Hand-pumps as reservoirs for microbial contamination of well water

Andrew S. Ferguson, Brian J. Mailloux, Kazi M. Ahmed, Alexander van Geen, Larry D. McKay and Patricia J. Culligan

ABSTRACT

The retention and release of total coliforms and *Escherichia coli* was investigated in hand-pumps removed from tubewells tapping a faecally contaminated aquifer in Matlab, Bangladesh, and from a new hand-pump deliberately spiked with *E. coli*. All hand-pumps were connected to reservoirs of sterile water and flushed. Faecal coliforms were observed in the discharge from all three of the previously used hand-pumps, at concentrations comparable to levels measured in discharge when they were attached to the tubewells. During daily flushing of one of the previously used hand-pumps, the concentration of total coliforms in the discharge remained relatively constant ($\approx 10^3$ MPN/100 mL). Concentrations of *E. coli* in the pump discharge declined over time, but *E. coli* was still detectable up to 29 days after the start of flushing. In the deliberately spiked hand-pump, *E. coli* was observed in the discharge over 125 days ($t_{50} = 8$ days) and found to attach preferentially to elastomeric materials within the hand-pump. Attempts to disinfect both the village and new hand-pumps using shock chlorination were shown to be unsuccessful. These results demonstrate that hand-pumps can act as persistent reservoirs for microbial indicator bacteria. This could potentially influence drinking water quality and bias testing of water quality.

Key words | Bangladesh, chlorination, faecal indicator bacteria, groundwater monitoring, hand-pumps, water supply

Andrew S. Ferguson (corresponding author)
Patricia J. Culligan
Civil Engineering and Engineering Mechanics,
Columbia University,
605 Seeley W. Mudd,
500 West 120th Street,
New York, NY 10027-6902, USA
E-mail: *asf747@gmail.com*

Brian J. Mailloux
Department of Environmental Science,
Barnard College,
New York, NY 10025-6598, USA

Kazi M. Ahmed
Department of Geology,
University of Dhaka,
Dhaka 1000, Bangladesh

Alexander van Geen
Lamont-Doherty Earth Observatory,
Columbia University,
Palisades, NY 10964, USA

Larry D. McKay
Department of Earth and Planetary Sciences,
University of Tennessee,
Knoxville, TN 37996, USA

INTRODUCTION

Hand-pumped tubewells offer affordable access to shallow groundwater and provide the main groundwater extraction technology for villages in many developing countries across Africa, Asia and the Pacific. The microbial quality of groundwater pumped from tubewells is usually better than in unprotected surface water, but in many cases well discharge may still contain significant levels of faecal indicator bacteria (FIB) such as faecal coliforms and *Escherichia coli* (Islam *et al.* 2001; Hoque *et al.* 2006; Leber *et al.* 2011; van Geen *et al.* 2011). Such FIB are widely used surrogates because of the cost, equipment and time required to detect actual faecal pathogens. In Bangladesh, these include pathogenic *E. coli*, *Shigella*, *Vibrio* and rotavirus, all of which have been frequently isolated from hospital patients (Albert *et al.* 1999). The occurrence of faecal pathogens in groundwater has been reported in Canada (Howard 2006) and the US (Hunt *et al.* 2010; Gibson & Schwab 2011), although in both cases groundwater samples were not obtained using hand-pumped tubewells. In rural South Africa, Momba *et al.* (2006) report the occurrence of enteropathogenic *E. coli* and toxigenic *V. cholerae* in groundwater pumped using a rotary hand-pump.

Microbial contamination of groundwater is usually attributed to infiltration of water containing human or livestock faeces into the underlying aquifer. However, in areas employing hand-pumped tubewells part of the contamination might also be due to microbial attachment to surfaces inside the pump or the well casing, or

doi: 10.2166/wh.2011.106

contamination of the water used for periodic priming of the hand-pumps. Determining the route/pathways of contamination is important as they may differ greatly in terms of type and frequency of occurrence of disease-causing microorganisms (pathogens). Furthermore, the effectiveness of well disinfection methods, such as 'shock chlorination' (Luby *et al*. 2006) will likely vary depending on whether the contaminants are mainly in the pump, the well casing or the aquifer surrounding the well. The formation of biofilms within hand-pumps has previously been reported in relation to corrosion (Ibe *et al*. 2002). However, there remains a lack of data detailing the occurrence of coliforms in hand-pumps or the effectiveness of shock chlorination for removing these contaminants, both of which are addressed in this study.

