Risk of diarrhoea from shallow groundwater contaminated with enteropathogens in the Kathmandu Valley, Nepal

Sadhana Shrestha, Eiji Haramoto, Rabin Malla and Kei Nishida

ABSTRACT

Shallow groundwater is the main water source among many alternatives in the Kathmandu Valley, Nepal, which has a rapidly growing population and intermittent piped water supply. Although human pathogens are detected in groundwater, its health effects are unclear. We estimated risk of diarrhoea from shallow groundwater use using quantitative microbial risk assessment. *Escherichia coli*, *Giardia* cyst and *Cryptosporidium* oocyst levels were analysed in dug and tube wells samples. *E. coli* concentrations were converted to those of enteropathogenic *E. coli* (EPEC). Risks from EPEC in dug wells and from *Cryptosporidium* and *Giardia* in both dug and tube wells were higher than the acceptable limit ($<10^{-4}$ infections/person-year) for both drinking and bathing exposures. Risk from protozoan enteropathogens increased the total risk 10,000 times, indicating that ignoring protozoans could lead to serious risk underestimation. Bathing exposure considerably increased risk, indicating that it is an important pathway. Point-of-use (POU) water treatment decreased the risk six-fold and decreased risk overestimation. Because removal efficiency of POU water treatment has the largest impact on total risk, increasing the coverage and efficiency of POU water treatment could be a practical risk management strategy in the Kathmandu Valley and similar settings.

Key words | exposure pathways, health impact, Monte Carlo simulation, point-of-use water treatment, probabilistic analysis, sensitivity analysis

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INTRODUCTION

Diarrhoea is the second leading cause of healthy time lost due to illness (72.8 million disability-adjusted life years) worldwide (WHO 2008). In Southeast Asia, 8% of total deaths and 38% of deaths in the children under the age of 5 are caused by diarrhoea (WHO/UNICEF 2009). In Nepal, incidence of diarrhoea among the children increased from 378 per 1,000 in 2007 to 598 per 1,000 in 2009 (MoHP 2008/2009; MoHP 2009/2010). Among total cases recorded in hospitals of the Kathmandu Valley, 30% were for diarrhoea, and there is a possibility of diarrhoea outbreaks every year (MoHP 2009/2010). Diarrhoea occurs more commonly when there is a shortage of clean water for drinking, cooking, cleaning and basic hygiene.

The Kathmandu Valley has five municipalities and 114 village development committees (Pradhan *et al.* 2007). Like many urban areas in developing countries, municipalities of the Kathmandu Valley receive intermittent piped water supply (in most areas <4–7 h/week) (ADB 2010). Groundwater has been the major source for drinking and domestic activities mainly for the poorest urban households in many Asian countries including India and Bangladesh (IIED 2010). In the Kathmandu Valley, the percentage of households that use groundwater increases from 48% in the wet season to 55% in the dry season in the valley and the water is used for drinking or bathing or both (Yoden 2012). A significant percentage of groundwater is contaminated with faecal indicators (in the valley as well as in

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other Asian cities) (Prasai *et al.* 2007; Warner *et al.* 2008; Pant 2011; Van Geen *et al.* 2011; Pujari *et al.* 2012) and human pathogens (Haramoto *et al.* 2011).

Cases of diarrhoea are more likely to be caused by groundwater pollution in places with extensive utilisation of this water source, but studies reflecting such causal relationships are rare in developing countries in Asia. So to visualise the existing situation, risk of diarrhoea from exposure to current level of groundwater microbial pollution should be estimated. Predicting the risk is a part of managing the health-related microbiological quality of water (Haas *et al.* 1999). However, it is difficult to estimate the risk from the use of groundwater using epidemiological studies because it is hard to confirm whether the cases are from piped water or from groundwater use. Recently, in many countries, quantitative microbial risk assessment (QMRA) has become a standard for assessing the public health risk from microbial pathogens.

