (if present). The owners provided the well screen depth, which they usually recall accurately within 3 to 6 m (10 to 20 feet) (van Geen et al. 2003).

Surface water samples were randomly collected from 4 to 10 ponds during both sampling seasons at Sites B and C to provide broad geographic coverage of the village. During the March trip, the ponds selected were among the few that contained any water.

Well Sampling Procedure

Additional sampling was carried out to constrain the variability of contamination over time. Fourteen wells with a range of *E. coli* concentrations were sampled repeatedly over the course of the March 2007 dry season trip to determine the variability of measurements taken on different days during the sampling period. Fifteen wells were also sampled during the wet and dry season to understand the effect that short-term pumping of varying volumes of water had on *E. coli* concentrations.

Before collecting a sample, at least 3 and no more than 10 well volumes of water were purged from the 4-cm (1.5″) wells using the well's dedicated hand pump at a rate of approximately 30 to 40 L/min. The amount of water purged was recorded by collecting water in buckets of known volume. Duplicate 100 mL samples for *E. coli* and total coliform cultures were collected in sterile vials containing sodium thiosulfate and were stored in a cooler for up to 12 h before processing.

Enumeration of *E. coli*

The most probable number (MPN) of *E. coli* (and total coliforms) was enumerated using a standard U.S. EPA-approved commercial culture kit (Colilert, IDEXX Laboratories Inc., Westbrook, Maine) according to the manufacturer's directions. The specified amount of Colilert reagent was added to the 100 mL water samples. The reagent contains two carbon sources that are selectively metabolized by either all coliforms or *E.coli*, turning yellow or fluorescent, respectively, when an indicator is cleaved during metabolism. The samples were poured and distributed into 51- or 97-compartment trays, which were then heat sealed and incubated for 24 h at 35°C. After this period, the number of positive yellow and fluorescent compartments, indicating that a minimum of one indicator organism was initially present, were counted and converted to a MPN for total coliforms and *E. coli*, respectively. Two to three sterile water samples were analyzed as blanks during each sampling period.

Data Analysis

Well and pond sample GPS coordinates were overlaid onto an IKONOS satellite image of Araihazar at 4 m spatial resolution taken in November 2000 (van Geen et al. 2003). The boundaries of the two villages, as indicated by areas of tree cover compared with surrounding fields, and village pond locations were traced visually (Figure 2).

A well sample was considered positive for *E. coli* if both replicates contained detectable *E. coli*, which is greater or equal to 1 colony forming unit/100 mL (cfu/100 mL) (Embrey and Runkle 2006). In cases when multiple replicate pairs were collected for time series measurements, then if at least one replicate pair was *E. coli* positive, the well was also considered positive. Pearson chi-square tests of the categorical data (e.g., *E. coli* positive or negative) were used to assess variation in the number of *E. coli* positive wells for different sites, seasons, and concrete platform conditions (Francy et al. 2006).

Sample and well replicate concentrations were averaged, with a value of half the detection limit (0.5 cfu/100 mL) substituted to include nondetect data. Since the data were not normally distributed, the Wilcoxon nonparametric rank sum test was used to assess significant differences in *E. coli* concentrations grouped by site and season (Helsel and Hirsch 2002; Francy et al. 2005).

Results

Consistency of *E. coli* as an Indicator

This study relies primarily on *E. coli* as an indicator of fecal contamination during the wet and dry season. Seventy percent of tube wells (168 of 247 wells) were sampled in duplicate. *E. coli* concentrations fell outside the 95% confidence interval (CI) of its duplicate pair in 8% of these samples (14 of 168 samples). *E. coli* straddled the detection limit (1 cfu/100 mL) in 13% of duplicate pairs (22 samples). However, in only 4 of these 22 pairs did the *E. coli*-positive sample concentration fall outside the upper 95% CI (3.7 cfu/100 mL) of the nondetect sample. Sample blanks were always below detection for both *E. coli* and total coliforms.

During dry season sampling, 14 shallow wells were sampled over several days (Figure 3a). *E. coli* concentrations in three of these wells crossed the detection limit from below or above during the time series, although *E. coli* levels in only one positive well sample varied outside the 95% CI of the nondetect samples. In addition, two wells had consistently high concentrations of *E. coli*, two wells contained *E. coli* between 1 and 10 cfu/100 mL, and seven wells stayed below detection.

During both sampling periods, samples were collected at varying purge volumes from a total of 15 wells. These wells were continuously pumped and samples were collected at regular purge volume intervals (Figure 3b). Two wells contained consistently high *E. coli* concentrations, one well varied in concentration from 1 to 10 cfu/100 mL, and five wells remained below detection. Samples from seven wells crossed the detection limit threshold while purging. Samples from six of these seven wells, collected at both low (<100 L) and high (>250 L) purge volumes, varied between nondetect and concentrations ranging from 1 to 10 cfu/100 mL. *E. coli* concentrations in samples from one of the seven wells ranged from nondetect to 32 cfu/100 mL; however, this range was spanned while sampling at purge volumes between 0 and 40 L. During general surveying, samples were never collected at purge volumes less than 60 L (about three well volumes) or greater than 250 L (about 10 well volumes).