Traditionally fabricated from cast iron and prone to rust, hand-pumps offer a large surface area which over time could harbour microorganisms (Ibe *et al*. 2002) such as FIBs and/or pathogens either attached directly to the pump surfaces or incorporated into biofilms. Biofilms are described by O'Toole *et al*. (2000) as communities of microorganisms encapsulated by extracellular polymeric substances, which offer increased protection against predation, physical or chemical attack. Biofilms may also provide havens for waterborne pathogens that are attached to or incorporated into the biofilm (Faruque *et al*. 2006), and hence can have a significant impact on measurements of water quality. Hand-pumps, especially those that contain biofilms, may provide suitable environments for growth of FIB such as coliforms or *E. coli* (LeChevalier *et al*. 1987; Banning *et al*. 2003; Pote *et al*. 2009). Therefore if this occurs, water quality assessments based on samples from the pumps may overestimate the risk of occurrence of waterborne pathogens, which do not replicate outside of their human or animal host.

The main objectives of this study were: (1) to evaluate the influence of pre-existing microbial reservoirs in tubewell hand-pumps on the occurrence of indicator bacteria (total coliforms and *E. coli*) in pumped water; and (2) to evaluate the effectiveness of shock chlorination to remove reservoirs of total coliforms from hand-pumps. Experiments were undertaken using three previously used hand-pumps taken from tubewells in rural Bangladesh, as part of an ongoing study of microbial groundwater quality (van Geen *et al*. 2011), and one new hand-pump. Owing to the large number of hand-pumps used for groundwater extraction across South and South-east Asia, the results of this study will have broad implications for the assessment of microbial water quality and investigations of microbial transport in aquifers in these regions.

MATERIALS AND METHODS

MPN analysis of faecal indicators

All groundwater samples were analysed for total coliforms and *E. coli*, using the MPN based Colilert™ assay (IDEXX Laboratories, Inc.). The presence of *E. coli* is assumed to be indicative of faecal contamination from a human or animal source and is used as a surrogate for pathogens. Total coliforms are a general indicator of microbial groundwater quality, but are not considered a true indicator of faecal pollution (Payment & Locas 2011). Assays were performed according to the manufacturer's instructions. Briefly, water samples (100 mL) were amended with reagent and gently mixed to dissolve the media. The contents were transferred into sterile Quanti-Tray 2000 trays, heat sealed, and incubated at $35 \pm 0.5\,^{\circ}$C for 24 h. When necessary, dilution of the water samples with ultrapure water was carried out in order to avoid exceeding the maximum detection limit of the Colilert™ assay. In order to discount contamination during the assay, one negative control was analysed during each sampling period using bottled drinking water in Bangladesh and ultrapure water at Columbia University (US). The negative control samples did not indicate the presence of total coliforms or *E. coli* (i.e. <1 organism per 100 mL). The MPN solution of Hurley & Roscoe (1983) was used to determine an MPN/100 mL and respective 95% confidence intervals.

Field site hand-pump replacement

In July 2009, three household tubewells (well no. 21602, 21612 and 21613) located in Sardarkandi village, Bangladesh (Matlab *upazilla*; 23.352° N; 90.656° E), were selected for hand-pump replacement and testing. Sardarkandi is located ≈65 km south-east of Dhaka city, within an embankment, built for flood protection, and sits on a 3–6 m clay layer underlain by a shallow sandy aquifer. All three tubewells had been installed using the traditional hand-flapper drilling method (Horneman *et al.* 2004; Leber *et al.* 2011) with PVC casings (3.8 cm diameter) and well intakes located in the shallow sand aquifer at depths ranging from 10 to 16 m. There were no concrete platforms around the base of the tubewells selected for this study and all were equipped with single-action cast iron hand-pumps (Fraenkel 1986) (Figure 1). Throughout rural Bangladesh, primitive hanging or pit latrines are often located within 10 m of tubewells and are a likely source of groundwater contamination. Domestic

Figure 1 | Tubewell no. 21602 (a) with original hand-pump attached and (b) replaced with a new hand-pump.

and farm animals also live within villages and provide additional sources of faecal waste. Previous monitoring has indicated that groundwater samples from such locations frequently contained *E. coli* and total coliforms (Leber *et al.* 2011; van Geen *et al.* 2011). Prior to removal of the pumps from the wells, they were purged by hand-pumping for three well volumes (typically 41 to 74 L at a rate of ≈0.5 L/s), and duplicate groundwater samples (100 mL) were collected in sterile Colilert™ bottles from the pump outlet (Table 1). No sediment was observed in the collected samples. The original hand-pumps were then removed and replaced with new hand-pumps purchased from a local distributor (Figure 1). Water samples were transported to the field laboratory in a cooler at 4 °C and analysed for total coliforms and *E. coli* using the MPN-based Colilert™ assay within 8 h.

