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International Journal of Environmental Health Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cije20

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Available online: 03 May 2011

To cite this article: Maria Tereza Pepe Razzolini, Mark H. Weir, Maria Helena Matte, Glavur Rogerio Matte, Licia Natal Fernandes & Joan B. Rose (2011): Risk of *Giardia* infection for drinking water and bathing in a peri-urban area in São Paulo, Brazil, International Journal of Environmental Health Research, 21:3, 222-234

To link to this article: http://dx.doi.org/10.1080/09603123.2010.533367

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International Journal of Environmental Health Research
Vol. 21, No. 3, June 2011, 222–234

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Risk of *Giardia* infection for drinking water and bathing in a peri-urban area in São Paulo, Brazil

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(*Received 8 June 2010; final version received 1 October 2010*)

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A high incidence of waterborne diseases is observed worldwide and in order to address contamination problems prior to an outbreak, quantitative microbial risk assessment is a useful tool for estimating the risk of infection. The objective of this paper was to assess the probability of *Giardia* infection from consuming water from shallow wells in a peri-urban area. *Giardia* has been described as an important waterborne pathogen and reported in several water sources, including ground waters. Sixteen water samples were collected and examined according to the US EPA (1623, 2005). A Monte Carlo method was used to address the potential risk as described by the exponential dose response model. *Giardia* cysts occurred in 62.5% of the samples (<0.1–36.1 cysts/l). A median risk of 10^{-1} for the population was estimated and the adult ingestion was the highest risk driver. This study illustrates the vulnerability of shallow well water supply systems in peri-urban areas.

Keywords: *Giardia*; risk assessment; drinking water; shallow wells; peri-urban area

Introduction

Waterborne diseases are reported worldwide and are a major cause of morbidity and mortality with significant social and economic costs, particularly for the developing world (World Health Organization [WHO] 2003; WHO/United Nations Children's Fund [UNICEF] 2005). Due to the lack of adequate water supply and sanitation, people living in sub-standard settlements are often exposed to unsafe water which can be contaminated with pathogens (Teixeira et al. 2007). According to the WHO, in developing countries it was estimated that 94% of the diarrheal disease burden was associated with risk factors such as unsafe drinking-water and poor sanitation and hygiene (WHO 2006). In Brazil, infectious diseases were responsible for 5.1% of deaths and are the fifth most common cause of mortality, according to the Brazilian Health Ministry (Brasil 2004). From 1995–1999, patients due to environmentally-caused diseases occupied 3.4 million hospital beds (Brasil 2004).

The protozoan parasite *Giardia* has been described as an important waterborne pathogen and is associated with severe gastrointestinal illness (giardiasis). Karanis

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ISSN 0960-3123 print/ISSN 1369-1619 online
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DOI: 10.1080/09603123.2010.533367
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et al. (2007) carried out a study and reported on 325 water-associated outbreaks of parasitic protozoa worldwide, verifying that *Giardia duodenalis* and *Cryptosporidium parvum* likely presented the largest number of recorded outbreaks.

Giardia develops cysts which are excreted in feces which provide the organism protection from environmental stresses. Cysts have been found in several water types in numerous studies, including wastewater, surface waters, spring waters, recreational waters, ground waters and drinking water (Solo-Gabriele et al. 1998; Franco et al. 2001; Rose et al. 2002; Hachich et al. 2004; Briancesco and Bonadonna 2005; Ryu and Abbaszadegan 2008; Muchiri et al. 2009). Important issues regarding *Giardia* cyst presence in waters includes its resistance to conventional methods of disinfection, high persistence in the environment and low infectious dose (WHO 2002). For these reasons, the presence of *Giardia* cysts in source water and particularly in drinking water represents a public health concern.

Groundwater is often thought of as being protected from these larger cysts. However, some researchers have reported the presence of this parasite in groundwater samples, and previous giardiasis outbreaks have been associated with groundwater sources. In 2007, Karanis et al. reported that giardiasis cases were associated with contaminated wells in Colorado, South Dakota and Florida. Daly et al. (2009) carried out a study which suggested that groundwater was the source of the giardiasis outbreak in New Hampshire in 2007. In water surveillance studies, Hancock et al. (1998) found that 12% of the 199 sites analyzed in the United States were contaminated with *Cryptosporidium*, or *Giardia* or both. Solo-Gabriele et al. (1998) reported *Giardia* and *Cryptosporidium* presence in a well that supplies water for San Pedro Sula (Honduras). Karanis et al. (2006) assessed water quality of private wells in Russia and Bulgaria, used mostly to irrigate gardens; and reported that out of 18 samples analyzed two (11.1%) and three (16.7%) were positive for *Giardia* and *Cryptosporidium*, respectively.