Total coliforms were enumerated even though they represent too broad a class of bacteria to provide a reliable indication of fecal contamination, especially in tropical countries (WHO 2006). In spite of this, MPNs for total coliform and *E. coli* are reasonably well correlated across a wide range of concentrations ($R^2 = 0.72$; Figure 4). Outliers often consist of samples with detectable levels of total coliform but undetectable *E. coli*.

Overall, enumeration of *E. coli* proved to be replicable and consistent over the course of sampling on time scales of seconds, minutes, and days. Most of the variability in *E. coli* concentrations fell within expected 95% CIs, or, in a few cases, at least within an order of magnitude.

E. coli by Village and by Season

Detection

Overall, *E. coli* was detected in at least one season in 43% of the shallow wells sampled (41 of 96 wells). Considering only the 57 shallow wells sampled during both seasons, 28% were below the detection limit of 1 cfu/100 mL for *E. coli* during both sampling periods. The majority (60%), however, contained elevated concentrations in the wet season but were below detection in the dry season. Only one well contained detectable *E. coli* in the dry season and not the wet season. There is no clear spatial pattern in the distribution of *E. coli* contamination in either village (Figure 5), although elevated wet season values at Site C appear grouped around the north-central portion of the village. At both sites, *E. coli* concentrations in well water vary between detect and nondetect across distances less than 100 m.

At both Sites B and C, the proportion of shallow wells positive for *E. coli* was significantly higher ($p < 0.05$) during the wet season (61%) than during the dry season (9%) (Figure 6). During the wet season, a significantly higher proportion of shallow wells contained *E. coli* at Site C (72%) compared with Site B (43%). During the dry season, although the proportion of wells with detectable *E. coli* was still higher at Site C (12%) than at Site B (5%), these differences were not statistically significant ($p > 0.05$). Overall, wells at Site C had about twice as a high a probability of containing detectable *E. coli* compared with wells at Site B.

Concentration

Of the 41 shallow wells that contained *E. coli* during at least one season, *E. coli* concentrations were above 100 cfu/100 mL in 2 wells, between 10 and 100 cfu/100 mL in 3 wells, and between 1 and 10 cfu/100 mL in 36 wells. All wells with *E. coli* concentrations above 10 cfu/100 mL were located at Site C.

During the wet season, the median *E. coli* concentration in shallow wells at Site C was 1.8 cfu/100 mL (Table 1). For shallow wells at Site B, the median value was below the detection limit of 1 cfu/100 mL, but the geometric mean (1.2 cfu/100 mL) was above the detection limit. During the dry season, the median and geometric mean at both sites were below the detection limit. Although *E. coli* levels in nearly all wells were below detection during the dry season, the two highest overall concentrations in any shallow well (388 and 127 cfu/100 mL) occurred during the dry season at Site C and were an order of magnitude higher than the maximum wet season value at Site C (24 cfu/100 mL). Overall, wet season shallow well *E. coli* concentrations were significantly

