



The public health significance of latrines discharging to groundwater used for drinking



P. Ravenscroft^a, Z.H. Mahmud^b, M. Shafiqul Islam^b, A.K.M.Z. Hossain^b, A. Zahid^c,
G.C. Saha^d, A.H.M. Zulfiquar Ali^e, Khairul Islam^f, S. Cairncross^g, J.D. Clemens^b,
M. Sirajul Islam^{b,*}

^a Independent Consultant, Cambridge, UK

^b International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), 68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka, 1212, Bangladesh

^c Bangladesh Water Development Board, Green Road, Dhaka, Bangladesh

^d Dhaka University of Engineering and Technology, Shimultoly Road, Gazipur, Bangladesh

^e Department of Soil, Water & Environment, University of Dhaka, Dhaka, 1000, Bangladesh

^f WaterAid Bangladesh, House 97/B, Road No 25, Block A, Banani, Dhaka, 1213, Bangladesh

^g Department of Disease Control, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK

ARTICLE INFO

Article history:

Received 18 April 2017

Received in revised form

17 June 2017

Accepted 18 July 2017

Available online 19 July 2017

Keywords:

Faecal coliforms

Latrines

Groundwater pollution

Bangladesh

Drinking water

Risk

ABSTRACT

Faecal contamination of groundwater from pit latrines is widely perceived as a major threat to the safety of drinking water for several billion people in rural and peri-urban areas worldwide. On the floodplains of the Ganges-Brahmaputra-Meghna delta in Bangladesh, we constructed latrines and monitored piezometer nests monthly for two years. We detected faecal coliforms (FC) in 3.3–23.3% of samples at four sites. We differentiate a near-field, characterised by high concentrations and frequent, persistent and contiguous contamination in all directions, and a far-field characterised by rare, impersistent, discontinuous low-level detections in variable directions. Far-field FC concentrations at four sites exceeded 0 and 10 cfu/100 ml in 2.4–9.6% and 0.2–2.3% of sampling events respectively. The lesser contamination of *in-situ* groundwater compared to water at the point-of-collection from domestic wells, which itself is less contaminated than at the point-of-consumption, demonstrates the importance of recontamination in the well-pump system. We present a conceptual model comprising four sub-pathways: the latrine-aquifer interface (near-field); groundwater flowing from latrine to well (far-field); the well-pump system; and post-collection handling and storage. Applying a hypothetical dose-response model suggests that 1–2% of the diarrhoeal disease burden from drinking water is derived from the aquifer, 29% from the well-pump system, and 70% from post-collection handling. The important implications are (i) that leakage from pit latrines is a minor contributor to faecal contamination of drinking water in alluvial-deltaic terrains; (ii) fears of increased groundwater pollution should not constrain expanding latrine coverage, and (iii) that more attention should be given to reducing contamination around the well-head.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Graham and Polizzotto (2013) draw attention to the ‘widespread global reliance on both pit latrines and groundwater ...’ and estimate that 1.77 billion people worldwide use pit latrines, a figure that will increase in line with achieving Sustainable Development Goal (SDG) 6.2: safe sanitation for all. While expressing caution about

the variable quality of previous studies of the risks of latrines to rural water supplies, they warn that areas with shallow groundwater and prone to flooding, conditions common in S and SE Asia, present the greatest risks to health. It is estimated that 38% of improved water sources globally are contaminated by faecal indicator bacteria (FIB; Bain et al., 2014), and that untreated groundwater is a major source of enteric disease globally and that the proven disease burden is only ‘the tip of the iceberg’ (Murphy et al., 2017). Bangladesh is a country that conforms to this pattern: untreated groundwater supplies >90% of the rural population, with FIB detected in around 40% of all supplies at the point of collection

* Corresponding author.

E-mail address: sislam@icddr.org (M.S. Islam).

(PoC), rising to about 60% at the point of use (PoU; Hoque, 1998; Ravenscroft et al., 2014; BBS/UNICEF, 2015). Increases in contamination from PoC to PoU have been noted elsewhere and already drives hygiene education programmes (e.g. Trevett et al., 2005; UNICEF, 2012). The many reviews of the effects of on-site sanitation on groundwater quality (e.g. Lewis et al., 1982; Cave and Kolsky, 1999; Lawrence et al., 2001; Foppen and Schijven, 2006; Graham and Polizzotto, 2013 and references therein) draw heavily on short-term monitoring of existing water wells near existing latrines and equate, incorrectly we believe, the intrinsic quality of groundwater with the quality of the water collected from wells. The conventional wisdom that inadequate spacing of pit latrines and wells is a major contributor to faecal contamination of drinking water has long directed public health authorities to mandating spacing criteria, typically 10–50 m, although the scientific basis and efficacy of these rules are often open to question (Graham and Polizzotto, 2013). We seek to show that the application of the precautionary principle to cases of uncertain attribution of the cause of faecal contamination in water wells leads to disproportionate attention being given to spacing as a control measure.

Concern over the severity of the risks of on-site sanitation polluting groundwater-sourced drinking water has fluctuated, mirroring renewed drives for safe water and sanitation such as the Millennium Development Goals, the SDG's and for example, India's Total Sanitation Campaign which aims to build 12 million latrines by 2019 but has been criticised for potentially increasing pollution of groundwater and drinking water. For example, on alluvial terrain in India's Odisha State, Daniels et al. (2016) report '*strong evidence of protozoa contamination of shallow groundwater from pour-flush latrines within 15m*' and conclude that contamination of groundwater used for drinking is correlated with faecal loading of latrines, literacy, livestock, damaged well-heads and antecedent rainfall. Daniels et al. (2016) predict that achieving 100% latrine coverage will result in a 1.9–4.1 times increase in protozoan contamination. Odagiri et al. (2016) also in Odisha State and Sorensen et al. (2016) in Bihar State both predict increased microbial contamination of shallow groundwater from increased adoption of latrines, suggesting a trade-off between sanitation and water supply objectives (i.e. SDG 6.1 and 6.2).

There is a potential contradiction between the conventional views that, on the one hand, perceive groundwater as being intrinsically safe and, on the other hand, perceive groundwater as being intrinsically at risk from latrines, although this difference may be partly explained by pit latrines by-passing the soil zone. It is a widely accepted norm that the immediate vicinity of hand-pumped tubewells are vulnerable to pollution, and much effort is given to reducing this risk through sanitary inspections and their incorporation into Water Safety Plans (WHO, 2011). Unfortunately, sanitary inspection scores have little ability to predict bacterial contamination of well water (e.g. Hoque, 1998; Luby et al., 2008; Ercumen et al., 2017; Misati et al., 2017). A possible explanation for this poor correlation is that the contents of the standard WHO sanitary inspection are necessary but not sufficient because it excludes factors such as well construction, the survival and growth of bacteria inside pipes and pumps, and the use of dirty priming water (Ferguson et al., 2011; Hoque, 1998; Knappett et al., 2012a).

The primary objectives of this study were to understand the migration of faecal bacteria away from latrines under different hydrogeological settings and thereby produce better guidelines for siting and construction, at least for alluvial terrains. However, recognising that the causes of faecal contamination of drinking water are more nuanced than simple spacing, we also sought to isolate factors such as operation and maintenance and hygiene practices from the migration of contaminants in groundwater. To do this, we constructed and monitored new latrines and dedicated

piezometers at households with no existing latrine at four sites in rural Bangladesh monthly over two years. We interpret these results in the light of drinking water surveys conducted by ourselves and others and studies of alternative pollution pathways to assess the relative contributions of different sub-pathways from the latrine to the point of water consumption.