Flushing experiments using previously used hand-pumps

Following hand-pump replacement, the three previously used hand-pumps were transported to the field laboratory, fitted with a new down pipe, and connected to a reservoir within 8 h (Figure 2(a)). The reservoir contained 55 L of groundwater, obtained from a tubewell adjacent to the field laboratory, which had been filter sterilized (0.2 μm nitrocellulose filter, Cole-Parmer) and was determined to

Table 1 | Hand-pumps and analyses

Hand-pump no. 21602	21612	21613	New pump
Tested water	Tested water	Tested water	Transported to Columbia University Lab
Removed hand-pump from tubewell	Removed hand-pump from tubewell	Removed hand-pump from tubewell	Purposely contaminated
50 L flush with clean water in field lab	50 L flush with clean water in field lab	50 L flush with clean water in field lab	35 L flush with clean water
Transported hand-pump to Columbia University Lab			Flushed and monitored for 55 days
Flushed and monitored for 46 days			Dismantled and swabbed
Bleached by soaking			Bleached by pumping
Conservative tracer			Flushed and monitored for 62 days

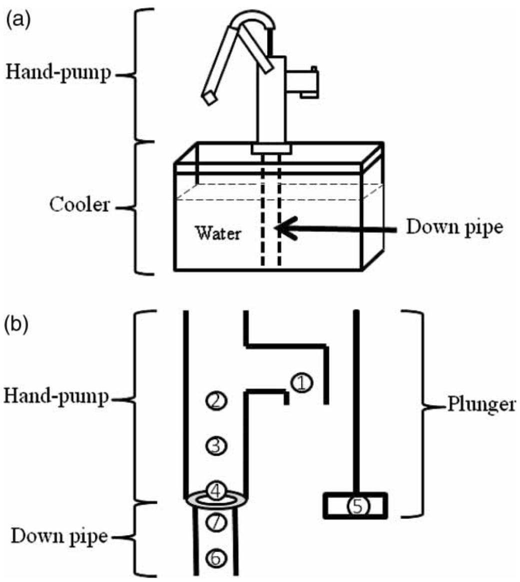

Figure 2 | (a) Schematic of laboratory hand-pump experiment; and (b) location of swabs (1–7) taken from the internal surface of the new hand-pump.

be free (<1 organism/100 mL) of total coliforms and *E. coli*. Water was pumped from the reservoirs using the previously used hand-pumps and duplicate water samples (100 mL) were collected from the hand-pump discharge following the 1st and 20th strokes (1 stroke ≈1 L). Pumping continued for a total of 50 strokes at an approximate rate of 0.5 L/s. Water samples were also collected directly from the reservoirs pre and post flush. All samples were analysed for total coliforms and *E. coli*.

The inlet and outlet of one of the hand-pumps (no. 21602) was capped before being boxed and transported over a period of 4 days to the US for additional experiments at Columbia University (Table 1). In the laboratory, flushing experiments were carried out using the same hand-pump and reservoir configuration as previously used in Bangladesh (Figure 2(a)). For these experiments, the pumps were flushed at an approximate rate of 0.5 L/s with 50 L of artificial groundwater (AGW) at pH 7.0, which was free of total coliforms and *E. coli*, and contained 0.12 g/L sodium bicarbonate and 0.16 g/L calcium chloride. The flushing continued daily over a period of 46 days with periodic analysis of the hand-pump discharge (1st and 20th strokes) and reservoir (pre and post flush) for total coliforms and *E. coli*. Following each daily flushing cycle the cooler (see Figure 2(a)) was cleaned three times using ultrapure water, dried and refilled with 55 L of sterile AGW. The periodic analysis showed that the reservoir was free of total coliforms and *E. coli* prior to flushing events. On day 47 the pump was removed and shock chlorinated as described below. In this configuration, any indicator bacteria contained in the water exiting the hand-pump had to originate from within the pump since the inlet cooler water was changed daily and determined to be uncontaminated. If at the end of each daily pumping cycle the inlet cooler water contained indicator bacteria, this source of contamination most likely was attributed to backflow from the pump caused by leaky valves and gaskets.