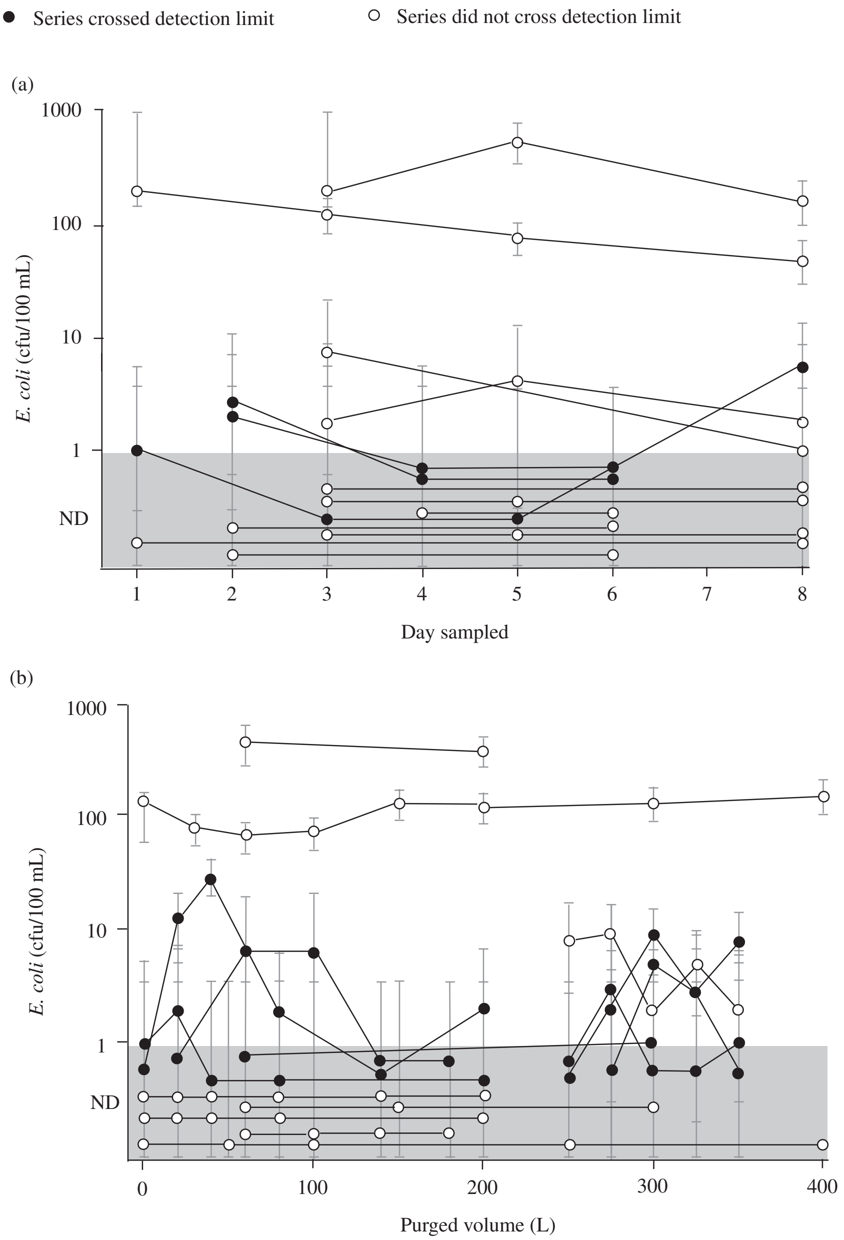

Figure 3. (a) Time series of 14 individual wells sampled during multiple days in March 2007 (dry season) at both Sites B and C. Filled circles indicate the three wells that crossed nondetect threshold (1 cfu/100 mL). *E. coli* concentrations in samples from the remaining 11 wells stayed relatively consistent during the week (seven wells stayed nondetect; two stayed within the 1 to 10 cfu/100 mL range, and two in the 100 to 1000 cfu/100 mL range). At least 60 L and no more than 250 L were purged prior to sampling during the general surveying of shallow wells. (b) Time series of samples collected at varying purge volumes while continuously pumping a total of 15 wells during both the August 2006 (wet season) and March 2007 (dry season). Filled circles indicate the seven wells that crossed the detection limit threshold while purging. Five other wells stayed below the detection limit, while one varied between 1 and 10 cfu/100 mL and two stayed in the 100 to 1000 cfu/100 mL range. Because similar variation was seen at both low and high purge volumes, well pumping probably did not affect *E. coli* concentrations in any systematic direction.

higher than in the dry season ($p < 0.05$). The difference in *E. coli* concentrations at Sites B and C, however, are not significant ($p > 0.05$), even though the range of concentrations is larger at Site C than at Site B.

Ponds

Surface water was collected from ponds because they are a potential source of fecal pathogens for shallow aquifers. The geometric mean and median *E. coli* concentrations in the pond samples (Table 1) were twos of magnitude higher ($p < 0.05$) in the dry season ($\sim 10^4$ cfu/100 mL) than in the wet season ($\sim 10^2$ cfu/100 mL), and, with two exceptions, at least an order of magnitude higher than any tube well samples. There was no significant difference in *E. coli* levels for pond water samples at Sites B and C ($p > 0.05$).