An essential parameter of risk estimation using the QMRA method is the pathogen removal efficiency of water treatment methods. Post-source contamination is very prevalent in developing countries, and point-of-use (POU) water treatment methods are widely practised at the household level to safeguard against this. In order to simulate household scenario, it is necessary to incorporate POU water treatment methods into the QMRA. Groundwater has been extensively used for bathing, and this is an important transmission pathway through accidental water ingestion (Pruss *et al.* 2002) because bathing water is usually untreated. Therefore, in addition to the risk from drinking, risk from the bathing pathway should also be estimated. Previous studies have reported less microbial contamination in tube wells than with dug wells (Maharjan 2005; Warner *et al.* 2008; RDI 2008; Uy *et al.* 2010; Barthiban *et al.* 2012), and it is useful to determine whether tube well water is safer. Therefore, we aimed to estimate the risk from both types of wells. Point estimates of risk cannot convey variability of estimated risk. Therefore, probabilistic analysis was carried out by coupling QMRA with Monte Carlo simulations (MCS), a technique that has recently been widely used for similar studies (Razzolini *et al.* 2011; Sato *et al.* 2013).

MATERIALS AND METHODS

Study area and sample collection

The study area was the Kathmandu Valley, which has an area of 665 km^2 and had a population of 2.51 million people in 2011 (CBS 2012). Groundwater samples were collected from dug and tube wells during the dry seasons of year 2009 and 2012 and the wet seasons of the years 2009 through 2012. Sixty-seven and 110 samples from dug and tube wells, respectively, were analysed for *Escherichia coli*. Analysis of *Cryptosporidium* oocysts and *Giardia* cysts was carried out for 22 dug well and 15 tube well samples during the wet seasons only. The locations of sampling sites are shown in Figure 1.

Microbial analysis

E. coli in the dry season samples from 2009 was measured by the membrane filtration method using mColiBlue broth (Merck Millipore, Billerica, MA, USA), whereas that in other samples was tested by the most probable number (MPN) method using the Colilert reagent (Idexx Laboratories, Westbrook, ME, USA). Details of the procedures have been described elsewhere (Shrestha *et al.* 2014). These analyses were performed in the laboratory in Kathmandu, immediately after field sampling. Because enteropathogenic *E. coli* (EPEC), a pathogenic strain, constitutes 8% of the total *E. coli* population in water (Levine *et al.* 1987), the concentration of *E. coli* has been converted to that of EPEC. *Cryptosporidium* oocysts and *Giardia* cysts were detected by immunomagnetic separation using Dynabeads GC combo (Invitrogen, Carlsbad, CA, USA) and an immunofluoresence essay using Easy Stain (BTF, North Ryde, Australia). The details of these procedures have been described elsewhere (Haramoto *et al.* 2011). According to the protocols of the above-mentioned procedures, the value for not detected samples was taken to be <1 and the lower detection limit (DL) was taken to be 1. A small number of samples from the *E. coli* analysis and several samples from the protozoan analyses had left-censored observations, samples with concentrations below the theoretical lower DL. In order to represent