In Brazil, there are no official data regarding giardiasis outbreaks but some health studies show high prevalence of infection especially in areas with poor sanitary conditions. Gross et al. (1989) reported a giardiasis prevalence of 17.9% in two low-income urban communities in Belo Horizonte (MG/Brazil). Later, Teixeira et al. (2007) conducted a study that showed giardiasis prevalence was 18% among children in a sub-standard settlement in Juiz de Fora (MG/Brazil). Prado et al. (2003) studied *Giardia* cysts prevalence in the feces of pre-school children (2–45 months) in the city of Salvador (BA/Brazil) and found 13.7% (95/694) of them were infected with *Giardia duodenalis*. According to Mascarini and Donalísio (2006) there is high prevalence of giardiasis among children who attend daycare centers in Brazil, mainly in poor areas.

Quantitative Microbial Risk Assessment (QMRA) is a valuable tool to estimate the risk of infection from exposure to pathogens (Gale 1996; Haas et al. 1999; Soller 2006) and to predict the relative magnitude of the potential problems by estimating the possible number of people affected, in a specific location, over time in this case via the consumption of contaminated drinking water (Rose et al. 1991; Haas et al. 1993; Hunter et al. 2003; Soller and Eisenberg 2008). The advantages of using this kind of analysis are that by estimating impacts of exposure to pathogens, expressed quantitatively in terms of probability of infection, morbidity and/or death, this assists in addressing the management options which will best focus on health protection, risk reduction and in addressing the uncertainty and drivers of the data which are prominent in the risk.

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The objective of this study was to utilize a QMRA approach for examining public health risks associated with the use of shallow wells in a peri-urban area of the Metropolitan Region of São Paulo (MRSP), Brazil, for a population of about 2,000. These wells, used as drinking water sources, are located where sanitation has been observed as inadequate and the risk of parasitic infections via groundwater was a significant concern. Therefore, it was considered likely that the residents were at an increased risk of exposure to pathogens from their drinking water.

The Monte Carlo method was used in this study and allows for repeated random sampling, computing results of a model or models over many multiple iterations. The Monte Carlo method relies on probability distributions which are used to describe the variables deemed uncertain. In the case of this work the uncertainty of the following variables was described with probability distributions:

(1) Exponential dose response k parameter;
(2) Dose of cysts to adults and children from both bathing and drinking;
(3) Concentrations of cysts in the well.

The Monte Carlo method allows for a more complete picture of the risks to the population and the likely factors driving this risk (Rubinstein and Kroese 2008). It also allows for the capture and control of the variability (seasonal or otherwise) of the environmental sampling data, by overlaying a probability distribution in order to describe these values, in the model (dose response function).

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Materials and methods

Area characterization

Peri-urban settlements irregularly established in the Protection Water Catchment Area in the MRSP (south-eastern Brazil), were identified as areas of concern when poor sanitary conditions were observed. This area has approximately 2,000 inhabitants. Some treated water is supplied by truck-tanks and transferred to collective reservoirs, this is intended to provide water for 5–7 families, and however, this is not enough to meet the community water demand. This situation has forced people to look for alternative water sources by digging shallow wells (depth of 3–5 m) in their backyards. These wells are used as drinking water sources and for household activities such as cooking, cleaning and bathing. The sewage is disposed in septic tanks, in basic latrines or directly into water bodies. It should be emphasized that neither the wells nor the septic tanks were built following any sanitary criteria.

Sampling and analysis

A total of 16 water samples were collected from wells during four months spanning winter and spring (July, September, October and November 2008). Sampling was performed according to the US EPA – Method 1623 (2005). Ten liters of water were collected using sterile disposable containers and were kept chilled during the transportation and examined within a 24-h period.