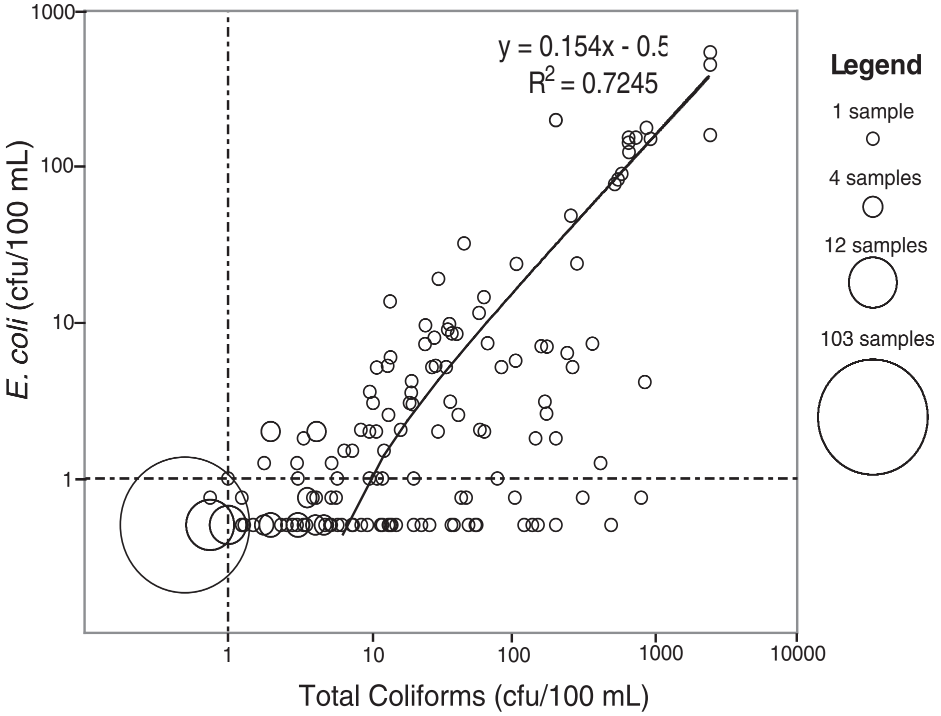

Figure 4. Relationship between *E. coli* and total coliform concentrations measured in the same sample from shallow and deep wells. A linear fit is shown as a solid line ($R^2 = 0.72$). The area of the circle is proportional to the number of wells plotted at the data point. Note the log-log scale. Most of the data points that are not well correlated occur when there is detectable levels of total coliforms, but nondetect *E. coli*. This is probably the result of naturally occurring total coliforms in groundwater.

Platform Condition

Forty-two percent of shallow wells sampled had concrete platforms in good condition (Table 2). Another 16% of platforms were visibly cracked or broken, and 29% of wells had no platform (this information was not recorded at 13% of the wells). There was no effect of platform grouping on *E. coli* concentration or positive/negative determination ($p > 0.05$). Of the three shallow wells with highest *E. coli* concentrations in the data set, one had a good platform, one had a broken platform, and the other had none.

Well Depth

E. coli concentration generally decreased as the depth to the well screen increased (Figure 7). Surface water samples contained *E. coli* concentrations two to five orders of magnitude higher than shallow well samples. The exceptions are the two highest *E. coli* concentrations (388 and 127 cfu/100 mL) from two shallows wells sampled at Site C during the dry season. The average depth of shallow wells sampled at Site C was 9 ± 4 m (30 ± 12 feet), while the average sampled well depth at Site B was 15 ± 3 m (50 ± 9 feet).

Of the sampled tube wells deeper than 20 m (60 feet), including some at Site B and the community wells throughout Araihazar, a total of 12% contained detectable *E. coli* (7 of 55 wells), and three-quarters of these were sampled during the wet season. Three of 8 wells between 20 and 30 m (65 and 100 feet) deep contained *E. coli*. At depths from 30 to 60 m (100 to 200 feet), 11% of wells (3 of 27) contained *E. coli* at a maximum value of 3.3 cfu/100 mL. One well (of the 20 sampled wells) greater than 60 m (200 feet) deep contained detectable levels of *E. coli*. These deepest wells are all village community wells considered the most reliable source of safe water in Araihazar (Ahmed et al. 2006; van Geen et al. 2007).

Discussion

Overall Microbial Quality

The new data presented here confirms that the microbial quality of groundwater is vastly better than that of pond water, as indicated by the orders-of-magnitude difference in *E. coli* concentrations. Nevertheless, groundwater pumped from tube wells is clearly not free of indicator organisms and their distribution appears to be correlated to both seasonal and geological factors. The 43% of wells that were positive for *E. coli* at least once indicate that oftentimes groundwater in Araihazar does not meet the Bangladesh or World Health Organization guideline for *E. coli* as a fecal indicator of contaminated drinking water (0 cfu/100 mL, i.e., nondetect). The relative health risk indicated by *E. coli* in shallow wells can be approximated by power of 10 increases in concentrations (WHO 1997). In that context, the majority of shallow wells containing *E. coli* positive shallow wells (36 of 41) at 10^0 to 10^1 cfu/100 mL are considered relatively low risk. For additional perspective, it is worth pointing out also that that even groundwater with initially low levels of *E.*