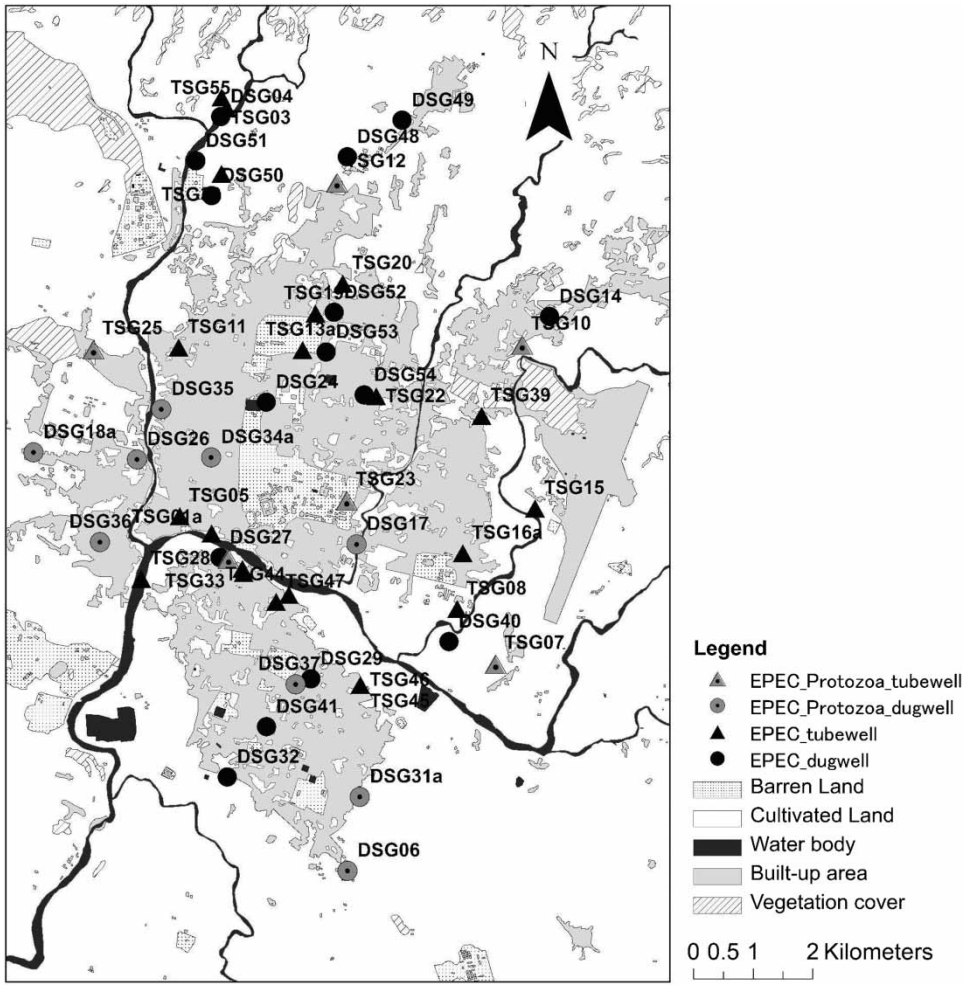

Figure 1 | Distribution of sampling sites. DSG: Dug well; TSG: Tube well.

such samples, half of the lower DL, 0.5, was used (Sato *et al*. 2013).

Risk estimation

QMRA described by Haas *et al*. (1999) was followed in this study. The QMRA framework consists of four steps: hazard identification, exposure assessment, dose–response relationship and risk estimation.

Hazard identification

Among bacterial enteropathogens, EPEC has been reported to be the most prevalent and among protozoan enteropathogens, *Giardia* has been reported to be the most prevalent, followed by *Cryptosporidium*, in both children (Ono *et al*. 2001; Uga *et al*. 2004; Ansari *et al*. 2012) and adults (Pandey *et al*. 2002) in the valley.

Exposure assessment

This step determines the number of organisms ingested in a single exposure, dose (Haas *et al*. 1999). We have considered two exposure pathways: drinking and bathing. A questionnaire survey was administered to 320 households (age, 18–55 years) and the questions included water ingestion rate per person per day and bathing frequency. For the survey, the Kathmandu Valley was divided into three groups based on ADB (2010) according to piped water supply hours: group A (>7 hours/week), group B (4–7 hours/week),

group C (<4 hours/week). Within each group, four administrative units were randomly chosen and within each unit around 26 or more households were then randomly selected.

Three crucial parameters included in risk estimation in this study were:

Concentration of enteropathogens (C): The concentration of EPEC, *Cryptosporidium* oocysts and *Giardia* cysts were estimated using the analysis procedures described above.

Water ingestion rate (V): In this study there were two types of water ingestion rates considered depending on the exposure pathways. First was the voluntary water ingestion rate for the drinking pathway which varies depending on dietary behaviour, cultural factors and climatic conditions. Therefore, this rate should be based on surveys in the local area. We used a questionnaire survey to determine the rate and included questions about the amount of any kind of fluid, boiled water and plain water consumed per day.

Second was the involuntary water ingestion rate for the bathing pathway and it was assumed to be 100 mL per bath as used by Steyn *et al.* (2004).

Removal efficiency of treatment method (R): About 67% of households in the Kathmandu Valley used several kinds of POU water treatment methods (Shrestha *et al.* 2013). Ceramic water filter (CWF) is one of the most commonly used POU water treatment methods in Nepal (Low 2002; Lamichhane 2013). The effectiveness of the CWF decreases when filter unit is not cleaned regularly and flow rate decreases along with the time although hygiene is maintained (Sobsey *et al.* 2008). Therefore, although new CWF can have >95% removal efficiency for *E. coli* (Low 2002), CWFs used in households could have reduced efficiency. So in order to depict household scenario we assumed removal efficiency to be 0.2 log (37%) for EPEC (Bielefeldt *et al.* 2009) and 1.58 log (97%) for *Cryptosporidium* oocysts and *Giardia* cysts (Clasen & Menon 2007). It was assumed that no treatment method was applied to bathing water.