At the end of the experiment, 50 L of AGW containing 100 mg/L bromide (Br), a conservative tracer, was pumped through one hand-pump (no. 21602). AGW containing no Br was then flushed through the hand-pump until Br was not detected. The goal was to determine the residence time of a non-biological conservative tracer within the hand-pump. Br concentrations were determined by ion chromatography on a Dionex ICS2000 with an AS-18 column (Dionex, Sunnyvale, CA). The detection limit was 0.1 mg/L Br.

Flushing experiment using a purposely contaminated new hand-pump

A new hand-pump was purchased from a local distributor in Bangladesh and then transported to Columbia University for laboratory experiments to investigate microbial retention in the pump under controlled conditions (Table 1). The hand-pump and reservoir configuration is shown in Figure 2(a). The hand-pump was attached to a reservoir containing 50 L of sterile AGW and pumped 35 times at an approximate rate of 0.5 L/s. Initial water samples (100 mL) were collected from the reservoir and hand-pump discharge (1st and 20th strokes), which showed no detectable levels of total coliforms or *E. coli*. The hand-pump was then purposely contaminated by flushing 35 L of AGW containing 1.5×10^6 MPN/100 mL *E. coli* (ATCC#700891) (Debartolomeis & Cabelli 1991) which had been grown for 24 h in LB at 37 °C. Prior to flushing, the contaminated AGW was sampled (1.5 L) from the reservoir, aliquoted into separate 50 mL centrifuge tubes and stored in the dark at room temperature. The batch survival of *E. coli* from the centrifuge tubes was then determined through routine sacrificial sampling.

After contamination of the hand-pump, the reservoir was cleaned and rinsed three times using ultrapure water, dried and refilled with 50 L of sterile AGW. Water samples (100 mL) were then collected from the reservoir pre- and post-flush and from the hand-pump effluent following the 1st, 10th and 20th strokes and analysed for total coliforms and *E. coli*. Flushing continued for a total of 35 strokes before the reservoir was again cleaned and refilled with 50 L of AGW. The hand-pump was then left for a period of 24 h until samples were collected again from the reservoir and the hand-pump effluent. Hand-pump flushing continued daily for 125 days with frequent determination of total coliforms and *E. coli* within the reservoir (post-flush) and hand-pump effluent. Prior to each pumping cycle the reservoir was sampled and determined to contain no detectable levels of total coliforms and *E. coli*. On day 55 the hand-pump was dismantled and the internal surface swabbed in seven locations using sterile cotton swabs (Figure 2(b)). Each swab was then placed into 100 mL of ultrapure water and agitated for 10 min before being analysed for total coliforms and *E. coli*. On days 56, 61 and 63 attempts were made to disinfect the hand-pump before the pump was returned to the routine flushing cycle.

Shock chlorination

The chlorination procedure employed in this study used Clorox® bleach containing 5.25% sodium hypochlorite. A free chlorine concentration of 200–400 mg/L and a minimum contact time of two hours is typically recommended for shock chlorination (UNICEF 2005; Kjaergaard *et al.* 2007), although shorter contact times have been reported (Luby *et al.* 2006). After 46 days of flushing with sterile AGW, previously used hand-pump no. 21602 was detached from the reservoir and dismantled prior to shock chlorination. On days 47 and 50, this hand-pump was totally immersed and soaked for 2 h in ultrapure water containing enough bleach to achieve 1 and 6 g/L free chlorine, respectively. On day 52 the inside of the hand-pump was scrubbed using a wire brush and totally immersed and soaked for 24 h in ultrapure water containing 12 g/L free chlorine and 1% acetic acid. No chlorination procedure took place on day 53. Following each shock chlorination event the hand-pump was washed and rinsed three times in ultrapure water, air dried, reassembled, and reattached to the reservoir. The reservoir was then pumped using the procedure described for the earlier tests and samples of pump discharge were collected following the 1st and 20th strokes. Samples were also collected directly from the reservoir before and after pumping to measure FIB.

The new hand-pump, which had been purposely contaminated with *E. coli* and flushed as described previously, was shock chlorinated on days 56 (0.2 g/L free chlorine), 61 (0.4 g/L) and 63 (1 g/L) through the addition of bleach to 50 L ultrapure water contained in the reservoir. On each occasion, the chlorinated water was circulated by pumping for 30 min in an attempt to achieve a contact time similar to that employed by Luby *et al.* (2006). The reservoir was then washed and rinsed three times with ultrapure water, filled with 50 L of sterile AGW, and flushed through the hand-pump. Flushing occurred three times (total volume 105 L), before water samples (100 mL) were taken for assays of total coliforms and *E. coli* from the reservoir pre- and post-flush and from the hand pump effluent following the 1st, 10th and 20th strokes. The hand-pump was returned to the routine pumping cycle after each shock chlorination.