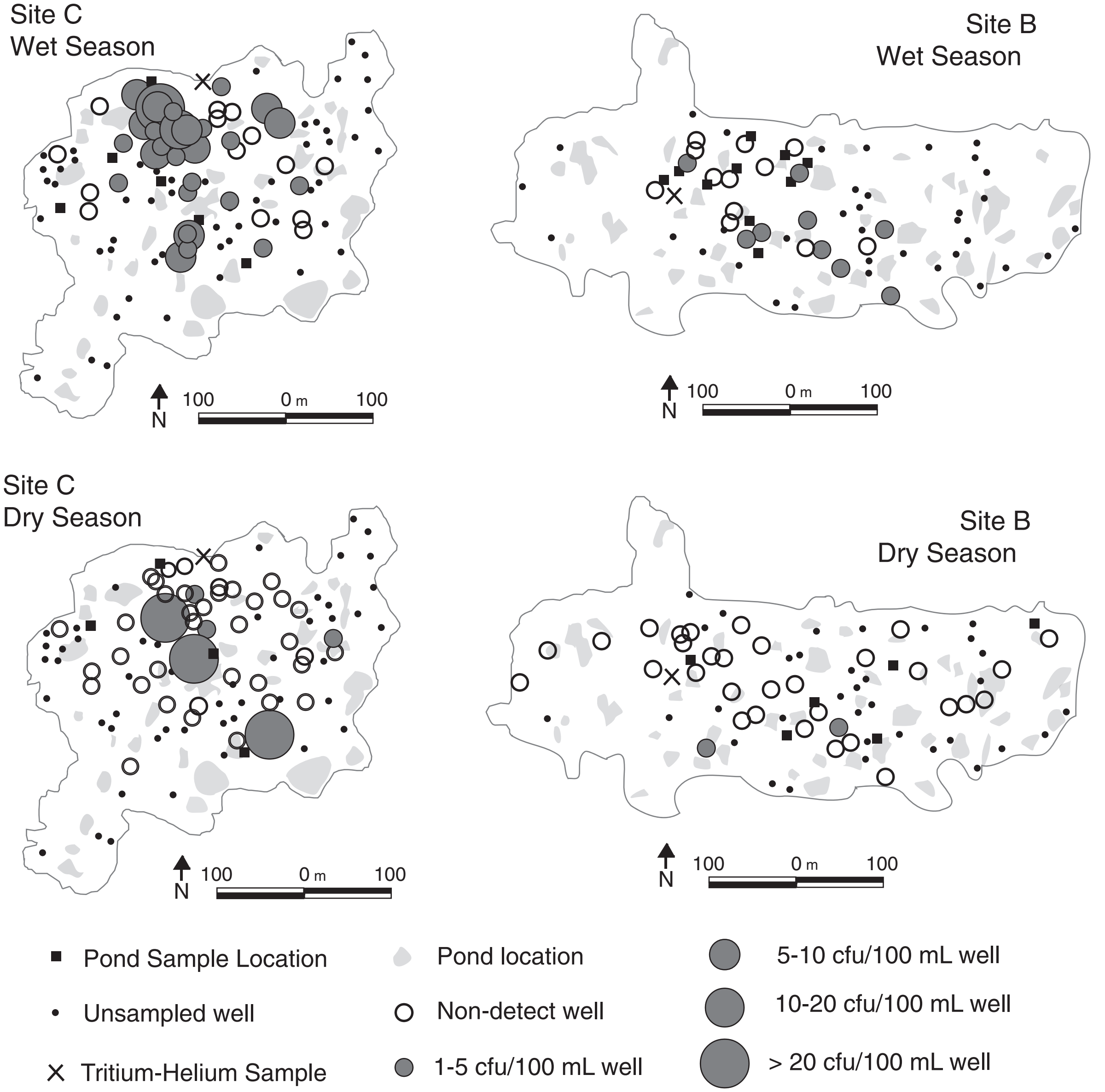

Figure 5. Distribution of average *E. coli* concentrations in shallow wells (<20 m/65 feet) at Sites C and B, grouped by sampling period. Although there is no clear spatial pattern, some elevated *E. coli* levels appear to be grouped in the north-central area of Site C during the wet season. Using a November 2000 IKONOS satellite image, pond locations were traced and village outlines were drawn on the basis of a transition from tree cover to open fields. The locations of wells not sampled for *E. coli* are based on a 2000 through 2001 arsenic survey (van Geen et al. 2003) and are shown to illustrate the density of shallow tube wells in these villages.

coli can become significantly contaminated during storage (Hoque et al. 2006). Deeper wells contained detectable levels of *E. coli* less frequently, and, with one exception, community wells deeper than 61 m (200 feet) all met the WHO guideline. The presence of a concrete platform did not appear to affect the likelihood of *E. coli* detection in a given well. Luby et al. (2008) also found that a tube well's overall sanitary score, including platform condition, did not correlate with the presence of fecal indicator bacteria.

That *E. coli* was detected in 43% of domestic wells is unsurprising. Even in the United States, 8% of 400 sampled domestic wells sampled nationwide contained cultivable *E. coli* (DeSimone et al. 2009). In countries like Bangladesh, India, Nepal, and in Central Africa, previous well surveys conducted in rural areas with minimal sanitary infrastructure have also reported the occurrence of fecal indicator bacteria in a substantial proportion if not a majority of drinking water wells, especially during the rainy season (Lindskog et al. 1988; Hoque et al. 2006; Bordalo and Savva-Bardalo 2007; Prasai et al. 2007; Chitanand et al. 2008; Luby et al. 2008).

There was some variability, mostly within the range of the 95% CIs, in the distribution of *E. coli* at different sampling times, by purge volume and by day. But these changes followed no clear direction or pattern.