The dose of microorganisms per exposure can be calculated by Equation (1)

$$\text{Dose } (d) = C \times V \times 10^{-R} \tag{1}$$

Dose–response relationship

A beta-poisson dose–response model (Equation (2)) was used for EPEC (Haas *et al.* 1999), and an exponential dose–response model (Equation (3)) was used for *Cryptosporidium* (Dupont *et al.* 1995) and *Giardia* (Rose *et al.* 1991) to compute the risk of infection/day or event (P_d)

$$P_d = 1 - \left[1 + d/N_{50} \times \left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \tag{2}$$

$$P_d = 1 - \exp^{(-r \times d)} \tag{3}$$

where d = number of pathogens per exposure, N_{50} = average infecting dose (8.60×10^7), α = parameter of probability function (0.1778) (Haas *et al.* 1999), r = organism specific infectivity (0.01982 for *Giardia* (Rose *et al.* 1991) and 0.004202 for *Cryptosporidium* (Dupont *et al.* 1995)).

Risk estimation

First a set of random values was extracted from the probability distributions of the enteropathogen concentration and water ingestion rate. Then d was calculated (Equation (1)) and P_d was obtained from the dose–response model (Equations (2) & (3)). Annual risk of infection (P_a) was calculated by repeating the equations N times (Equation (4))

$$P_a = 1 - (1 - P_d)^N \tag{4}$$

Here, N is 365 for the drinking pathway and 104 for the bathing pathway (based on the questionnaire survey). For EPEC risk estimation, $N = 183$ for each season for the drinking pathway and 52 for each season for the bathing pathway. Two seasonal data were combined to get P_a (Equation (5))

$$P_a = 1 - \left(1 - P_{d,\text{dry season}}\right) \times \left(1 - P_{d,\text{wet season}}\right) \tag{5}$$

Subsequently, MCS was performed and these calculations were iterated 10,000 times to obtain the distribution of the P_a. The iteration was found to be adequate to obtain stable results. After estimating P_a (Equation (5)), we estimated combined annual risk of diarrhoea (P_{combined}) from

all three enteropathogens from each pathway (Equation (6)). Finally, we estimated total annual risk of infection (P_{total}) from dug wells and from tube wells by combining all enteropathogens and exposure pathways (Equation (7)). In this study, the unit of risk of infection is infection/person-year

$$P_{combined} = 1 - (1 - P_{a,EPEC}) \times (1 - P_{a,Cryptosporidium}) \times (1 - P_{a,Giardia}) \quad (6)$$

$$P_{total} = 1 - (1 - P_{a,drinking}) \times (1 - P_{a,bathing}) \quad (7)$$

An acceptable limit of risk proposed by United States Environmental Protection Agency, $<10^{-4}$ infections/person-year from waterborne exposure through potable water, was applied for performing risk characterisations. Here infection is assumed to be equivalent to diarrhoea. We used median risk to describe our results. Although we could compare our results with WHO reference level of risk by estimating disease burden using disability-adjusted life years, we lack the necessary information at present and we hope to assess it in the near future.

Sensitivity analysis

Sensitivity analysis determines the relative impact of various parameters on the computed output (Haas *et al.* 1999). Sensitivity analysis was performed in order to estimate the 'relative impact' of *C, V* and *R* on risk results according to the method described by Haas *et al.* (1999) and followed by Sato *et al.* (2013). The rank correlation coefficients (Spearman rank correlation coefficient) between every parameter (*C*, *V* and *R*) and estimated annual risk were calculated. Then the contribution to the variance of risk was estimated by squaring the rank correlation coefficients for the parameters and normalising to 100%. The contribution to the variance is an approximated method to estimate the percentage of the variance in the risk due to each parameter (Sato *et al.* 2013). For risk estimation, *R* was considered as constant but in sensitivity analysis, we created minimum and maximum values of *R* by decreasing and increasing 50% to the constant value respectively and then generated 10,000 random numbers for MCS. For sensitivity analysis only, we ran MCS once again with random numbers for *C, V* and *R* and thus the estimated risks were different from that derived from the procedure described in previous sections. Because of this limitation on the values of *R*, numerical interpretation was not done.