RESULTS

Flushing experiments with previously used hand-pumps

Prior to hand-pump replacement, the initial concentration of total coliforms and *E. coli* in water pumped from three tubewells (no. 21602, 21612 and 21613) ranged from 328 to 2,400 MPN/100 mL, and 6 to 205 MPN/100 mL, respectively. In the field laboratory in Bangladesh, flushing 50 L of filter sterilized groundwater through the previously used hand-pumps resulted in continued contamination of all samples of the discharge water with total coliforms and *E. coli* (Figure 3(a) and (b)). Differences in microbial concentrations between samples collected on the 1st or 20th stroke for each pump were small in all but one case (*E. coli* in discharge from hand-pump no. 21612). Concentrations of total coliforms in the discharge from the hand-

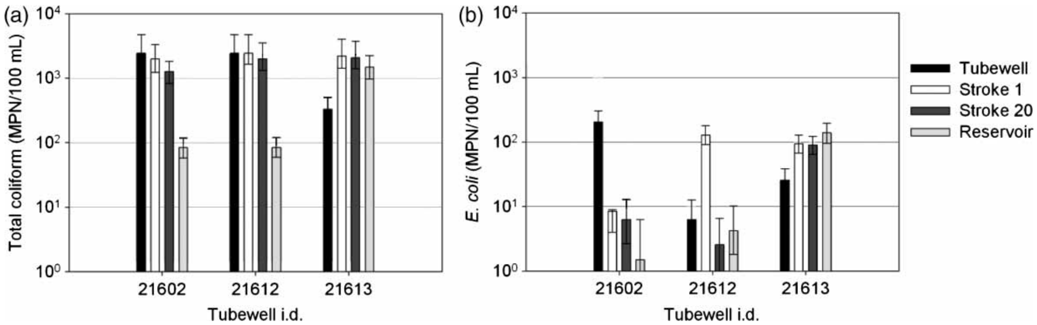

Figure 3 | Concentration of (a) total coliforms and (b) *E. coli* in discharge from the previously used hand-pumps prior to removal from their corresponding village tubewell, and following their attachment to reservoirs containing 50 L of filter-sterilized groundwater. Discharge from the reservoir-attached samples was collected on the 1st and 20th hand-pump stroke. Samples were also collected directly from the reservoirs after pumping. Error bars represent 95% confidence intervals.

pumps attached to the uncontaminated reservoirs (Figure 3 (a)) were similar to or higher than concentrations measured in the same pumps while they were attached to the tubewells. This was also the case for *E. coli* concentrations in pumps no. 21612 and 21613, but not for pump no. 21602, where concentrations in the discharge from the uncontaminated reservoir were approximately a factor of 20 times lower than measured when the pump was mounted on the tubewell (Figure 3(b)). When the pumping cycle ended, FIB were also detected in the previously uncontaminated reservoir water. This was almost certainly due to backwash from the previously used pumps. For pumps no. 21602 and 21612, the *E. coli* concentrations in the reservoir were substantially lower than observed in pump discharge, but for pump no. 21613 the concentrations in the reservoir were slightly higher than in the pump discharge.

Hand-pump no. 21602 was sampled over a period of 46 days while attached to a reservoir of sterile water in the US laboratory. Concentrations of total coliforms in discharge from this pump fluctuated slightly, but remained relatively constant at $\approx 10^3$ MPN/100 mL (Figure 4(a)). The levels of total coliform contamination were only slightly lower than those concentrations observed in discharge from the flushing experiments in Bangladesh or in discharge when it was still mounted on the tubewell (Figure 3(a)). Concentrations of total coliforms in the reservoir (which was emptied, cleaned and refilled daily with AGW containing no indicator bacteria) reached a peak value of 28 MPN/100 mL after 8 days of flushing, and then declined to below the detection limit (<1 organism/100 mL) by 29 days.