2. Methods and materials

2.1. Study sites

Following a pilot study on older (Pleistocene) sediments of the Barind Tract and Holocene sediments of the Chittagong Coastal Plain (Islam et al., 2016; Table S1), we selected four (Phase 2) sites on the Ganges – Brahmaputra – Meghna delta to reflect the diversity of recent floodplain environments in Bangladesh (Fig. 1; Brammer, 2012; Rahman and Ravenscroft, 2003). The village and household selection were carried out considering social and logistical factors as detailed by Islam et al. (2017a) and included the requirement that collaborating households did not already have their own latrine and that shallow groundwater was not contaminated by arsenic above the national standard (50 µg/L). At our study sites, domestic water supplies are drawn dominantly from manually-drilled, privately-owned tubewells a few tens of metres deep and equipped with suction-mode (e.g. the UNICEF Nr 6) hand pumps. Most households use a latrine of the same type as constructed under the study; open defecation is rare at all sites. Pirganj, on the Tista Fan and Shambaganj on the Old Brahmaputra floodplain (Rangpur and Mymensingh districts respectively) have sandier, more permeable sediments with less organic matter and deeper water tables. Jajira (Shariatpur District) and Paikgachha (Khulna District) lie respectively on the River and the Tidal floodplains of the Ganges (Brammer, 2012), formed of finer sediments, albeit with different internal sedimentary structures, and shallower water tables. All sites have surface aquitards 3–5 m thick within which the latrine was constructed. However, the grain sizes of the sediments to depths of 5 m bgl differ: sandy at Pirganj and Shambaganj but dominated respectively by silt and clay at Jajira and Paikgachha (Fig. S1). Only at Paikgachha (8 m) is there a lower aquitard of sufficient thickness (>0.5 m) that might divide the upper 30 m of sediment into separate aquifers.

All sites are subject to a tropical monsoonal climate. Mean annual rainfall is typically around 2000 mm, and mean annual temperature is about 25 °C with monthly means ranging from 18 to 30 °C (Brammer, 2012). There is significant pumping from shallow tubewells (tens of metres deep) for irrigation in the vicinity of the Shambaganj and Pirganj sites and may cause temporary changes in flow direction between February and April each year. No irrigation wells were identified that could affect either the Jajira or Paikgachha sites.

2.2. Monitoring networks and sampling and analytical protocols

New pit latrines were manually excavated to a depth of 2.5 m and completed with 1.5 m diameter concrete rings as per local practice. At each site, between 25 and 38 PVC piezometers (with 38 mm diameter, 3 m long screens) were installed using the local manual-percussive technique (e.g. Ali, 2003) in a cruciform pattern in four layers at depths of 4–30 m and radial distances of 2–10 m. The first monitoring horizon was selected to be as close as practical to the water table to intercept any shallow pathway, should it exist, and was completed in a presumed low permeability (silt or sandy silt) layer since none of the latrines discharged directly into an aquifer. The second monitoring horizon, typically 5–10 m bgl, was selected to represent water quality at top of the first aquifer. The

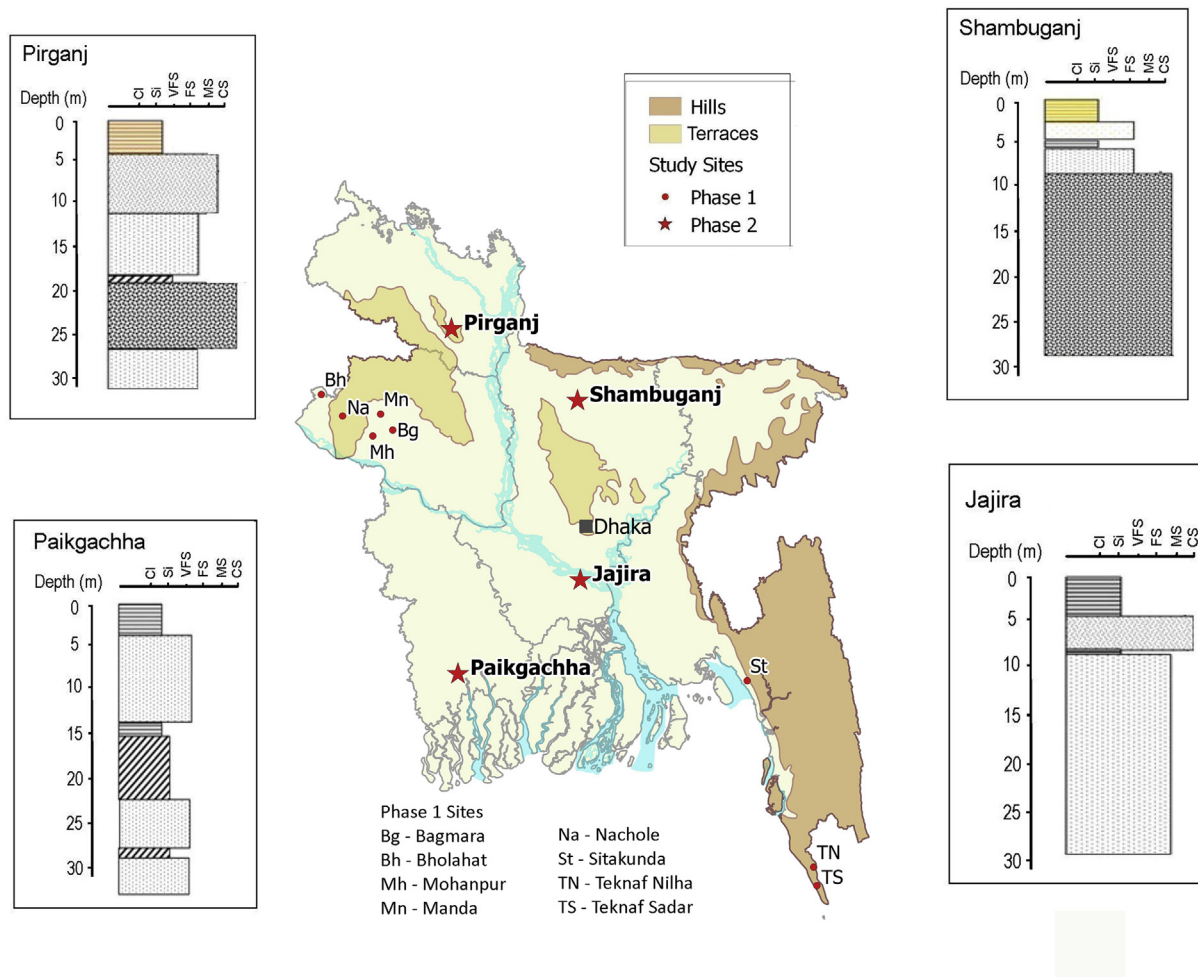


Fig. 1. Location of study sites. The Phase 1 (Pilot Study) sites are located either on or around the Pleistocene Barind. Tract on the Chittagong Coastal Plain; the Phase 2 sites are located on the floodplains of the main Ganges – Brahmaputra – Meghna river system. The annotations show the lithological profiles at one of the deepest piezometers at each site where lithological codes are Cl – clay, FS – fine sand, MS – medium sand, CS – coarse sand.

third horizon was selected to identify any significant vertical migration of bacteria into the aquifer. The deepest monitoring horizon, typically 25–30 m bgl, was selected to represent the deepest credible depth for bacterial migration, or to represent water quality in a second aquifer if encountered. Immediately after completion, the borehole annulus was sealed from the surface with a sand-cement slurry, and in any lower aquitard by carefully dropping hand-rolled balls of bentonite clay, to prevent percolation of surface water or inter-aquifer leakage. The layout of piezometers was based on a conceptual site model developed from a test borehole, water features survey, estimation of groundwater flow direction(s), examination of land use and discussions with residents. Concerns over possible cross-contamination in the Pilot Study prompted a change in the sampling procedure from a conventional hand-pump to a peristaltic pump. Between and/or immediately before sampling each piezometer, the hoses were flushed with water was hot enough to sterilise the tube and the outside of the pipe was also properly cleaned.