Samples were concentrated using the IDDEX FiltaMax® filter with a controlled flow between 1 and 4 l/min. After this step, the elution was done with 600 ml of

phosphate buffered saline (PBST) solution processed in a stomacher for 5 min. This procedure was performed twice. Then, the eluate obtained was centrifuged at 2500 rpm for 15 min to obtain a pellet for processing by the immunomagnetic separation (IMS). The IMS step was run using the commercial kit Dynabeads GC-Combo (Dynal Biotech,UK) as described in the Method 1623 (US EPA 2005). Following this step, the sample was stained using fluorescent antibody A100FLR-1X (Waterborne, USA) according to manufacturer's instructions with a counter DAPI stain. Finally, cysts were enumerated with an epifluorescence microscope (BX-60, Olympus Optical Co., Ltd, Tokyo, Japan). The enumeration of *Giardia* cysts was undertaken by microscopic enumeration using the fluorescence produced by the cyst wall-based antibody stain, DNA stain and differential interference microscopy (FITC, DAPI and DIC examination), respectively (US EPA 2005). The results were expressed in cysts/l. Positive and negative controls were also prepared.

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Quality control: Precision and recovery

In order to obtain the recovery using this method, five independent spiking experiments were performed by filtering 10 l of contaminated reagent water. Spiking suspensions of 100 cysts of *Giardia* (EasySeed™, BTF Precise Microbiology, Australia). The analytical procedure was the same described above. The percentage recovery efficiency (R) was calculated according to Equation 1.

$$R = (\text{cysts recovered}/\text{number of cysts spiked}) \times 100 \tag{1}$$

The mean percentage of recovery and the standard deviation (SD) were calculated for *Giardia*. The interference of the sample matrix was investigated. Volumes of 10 l of the water sample from the wells were collected and spiked with a suspension containing 100 cysts of *Giardia* (EasySeed™, BTF Precise Microbiology, Australia). The analytical procedure was the same as described above. This procedure was done once in view that the frequency recommended by EPA is one Matrix Spike (MS) sample per 20 samples field samples. The percentage recovery ($R\%$) was calculated according to Equation 2, knowing the percent recovery (R).

$$R = \left[\left(N_{sp} - N_{ns}\right)/T\right] \times 100 \tag{2}$$

where: N_{sp} = Number of cysts counted in spiked samples; N_{ns} = number of cysts counts in not spiked samples; and T represents the number of cysts spiked (100 cysts of Giardia).

The mean percentage of recovery and the relative standard deviation (RSD) were determined for *Giardia*. The percentage of recovery of the method ($n = 5$) was 35.8% and relative standard deviation (RSD) was 10.6% for *Giardia* cysts According to US EPA (2005), the initial precision and recovery criteria for *Giardia* should range from 24–100% and the maximum RSD for *Giardia* cysts should be 49%. Therefore data obtained in this study are in accordance with EPA's recommendation. In relation to well water samples spiked and unspiked, the recovery obtained was 37% for *Giardia*.

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Giardia *genotyping*

Giardia genotyping of three samples of well water was undertaken. Sample concentration (10 l) was carried out according to Araújo et al. (2006), which consists of filtration and centrifugation steps. Millipore membranes and a manifold (Millipore® Brasil) were employed. The material captured from the filter was all re-suspended in one tube and washed with ultra pure water (Milli Q®). The material was centrifuged (7.428 g/15′/24°C) and supernatant was discharged. The pellet obtained after the concentration step was purified by flotation in zinc sulphate solution (33%) according to Faust et al. (1938), with some modifications. Briefly, the pellet was washed with ultra pure water (Milli Q®). Supernatant was discharged after centrifugation (7.428 g/15′/24°C). Ten milliliters of zinc sulphate solution was added. After homogenization, tubes were centrifuged (3.714 g/3′/24°C). The pellet was removed with a 10 ml pipette. The floated supernatant left in the tube (2 ml) was washed twice with ultra pure water (Milli Q®), and then re-suspended in ultra pure water (Milli Q®). Genomic DNA was extracted using phenol-chloroform-isoamilic alcohol modified protocol (Araújo et al. 2006), with small modifications

PCR mix consisted of 25 ng of DNA, 10× buffer, 2 mM of $MgCL_2$, 1% of polyvinylpyrrolidone (PVP), 300 μM of deoxynucleoside triphosphate mix, 0.5 μM of each primer, 1.25U of *Tth* DNA polymerase (Biotools® Madrid, Spain) and ultra pure water (MilliQ®) to complete the final volume of 25 μl. In the second amplification, PCR mix was maintained except that PVP was not added. Amplification primers and conditions are described in Table 1.

