Fecal Contamination of Shallow Tubewells in Bangladesh Inversely Related to Arsenic


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Supporting Information

ABSTRACT: The health risks of As exposure due to the installation of millions of shallow tubewells in the Bengal Basin are known, but fecal contamination of shallow aquifers has not systematically been examined. This could be a source of concern in densely populated areas with poor sanitation because the hydraulic travel time from surface water bodies to shallow wells that are low in As was previously shown to be considerably shorter than for shallow wells that are high in As. In this study, 125 tubewells 6–36 m deep were sampled in duplicate for 18 months to quantify the presence of the fecal indicator Escherichia coli. On any given month, E. coli was detected at levels exceeding 1 most probable number per 100 mL in 19–64% of all shallow tubewells, with a higher proportion typically following periods of heavy rainfall. The frequency of E. coli detection averaged over a year was found to increase with population surrounding a well and decrease with the As content of a well, most likely because of downward transport of E. coli associated with local recharge. The health implications of higher fecal contamination of shallow tubewells, to which millions of households in Bangladesh have switched in order to reduce their exposure to As, need to be evaluated.

INTRODUCTION

Filtration through aquifer sands reduces the likelihood of fecal contamination of groundwater by orders of magnitudes compared to surface water. There is growing evidence, however, that even sandy aquifers, and not merely aquifers contained within fractured rocks or karst deposits, are vulnerable to fecal contamination.1 In Bangladesh and neighboring countries with large sedimentary basins draining the Himalayas, much of the attention has focused instead on elevated levels of arsenic (As) in shallow groundwater pumped from tubewells installed by millions of households.2 This is justified because the burden of disease caused by drinking tubewell water containing As concentrations often 10–100 fold higher than the WHO guideline of 10 μg/L will be significant for decades to come.3 There is concern, however, that certain forms of As mitigation could lead to the substitution of exposure to As with exposure to fecal contaminants.4–6 This study evaluates this possibility for well-switching, the most effective form of As mitigation to date.6

The present study focuses on the possibility of risk substitution caused by switching from a shallow (defined here as <36 m deep) private well that is high in As to a neighboring private well that is also shallow but low in As. Unless a deep well that is low in As has been installed nearby by the government or a nongovernmental organization, this has been the response of millions of households in Bangladesh after being informed that their well was high in As.7–9 Dating of groundwater using the 3H–3He method within a region with spatially variable As has previously demonstrated that groundwater is typically considerably younger in shallow low-As
wells than in shallow high-As wells. Further investigation has shown that differences in the age (and therefore As content) of shallow groundwater are related to the grain size of surface soils that cap the underlying aquifer. Shallow groundwater can be <1 year old in villages where the sandy aquifer essentially extends to the surface and >10 year old at the same depth in neighboring villages where the shallow aquifer is instead capped by fine-grained clays and silts. Because attenuation of fecal contamination along a flowpath that starts in a shallow recharge zone depends on a combination of retention and die-off, this raises the possibility that shallow aquifers containing young/low-As groundwater could be particularly vulnerable to microbial contamination. Such a contrasting influence of local geology on E. coli and As contamination of shallow aquifers was recently investigated in a pilot study comparing two villages of Bangladesh during the dry season and the wet season. The present study takes this analysis further by comparing E. coli and As variability within villages rather than between villages and by taking into account local variations in population density.

**METHODS**

**Study Sites.** Two densely populated and geologically comparable rural areas of Bangladesh were selected for this study (Figure 1a). The village of Char Para (23.796° N, 90.629° E) in Arahazar upazilla is located on a sand bar rising 1–3 m above surrounding fields and the meander of a small stream to the south (Figure 1b). Local recharge is likely an important factor that maintains As concentrations in shallow groundwater at generally low levels in the center of Char Para, with As concentrations increasing toward the periphery of the village. Several hundred latrines scattered throughout the villages and approximately fifty ponds, many of which receive discharge from latrines, are potential sources of fecal pollution to the shallow aquifer of Char Para. In Matlab upazilla (Figure 1c), the six villages of Barahaldia (23.370° N, 90.646° E), Sardarkandi (23.352° N; 90.656° E), Shakharipara (23.356° N, 90.646° E), Farazikandi (23.360° N, 90.637° E), Namapara (23.361° N, 90.628° E), and Shankibhanga (23.370° N, 90.623° E) cover a range of depositional environments and groundwater As concentrations. The rural sanitation infrastructure in the region ranges from simple septic tanks built of concrete rings to defecation directly into ponds and does not differ markedly between Arahazar and Matlab. The location of each household within the study areas was recorded with hand-held GPS units to calculate the population residing within a given distance from each monitored well (Supporting Information).

