Fecal contamination of shallow tubewells in Bangladesh inversely related to arsenic

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Supporting Information
The village of Char Para covers a 0.3 km² area in Araihazar upazilla, 25 km northeast of Dhaka, where Columbia University and the University of Dhaka have studied the origin and health impacts of high As in groundwater since 2000. The second site comprises six villages distributed across a 25 km² portion of Matlab upazilla, 45 km southeast of Dhaka, where the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB), has been conducting research for three decades on a range of public health issues including As in groundwater. The following paragraphs provide additional information on data collection methods as well as a test of As toxicity to *E. coli*.

**Household survey.** In July-August 2008 and 2009, the size of 243 households in Char Para and 1236 households in the six Matlab villages living within a radius of up to ~200 m of the monitored wells was recorded during a systematic survey. The location of each household was recorded with a precision of ~1 m using a hand-held differential GPS unit linked to a local base station. From this information, the population residing within distances of 25, 50, 100, and 200 m of each monitored well was calculated. Households were also asked the depth of their well and whether they drink from a particular well, to distinguish this specific use from larger volumes of water pumped for washing and cleaning. The head of the household typically remembers the number of 6-m PVC pipe section that went into the construction of a well because he paid for them. The depth estimates provided can vary by 3-9 m when the owner is asked on separate occasions, however.

**Precipitation** Daily rainfall was measured in Barahaldia village with a HOBO weather station (ONSET, Bourne, MA, USA). For estimating precipitation before the weather station was installed and in April-June 2009 when the tipping-bucket sensor was clogged, we used precipitation data from Chittagong, 200 km southeast of Dhaka (Pattenga station; [http://www7.ncdc.noaa.gov/CDO/dataproduct](http://www7.ncdc.noaa.gov/CDO/dataproduct)), the nearest station with a complete record covering the period that is available. Gaps were filled on the basis of the Chittagong data and the relationship between daily precipitation at the two sites obtained by linear regression of the two time series where they overlap in time.

**Well sampling.** The location of each monitored well was measured by differential GPS. Before sampling, the hand-pump was used to withdraw ~50 L of water at a rate of 30-40 L/min, irrespective of well depth. The volume pumped corresponds to about one well volume for a 12 m deep well constructed with 1.5 in. (3.8 cm) ID diameter PVC pipe. The occasional need to pour water into the hand-pump for priming, typically from a nearby pump or stored water from the same pump, was recorded. Consent was obtained from a senior member of the household owning the well before a time series was initiated for this study, which was approved by the Institutional Review Boards of ICDDRB and Columbia University.
Microbial determinations. Colilert reagent was added to each 100 mL of well water sample and 2-3 samples of bottled water included as blanks at the end of each sampling day. The reagent contains a carbon source that is selectively metabolized by total coliforms and E. coli. For total coliforms, the media turns yellow under ambient light; for E. coli the media also turns fluorescent blue under ultraviolet light. Samples are subdivided into 49 large and 48 small wells (Quanti-Tray/2000) and incubated for 24 hours at 35°C. Wells that turn both yellow and fluoresce were counted as positive for E. coli. The number of large and small fluorescent wells for individual samples was converted to an MPN and a 95% confidence interval (CI) within the quantifiable range of <1 (non-detect) to a maximum of 2419 MPN/100 mL (20). Of the 105 occasions when 95% CIs do not overlap, only 25 pairs of incubations of water from the same well indicate a detectable level of E. coli in one and not in the other. E. coli was not detected in either duplicate on 1352 (57%) of all sampling occasions. E. coli concentrations in well water exceeded the maximum of 2083 MPN/100 mL that can be enumerated with two QuantiTrays on only 12 occasions. On the basis of parallel determinations of specific pathogens in well water (A. Layton, U. Tennessee, Knoxville, in preparation), wells were categorized according to the frequency of E. coli detection over the sampling period.

Between 112 and 124 wells were sampled for E. coli analysis 17 to 18 times from May 2008 to October 2009 (only 85 wells from Matlab were sampled in November 2009). Wells could not be sampled on some occasions because of a malfunctioning or detached hand-pump. In order to avoid biasing the time series data to those months that were sampled, monthly data for each well were first categorized in intervals of E. coli concentrations of <1 (i.e. non-detect), >1, and >10 MPN/100 mL. For each month of the year that was sampled twice, the occurrence of E. coli was then averaged (e.g. set to 0.5 if E. coli was detected one in a well one year and not the other).