Nested PCR product was resolved with 1.8% agarose gel electrophoresis, prepared with 1× tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide. A 100 bp DNA Ladder Plus (Fermentas) was included as a size marker. Gel images were obtained by UV transillumination and captured by a gel documentation system (Epi Chemi II Darkroom and Software Labworks – UVP).

Following successful PCR, the products were purified (Ilustratm GFXtm PCR DNA and Gel Band Purification Kit, GE® Healthcare, UK). Molecular clone and sequencing were done as described by Ivanova et al. (2001). Analyses of the 862 position (position 659–1485 from the start of the gene) of the *gdh* coding gene sequences of the clones were compared with their closest relatives in the GenBank database by BLASTN searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Further phylogenetic and molecular evolutionary analyses were performed with the MEGA 4.1 program (Tamura et al. 2007).

Table 1. Amplification primers and conditions for PCR analysis.

Step	Primers (Souza et al. 2007)	Conditions
First 1190 bp	GdhR (forward) 5′CGAGCGCGAGCCGAAGTATATCC3′ #579II (reverse) 5′GATGTTYGCRCCCATCTGRTAGTTC3′	95°C for 7 min ("hot start"); 40 cycles at 95°C for 2 min, 50°C for 1 min and 72°C for 1.5 min; 72°C for 5 min.
Second 890 bp	GDH-FI (forward) 5′AAYGAGGTYATGCGCTTCTGCCA 3′ #579II (reverse) 5′GATGTTYGCRCCCATCTGRTAGTTC3′	94°C for 2 min; 30 cycles at 94°C for 35 s, 56°C for 35 s and 72°C for 1 min; 72°C for 5 min.

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Risk assessment

In order to determine the health risk impacts from use of the well water, a QMRA was performed. In the QMRA the exposure was defined as ingestion of the water either inadvertently through bathing or directly via drinking. The concentrations of *Giardia* cysts in water from wells were used to estimate the dose and then the daily probability of infection (P_i) and annual probability of infection (P_a).

The rate of daily drinking water ingestion was assumed to be 2 l per day for adults and 1 l per day for children (US EPA 1980, 1991). Water ingestion during the bathing was assumed to be 16 ml per 45 min of bathing for adults while for children was 37 ml per 45 min, based on study carried out by Dufour et al. (2006) which estimated ingestion of water during swimming activities.

The exponential dose response model (Equation 3) was found to be the best at depicting the dose response relationship for *Giardia* cysts (Rose et al. 1991).

$$P_i = 1 - \exp(-k \cdot dose) \tag{3}$$

where P_i is the daily probability of infection, k is the best-fit parameter that describes the probability of cysts initiating an infection, which for *Giardia* is 0.01982 (with upper and lower 95th confidence intervals of 0.009798 and 0.03582, respectively) (Rose et al. 1991). For the exponential model dose must be in cysts number, which is determined using the cyst concentration in the water multiplied by the daily consumption value previously discussed.

In this study, it was assumed that all cysts were viable, even though the method used to determine the parasite does not allow knowing about the viability. However, method assessment demonstrates that only 35.8% of the cysts present are likely recovered. Thus while one assumption of viability may overestimate the risk, the method recovery underestimates the risk.

The daily risk was also subjected to a Monte Carlo uncertainty analysis. The Monte Carlo method uses random sampling form probability distributions which describes the uncertain variables in the model. In this case the model is the dose response model (Equation 3). The uncertain variables for this analysis included the total dose of *Giardia* cysts which are ingested by children and adults during bathing or drinking, as well as the exponential dose response parameter.

Table 2 shows the probability distributions used in the Monte Carlo method, which was run for 10,000 iterations and summarizes the dose-response model used in

Table 2. Probability distributions and parameters used in the Monte Carlo uncertainty analysis.