**Precipitation and Water Levels.** Daily rainfall was measured in Barahaldia village starting in June 2008; data gaps were filled on the basis of rainfall data from Chittagong (Supporting Information). Fluctuations in the level of surface water and groundwater were recorded starting in September 2008 by installing data-logger pressure transducers (Solinst, Georgetown, ON, Canada) in a pond and a shallow well in Barahaldia, respectively. Pond water levels were only occasionally measured in Char Para in 2009, but groundwater levels were measured with an electric tape over the entire sampling period.

**Well Sampling and As Analysis.** Starting in May 2008, 33 and 92 shallow household wells in Char Para and the six Matlab villages were sampled quasi-monthly until October and November 2009, respectively. Duplicate 100 mL samples of well water were collected for microbial analysis in sterile vials and stored in the dark for up to 8 h before processing. Water was also collected without filtration in 20 mL scintillation vials with PolySeal caps. These samples were acidified to 1% HCl with high-purity acid at least one week prior to analysis by high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS). Internal consistency standards included with each run indicate a reproducibility better than 3% (1 standard deviation) in the 50–500 µg/L range of As concentrations.

**Microbial Determinations.** The most probable number (MPN) of total coliforms and E. coli in well water samples was determined using a U.S. EPA-approved commercial culture kit (Colilert, IDEXX Laboratories, Inc., Westbrook, Maine) according to the manufacturer’s directions. To combine duplicate measurements, positive wells in both trays were summed and converted to a MPN and 95% CI on the assumption that E. coli were homogeneously distributed in well water. In this study, a sample was considered positive for E. coli if both replicates contained detectable E. coli (i.e., ≥ 1 MPN/100 mL). To determine the significance of a difference in the proportion of wells with detectable E. coli for...
different categories of wells, we used the approximation $$\pm 2 \left[ \frac{p(1-p)}{n} \right]^{1/2}$$ to estimate 95% confidence intervals (CIs), where $$p$$ is the proportion of months when E. coli was detected for the group of wells (a binary outcome) and $$n$$ is the number of months when well water from the group of wells was analyzed.\(^\text{22}\) Further details about the surveys, sampling, and measurements, as well as a test of As toxicity to E. coli in groundwater collected from a subset of wells, are provided in the Supporting Information.

\section*{RESULTS}

**Precipitation and Water Levels.** The precipitation pattern in 2008 and 2009 was typical of the monsoonal climate of the region, with heavy rainfall during the months of April through October and little if any rain during the remaining months (Figure 2a). In response, water levels in ponds monitored inside the embankment in Matlab and Char Para varied by $$\sim$$1 m between the dry and wet season. Relatively deep ponds were selected for monitoring; many ponds dry out entirely during winter. The seasonal range in groundwater levels measured within the embankment in Matlab is somewhat wider ($$\sim$$1.5 m), more so outside the embankment (2.5 m) and even more so in Char Para (4 m). The difference between surface and groundwater levels is a roughly constant 0.5 m downward within the embankment in Matlab over much of the year (downward head gradient of 0.05 given the 10 m depth of the monitoring well), with the exception of March and April when groundwater and pond levels were essentially the same (Figure 2a). The limited pond data available from Char Para indicate a more pronounced difference of 2 m between the pond and groundwater (downward gradient of 0.2) in June 2009 and no significant gradient later in August through October.