Water samples were collected in 500 ml autoclavable Nalgene plastic bottles which were autoclaved each time before sampling, and after sampling kept in insulated boxes with ice packs to maintain the temperature between 4 and 8 °C and transported to the Environmental Microbiology Laboratory of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) at

Dhaka. The collected water samples were analysed within 24 h of collection following procedures described earlier (Islam et al., 2001, 2016). Analyses enumerated faecal coliforms (FC), *E. coli* and faecal streptococci; however, because the patterns of these parameters are essentially the same (Islam et al., 2017a) we restrict our presentation to faecal coliforms. Water samples were also tested in the icddr,b laboratory in Dhaka for pH and electrical conductivity monthly, and chloride and nitrate quarterly (Islam et al., 2017b). Sampling was conducted in 24, approximately monthly, sampling events. This interval is three times less than the putative maximum survival period of FC's outside the gut (e.g. Lewis et al., 1982). During each sampling event, which required at least two days on site, the depth to groundwater was measured in every piezometer using a standard electrical dip meter. In categorising faecal risk, we follow the WHO (2011) classification where very low, low, medium, high and very high risk correspond to 0, 1–10, 11–100, 101–1000 and > 1000 cfu/100 ml.

2.3. Limitations

This paper does not describe the latrine-derived chemical contamination by nitrate and chloride or other FIB, which are discussed by Islam et al. (2017a, b) who also present a detailed analysis of the environmental factors that explain intra- and inter-site

variations and hence siting criteria. The study did not consider the subsurface transport of protozoa, viruses or pathogens in general, the influence of pit emptying, or household water treatment.

3. Results

Lateral and vertical head differences in monthly measurements of groundwater elevation within sites were generally negligible, rarely exceeding a few centimetres and usually within the measurement error. However, differences between sites are significant (Fig. 2). At Paikgachha, the most down-basin site where the grain size is finest, the piezometric surface at all depths was always above the base of the latrine. At Jajira, the water table rises above the base of the latrine for about half the year, whereas at the northern sites with sandy soil and subsoil, Shambuganj and Pirganj, the water table intersects the base of the latrine for a short period in the monsoon, if at all.

Analyses of faecal coliforms for each sampling event at each piezometer at each site are presented in the Supporting Information (SI S3) and summarised in Table 1 and Fig. 3. The spatial and temporal distributions of FC are illustrated in Figs. 4 and 5 and the Supporting Information (SI S4). From north to south, there are progressive increases in the overall detection rate (3.3% at Pirganj and 23.3% at Paikgachha) and the proportions of samples with medium, high and very high levels of contamination. Everywhere the magnitude and frequency of FC detections decline rapidly with distance from the latrines. In the shallowest piezometers, at a radial distance of 2 m, FC concentrations typically ranged between 10^2 and 10^4 cfu/100 ml and almost always detected, but beyond a straight line distance of about 5 m (including vertical and horizontal dimensions) FC are undetectable more than 90% of the time and rarely exceed 10 cfu/100 ml. Beyond about 5 m, such detections as occur are not continuous in space or time (SI S4). Moreover there are no consistent concentration gradients or dominant directions of contaminant transport.

Fig. 5 illustrates the discontinuous, multi-dimensional spatial distribution of faecal bacteria around latrines, and in particular the very low levels of contamination beyond the inner ring of piezometers, even at the most contaminated sites and in the most contaminated events. At Paikgachha, the most contaminated site, FC were detected at least once at every piezometer but, beyond the shallowest layer the frequencies and maximum levels of

contamination are very low, and in most of the deeper (≥ 10 m) piezometers FC never exceed 10 cfu/100 ml. At Paikgachha, the sampling event with the most detections occurred in the middle of the dry season (December 2014; Fig. 5a). Here, the shallowest piezometers at 2 m distance are severely contaminated, but the FC concentration was only 15 cfu/100 ml at a 4 m radial distance. At a depth of 10 m, the maximum FC concentration was only 7 cfu/100 ml, and at eight out of thirteen piezometers FC were not detected. Evidently, the distribution of FC detections in the far-field does not follow an obviously rational pattern. For example, more detections were found in the 20 and 27 m deep piezometers although the maximum concentration was just 6 cfu/100 ml. By contrast with Paikgachha, the most-contaminated sampling event at Shambuganj occurred in the late monsoon (September 2015; Fig. 5b). In the shallowest layer, FC concentrations were mostly a few hundred cfu/100 ml at a 2 m radial distance and in one piezometer FC's were not detected. At depths of 8 and 18 m, FC's were detected in only 4 and 3 out of the 13 piezometers in each horizon respectively; and at 25 m there were no detections. In the best instrumented horizons (10 m at Paikgachha; 8 and 18 m at Shambuganj) there is no indication of either lateral concentration gradients or continuity of contamination, and on some limbs contamination was detected only in the most distant piezometer. The far-field monitoring results at Paikgachha, Shambuganj and the other sites indicate the distribution of faecal bacteria do not resemble the classical plume that is assumed in solute transport theory, whether in terms of direction, continuity or concentration gradient.

4. Discussion

Monthly monitoring at four experimental latrines over two years revealed a consistent pattern of bacterial migration, characterised by what we term near-field and far-field contamination (Table 2). The near-field extends for only a few metres from the latrine and contains high concentrations of FC that are spatially and temporally continuous. In the far-field, by contrast, FC detections are rare, with concentrations mostly ≤ 10 cfu/100 ml and where bacterial contamination is spatially and temporally discontinuous, displaying neither concentration gradients nor relation to either inferred flow directions or seasonal fluctuations. Transport in the far-field may involve periodic detachment of sediment-bound bacteria and/or the operation of 'two populations' as suggested by the column-modelling studies of Feighery et al. (2013) who inferred the presence of a minor second population which could be transported up to 10 m. This is similar to the pioneering observations of Caldwell and Parr (1937), Dyer (1941) and Dyer et al. (1945) who found that the migration of faecal bacteria is quickly limited to a few metres, equivalent to our near-field. Longer-range migrations, much cited in literature reviews, may be equivalent to our far-field detections but capture neither the nature of risk nor adequately describe the nature of advective transport of bacteria through aquifers.

The differences in faecal contamination between sites are open to various environmental explanations that are discussed in detail by Islam et al. (2017b). We consider that differential faecal loading is an unlikely explanatory factor because each latrine received the product of a single family. Near-field contamination is greatest where the unsaturated zone below the latrine is thin, transient or absent, suggesting that bacterial survival or transport is facilitated in the saturated zone. However, any causal interpretation is subject to confounding by inter-related factors including higher clay and organic matter content of sediments and, at Paikgachha, by saline pore water which favours preservation of bacteria (e.g. Miller et al., 1984). Conversely, the sites with deeper water tables are also

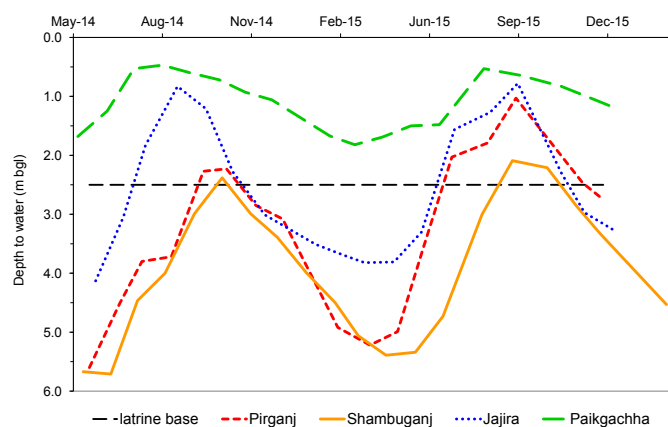


Fig. 2. Water table hydrographs at the four experimental sites. Each hydrograph represents monthly measurements of the depth to water in a 10 m deep piezometer at a radial distance of 2 m from the latrine. Except in a few very shallow piezometers which briefly run dry in the dry season, the choice of piezometer makes a difference of no more than a few centimetres and usually less. The depth of the latrine base is approximately the same at all sites.

Table 1
Faecal Coliform detections at four sites. All measurements are units of colony forming units per 100 ml of water (cfu/100 ml) analysed at the icddr, Environmental Microbiology laboratory in Dhaka. The number of samples excludes baseline and duplicate samples.