		Distributin parameters		
Uncertain parameter	Distribution	Minimum	Maximum	Likeliest
k unitless	Triangular	0.009798	0.03582	0.01982
Drinking ingestion dose to adults*	Triangular	8.3	30.5	19.4
Drinking ingestion does to children*	Triangular	4.2	15.2	9.7
Bathing ingestion dose to adults*	Triangular	0.6	2.4	1.5
Bathing ingestion dose to children*	Triangular	1.5	5.5	3.5

*Units of cysts.

this study to estimate the probability of *Giardia* infection (P_i) posed by ingestion of water from wells. For these distributions, the confidence intervals were used to determine the parameter values for the triangular distributions. The triangular distribution was chosen due to the wide applicability of the distribution, and the more realistic nature than using a uniform distribution, since there were insufficient data points to fit a distribution. The annual risk was performed and was determined using Equation 4.

$$P_a = 1 - (1 - P_i)^n \tag{4}$$

where P_a is the annual risk, P_i is the daily risk and n is the number of exposures (365 days).

Results and discussion

This study addressed the water quality of an at risk community for parasites using a minimal sampling approach over a short time frame in order to provide information and recommendations as quickly as possible for public health. Table 3 shows the results obtained for *Giardia* cysts concentrations in 16 water samples collected from the wells in the peri-urban area in the MRSP. A high percentage of samples were positive, 62.5% (10/16) were found to have *Giardia* cysts and the concentrations ranged from <0.1–36.1 cysts/l with a mean of 9.7cysts/l. This level of pollution initiated an immediate public health concern and further analyses were warranted on the types of *Giardia* present (indicating the source of the contamination and the risk of human infection).

Table 3. *Giardia* cysts concentration detected in well water samples from a peri-urban area in the metropolitan region of São Paulo, 2008.

Date of collection	Wells	Concentration of *Giardia* cysts/l[1]
07/28/2008	Well 1	<0.1
07/28/2008	Well 2	<0.1
07/28/2008	Well 3	5.0
09/01/2008	Well 4	2.8
09/01/2008	Well 5	28.0
09/29/2008	Well 6	33.2
09/29/2008	Well 7	36.1
10/13/2008	Well 8	18.6
10/13/2008	Well 9	15.0
10/13/2008	Well 10	10.0
10/13/2008	Well 11	4.0
11/02/2008	Well 12	2.5
11/10/2008	Well 13	<0.1
11/10/2008	Well 14	<0.1
11/24/2008	Well 15	28.0
11/24/2008	Well 16	<0.1
Mean		9.7
Standard Deviation		12.67
CI 95th (Lower/Upper)		4.16/15.24.

[1]Detection limit=0.1 cysts/l.

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The PCR analyses performed yielded genotype A group II with sequences in the Genbank as follows: Three sequences GQ503142 a GQ503144 and seven sequences GQ503145 a GQ503151. Most studies have found that this assemblage is associated with human infections (Amar et al. 2002; Monis et al. 2003; Cacció et al. 2005). Thus, the probable source of *Giardia* contamination in the water wells is from human excreta and represents a high risk associated with the further spread of human infections.

Estimating the risks of infection was of interest for the population using this well water. Drinking represented the greatest exposure and highest potential for risk. However, the population was using the water for bathing and there was little understanding of the risk that this activity might carry with it when using contaminated water. The calculated risk estimates, P_i and P_a are shown in Table 4, bounded by the 95% confidence interval. The estimated risk for adults bounded within 1.74×10^{-1} and 5.02×10^{-1} with a mean of 3.2×10^{-1}, while for children the probability of infection presented a slight fall within the bounds of 9.10×10^{-2} and 2.94×10^{-1} with a mean of 1.76×10^{-1}. Bathing risks were 100 times lower on a daily basis but if the contaminated water is used year round even bathing increased the risk. Drinking water ingested by adults was higher is the primary driver of the risk. Interestingly, during bathing it is estimated that children will actually swallow more water than adults, thus risks are slightly higher for children during bathing compared to adults.