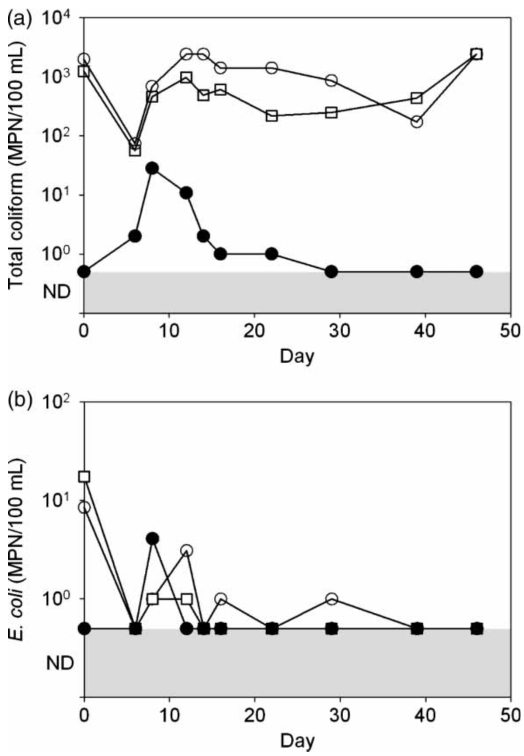

Figure 4 | Timeseries of (a) total coliforms and (b) *E. coli* concentrations within the pumped water discharge of the previously used hand-pump (no. 21602). Sterile artificial groundwater (50 L) was flushed daily through the hand-pump. Water samples were collected from the 1st (○) and 20th (□) strokes and directly from the reservoir after pumping (•). The reservoir was cleaned and refilled with sterile AGW after each sampling event.

E. coli concentrations in the pump discharge (Figure 4(b)) were lower and more variable than total coliform concentrations. *E. coli* levels declined during the experiment, with the last measurable *E. coli* concentration occurring after 29 days of flushing. Concentrations of *E. coli* in the reservoir were below the detection limit (<1 organism/100 mL) for all samples, except one that was collected after 8 days of flushing.

The conservative tracer, Br (100 mg/L), was flushed (50 L) through the pump to examine the differential effects of a biological and a dissolved tracer. The concentration of Br was below the detection limit of 0.1 mg/L following flushing the hand-pump with 80 L AGW. No extended tailing was observed with the Br tracer.

Flushing experiment with a purposely contaminated new hand-pump

Following the deliberate contamination of a new hand-pump, *E. coli* concentrations in the pump discharge rapidly declined from 10^6 MPN/100 mL to $<10^3$ MPN/100 mL over the first 3 days, increased to 10^4 MPN/100 mL on day 5, and then slowly decreased over the next 55 days with a half-life (t_{50}) of 8 days (Figure 5). The concentration of *E. coli* in the reservoir after flushing was below the detection limit (<1 organism/100 mL) on all occasions apart from day 0 (835 MPN/100 mL) following the replacement of the contaminated AGW source, day 5 (34 MPN/100 mL), and day 7 (16 MPN/100 mL). Swabs taken from seven different locations within the hand-pump (Figure 2(b)) on day 55 revealed the presence of *E. coli* at two locations, positions 4 (613.1 MPN/swab) and 5 (95.9 MPN/swab). Both positions were associated with the rubber seals on the foot valve (position 4) and plunger (position 5). No total coliforms or *E. coli* were detectable at any other locations within the pump. Subsequent reattachment of the hand-pump to the reservoir on day 55 resulted in an increase in *E. coli* concentrations in the pumped effluent from 10^2 to 10^3 MPN/100 mL. The initial decline in *E. coli* concentration was attributed to the removal of the contaminant source and ensuing washout of loosely attached microorganisms. This washout trend was expected to continue as the action of vigorously flushing water diluted and displaced any microorganisms within the hand-pump. However, *E. coli* continued to discharge from the hand-pump over a period of 125 days, even after attempts to sanitize the hand-pump between days 56 and 63. Batch microcosm tests in the AGW (Figure 5) showed culturable *E. coli* survived for a period of 63 days ($t_{50} = 3$ days) indicating that the observed long-term persistence of *E. coli* in hand-pump discharge is somehow related to conditions present inside the pump assembly.