Site	GPS Coordinates	Elevation (m asl)	Nr of Piezometers	Nr of Samples	Faecal Coliforms (cfu/100 ml)		
					Median	>0	>10
Pirganj, Rangpur	E: 89.20513 N: 25.29444	26.4	30	626	0	3.3%	0.8%
Shambuganj, Mymensingh	E: 90.27130 N: 24.455778	13.5	34	842	0	10.5%	2.5%
Jajira, Shariatpur	E: 90.14381 N: 23.24308	7.2	32	737	0	13.7%	6.7%
Paikgachha, Khulna	E: 89.18509 N: 22.36135	1.4	27	575	0	23.3%	19.0%

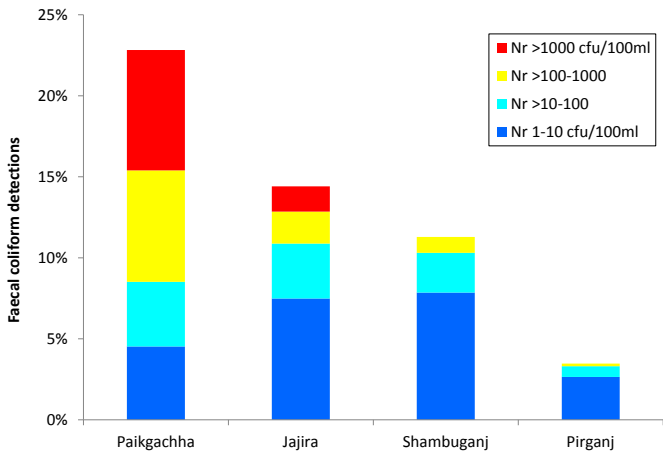


Fig. 3. Histogram of faecal coliform detections at piezometers at four sites. The four concentration classes correspond to the WHO classification of low, medium, high and very high microbial risk.

underlain by sandier sediments (Fig. 1) and less organic matter.

Much was learned about latrines and groundwater pollution 70–80 years ago through the detailed field investigations of Elfreda Caldwell and colleagues in the southern USA and Brian Dyer and colleagues in the Indian Subcontinent. Notwithstanding mention in some reviews (e.g. Lewis et al., 1982), this knowledge, which remains valid, has unfortunately been ‘lost’ to recent researchers. Our findings replicate and revalidate the findings of both groups regarding the very limited transport of bacteria in the subsurface. Caldwell and Parr (1937) and Caldwell (1938a, b) tested about 20,000 water samples from around five experimental bored latrines on the coastal plain of Alabama. Initially, *E. coli* reached radial distances of 3 and 5 m in a few days. After two months, *E. coli* were detected in 90% of 5 m wells; 40% of 8 m wells for one month; and occasionally at 11 m for 10 days. After five months, *E. coli* was not detected at 3 m, and after 7 months was not detected 1.5 m from the latrine, excepting temporary resurgences following rises in the water table. The authors hypothesised an attenuation mechanism equivalent to a schmutzdecke in parallel with the changes inside the latrine. Their work included an early discovery of hand-pumps

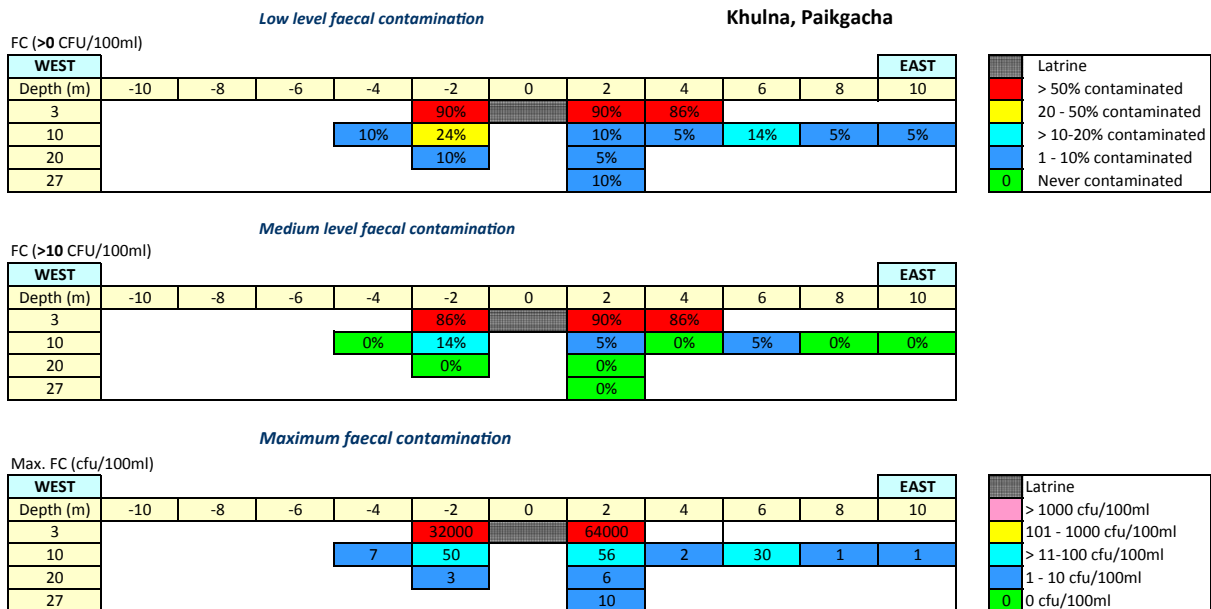


Fig. 4. Pseudo-sections illustrating faecal contamination along one axis of piezometers at Paikgachha, the most contaminated site. The top row of numbers shows the distance in metres from the latrine and corresponds to the fixed lateral spacing of piezometers. The rows correspond to the pre-defined depths at which piezometer screens are installed. Blank cells on the figure are where no piezometer is installed. Contamination is represented in three alternative ways: (a) the percentage of monthly samples exceeding 0 cfu/100 ml; (b) the percentage of monthly samples exceeding 10 cfu/100 ml; and (c) the maximum value of FC determined at each piezometer on any occasion.