Statistical analysis

The probability distributions of the parameters were determined and 10,000 random values of the parameters were produced by using EasyFit 5.5 Professional software. Chi-square goodness of fit was assessed at the significance level of 0.05 and 0.01. The data sets whose null hypothesis (data distribution fits the assumed distribution) had been rejected using the chi-square test were reassessed for the Anderson–Darling test at the same significance level.

Microsoft Office Excel 2007 was used to perform MCS for risk estimation. In order to compare risk between individual pathogens, independent samples *t*-tests were performed on risk estimated from MCS but not on the observed data. Similarly the independent samples *t*-test was used to compare risks between dug well and tube well. The same test was also used to compare risk between the drinking and bathing pathways. Independent samples *t*-test and Spearman's rank correlation were performed using the Statistical Package for Social Studies version 21 (SPSS Inc., USA).

RESULTS

Detection of enteropathogens and probability distribution

On an average, 85% of dug wells and 48% of tube wells exceeded the WHO guideline for drinking water (WHO 2011) for *E. coli* (0 MPN/100 ml). Among dug wells, 32 and 37% were positive for *Cryptosporidium* oocysts and *Giardia* cysts respectively. Among tube wells, 7 and 13% were positive for *Cryptosporidium* oocysts and *Giardia* cysts respectively. The maximum and minimum concentrations as well as best fitted probability of enteropathogens are shown in Table 1.

Water ingestion rate and probability distribution

Water ingestion rate best fitted with lognormal distribution (3P) ($\mu = 1.0095$; $\gamma = 1.47$; $\sigma = 0.3$) and the goodness of fit

Table 1 | Descriptive statistics of enteropathogens

Enteropathogens	Seasons	Min.[a]	Max.	Mean	Fitted distribution	Goodness of fit test
EPEC-DW (MPN/100 ml)	Dry	<1	184	14	Lognormal ($\mu = -2.41$, $\sigma = 2.2$)	Chi-square*
	Wet	<1	1,589	119	Lognormal ($\mu = 5.05$, $\sigma = 2.6$)	Chi-square*
EPEC-TW (MPN/100 ml)	Dry	<1	45	1	Gamma ($\alpha = 0.04$, $\beta = 397$)	Chi-square*
	Wet	<1	90	5	Lognormal ($\mu = -1.08$, $\sigma = 1.8$)	Chi-square**
Cryptosporidium-DW (oocyst/L)	Wet	<1	21	2	Gamma ($\alpha = 0.27$, $\beta = 8.69$)	Chi-square*
Cryptosporidium-TW (oocyst/L)	Wet	<1	22	2	Pareto ($\alpha = 4.06$, $\beta = 0.55$)	Anderson–Darling*
Giardia-DW (cyst/L)	Wet	<1	58	6	Generalised Pareto ($\mu = 0.18$, $\sigma = 0.88$, $k = 0.84$)	Chi-square*
Giardia-TW (cyst/L)	Wet	<1	22	2	Pareto ($\alpha = 2.5$, $\beta = 0.55$)	Anderson–Darling*

[a]<1 is written for no detection; DW: Dug well; TW: Tube well; μ, γ: location parameter; σ: scale parameter; α, β, κ: shape parameter; *: p-value <0.05; **p-value <0.01.

test was the chi-square (p-value <0.05). Almost all the respondents were drinking plain water without boiling, but we assumed that people used CWF to treat drinking water.

Risk of diarrhoea from dug well water

Figure 2 summarises estimated risks of diarrhoea from dug well water. The median risk from EPEC through the bathing pathway (0.0001 infections/person-year) was within the acceptable limit (<10–4 infections/person-year) but half of the distribution was still higher than the limit. The median risk from *Giardia* through drinking (0.2093 infections/person-year) and through bathing (0.1911 infections/person-year) was the highest among all risks and that from EPEC through both pathways was the lowest.

The combined risk from all three enteropathogens was 0.3103 infections/person-year from the drinking pathway and 0.2746 infections/person-year from the bathing pathway. Because the risk from *Giardia* was much higher than that from other enteropathogens, the combined risks were dominated by *Giardia*. The total risk was 0.5146 infections/person-year from using dug well water, which is approximately 10^3 times higher than the acceptable limit.