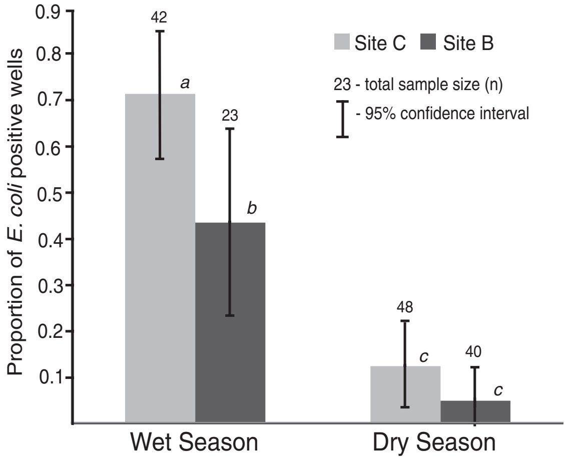

Figure 6. Proportion of wells with detectable *E. coli*, grouped by site and by season. Letters "a," "b," and "c" refer to statistically different groupings (Pearson chi-square test, $p < 0.05$). In the wet season, significantly more wells were *E. coli*-positive than in the dry season. Site C also contained significantly more *E. coli*-positive wells than Site B during the wet season. During the dry season, this association held, although the difference was not significant.

Pumping the well for short periods did not systematically affect *E. coli* concentrations. The observed variability is, therefore, probably the result of minor variations in microbial distribution, transport, or survival of *E. coli* in the subsurface. Considering the order of magnitude consistency of *E. coli* concentrations in samples collected from the same well over a range of time scales, the microbiological water quality appears to be relatively uniform in the subsurface over relatively short spatial scales.

Seasonal Differences

The rate, source, and travel path of recharge water in Bangladesh is complicated by the seasonality of the monsoon and the use of groundwater for irrigation during the dry season. At both sites in Araihazar, more shallow wells contained *E. coli* at higher concentrations during the wet season than in the dry season. As sampling occurred during a year with little flooding, this is not likely to be an artifact of direct contamination with surface water. At the end of the wet season, however, the vertical distance from the surface to the saturated zone is reduced to approximately 1 m (Figure 1a). The shortened travel distance through unsaturated sands, which are particularly effective at removing microorganisms, could be a major factor leading to the high proportion of *E. coli* contamination (Crane and Moore 1984). During the wet season, pit latrines as well as ponds may also come close to intersecting the saturated zone. Finally, direct runoff and infiltration of water contaminated by livestock feces could be a contributing factor. Previous studies carried out in Araihazar have shown that downward hydraulic gradients occur during the transition from the dry season to early monsoon season (Stute et al. 2007; Aziz et al. 2008). This may be a time when microbial pathogens contained in surface soil, latrines, and ponds built up during drier months are washed deeper into the vadose zone. Rising groundwater levels may then mobilize these contaminants into the saturated zone, as suggested by Hoque et al. (2006).

In the dry season of 2007, the wells were sampled before any significant rainfall. Risks for higher *E. coli* contamination during the dry season include potentially a higher percentage of recharge originating from concentrated sources of *E. coli*, such as pond water or latrines. While the pond samples indicate that surface water *E. coli*

Table 1
E. coli Concentrations in Shallow Wells and Ponds, Grouped by Site and by Season

	Shallow Wells				Ponds			
	Site C Coarse-Grained Avg. Arsenic = 6 μg/L Avg. Depth = 9 m		Site B Fine-Grained Avg. Arsenic = 280 μg/L Avg. Depth = 15 m					
	Wet	Dry	Wet	Dry	Site C	Site B	Wet Season	Dry Season
Median (cfu/100 mL)	1.8	0.5 (ND)	0.8 (ND)	0.5 (ND)	455	910	214	9100
Geometric mean (cfu/100 mL)	1.9	0.8 (ND)	1.2	0.5 (ND)	1370	1020	294	10,040
Max. (cfu/100 mL)	23.9	388.6	5.3	4.9	2,420,600	21,100	3450	2,420,600
Wells/ponds sampled (*n*)	42	48	23	40	10	16	16	10
Statistical comparisons **indicates $p < 0.05$	Site C wet vs. Site C dry** Site B wet vs. Site B dry** Site C wet vs. Site B dry Site C wet vs. Site B wet				Wet season ponds vs. dry season ponds** Site C ponds vs. Site B ponds			

Note: The Wilcoxon nonparametric rank sum test was used to assess significant differences ($p < 0.05$). In shallow wells, *E. coli* concentrations during the wet season were significantly higher than in the dry season. In ponds, *E. coli* concentrations were significantly higher during the dry season. Between the two villages, there was no significant difference in average *E. coli* concentrations in shallow wells or ponds.

concentrations are significantly higher during the dry season, shallow wells were generally below detection for *E. coli* (with the exception of three wells at Site C). Thus, it does not seem that these concentrated surface sources of pathogens directly affected the shallow wells. Without a detailed hydrological study, it is unclear to what extent irrigation pumping in surrounding fields drawing groundwater away from the villages during the dry season (Harvey et al. 2006) affected the distribution of microbial contaminants in shallow aquifers.