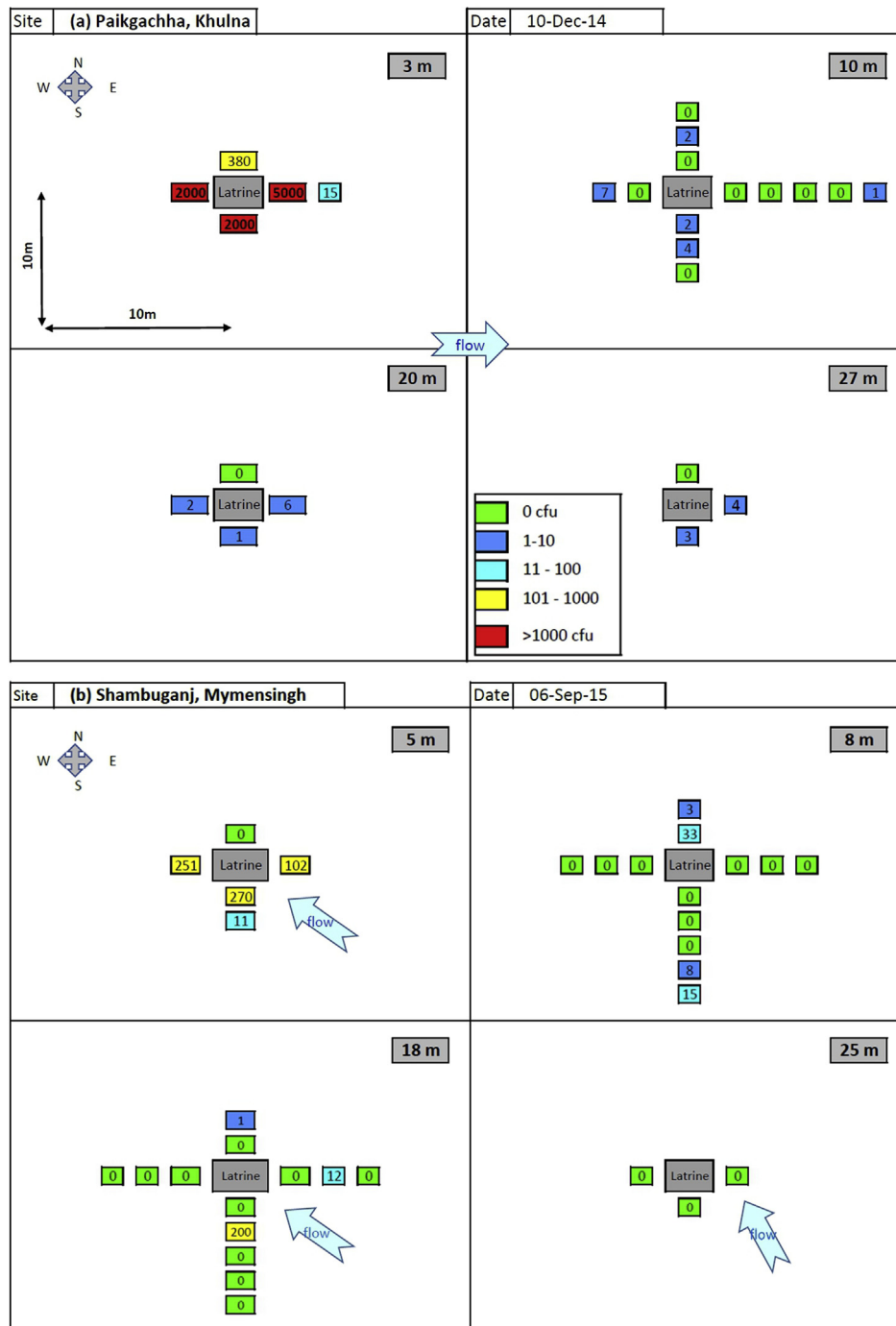


Fig. 5. Distribution of faecal coliforms (in cfu/100 ml) in the most contaminated sampling events at (a) Paikgachha in December 2014 and (b) Shambaganj in September 2015. The four quadrants show the four depth horizons at which piezometers are installed. The geometric alignment in the figure follows the nominal (north, south, east west) arrangement of the axes of the piezometer nests.

acting as bacterial reservoirs (Parr and Caldwell, 1933). At experimental bored latrines on alkaline-alluvium at Lahore (Pakistan), Dyer (1941) monitored hand-pumped wells at radial distances of 1.5–10 m, concluding that faecal bacteria travelled > 1.5 m but < 3 m in the direction of flow. Extending these investigations to an alluvial site near Kolkata (India), Dyer and Bhaskaran (1943, 1945) and Dyer et al. (1945) found that *E. coli* were detected at radial distances of 3 m for 2 months and abundant at 1.5 m, but virtually absent by the end of a year.

Recent studies in Bangladesh (Table 3) enhance the significance

of our findings. Irrespective of whether pump spouts are disinfected, 20–50% of tubewell water samples are contaminated by low levels of FC (and other FIB) at the point of collection, and significantly more at the point of use. Moreover, a high proportion of these wells were a few tens to a few hundreds of metres deep; depths to which faecal bacteria could not survive if transported by normal groundwater flow. Correlations of tubewell contamination with sanitary inspections scores have low explanatory power. Some correlations with distance to latrines (e.g. Escamilla et al., 2013) suggest associations over tens of metres, although our data do not

Table 2
Summary of Near- and Far-Field contamination characteristics at four sites. All detections refer to faecal coliforms in cfu/100 ml. Note, in the far-field, all median FC concentrations are zero. At Pirganj and Shambaganj the true near-field does not reach the 2 m (radially) distant piezometers; there is a zone with slightly elevated frequencies of detections ($\leq 20\%$) extending about 4 and 8 m respectively. Also, breakthrough time at these sites were probably affected by the fall in the water table in the dry season below the shallowest piezometers causing downward migration of pollution that did not reach the second monitored horizon.

Site	Near-field			Far-field					
	Typical high and median FC conc'n	Typical frequency		Lateral & vertical extent (m)	max. FC (cfu/100 ml)	FC frequency		Migration distance, m	
		FC > 0	FC > 10			FC > 0	FC > 10	FC > 0	FC > 10
Pirganj, Rangpur	100; median 0	16%	8.0%	<1.7 m	20	2.4%	0.2%	11	4.4
Shambaganj, Mymensingh	200–600; median 0	17.1%	9.4%	L < 2.2 m; (7 m)	500	9.4%	1.3%	28	6.5
Jajira, Shariatpur	4000–20,000; median 0	69%	59%	L < 2 m; (8 m)	92	9.6%	2.3%	23	11
Paikgachha, Khulna	1500 - >80,000; median 560	93%	92%	c. 5 m	56	7.0%	1.6%	28	8.7

support such inferences. Greater faecal contamination in the wet season appears to be near universal (Kostyla et al., 2015) and a few studies infer that heavy antecedent rainfall promotes contamination events (e.g. Wu et al., 2016). Although there is some indication of greater near-field contamination in the monsoon, we find no correlation between contamination events and 3-day or 7-day antecedent rainfall (SI-S4).

Parr and Caldwell (1933), Hoque (1998), Knappett et al. (2012a) and Ferguson et al. (2011) draw attention to the role of the borehole - well - pump system in contaminating drinking water (Table 3) including: (i) dirty priming water; (ii) leaking casing joints; (iii) cement grouting of the borehole annulus reducing contamination of shallow wells; and (iv) elastomeric components of hand-pumps acting as bacterial reservoirs. The high frequencies of faecal contamination in the pilot study tubewells (Islam et al., 2016; SI-1) and piezometers compared to piezometers in this study (sampled with a peristaltic pump) support the conclusion that the in-situ microbiological quality of groundwater is much superior to that

of well water. The impersistent effect of spot chlorination (e.g. Luby et al., 2006) may thus be explained by factors other than pervasive contamination of the aquifers.

Combining our study data for near- and far-fields with published data supporting pervasive recontamination, we propose (Fig. 6) an extended conceptual model of faecal contamination of drinking water that distinguishes four sub-pathways: (P1) leakage and biofiltration of faecal waste at the latrine-aquifer interface and near-field; (P2) leachate migration through the far-field to the borehole; (P3) from the face of the borehole to the pump spout; and (P4) from point of collection to point-of-use. The overall source-pathway-receptor route is characterised by a large and rapid decline of faecal bacteria in the near-field followed by slow-attenuation of low-level contamination in the far-field and then progressive increases in faecal bacteria between the point of entry to the well and the point of consumption due to recontamination from non-latrines sources. Sub-pathways P2 and P4 have long been recognised and have given attention through siting criteria and

Table 3
Key Findings from recent studies of water well pollution and latrines in Bangladesh and West Bengal (India).