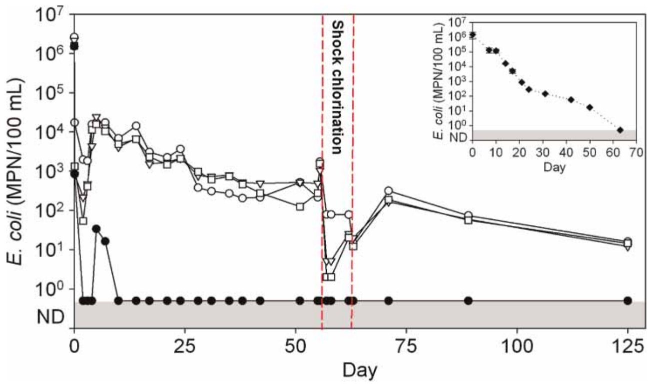

Figure 5 | Concentration of *E. coli* within the effluent of a purposely contaminated new hand-pump, before (days 0–56), during (day 56, 61 and 63) and after (days 63–125) shock chlorination. *E. coli* concentrations are given for the reservoir post flush (•) and from the hand-pump effluent following the 1st (○), 10th (Δ) and 20th (□) strokes. Concentrations of sodium hypochlorite used to shock chlorinate were 0.2 g/L (day 56), 0.4 g/L (day 61) and 1 g/L (day 63). Insert shows *E. coli* concentration (◆) in static batch microcosms.

Shock chlorination

Following the shock chlorination of hand-pump no. 21602 and after the flushing experiments in the US laboratory, concentrations of total coliforms in discharge from the pump remained constant or declined only slightly after the first two treatments by soaking in bleach solution (days 47 and 50 on Figure 6). A substantial decline in total coliform concentrations within the pump discharge (including undetectable levels in the sample collected after 20 pump strokes) occurred on day 52 following a more aggressive treatment with chlorine bleach, acetic acid and vigorous scrubbing of internal pump surfaces (Figure 6). However, on day 53, just one day after the aggressive decontamination treatment, total coliform concentrations measured in the pump discharge increased rapidly with the values measured after the 1st and 20th stroke at 219 and 36 MPN/100 mL, respectively. Concentrations of *E. coli* in the discharge from hand-pump no. 21602 were below the detection limit (<1 organism/100 mL) throughout the chlorination treatment tests. In relation to the new hand-pump, a 30-minute hypochlorite flush on days 56 (0.2 g/L free chlorine), 61 (0.4 g/L) and 63 (1 g/L) caused only an initial decrease

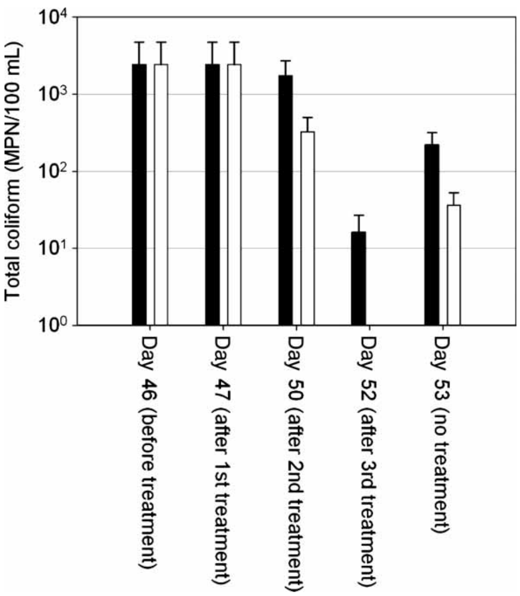

Figure 6 | Concentration of total coliforms in discharge from hand-pump no. 21602 on day 46 (before treatment), day 47 (after the first round of shock chlorination), day 50 (after the second round of shock chlorination), day 52 (after the third round of shock chlorination, combined with acid soaking and aggressive scrubbing) and day 53 (no treatment). Enumeration of total coliforms was determined following the 1st (■) and 20th (□) strokes. Error bars represent 95% confidence intervals.

in levels of culturable *E. coli*, measured in the pump discharge, and subsequent recovery to levels observed prior to shock chlorination (Figure 5).

DISCUSSION

Influence of hand-pumps on groundwater quality

Flushing tests carried out using both the previously used hand-pumps, which had been removed from the village tube-wells, and the purposely contaminated new hand-pump, indicate that hand-pumps can act as reservoirs for coliform microorganisms. They also show that microbial contamination from the pumps can move backwards out of the hand-pump and into the reservoir of uncontaminated water (and presumably down the well casing, if the pump was mounted on a well). The results of the 46-day flushing test carried out in the US using one of the previously used pumps are consistent with the results of the flushing tests carried out in Bangladesh. Furthermore, they show that the microbial contamination in the hand-pumps can be persistent over pumped volumes (up to 2,300 L) that are many times larger than would typically be purged from a well prior to sampling. In addition, the conservative tracer experiments show that non-attaching tracers are rapidly purged from hand-pumps indicating that the observed trends are not due to mixing with pockets of stagnant water within the pump. A single spiking event involving contamination of a new hand-pump with high concentrations of *E. coli* was sufficient to cause contamination of discharge for a period of 125 days (4,375 L of flushing) after the original contaminant source was removed. Indeed, it appears that the long-term persistence of *E. coli* in the pump discharge (compared with the 3-day half-life of *E. coli* in batch tests in the AGW) may be related to conditions in the pump that enhance survival or growth of *E. coli*.