**Fecal Indicator Distribution.** Groundwater was sampled from the monitored tubewells in duplicate on a total of 2389 occasions between May 2008 and November 2009. The 95% confidence intervals (Supporting Information) for duplicate samples overlap in 96% of all cases. E. coli was detected 1023 times in duplicate samples, i.e., on 43% of all sampling occasions. Average E. coli concentrations calculated from duplicates were within ranges of $1-10$, $10-100$, and $100$ to over the maximum quantifiable of 2000 MPN/100 mL on $710$ (30%), $223$ (9%), and $90$ (4%) sampling occasions, respectively.

The frequency of E. coli detection ($>1$ MPN/100 mL) over a year was calculated for each of the 125 wells having data available for at least 10 out of 11 months of the year (no wells were sampled in December 2008), averaging the result if a well was sampled in the same month in 2008 and 2009. Such averaging avoids
over-representing those months that were sampled twice in the annual mean. Not a single well remained free of \(E. coli\) in duplicate samples throughout the sampling period. \(E. coli\) was detected between four and six months of the year in a majority of the wells (67 out of 125, i.e., 54%). \(E. coli\) was detected no more than four months of the year in only 25 (20%) of the total of 125 wells monitored. Groundwater pumped from the remaining 33 (26%) most-impacted wells contained detectable levels of \(E. coli\) between six and nine months of the year. Annually averaged \(E. coli\) detection frequencies for the entire set of 125 wells do not follow any clear geographic pattern (Figure 1b, c). Wells frequently contaminated with \(E. coli\) are interspersed with less contaminated wells within individual villages, including the more densely sampled villages of Char Para in Araihazar and Bara Haldia in Matlab.

**Well Arsenic.** Unlike \(E. coli\) concentrations, the As content of groundwater changed relatively little over time. Of the 116 wells from which two samples collected in August 2008 and March 2009 were analyzed by HR ICP-MS (the remaining 9 wells were analyzed for As only once), concentrations in the \(<1–500 \mu g/L\) range varied in all but 4 cases by less than 25% or less than 30 \(\mu g/L\). The group of 125 shallow wells that were monitored is divided roughly equally between average As concentrations below the WHO guideline of 10 \(\mu g/L\) \((n = 37)\), within the 10–50 \(\mu g/L\) \((n = 49)\) range, and above the Bangladesh standard of 50 \(\mu g/L\) \((n = 39)\). The distribution of As is mixed in the villages of Char Para in Araihazar and Bara Haldia in Matlab that were densely sampled (Figure S1 of the Supporting Information). Low-As wells were specifically targeted in the five other villages of Matlab where high-As wells otherwise dominate.18

**E. coli Time Series.** Concentrations of \(E. coli\) in individual wells were highly variable from one month to the next across the entire range of annual frequencies of \(E. coli\) detection. \(E. coli\) concentrations occasionally exceeded 100 MPN/100 mL in wells for which \(E. coli\) was undetectable for much of the year. Conversely, \(E. coli\) levels were at times undetectable for wells typically containing 10 MPN/100 mL \(E. coli\). Despite the variability of \(E. coli\) levels, there is a broad seasonal pattern defined by the entire set of wells (Figure 2b). In July–November 2008 and again in June 2009 and in September–November 2009, more than half the wells contained detectable levels of \(E. coli\). This contrasts with May 2008 and March 2009 when \(E. coli\) was detected in less than one-quarter of the wells.

The proportion of wells with detectable \(E. coli\) containing up to 10 \(\mu g/L\) As was greater than for wells with >50 \(\mu g/L\) As for 15 out of a total of 18 months (Figure 2b). For wells containing 10–50 \(\mu g/L\), this was the case for only 10 out of 18 months. Considering months individually, the 95% CIs for the proportion of wells with detectable \(E. coli\) generally overlap, although not in August 2008 when \(E. coli\) was detected in 70 ± 15% \((n = 37)\) of wells with As concentrations up to 10 \(\mu g/L\) and in 33 ± 15% \((n = 39)\) of wells with >50 \(\mu g/L\) As. There is a systematic decline in the proportion of wells with detectable \(E. coli\) across the three categories of As concentrations during four months of the dry season (January–April) and as well as four of months of the wet season (May–August), but the differences are statistically significant for both seasons only between the lowest and the highest As category (Figure 3a).