Subject	Findings/Observations	Reference(s)
FIB in water wells	With or without sterilisation of the pump spout: <ul style="list-style-type: none"> • 30–50% of shallow tubewells are contaminated at point of collection; • 27–50% of deep tubewells (>150 m) are contaminated at point of collection (authors suggest dirty pump priming water as a factor); • 76–94% of ring wells contain FIB are contaminated by FIB at point of collection, and much more frequently in higher risk classes than in tubewells. 	Ahmed et al. (2005); Ercumen et al. (2017); Hoque (1998); Leber et al. (2011); Ravenscroft et al. (2014); BBS/UNICEF (2015)
Platforms and annular space sealing	The presence of a platform made no difference to <i>E. coli</i> detection, but cement sealing of the annulus significantly reduced the frequency and magnitude of contamination.	Knappett et al. (2012a)
Handpumps as bacterial reservoirs	Hand-pumps removed from the field and a new handpump spiked with <i>E. coli</i> were flushed with sterile water; the field pumps produced FIB for at least 29 days, and the spiked pump for 125 days.	Ferguson et al. (2011)
Well disinfection	Disinfection by shock chlorination does not prevent the recontamination/regrowth of FIB.	Luby et al. (2006); Ferguson et al. (2011)
Latrine and site drainage.	Associations regarding distance to latrine or pollution source are weak, absent or have improbably large correlation distances. Correlations with Sanitary inspection scores generally absent. However, at 12 sites in West Bengal, FC migrations of 7 m laterally and 6.5 m vertically were inferred.	Banerjee (2011); Hoque (1998); Ercumen et al. (2017); Escamilla et al. (2013); Wu et al. (2016).
Impact of contaminated ponds on groundwater.	Low-permeability, biologically active 'skins' greatly restrict leakage to groundwater except following heavy rain and in the early monsoon.	Knappett et al. (2012b)
Rainfall and seasonality	Contamination of tubewells is greatest during the monsoon. One study reported a small but significant relationship with heavy antecedent rainfall.	Lawrence et al. (2001); Ahmed et al. (2005); Luby et al. (2008); Leber et al. (2011); Wu et al. (2016)
Transport of FIB through soil columns	An average decay rate of 0.03 log ₁₀ /day was measured in Column experiments and modelling required a two-population model where most bacteria are removed in the first metre but the second population could migrate up to 10 m.	Feighery et al. (2013)
Sediment binding of FIB	In a peri-urban area, sediments adsorbed 10 ³ –10 ⁴ cfu/g of FIB to a depth of at least 10 m.	Lawrence et al. (2001)

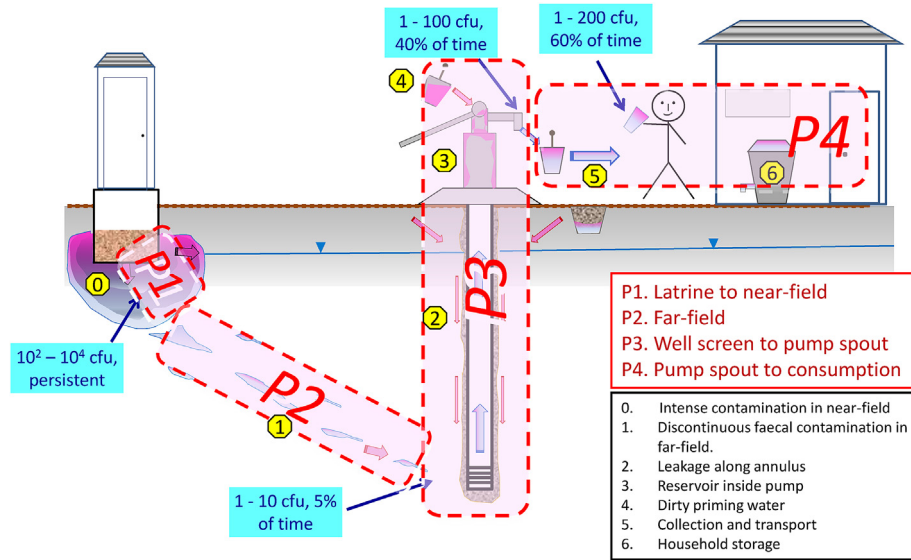


Fig. 6. Cartoon depicting an extended conceptual model of faecal contamination of groundwater-derived drinking water. See text for explanation.

hygiene education respectively. On the other hand, sub-pathway P3 has been largely neglected and comprises at least four sources of contamination: (a) dirt or drilling fluid additives such as cow dung introduced during construction; (b) faecal contamination infiltrating beneath platforms and along the borehole annulus to enter through the screen or leaking joints; (c) dirty priming water; and (d) biofilms on elastomeric components of hand-pumps that can harbour faecal bacteria for more than a hundred days.

4.1. Estimation of potential disease burden

Extreme contamination in the near-field is well-known although the rate of attenuation is less well appreciated. The public health significance of the subsequent sub-pathways is indicated by the increasing frequency and magnitude of contamination from far-field groundwater to the point-of-use. Actual morbidity risks will depend on the particular pathogens present, the frequency of occurrence, concentration, and their dose-response functions. For illustrative purposes, we use the degree of faecal contamination on each sub-pathway to estimate the corresponding relative disease burden by comparing the behaviour of real pathogens to that of the hypothetical enteric bacterium, *Bacterium experimentus* of Briscoe (1984) which follows a perfect log-linear dose-response function with a probability of infection ($P_{inf,i}$) defined as

$$P_{inf,i} = 0.5 * \log_{10}(\text{dose})$$

where the dose is the concentration of the pathogen and $P_{inf,i}$ is limited to a maximum value of 1.0. We assume that the dose of *B. experimentus* in a given time unit (e.g. a day) is directly proportional to the median concentration of FC in each sub-pathway, where we estimate a hypothetical ‘dose’ by summing the products of the proportions of samples contaminated and the median FC concentration in each WHO risk class. The input data on the proportions and concentrations in each risk class and for each sub-pathway are taken either from this study or the quality control data set (n = 109) of the 2013 Multiple Indicator Cluster Survey (BBS/UNICEF, 2015, Table 4).

For each sub-pathway, we sum the weighted probabilities to calculate an attributable risk (AR) for the sub-pathway of interest (P_i) as:

Table 4

Input data for the base case for pathogenic disease burden estimation. Data for the far-field are taken from this study; and other data are taken from quality control data set from BBS/UNICEF (2015). All samples were collected by ICDDR,B staff and analysed in the ICDDR,B Environmental Microbiology Laboratory in Dhaka.

WHO Risk Class	Far-field Groundwater		Point of Collection		Point of Use	
	Median FC	Prop'n	Median FC	Prop'n	Median FC	Prop'n
Very low	0	93%	0	77%	0	30%
Low	2	5.7%	3	10%	4	28%
Medium	20	1.3%	18	8%	60	26%
High	350	0.1%	300	3%	294	13%
Very High	0	0%	2300	2%	1800	3%

$$AR_i = pn_i * P_{(inf,i)}$$

where pn_i is the proportion of samples in that risk class. Thus AR_{i-1} , the attributable risk from the preceding sub-pathway, is subtracted from AR_i to calculate the incremental attributable risk (IAR) arising from sub-pathway P_i alone. Dividing the increment of attributable risk by the total attributable risk at the point of consumption provides an estimate of the proportion of diarrhoeal disease attributable to bacteria originating in each of sub-pathways P2 to P4.

The calculated proportions of attributable disease for the base case are 1.3%, 28.8% and 69.9% (Table 5). A sensitivity analysis was conducted, initially applying 50% increases and reductions to the proportions of samples contaminated, median concentrations, and $\pm 200\%$ variations in P_{inf} . Subsequent checks considered reducing the dose by a factor of ten, and adjusting the infectivity of Briscoe’s putative pathogen ($P_{inf}, 0.2 * \log_{10}(\text{dose})$) to result in a 100% probability of infection when consuming 2 L of water containing 5000 cfu/100 ml of FC. The resulting ranges of disease risk attributable to each sub-pathway were: 0.3–4.1% in the far-field, 27.4–32.6% at point of collection and 66.0–71.0% at point of use.

The use of Briscoe’s hypothetical pathogen in our calculations does not contradict our questioning of his argument about the interaction of different transmission routes (Cairncross, 1987). Our calculations make the conservative assumption of no bacterial die-off on subsequent sub-pathways which, if included, would reduce the risks originating from the upstream sub-pathways even further. Uncertainty arises because we combine our site data with national

Table 5
Relative risks for *Bacterium experimentus* in the aquifer – well – household system.

Sub-pathway	Attributable Risk	Incremental Attributable Risk	Attributable Risk at PoU
Latrine to Well entry	0.017	0.017	1.3%
Well entry to PoC	0.385	0.368	28.8%
PoC to PoU	1.276	0.892	69.9%

data; nevertheless, we consider the sensitivity analysis renders the qualitative conclusions robust and serves the purpose of driving improvements in sanitary practice.