Although far fewer shallow wells showed signs of fecal contamination during the dry season, users of three wells were at the highest risk. These exceptions may reflect a more direct link with a contaminated source during the dry season or faulty well construction, such as a crack in the well casing or an unsealed well. While the

Table 2
The Condition or Presence of the Well Platform Protecting the Surface Surrounding the Well Did Not Affect the Likelihood of Detecting *E. coli* (Pearson Chi-Square Tests, $p > 0.05$)

Platform Condition	Number of Wells	Number of Samples (Two Seasons)	*E. coli*-Positive Well Samples
None	28	45	15 (33%)
Broken	15	26	11 (22%)
Good	40	66	17 (25%)

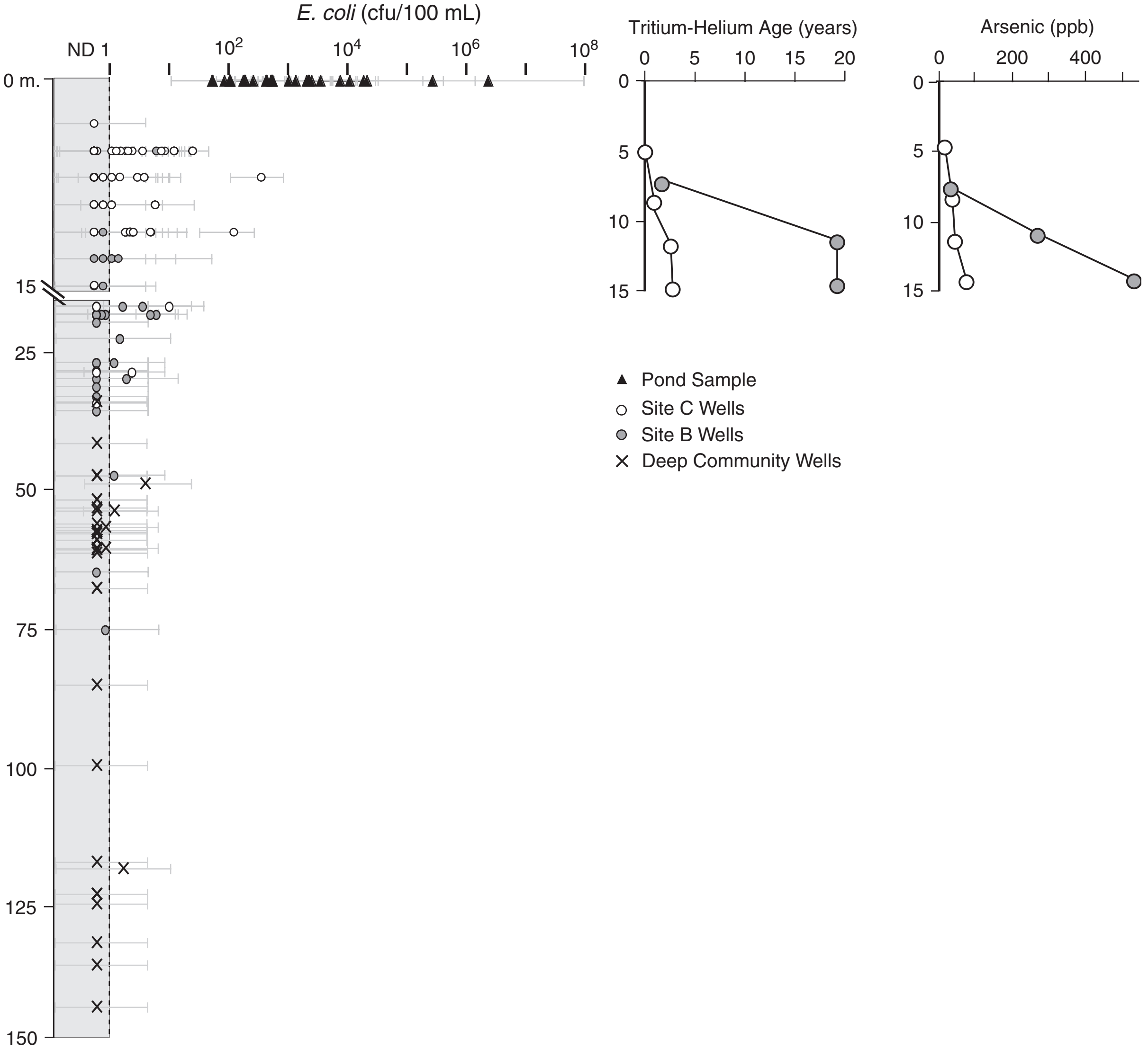

Figure 7. Depth profile of *E. coli* concentrations in pond, shallow well (<20 m/65 feet), and deeper well (>20 m/65 feet) samples. Concentration declines by orders of magnitude from the surface water samples to the shallowest of ponds, with the exception of two wells at Site C. Chart illustrates that Site B wells are generally deeper than Site C wells. Insets show that groundwater age, based on tritium-helium age dating (Stute et al. 2007), and arsenic levels. At a depth of about 10 m, the arsenic is higher at Site B than at Site C and the water is significantly older. Deep community wells rarely contain *E. coli*.

presence of a platform did not appear to have an effect on whether a well was contaminated, preferential flow of contaminated surface water to the well cannot be ruled out, especially in the case of wells anomalously high in *E. coli*.