**Potential Confounding Factors.** For 43 wells out of the set of 125 that were monitored, priming was required on 6–38% of the sampling occasions but was not systematically associated with higher rates of \(E. coli\) detection across the three categories of As concentrations (Figure 3b). A concrete platform prevented standing water from seeping right around the annulus of 65 of the 125 monitored wells but was broken or nonexistent for the remaining wells. This potential source of contamination was again not associated with systematically higher \(E. coli\) levels across the three categories of As concentrations, however (Figure 3c). Unlike the previous pilot study conducted in two villages within Araihazar,16 there is no systematic pattern in the depth distribution of wells monitored within the study area. The proportion of wells meeting the Bangladesh standard of 50 \(\mu g/L\) As is higher for wells shallower than the average of 14 m (86%) compared to deeper wells (46%), however. Despite this association between As and depth, the data show no detectable effect of well depth on the relation between As and detection of \(E. coli\) (Figure 3d).

**Population Density.** The household survey counted a total population of 7077 distributed across 1479 households living within a radius of up to ~200 m of the monitored wells. Two-thirds of the households are composed of up to 5 members; the remaining third of households range from 6 to 19 members in size. On average, 20 people live within a distance of 25 m of a monitored well, and the population roughly triples for every doubling of the distance from each well. The average population living within a distance of 25 m of a monitored well is somewhat higher in Matlab (21) compared to Char Para (16).

Detection of \(E. coli\) was systematically compared to population density, taking into account both As levels and platform quality. The strongest correlation \((r^2 = 0.42, p = 0.004)\) is observed between \(E. coli\) detection during the wet season and population density within 25 m of a well for the subset of 18 wells with a good platform and As > 50 \(\mu g/L\) (Figure 4). The relationship is more scattered but still significant for 29 wells with a good platform containing 10–50 \(\mu g/L\) As \((r^2 = 0.28, p = 0.003)\). There is no relationship between \(E. coli\) detection and population density for...
Figure 4. Comparison of the detection frequency of *E. coli* as a function of population density within 25 m of a well with a good platform for three categories of As concentrations. Regression coefficients for other time spans and categories of wells are listed in Table S1 of the Supporting Information; the corresponding plots are in Figure S4 of the Supporting Information.

The relationship between *E. coli* detection in May–August and population density remains significant when considering all wells with As concentrations >50 μg/L (\(r^2 = 0.23, p = 0.002\)), but not when considering wells without a platform or with a broken platform only (Table S1 of the Supporting Information). Almost as striking as these correlations is the lack of any significant relationship for regressions of *E. coli* detection as a function of population density during the dry season or over the entire year, regardless of the As concentration or the presence of a platform. The spatial scale of the correlation is also quite restricted because the correlation between *E. coli* and population density is no longer significant for distances from a well ranging from 50 to 200 m (Supporting Information), even during the wet season and for the 18 wells with a good platform and As > 50 μg/L. The data suggest a local impact of population density on fecal contamination of shallow aquifers that manifests itself only during a particular time of the year and only for certain types of wells.

**DISCUSSION**

The high proportion of tubewells samples in Araihazar and Matlab that periodically contain detectable levels of *E. coli* is not a surprise. A number of previous studies relying on proven sampling and analytical methods have reported the presence of fecal indicators in groundwater in the Bengal Basin, and many more studies have been done so for other sedimentary basins in both developing and industrialized countries. The main finding of this study is the inverse relationship between fecal contamination and groundwater As, which has implications for microbial transport as well as As mitigation.

Arsenic toxicity could have provided an alternative explanation for the observed decline in *E. coli* detection frequency as a function of As. However, the incubations of contaminated groundwater clearly indicate that *E. coli* tolerates As concentrations orders of magnitude higher than ambient levels, at least in the short term (Table S2 of the Supporting Information). An effect on *E. coli* of chronic exposure to lower As levels cannot be ruled out but seems unlikely given this organism’s well-documented detoxification mechanisms.