4.2. Policy implications

Recent reviews and studies (e.g. Graham and Polizzotto, 2013; Daniels et al., 2016; Odagiri et al., 2016; Sorensen et al., 2016) have re-ignited an old debate as to whether increasing latrine coverage increases diarrhoeal disease due to increased groundwater contamination. Our results suggest that such warnings conflate the low-risk from groundwater contamination in the far-field with much higher risks associated with contaminated well water, and therefore fail to target the principal sources of microbial risk, which arise close to the well and post-collection. Thus, while recent initiatives to improve post-collection water handling (e.g. UNICEF, 2012) are fully justified, the attention given to well-latrine spacing is not proportionate to the associated risks. Further, increased groundwater contamination from expanding latrine coverage poses only a modest threat to drinking water quality compared to the risks associated with sub-pathways P3 and P4. To translate the benefits of improved post-collection hygiene practices into reduced mortality, more attention must also be given to reducing contamination at the well (cf. VanDerSlie and Briscoe, 1995). Moreover, the rare and low level of contamination found at depths of 10 m or more confirms that the traditional attention given to horizontal spacing is not only unwarranted but fails to understand the pathways of the widespread contamination of tubewells tens to a few hundreds of metres deep. Hence, provided modest vertical and horizontal spacing criteria are adhered to, measures to increase natural attenuation during groundwater flow from latrines will have little impact on the prevalence of diarrhoeal disease. Only measures that reduce contamination along the P3 and P4 sub-pathways will have a major impact on disease burden.

Possible low-cost interventions to reduce faecal pollution along the P3 sub-pathway include: (i) replacing cow-dung with bentonite clay as a drilling additive; (ii) cement grouting of the borehole annulus after sand packing; (iii) overnight shock chlorination on the day of well completion; (iv) maintaining a container of chlorinated water for priming purposes; (v) regular removal and cleaning of the pump head with disinfectant and a brush; (vi) more microbiological testing; and last but not least (viii) incorporation of the above into awareness raising in caretaker training and water safety plans.

5. Conclusions

Severe and spatially or temporally continuous faecal pollution of groundwater from pit latrines is largely restricted to a near-field that extends for a very few metres and passes rapidly into a far-field (FC typically <10 cfu/100 ml and <10% of the time) where faecal pollution is spatially and temporally discontinuous, lacks concentration gradients and is poorly correlated with groundwater flow. Contamination of far-field groundwater is less frequent and less severe than water collected from hand-pumped tubewells. Faecal contamination along a sequence of four-sub-pathways

comprising the latrine-groundwater interface or near-field (P1); flow through the far-field (P2); well screen to pump outlet (P3); and collection-to-consumption (P4) drops to a minimum in the far-field and then is progressively re-contaminated up to the point of use. Invoking the hypothetical pathogen *Bacterium experimentus*, we estimate that sub-pathways P2, P3 and P4 respectively contribute of the order of 1–2%, 29% and 70% of faecal risk at the point of consumption.

Tactically, the goal of safe drinking water (SDG 6.1) will only be realised if parallel action is taken to control risks in the much-neglected well-pump sub-system as well as reducing post-collection contamination. Strategically, our findings suggest that recent warnings of the reality and public health significance of increased groundwater pollution resulting from expanding latrine coverage appear exaggerated. Our findings are expected to apply to all alluvial-deltaic terrains, but may not apply in areas with fractured or fissured aquifers and thin soil cover.

Acknowledgements

The study was funded by the UK Department for International Development (DFID), through the Sanitation and Hygiene Applied Research for Equity (SHARE) Consortium led by the London School of Hygiene and Tropical Medicine (LSHTM) and WaterAid Bangladesh. icddr,b acknowledges with gratitude the commitment of these agencies to the research. icddr,b is grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing unrestricted support. We thank Professor Richard Carter for helpful comments on an early version of the script. We also thank two anonymous reviewers for their valuable and constructive suggestions. The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2017.07.049>.

References

- Ahmed, M., Shamsuddin, S., Mahmud, S., Rashid, H., Deere, D., Howard, G., 2005. Risk Assessment of Arsenic Mitigation Options (RAAMO). Arsenic Policy Support Unit, Local Government Division, Ministry of Local Government, Rural Development and Cooperatives, Bangladesh.
- Ali, M., 2003. Review of drilling and tubewell technology for groundwater irrigation. In: Rahman, A.A., Ravenscroft, P. (Eds.), *Groundwater Resources and Development in Bangladesh – Background to the Arsenic Crisis, Agricultural Potential and the Environment*. The University Press Ltd, Dhaka.
- Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T., Bartram, J., 2014. Faecal contamination of drinking-water in low-and middle-income countries: a systematic review and meta-analysis. *PLoS Med.* 11 (5), e1001644.
- Banerjee, G., 2011. Underground pollution travel from leach pits of on-site sanitation facilities: a case study. *Clean Technol. Environ. Policy* 13 (3), 489–497.
- BBS/UNICEF, 2015. Bangladesh Multiple Indicator Cluster Survey 2012–2013. Final Report, Bangladesh Bureau of Statistics and UNICEF Bangladesh, Progotir Pathy.
- Brammer, H., 2012. *Physical Geography of Bangladesh*. The University Press Ltd, Dhaka.
- Briscoe, J., 1984. Intervention studies and the definition of dominant transmission routes. *Am. J. Epidemiol.* 120 (3), 449–456.
- Cairncross, S., 1987. Ingested dose and diarrhea transmission routes. *Am. J. Epidemiol.* 125 (5), 921–922.
- Caldwell, E.L., 1938a. Pollution flow from a pit latrine when permeable soils of