Risk of diarrhoea from tube well water

Figure 3 summarises risks from tube well water. The median risk from EPEC in tube well water met the acceptable limit,

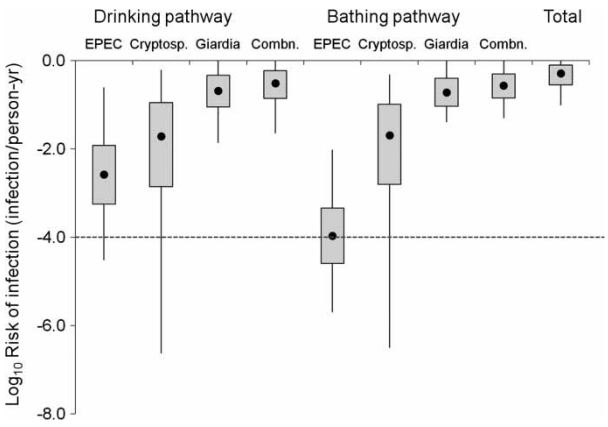

Figure 2 | Annual risk of diarrhoea from using dug well water. Boxes represent interquartile ranges, high and low lines represent 95th and 5th percentile values respectively, and dots represent median values. Cryptosp.: *Cryptosporidium*; Combn.: risk from three enteropathogens combined; Total: total risk from dug well water. Dotted line represents acceptable limit of risk.

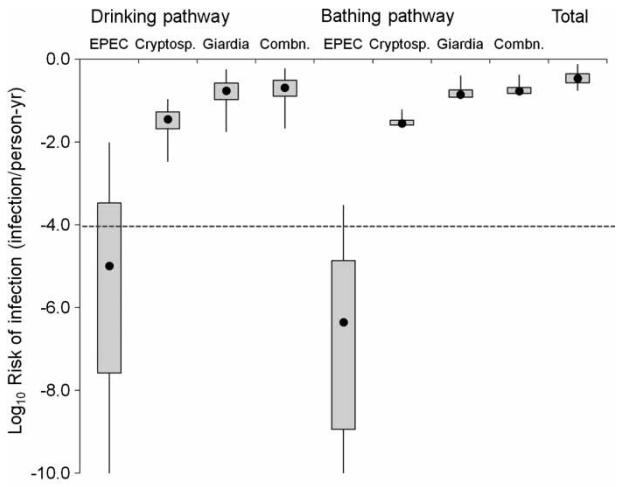

Figure 3 | Annual risk of diarrhoea from using tube well water. Boxes represents interquartile ranges, high and low lines represent 95th and 5th percentile values respectively, and dots represent median values. Cryptosp.: *Cryptosporidium*; Combn.: risk from three enteropathogens combined; Total: total risk from tube well water. Dotted line represents acceptable limit of risk.

from either exposure pathway but the risks from the remaining enteropathogens exceeded the acceptable limit through both exposure pathways. The highest risks were from *Giardia*: 0.1712 infections/person-year through drinking and 0.1392 infections/person-year through bathing. The combined risk from all three enteropathogens was 0.2043 infections/person-year through drinking and 0.1668 infections/person-year from bathing. Because the risk from *Giardia* was very much higher than that from other enteropathogens, the combined risks were dominated by that from *Giardia* in case of tube well water, similar to the case of dug well water. We estimated total risk from using tube well water by combining risks from all three enteropathogens through both exposure pathways. The total risk was 0.3428 infections/person-year, which exceeded the acceptable limit.

The total risk from using dug well water and from using tube well water exceeded the acceptable limit with the order of 10^4. Hence these results indicated high public health risk while using the valley's shallow groundwater.

Sensitivity analysis

The parameters considered for sensitivity analysis were enteropathogen concentration in the dry and wet seasons (C), water ingestion rate (V) and removal efficiency of treatment method (R) (Table 2). The values for the parameters in each row represent their contribution to the variance of risk from the enteropathogen in that row. Enteropathogen concentrations represent the largest contribution to variance of risk from EPEC in dug wells and tube wells in both dry and wet seasons. Removal efficiency represented the largest contribution to variance of risk from *Cryptosporidium* in dug wells and tube wells and that from *Giardia* in tube wells. There was little difference between the contribution of enteropathogen concentration (44%) and removal efficiency of treatment method (38%) on variance of risk from *Giardia* in dug wells. To summarise, enteropathogen concentration and removal efficiency contributed more to the variance of risk from EPEC and protozoa, respectively.