The results from the Monte Carlo are best demonstrated through Figure 1, which portrays the risks to the combined population (adults and children) from a combined exposure (drinking and during bathing). Figure 1 shows the distribution of the risk level (probability of infection on the x axis, running from 0.1–0.999) for the total exposed population (adults and children) versus the distribution of that risk (Y1 axis) via consumption of contaminated water with *Giardia* cysts, (with Y2 demonstrating the frequency of occurrences of the observed risk levels for 10,000 runs). In general, it is seen that the adults typically experience risks on infection greater than 0.50. Overall it was observed according to the model that there was a 3% chance that the risk was between 6 and 7×10^{-1}. Therefore it can be surmised that the total population is slightly skewed or likely to me more prone to a higher risk of infection, due to the influence of the adult ingestion volumes, as shown in the sensitivity analysis. This is highlighted again when viewing the distribution of risk level for just the children which had a 3% chance of the risk being between 2 and 2.6×10^{-1} due to exposure and the overall risk being skewed to the low risk region as is depicted in Figure 2.

In order to determine which of the variables is driving the risk a sensitivity analysis was performed as well. Figure 3 shows that adult ingestion is the highest driver to the risk for the population (55.6%). This result is not surprising due to the larger volume of water which they ingest on a daily basis if they use the wells for drinking water.

A high level of contaminated well water was found in the peri-urban in the MRSP, putting the population at risk of waterborne disease. As previously discussed our results are comparable with those in other developing regions of the world such as Honduras, Russia and Bulgaria (Solo-Gabriele et al. 1998; Karanis et al. 2006).

This study demonstrated how vulnerable the water supply system was in this specific area. Both, monitoring *Giardia* cysts presence in the well water and the estimated probability of infection due to ingestion the contaminated water with

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Table 4. Daily and annual probability of *Giardia* infection by drinking (WI) and bathing, WI(B), in contaminated well water.

	Daily probability of infection (P_i)				Annual probability of infection (P_a)			
	WI		WI(B)		WI		WI(B)	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Mean	3.20 (10^{-1})	1.76 (10^{-1})	3.08 (10^{-3})	7.12 (10^{-3})	9.99 (10^{-1})	9.99 (10^{-1})	6.76 (10^{-1})	9.20 (10^{-1})
Lower 95th	1.74 (10^{-1})	9.10 (10^{-2})	1.53 (10^{-3})	3.52 (10^{-3})	9.99 (10^{-1})	9.99 (10^{-1})	4.28 (10^{-1})	7.24 (10^{-1})
Upper 95th	5.02 (10^{-1})	2.94 (10^{-1})	5.57 (10^{-3})	1.28 (10^{-3})	9.99 (10^{-1})	9.99 (10^{-1})	8.70 (10^{-1})	9.91 (10^{-1})

WI = Wateringestion, WI(B) = Water ingestion during bathing.

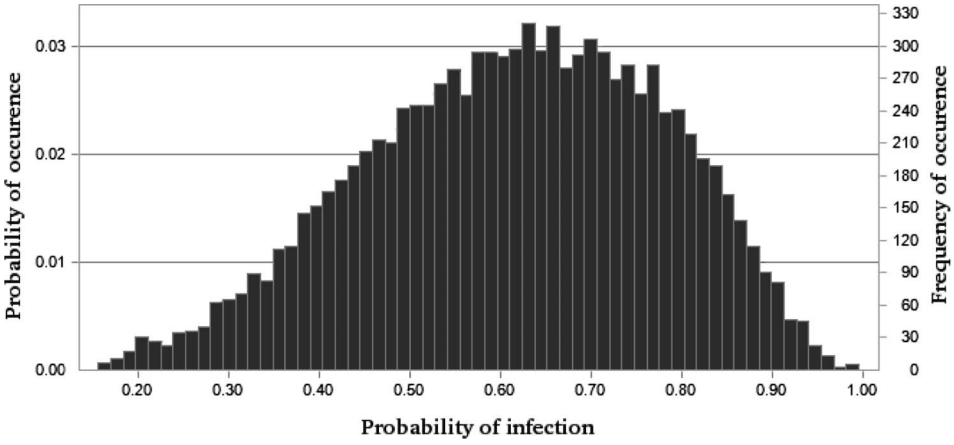

Figure 1. Risk estimation results from the Monte Carlo simulation with the combined population and combined exposure to water contaminated by *Giardia* cysts.