Given the groundwater ages that were measured, it is somewhat surprising to observe a marked seasonal variability in *E. coli* levels for shallow wells at Site B. For such shallow wells to respond seasonally, one might expect recharge from the surface to the well screen to occur also on time scales less than 1 year. This is the case at Site C but not at Site B, where groundwater at 7 m depth is already 1.6-year-old according to the ^{3}H-^{3}He measurements (Figure 4; Stute et al. 2007). Certain chemical properties of shallow groundwater at Site B were also shown to vary seasonally and this was tentatively attributed lateral displacement of groundwater of heterogeneous composition (Dhar et al. 2008). A similar explanation may hold for microbial contaminants distributed heterogeneously in the subsurface. *E. coli* is also known to survive and even grow in clay sediments (Burton et al. 1987; Mital et al. 2008). The overlying fine-grained sediments at Site B might therefore provide a reservoir of *E. coli* that are remobilized to a different extent as the level of the water table rises and falls seasonally.

Difference Between Villages

The effect of seasonal variations in the groundwater level on the presence of *E. coli* appears to be stronger than differences in hydrogeology between the two villages. However, reduced travel time from the surface to the well screen at Site C, where coarse sediments extend to the surface and recharge is relatively fast, is probably a factor contributing to the high proportion of wells containing detectable levels of *E. coli* (Figure 6). The fine sediments that cap the shallow aquifer at Site B, in contrast, may inhibit *E. coli* transport with infiltration more effectively by slowing groundwater flow and trapping microorganisms (Fontes et al. 1991; John and Rose 2005).

Well depth is another substantial difference between Sites C and B, however, with shallow wells, respectively, averaging 9 ± 3.7 m (30 ± 12 feet) and 15 ± 2.7 m (50 ± 9 feet) depth. Therefore, Site B wells could also less frequently contain *E. coli* because of the longer distance infiltrating water travels before reaching a tube well. The systematic difference in average well depth is tied to the contrasting geology at the two sites: the "shallow" wells are on average deeper at Site B precisely because the low hydraulic conductivity of the locally thick layer of fine sediments at Site B prevents their installation at approximately 10 m (30 feet). Without additional information, it is not possible to determine whether well depth or the permeability of surface soils, or some combination thereof, underlie the contrast in *E. coli* contamination of shallow wells between the two villages during the wet season. What is undisputable is that villagers relying on shallow wells at Site C are less exposed to health risks related to arsenic but are also more likely, during the wet season, to drink water contaminated with fecal matter.

In 2000 through 2001, the average concentration of arsenic in shallow wells sampled at Sites C and B was 6 and 280 μg/L, respectively, and is unlikely to have changed over time (van Geen et al. 2003). Arsenic toxicity to *E. coli* could therefore in theory explain the lower proportion of contaminated wells at Site B. This seems unlikely, however, given *E. coli*'s detoxification genes and the range of arsenic concentration in groundwater (Meng et al. 2004; Integrated Microbial Genomes 2008).

Conclusion

It is no surprise that the microbial quality of groundwater in villages of Bangladesh is vastly preferable to that of surface water. This study shows, however, that shallow tube wells are not necessarily an entirely safe drinking water source as a high proportion contained detectable levels of *E. coli*. For reasons that are only partially understood, hydrogeologic conditions that favor high levels of fecal contamination (unconfined aquifer, shallow water table, rapid recharge, and very young groundwater) generally seem to be the opposite of the conditions that favor high concentrations of geogenic arsenic in shallow aquifers. This probably applies to other areas of Bangladesh as household density, tube well protection, and sanitation practices in Araihazar are quite representative. Comparable proportions of wells contaminated with fecal matter and higher levels have been reported in similar settings worldwide.

The state of well platforms did not seem to be related to *E. coli* contamination. The dominant environmental control on the severity of microbial contamination of shallow tube wells appears to be precipitation and/or the depth of the water table. There may be a local overprint related to differences in geology, specifically whether or not shallow aquifers are capped by fine sediments. However, this effect could not be separated from that of well depth in the two villages that were studied.

In the numerous villages throughout Bangladesh most affected by arsenic, a large fraction of households use a shared community well installed by the government or an NGO that is often deeper than 90 m (300 feet) and low both in arsenic and, the present study shows, *E. coli* (Ahmed et al. 2006). By contrast, in those villages where wells are shallow and arsenic levels are low, households will be exposed to higher levels of microbial contamination for the foreseeable future. Whereas arsenic mitigation should remain the priority in affected regions throughout South and Southeast Asia, microbial contamination of shallow aquifers in densely populated sedimentary basins with limited sanitation infrastructure should be taken into account in long-term planning of improvements in the quality of rural water supply.

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