DISCUSSION

This study estimated the risk of diarrhoea due to the enteropathogens, EPEC, *Cryptosporidium* and *Giardia,* from exposure to shallow groundwater in the Kathmandu Valley. The exposure pathways considered were drinking and bathing. The risks from using dug wells either for drinking or for bathing or for both purposes were 10^3 times higher than the acceptable limit. Similarly, risks from using tube well water either for drinking or for bathing or for both purposes were also 10^3 times higher than the acceptable limit. These results indicate a severe public health concern for those who are using the valley's shallow groundwater. Hence there is an urgent need to implement risk reduction strategies. For the estimated risk from shallow groundwater

Table 2 | Contribution of parameters of risk calculation to the risk variance

	Contribution of parameters to the variance of risk (% contribution)					
	Dry season			Wet season		
Enteropathogens	Pathogen conc. (C)	Water ingestion rate (V)	Removal efficiency (R)	Pathogen conc. (C)	Water ingestion rate (V)	Removal efficiency (R)
EPEC-DW	0.94 (72)	0.29 (22)	0.08 (6)	0.94 (73)	0.29 (22)	0.07 (5)
EPEC-TW	0.97 (94)	0.05 (5)	0.01 (1)	0.99 (90)	0.08 (8)	0.02 (2)
Cryptosporidium-DW	NA	NA	NA	0.1 (11)	0.06 (6)	0.8 (83)
Cryptosporidium-TW	NA	NA	NA	0.03 (3)	0.26 (28)	0.65 (69)
Giardia-DW	NA	NA	NA	0.41 (44)	0.16 (17)	0.35 (38)
Giardia-TW	NA	NA	NA	0.05 (5)	0.26 (28)	0.61 (67)

NA: Not available.
DW: Dug well.
TW: Tube well.

to be reliable and useful for risk management in the valley or in similar settings, due consideration should be given to the following aspects.

Types of wells

The detection rates as well as concentrations of enteropathogens in our study were higher in dug wells than in tube wells (Table 1). Similar phenomena for faecal indicator bacteria were reported by Maharjan (2005) and Warner *et al.* (2008) in the valley, RDI (2008) and Uy *et al.* (2010) in Cambodia and Barthiban *et al.* (2012) in Sri Lanka. This might indicate that tube wells have comparatively better water quality. But in our study, risks of diarrhoea from *Cryptosporidium* and *Giardia* while using tube well water were higher than the acceptable limit for both drinking and bathing purposes. Therefore, even if tube wells have been widely reported to be less contaminated than dug wells, tube wells still represent a serious public health concern.

Enteropathogens

The only study that estimated risk of infection while using bore well water for drinking reported 10^{-5} infections/person-year from faecal coliforms (Emmanuel *et al.* 2009). This risk was similar to the risk from EPEC in our study through the same exposure pathway (10^{-5} infections/person-year). However, it was 100 times lower than the risk from *Cryptosporidium* (0.0350 infections/person-year) and 1,000 times lower than that from *Giardia* (0.1712 infections/person-year). The risk we obtained while combining the risks from EPEC with that from *Cryptosporidium* and *Giardia* was 10,000 times higher than the risk estimated by ignoring these protozoan enteropathogens, and the combined risk exceeded the acceptable limit. Thus, excluding infective enteropathogens in the risk estimation could lead to underestimation of the potential danger. Most of the positive wells in our study had very low concentrations of *Cryptosporidium* oocysts and *Giardia* cysts, but the estimated risks exceeded the guideline value of $<10^{-4}$ infections/person-year. These results indicated that even if protozoa are detected in relatively low concentrations and in fewer samples, they could produce higher health risks because of their high virulence, infectivity and environmental resistance.

In this study, risk could be over- or underestimated because we ignored pathogen infectivity, microbial die-off rate, recovery rate of microbial analysis and EPEC to *E. coli* ratio was variable and not specific to groundwater. Despite such limitations our study has uncovered important findings regarding risk related to groundwater use in the valley.