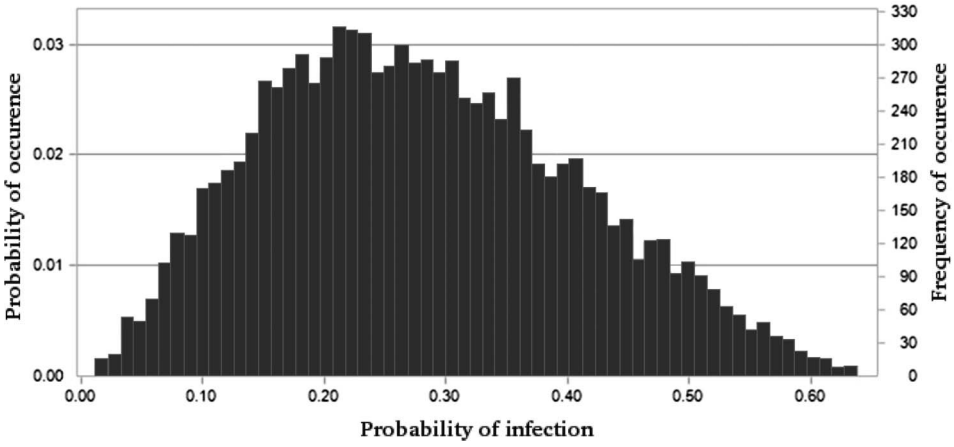

Figure 2. Risk to children from combined exposure (drinking and bathing) to water contaminated with *Giardia* cysts.

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Giardia show that there is a significant public health risk, even using this source for activities such as bathing when small volumes could be ingested. The probability of *Giardia* infection considering only water ingestion during bathing was estimated bounded within 1.53×10^{-3} and 5.5×10^{-3} with a mean of 3.08×10^{-3} for adults while for children the risk was bounded by 3.52×10^{-3} and 1.28×10^{-2} with mean of 7.12×10^{-3} (Table 4). It can be observed that the risk levels obtained for children and adults during bathing present the same magnitude. Yet the risk difference is not actually much higher, although children ingest higher volume of water, it brings concerns the impact on children's health. Moreover, it should take into account that children, especially in poor areas, are more vulnerable than adults.

As temporary measures, the population should be warned to boil or filter the water before using. If used for bathing, the community should be educated so as to avoid water ingestion. But, the ideal situation would be to provide an improved

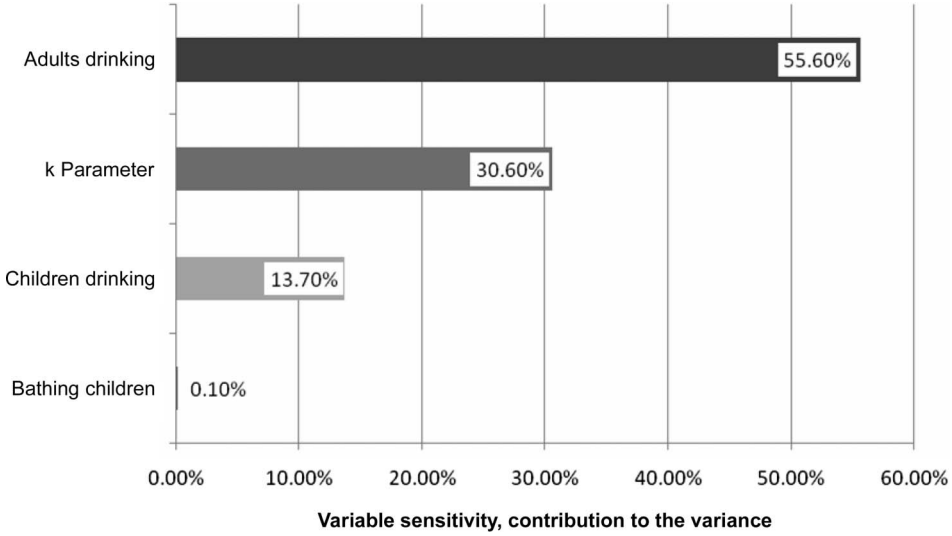

Figure 3. Sensitivity analysis of contribution to variance in the model for the variables in the model, results generated from the Monte Carlo simulation.

community water supply, filtration facility which could treat the ground water and provide disinfection resulting in reducing risks of infection and promoting health for this population.