- considerable depth exist below the pit. *J. Infect. Dis.* 62 (3), 225–258.
- Caldwell, E.L., 1938b. Studies of subsoil pollution in relation to possible contamination of the ground water from human excreta deposited in experimental latrines. *J. Infect. Dis.* 62 (3), 272–292.
- Caldwell, E.L., Parr, L.W., 1937. Ground water pollution and the bored hole latrine. *J. Infect. Dis.* 61, 148–183.
- Cave, B., Kolsky, P., 1999. Groundwater, Latrines and Health. WELL Study (163). London School of Hygiene and Tropical Medicine and WEDC Loughborough University, UK.
- Daniels, M.E., Smith, W.A., Schmidt, W.-P., Clasen, T., Jenkins, M.W., 2016. Modeling Cryptosporidium and Giardia in ground and surface water sources in rural India: associations with latrines, livestock, damaged wells, and rainfall patterns. *Environ. Sci. Technol.* 50 (14), 7498–7507.
- Dyer, B.R., 1941. Studies of ground water pollution in an alkaline alluvium soil. *Indian J. Med. Res.* 29 (4), 867–889.
- Dyer, B.R., Bhaskaran, T., 1943. Investigations of ground-water pollution. Part I. Determination of the direction and the velocity of flow of ground water. *Indian J. Med. Res.* 31 (2), 231–243.
- Dyer, B.R., Bhaskaran, T., 1945. Investigations of ground-water pollution. Part II. Soil characteristics in West Bengal, India, at the site of ground-water pollution investigations. *Indian J. Med. Res.* 33 (1), 17–22.
- Dyer, B.R., Bhaskaran, T., Sekar, C.C., 1945. Investigations of ground-water pollution. Part III. Ground-water pollution in West Bengal, India. *Indian J. Med. Res.* 33 (1), 23–62.
- Ercumen, A., Naser, A.M., Arnold, B.F., Unicomb, L., Colford Jr., J.M., Luby, S.P., 2017. Can sanitary inspection surveys predict risk of microbiological contamination of groundwater Sources? Evidence from shallow tubewells in rural Bangladesh. *Am. J. Trop. Med. Hyg.* 96 (3), 561–568.
- Escamilla, V., Knappett, P.S., Yunus, M., Streatfield, P., Emch, M., 2013. Influence of latrine proximity and type on tubewell water quality and diarrheal disease in Bangladesh. *Ann. Assoc. Am. Geogr.* 103 (2), 299–308.
- Feighery, J., Mailloux, B.J., Ferguson, A., Ahmed, K.M., Geen, A., Culligan, P.J., 2013. Transport of *E. coli* in aquifer sediments of Bangladesh: implications for widespread microbial contamination of groundwater. *Water Resour. Res.* 49 (7), 3897–3911.
- Ferguson, A.S., Mailloux, B.J., Ahmed, K.M., van Geen, A., McKay, L.D., Culligan, P.J., 2011. Hand-pumps as reservoirs for microbial contamination of well water. *J. Water Health* 9 (4), 708–717.
- Foppen, J., Schijven, J., 2006. Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. *Water Res.* 40 (3), 401–426.
- Graham, J.P., Polizzotto, M.L., 2013. Pit latrines and their impacts on groundwater quality: a systematic review. *Environ. Health Perspect.* 121 (5), 521–530.
- Hoque, B.A., 1998. Biological Contamination of Tubewell Water. Environmental Health Programme: International Centre for Diarrhoeal Disease Research Bangladesh, Dhaka.
- Islam, M.S., Siddika, A., Khan, M., Goldar, M., Sadique, M.A., Kabir, A., Huq, A., Colwell, R.R., 2001. Microbiological analysis of tube-well water in a rural area of Bangladesh. *Appl. Environ. Microbiol.* 67 (7), 3328–3330.
- Islam, M.S., Mahmud, Z.H., Islam, M.S., Saha, G.C., Zahid, A., Ali, A.Z., Hassan, M.Q., Islam, K., Jahan, H., Hossain, Y., 2016. Safe distances between groundwater-based water wells and pit latrines at different hydrogeological conditions in the Ganges Atrai floodplains of Bangladesh. *J. Health Popul. Nutr.* 35 (1), 26.
- Islam, M.S., Mahmud, Z.H., Islam, M.S., Zakir Hossain, A.K.M., Zahid, A., Ravenscroft, P., Saha, G.C., Zulfiquar Ali, A.H.M., Islam, K., Cairncross, S., Clemens, J.D., 2017a. Faecal Contamination of Monitoring Well Nests from Newly Established Pit Latrines in Bangladesh (in preparation).
- Islam, M.S., Mahmud, Z.H., Islam, M.S., Zakir Hossain, A.K.M., Zahid, A., Ravenscroft, P., Saha, G.C., Zulfiquar Ali, A.H.M., Islam, K., Cairncross, S., Clemens, J.D., 2017b. Relationship of Faecal Contamination of Monitoring Wells from Pit Latrine in Relation to Distance, Chloride and Nitrate Concentrations (in preparation).
- Knappett, P.S., McKay, L.D., Layton, A., Williams, D.E., Alam, M.J., Huq, M.R., Mey, J., Feighery, J.E., Culligan, P.J., Mailloux, B.J., 2012a. Implications of fecal bacteria input from latrine-polluted ponds for wells in sandy aquifers. *Environ. Sci. Technol.* 46 (3), 1361–1370.
- Knappett, P.S., McKay, L.D., Layton, A., Williams, D.E., Alam, M.J., Mailloux, B.J., Ferguson, A.S., Culligan, P.J., Serre, M.L., Emch, M., 2012b. Unsealed tubewells lead to increased fecal contamination of drinking water. *J. Water Health* 10 (4), 565–578.
- Kostyla, C., Bain, R., Cronk, R., Bartram, J., 2015. Seasonal variation of fecal contamination in drinking water sources in developing countries: a systematic review. *Sci. Total Environ.* 514, 333–343.
- Lawrence, A., MacDonald, D., Howard, A., Barrett, M., Pedley, S., Ahmed, K., Nalubega, M., 2001. Guidelines for Assessing the Risk to Groundwater from On-Site Sanitation. British Geological Survey. Commissioned Report CR/01/1422001.
- Leber, J., Rahman, M.M., Ahmed, K.M., Mailloux, B., van Geen, A., 2011. Contrasting influence of geology on *E. coli* and arsenic in aquifers of Bangladesh. *Ground Water* 49 (1), 111–123.
- Lewis, W.J., Foster, S.S., Drasar, B.S., 1982. The risk of groundwater pollution by on-site sanitation in developing countries. In: International Reference Centre for Wastes Disposal (IRCWD—now SANDEC) Report (01/82).
- Luby, S., Islam, M.S., Johnston, R., 2006. Chlorine spot treatment of flooded tube wells, an efficacy trial. *J. Appl. Microbiol.* 100 (5), 1154–1158.
- Luby, S., Gupta, S., Sheikh, M., Johnston, R., Ram, P., Islam, M.S., 2008. Tubewell water quality and predictors of contamination in three flood-prone areas in Bangladesh. *J. Appl. Microbiol.* 105 (4), 1002–1008.
- Miller, C.J., Drasar, B.S., Feachem, R.G., 1984. Response of toxigenic *Vibrio cholerae* O1 to physico-chemical stresses in aquatic environments. *J. Hyg.* 93 (3), 475.
- Misati, A.G., Ogendi, G., Peletz, R., Khush, R., Kumpel, E., 2017. Can sanitary surveys replace water quality Testing? Evidence from Kisumu, Kenya. *Int. J. Environ. Res. Public Health* 14 (2), 152.
- Murphy, H.M., Prioleau, M.D., Borchardt, M.A., Hynds, P.D., 2017. Review: epidemiological evidence of groundwater contribution to global enteric disease, 1948–2015. *Hydrogeol. J.* 25, 981–1001.
- Odagiri, M., Schriewer, A., Daniels, M.E., Wuertz, S., Smith, W.A., Clasen, T., Schmidt, W.-P., Jin, Y., Torondel, B., Misra, P.R., 2016. Human fecal and pathogen exposure pathways in rural Indian villages and the effect of increased latrine coverage. *Water Res.* 100, 232–244.
- Parr, L.W., Caldwell, E.L., 1933. Variation within the colon-aerogenes group as found in bacteriologic analysis of water from contaminated pumps. *J. Infect. Dis.* 53 (1), 24–28.
- Rahman, A.A., Ravenscroft, P., 2003. Groundwater Resources and Development in Bangladesh – Background to the Arsenic Crisis, Agricultural Potential and the Environment. Bangladesh Centre for Advanced Studies. University Press Ltd, Dhaka.
- Ravenscroft, P., Kabir, A., Hakim, S.A.I., Ibrahim, A., Ghosh, S.K., Rahman, M.S., Akhter, F., Sattar, M.A., 2014. Effectiveness of public rural waterpoints in Bangladesh with special reference to arsenic mitigation. *J. Wash Dev.* 4 (4), 545–562.
- Sorensen, J., Sadhu, A., Sampath, G., Sugden, S., Gupta, S.D., Lapworth, D., Marchant, B., Pedley, S., 2016. Are sanitation interventions a threat to drinking water supplies in rural India? An application of tryptophan-like fluorescence. *Water Res.* 88, 923–932.
- Trevett, A.F., Carter, R.C., Tyrrel, S.F., 2005. The importance of domestic water quality management in the context of faecal–oral disease transmission. *J. Water Health* 3 (3), 259–270.
- UNICEF, 2012. Water, Sanitation and Hygiene (WASH) in Schools. United Nations Children's Fund, New York. (Accessed 11 June 2016).
- VanDerslice, J., Briscoe, J., 1995. Environmental interventions in developing countries: interactions and their implications. *Am. J. Epidemiol.* 141 (2), 135–144.
- WHO, 2011. Guidelines for Drinking-water Quality, fourth ed. World Health Organization, Geneva.
- Wu, J., Yunus, M., Islam, M.S., Emch, M., 2016. Influence of climate extremes and land use on fecal contamination of shallow tubewells in Bangladesh. *Environ. Sci. Technol.* 50 (5), 2669–2676.