**Pathways of Fecal Contamination.** The proportionality observed between the frequency of *E. coli* detection in well water during the monsoon and population density for certain categories of wells suggests a link that could reflect variations in overlying source strength coupled with infiltration of surface contaminants into the aquifer. The population enumerated in the area surrounding each monitored well provides a plausible proxy for sources such as latrines because population and latrine density are closely related throughout rural Bangladesh. The assumption underlying much of the following discussion is that the features of the *E. coli* data reflect infiltration on a lateral scale of ~25 m (based on the decline in the significance of the correlations at greater distances) rather than along the annulus, even though tubewells are typically not grouted in Bangladesh. Such a contribution cannot be ruled out, although a dominant role seems unlikely given the observation that there is no systematic difference in *E. coli* detection for wells with and without a platform (Figure 2). We cannot rule out either the occasional contamination of well water due to backflow from the handpump, especially during the monsoon when hot and humid air could promote *E. coli* growth within a handpump.

Because *E. coli* generally die off rapidly in the subsurface, infiltration from a chronically contaminated surface source would have to occur within weeks to months, even if *E. coli* have been found in uncontaminated soils and can grow under some natural conditions. The presence of *E. coli* at 6–36 m depth would therefore require preferential flow paths of the type usually observed in karstic or fractured terrain and less so in sandy aquifers. On the other hand, only a very small fraction of contaminated surface water would be needed to attain an *E. coli* concentration of ~10 MPN/100 mL by mixing given that ponds often contain 100,000 MPN/100 mL. Whereas most bacterial transport studies at the field or lab scale indicate rapid filtration of *E. coli* and limited transport through sand, there is often also a small fraction of bacteria that does not attach to the sediment. Seasonal variability in the fraction of wells with detectable *E. coli* is consistent with rapid transport and harder to reconcile with long-lived *E. coli* that die off slowly and are gradually filtered along a flow path. Gradual filtration could also have been expected to produce a depth dependence of contamination with *E. coli*. This is not observed, at least within the 6–36 m depth range of the monitored wells (Figure 3d).

If preferential flow contaminates shallow aquifers in Bangladesh, why would the connection become apparent only during part of the year and only for certain types of wells? One possible explanation for the first observation is that decreasing groundwater levels during the dry season gradually disconnect the shallow aquifer from a vadose zone that is contaminated with *E. coli* due to surrounding latrines and contaminated ponds. Unsaturated conditions in the subsurface are known to inhibit bacterial transport relative to saturated conditions through various mechanisms. The minimum in *E. coli* detection also corresponds, however, to a period when surface and groundwater levels are essentially equal and, unlike other times of the year, there is no hydraulic gradient favoring the downward transport of fecal contaminants. The relative importance of groundwater level and flow direction cannot be determined from the available data. Even these two mechanisms cannot entirely explain the observed patterns because the frequency of *E. coli* doubled from March to April 2009, while the level of groundwater remained constant and the vertical hydraulic gradient close to zero over the same period (Figure 2). The onset of precipitation during
this transition, which could conceivably flush downward fecal matter accumulated in surface soils during the dry season, suggests yet another contributing and perhaps dominant factor that is consistent with a connection between \textit{E. coli} levels and population density during the wet season only.

Even if the physical forcing of rapid downgradient transport of \textit{E. coli} remains uncertain, the observed decline in detection frequency with increasing As concentrations is consistent with a hydrogeological feature of shallow Bangladesh aquifers previously established on the basis of $^{3}$H–$^{3}$He dating of groundwater relative to the time of recharge. The range of depths and As concentrations of shallow wells monitored in Char Para and Matlab are comparable to six nests of monitoring wells from Araihazar previously used to document a broad increase of As concentrations with groundwater age.\textsuperscript{10} This relationship has been linked to variations in the surface permeability of soil that modulate the rate of local recharge as well as the buildup of As in shallow aquifers.\textsuperscript{11} The available data from Araihazar indicate that shallow groundwater that is $<1$ year old and contains $<10 \mu g/L$ As is found in depositional environments where sandy aquifers essentially extend to the surface. Such aquifers would be particularly prone to microbial contamination, whereas fine-grained sediment overlying older aquifers with typically As concentrations $>50 \mu g/L$ As could be expected to offer some protection. The new data suggests such a hydrogeological connection might play a role even if the rapid preferential flowpaths leading to fecal contamination of shallow aquifers are probably not the same as those that set the bulk chemistry of groundwater, including As and $^{3}$H–$^{3}$He ages.$^{10}$