Other studies have shown variability in indicator bacteria concentrations with pumped volume in hand-pumped wells in Bangladesh (Knappett *et al.* 2011) and in Canada in wells with submersible pumps (Kozuskanich *et al.* 2011), but this is the first study that we are aware of that confirms that the hand-pump can play a major role in this variability. The retention and release of total coliforms and *E. coli* from the internal pump surface is not surprising considering the ability of microorganisms to attach to surfaces and/or incorporate with existing biofilms (LeChevalier *et al.* 1987; O'Toole *et al.* 2000; Banning *et al.* 2003). Elastomeric materials and rubber-coated valves, used in plumbing and water distribution systems, have previously been shown to promote biofilm formation through the release of biodegradable compounds (Rogers *et al.* 1994; Kilb *et al.* 2003; Bressler *et al.* 2009). The association of *E. coli* with the rubber foot valve and plunger seal indicates that such material within the hand-pumps could act as a point source for FIB contamination. Corrosion is also common in cast-iron hand-pumps and can offer a large surface area for microbial attachment (Ibe *et al.* 2002). The positive surface charge associated with metal corrosion could also potentially increase the initial attachment and retention of bacterial cells (Scholl *et al.* 1990). Biofilms are dynamic and eventual dispersal of attached bacteria, as part of their life-cycle, provides an abundant source of planktonic (free-living) microorganisms (O'Toole *et al.* 2000).

Our findings suggest that reservoirs of microorganisms in hand-pumps could potentially impact microbial water quality assessments in several ways: first, hand-pumped water could indicate microbial contamination even if the aquifer itself is not contaminated at the time of sampling, potentially distorting aquifer water quality assessment; and second, the pumps could act as reservoirs that retain pathogens from the aquifer and then slowly release them (Keevil 2002; Banning *et al.* 2003; Klayman *et al.* 2009; Shikuma & Hadfield 2010). This second statement is speculative, but if correct would tend to increase the duration of exposure to waterborne pathogens during a disease outbreak. This raises the need for additional research to determine whether hand-pumps can serve as reservoirs of pathogenic microorganisms, in addition to acting as reservoirs of indicator bacteria.

Hand-pump sanitation

Overall, shock chlorination proved to be a relatively ineffective method for reducing concentrations of coliforms from the hand-pump, even at free chlorine concentrations 60 times greater than recommended for well chlorination (UNICEF 2005; Luby *et al.* 2006; Kjaergaard *et al.* 2007). The rebound in indicator bacteria after the last treatment (day 52 and day 63 for the previously used and new hand-pumps, respectively) suggests the likelihood that the coliforms are present as biofilms, which can provide some protection against chemical attack (LeChevalier *et al.* 1988; Stewart *et al.* 2001).

The general strategy employed for tubewell sanitation in less economically developed countries is removal of the hand-pump, addition of bleach (200–400 mg/L free chlorine) to the well, a waiting period (minimum contact time of 2 h), and the reattachment of the hand-pump before flushing (UNICEF 2005; Kjaergaard *et al.* 2007). Previous research has demonstrated that the microbial quality of hand-pumped tubewell water was not improved following a 30 min shock chlorination procedure (Luby *et al.* 2006). This could be due to contamination of pumped water by the hand-pump, recontamination of the well by the pump (which are both consistent with the findings of this study), incomplete disinfection of the well casing, or recontamination of the well by microorganisms in the aquifer. Further research is required to fully assess the factors influencing microbial contamination of hand-pumped tubewells and to determine whether shock disinfection can be a useful strategy for improving water quality.

CONCLUSIONS

Our findings suggest that hand-pumps can be reservoirs for FIB, which could distort microbial assessment of water quality. It is presently unknown whether hand-pumps could potentially also harbour waterborne pathogens, especially in field settings, but further investigation appears warranted. Our study also indicates that shock chlorination is unlikely to be effective for cleansing this component of the water supply system.

ACKNOWLEDGEMENTS

We thank Md Jahangir Alam and Md Rezaul Huq of Dhaka University for their assistance during field work, and M. Yunus and P.K. Streatfield of ICDDR,B for their continued support. This work was supported by NIH/FIC R01 TW008066.

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First received 13 January 2011; accepted in revised form 8 June 2011. Available online 1 August 2011