Conversion of *E. coli*: protozoa concentration

The mean concentration of *Cryptosporidium* in dug wells in our study was 2 oocysts/L and the risk of diarrhoea while using dug well water for drinking was 0.0194 infections/person-year. Machdar *et al.* (2013) reported very low risk (8.2×10^{-5} infections/person-year) from *Cryptosporidium* through an identical pathway in Ghana. They estimated *Cryptosporidium* concentration (0.0038 oocysts/L) from *E. coli* concentration using an *E. coli*: protozoa ratio (10^6) derived from wastewater; this concentration of *Cryptosporidium* was very much lower than that in our study. *Cryptosporidium* is highly resistant to environmental conditions compared to other microorganisms, such as *E. coli* (Teunis *et al.* 1997). So it is probable that *Cryptosporidium* concentration was underestimated by Machdar *et al.* (2013). Because a small number of protozoan enteropathogens could result in considerably higher risk of diarrhoea, faulty estimation of the concentration could misrepresent the real scenario.

Exposure pathways

In our study the combined risks of diarrhoea from bathing exposure were 0.2746 and 0.1668 when using dug well water and tube well water, respectively. The only study which considered the bathing pathway also estimated a similar risk from *Giardia*, 0.6760 infections/person-year (Razzolini *et al.* 2011) from using shallow well water. The total risk of diarrhoea from dug and tube well water increased by 43 and 63%, respectively, when the bathing exposure pathway was added in this study. With the exception of a study by Razzolini *et al.* (2011), all QMRA studies ignored the bathing pathway for risk estimation. There is a strong perception among people that bathing water need

not to be as clean and safe as that for drinking. But our results show that there is a considerable public health risk even when using these contaminated sources for activities like bathing, when very small volumes of water could be ingested. Therefore, bathing should be considered as an important exposure pathway while doing health risk estimation studies of various water sources.

POU water treatment

The only two studies that estimated risk from pathogens in dug well water reported a risk of 0.9990 infections/person-year from *Giardia* (Razzolini *et al.* 2011) and 0.9970 infections/person-year from *E. coli* O157:H7 (Machdar *et al.* 2013). The respective mean concentration of *E. coli* O157:H7 was 30.4 MPN/100 mL in Machdar *et al.* (2013) and that of *Giardia* was 9.7 cysts/L in Razzolini *et al.* (2011). These mean concentrations were similar to those found in our study (EPEC = 66.5 MPN/100 mL; *Giardia* = 6 cysts/L) but the risks estimated were close to 1 infection/person-year, which was much higher than the respective risks estimated in our study (EPEC = 0.0027 and *Giardia* = 0.2093 infections/person-year). Because neither study considered the treatment method in risk estimation, risks could have been overestimated in both. In our study, when we excluded the POU water treatment method from risk estimation, the risk from *Giardia* increased from 0.2093 to 0.9999 infections/person-year and from 0.1712 to 0.9992 infections/person-year for dug well and tube well water respectively. Sato *et al.* (2013) considered conventional treatment methods while estimating risk from surface water in Brazil and neglected contamination of water in the distribution system and post-source contamination. In order to simulate the prevailing situation of household treatment of drinking water, the POU water treatment method should be incorporated into the risk estimation. In addition, it is recommended that various inexpensive POU water treatment methods should be incorporated in QMRA, and the focus should be on low income households and do comparative study.

Sensitivity of risk estimation

Pathogen concentration (dry and wet seasons) was the major contributing parameter for risk from EPEC, whereas removal efficiency was the major contributing factor for risk from *Cryptosporidium* and *Giardia* in both types of wells. Decreasing pathogen concentrations could require long-term planning. Total risks in both types of wells were driven by protozoan enteropathogens and hence removal efficiency was the major parameter for total risk variability. We focused on the CWF and hence advising people to use this method for POU water treatment and to properly maintain the equipment could serve as a practical risk management strategy on the local level, under the present scenario.

CONCLUSIONS

Our results showed that tube wells could also pose a serious risk of diarrhoea in spite of low contamination levels. When we included risks from *Giardia* and *Cryptosporidium*, the total risk increased by several thousand times. Thus, risk could be underestimated if such infective enteropathogens are excluded. In our study, the total risk of diarrhoea from shallow groundwater increased considerably when the bathing exposure pathway was included. Therefore, bathing should be considered as an important exposure pathway in addition to drinking. We estimated a very high risk of diarrhoea from shallow groundwater use, either for drinking or for bathing, which indicated a need for risk reduction strategies in the valley. We propose that household treatment should be included in risk calculations to decrease overestimation, especially in developing countries. In this study, POU treatment appeared to have the biggest impact on risk and hence increasing CWF's coverage and improving its efficiency could be a feasible risk management strategy on the local level in the Kathmandu Valley.

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