Unlike the relation to population density, there is no a priori reason to expect a linear relation between As concentrations and the frequency of \textit{E. coli} detection. Nevertheless, a bivariate regression of \textit{E. coli} detection over the entire year for all platforms combined ($n=125$) yields coefficients for population density ($p=0.03$) and As concentrations ($p=0.03$) that are both significant and of the expected sign. Including depth as an independent variable in the regression combining all wells does not affect the outcome, and the regression coefficient for depth is not significantly different from zero. In contrast to the present study, which takes advantage of variations within villages, the importance of depth or other potential village-level differences for fecal contamination could not be ascertained in the previous pilot study conducted in Araihazar.$^{16}$ Bivariate regressions of \textit{E. coli} detection frequency as a function of population density and As for wells without a good platform does not yield statistically significant results for any time of the year. This suggests that conditions conducive to fecal contamination such as low As concentrations (i.e., a sandy setting) or the lack of a protective platform obscure the impact of population density without markedly increasing the level of fecal contamination.

The main caveat of our interpretation is that highly localized microbial contamination along the annulus of the well cannot be ruled out. Such an extreme case of short-circuiting of the flow paths that control the bulk composition of groundwater could in fact potentially generate some of the observed patterns, including enhanced transport of \textit{E. coli} during the monsoon and some degree of proportionality of fecal contamination to local population density. If scaling around the annulus of a well after installation is influenced by the grain size of surface sediment, then more frequent contamination of low-As wells might be expected because surface permeability has been shown to affect the As content of shallow aquifers on a broader scale.\textsuperscript{11}

\textbf{Health Implications.} According to the WHO,\textsuperscript{35} drinking water containing 1–100 MPN/100 mL \textit{E. coli} carries a low to moderate risk of significant health effects. The results of extensive well sampling across time and space reported here confirm the widespread presence of \textit{E. coli} almost throughout the year, except for the driest months at levels of up to 100 MPN/100 mL. Of potential significance in the context of As mitigation is the finding that shallow tubewells that meet the WHO guideline of 10 $\mu g/L$ for As in drinking water are more likely to contain detectable levels of \textit{E. coli} and, therefore, potentially also pathogens.\textsuperscript{36} The significance of this finding is independent of the fact that the pathway for fecal contamination of shallow groundwater remains insufficiently understood. The relevance of drinking groundwater even moderately contaminated with fecal matter compared to other modes of contracting diarrheal disease in rural Bangladesh is presently unknown. At the same time, there is growing evidence of widespread and long-term disease caused by drinking well water containing high levels of As.\textsuperscript{37} In the absence of further knowledge about the health implications of the observed increase in fecal contamination of tubewell water, well-switching to reduce As exposure should therefore continue. Future mitigation campaigns, however, should be cognizant of the levels of microbial contamination of shallow tubewells documented in this study.

\section{ASSOCIATED CONTENT}

\section{Supporting Information.} Additional description of survey methods, a map of the distribution of As, maps of the distribution of \textit{E. coli} in January–April and May–August, As toxicity studies, comparisons of \textit{E. coli} and population density for different categories of wells and As concentrations, and the characteristics and \textit{E. coli} data for all 125 shallow wells that were monitored. This material is available free of charge via the Internet at http://pubs.acs.org.

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\section{ACKNOWLEDGMENT}

This study was supported by NIH/Fogarty International Center Grant 5R01TW8066-2, with additional funds provided by Superfund Research Program Grant 1 P42 ES10349. We thank Dhaka University students and Mohammad Rezaul Huq for their help during numerous field trips as well local field staff in Araihazar and Matlab. This is Lamont-Doherty Earth Observatory Contribution Number 7434.

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