As toxicity to E. coli. In August 2009, groundwater was collected from 5 tubewells known to contain high levels of E. coli in five sets of ten 100 mL sterile IDEXX bottles. Each but one of these bottles was amended to increase As(III) concentrations by 100 to 1,000,000 μg/L and incubated for 24 hours at 35°C. E. coli concentrations were determined as above. The median lethal As dose at which E. coli concentrations were halved (LC50) was determined by linear interpolation.

Concentrations of E. coli and As in well water selected for the toxicity test ranged from 70 to >2400 MPN/100 mL and 20-400 μg/L, respectively (Table S1). There was no detectable decline in E. coli concentrations at the end of the incubation until As concentrations above 10,000 μg/L were reached in the spiked samples. The median lethal As dose at which E. coli concentrations were halved (LC50) ranged four-fold from 50,000-200,000 μg/L and shows no clear relation to ambient As levels.
Table S1. *E. coli* detection and population within 25 m of well

| Platform       | As (µg/L) | Jan-Apr | May-Aug | Jan-Nov |
|----------------|-----------|---------|---------|---------|
|                | 0-10      | 0.12    | 0.07    | 0.01    |
| Good           | 10-50     | 0.04    | 0.28*   | 0.07    |
|                | >50       | 0.02    | 0.42*   | 0.11    |
|                | 0-10      | 0.00    | 0.00    | 0.01    |
| Poor or none   | 10-50     | 0.01    | 0.01    | 0.00    |
|                | >50       | 0.00    | 0.14    | 0.06    |
|                | 0-10      | 0.03    | 0.02    | 0.00    |
| All types      | 10-50     | 0.00    | 0.06    | 0.05    |
|                | >50       | 0.00    | 0.23*   | 0.08    |

*a*Regression coefficients for linear regressions of *E. coli* detection frequency in different months and different As concentrations.

* slope significantly different from zero at the p<0.05 level

Table S2. Tests of As toxicity to *E. coli*.

| Well ID | Initial *E. coli* (MPN/100 mL) | Well water As (µg/L) | *LC₅₀ E. coli* |
|---------|--------------------------------|----------------------|---------------|
|         | Natural                         |                       |               |
| 21769   | 68                              | 400-420              | 92,000        |
| 21749   | 1203                            | 260-370              | 130,000       |
| 21782   | 687                             | 86- 94               | 89,000        |
| 21759   | 2420                            | 80- 97               | 55,000        |
| 21800   | 285                             | 17- 18               | 200,000       |
Figure Captions

**Figure S1** Distribution of As in monitored wells of Char Par and Matlab. White circles show the location of households surrounding around each monitored well; black dots the location of previously surveyed wells.

**Figure S2** Detection of *E. coli* in monitored wells of Char Par and Matlab in January-April.

**Figure S3** Detection of *E. coli* in monitored wells of Char Par and Matlab in May-August.

**Figure S4** Comparison of the detection frequency of *E. coli* for time different time periods as a function of population density within 25 m of wells with different quality of platforms as well as different ranges of As concentrations.
Arsenic (µg/L) in wells 6-36 m:
- <10
- 10-50
- >50

Other wells and households are also shown in the map.
1 km

E. coli Jan-Apr wells 6-36 m
- <0.25
- 0.25-0.50
- >0.50
- other wells
- households

Char Para

Matlab

Figure S2
Char Para

Matlab

1 km

E. coli May-Aug
wells 6-36 m

<0.25

0.25-0.50

>0.50

other wells

households

Figure S3
Figure S4

Mean E. coli detection on Jan - Apr, good platform

Mean E. coli detection on May - Aug, good platform

Mean E. coli detection on Jan - Nov, good platform

Mean E. coli detection on Jan - Apr, no or broken platform

Mean E. coli detection on May - Aug, no or broken platform

Mean E. coli detection on Jan - Nov, no or broken platform

Mean E. coli detection on Jan - Apr, all wells

Mean E. coli detection on May - Aug, all wells

Mean E. coli detection on Jan - Nov, all wells

Population within 25 m