Conclusions

The results of this study demonstrate the vulnerability of shallow well water supplies in irregular settlements where people are exposed to waterborne pathogens. The daily risk as a probability of *Giardia* infection reflects a median risk to the population which is too high and not acceptable. Overall it can be shown that the highest risk is to the members of the population who are ingesting the larger volumes and using the wells as drinking water, thus it is can be recommended that this activity should be discontinued Public policies which provide an educational campaign, and access to water with improved quality will reduce the risk of infection and promote health for the people living in these areas.

Acknowledgements

We would like to thank FAPESP – Fundação de Amparo a Pesquisa do Estado de São Paulo (06/05011-7), CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico (200007/2009-2) for its financial support and also Center for Advancing Microbial Risk Assessment (CAMRA).

References

Amar CFL, Dear PH, Pedroza-Díaz S, Looker N, Linnane E, McLauchlin J. 2002. Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia duodenalis* in human feces. 2002. J Clin Microbiol. 40(2):446–452.

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Araújo RS, Carvalho TTR, Matté GR, Fernandes LN, Balsalobre LC, Matté MH. 2006. Modified method for detection of *Cryptosporidium* oocysts using DNA templates extracted from environmental samples. Revista Brasileira de Vigilância Sanitária. 3:3–4.

Brasil. 2004. Ministério das Cidades. Saneamento Ambiental 5. Cadernos MCidades Saneamento Ambiental. Brasília.

Briancesco R, Bonadonna L. 2005. An Italian study on *Cryptosporidium* and *Giardia* in wastewater, fresh water and treated water. Environ Monitor Assess. 104:445–457.

Cacció SM, Andrew Thompson RC, McLauchlin J, Smith HV. 2005. Unraveling *Cryptosporidium* and *Giardia* epidemiology. 2005. Trends Parasitol. 21(9):430–437.

Daly ER, Roy SJ, Blaney DD, Manning JS, Hill VR, Xiao L, Stull JW. 2009. Outbreak of giardiasis associated with a community drinking-water source. Epidemiol Infect. 15:1–10.

Dufour AP, Evans O, Behymer TD, Cantú R. 2006. Water ingestion during swimming activities in a pool: A pilot study. J Water Health. 04(4):425–430.

Franco RMB, Rocha-Eberhardt R, Cantusio Neto R. 2001. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw water from the Atibaia river, Campinas, Brazil. Rev Inst Med Trop S Paulo. 43(2):109–111.

Faust EC, D'Antoni JS, Odom V, Miller MJ, Peres C, Sawitz W, Thomen LF, Tobie JE, Walker JH. 1938. A critical study of clinical laboratory techniques for the diagnosis of protozoan cysts and helminth eggs in feces. I. Preliminary communication. Am J Trop Med. 18:169–183.

Gale P. 1996. Developments in microbiological risk assessment models for drinking water – a short review. J Appl Bacteriol. 81:403–410.

Gross R, Schell B, Molina MCB, Leão MAC, Strack U. 1989. The impact of improvement of water supply and sanitation facilities on diarrhea and intestinal parasites: A Brazilian experience with children in two low-income urban communities. Rev Saúde Públ. 23(3): 214–220.

Haas CN, Rose JB, Gerba C, Regli S. 1993. Risk assessment of virus in drinking water. Risk Analysis. 13:545–552.

Haas CN, Rose JB, Gerba C. 1999. Quantitative microbial risk assessment. 1st ed. New York: John Wiley & Sons, Inc.

Hachich EM, Sato MIZ, Galvani AT, Menegon JRN, Mucci JLN. 2004. *Giardia* and *Cryptosporidium* in source waters of São Paulo State, Brazil. Water Science Technol. 5(1):239–245.

Hancock CM, Rose JB, Callahan M. 1998. Crypto and *Giardia* in US groundwater. J AWWA. 90(3):58–61.

Hunter PR, Payment P, Ashbolt N, Bartram J. 2003. Assessment of risk. In: Dufour A, Snozzi M, Koster W, Bartram J, Ronchi G, Fewtrell L, editors. Assessing microbial safety of drinking water – improving approaches and methods. London, UK: OECD/WHO, IWA Publishing. p. 79–109.

Ivanova E, Romanenko LA, Matté MH, Matté GR, Lysenko, Simidu AMU, Kita-Tsukamoto K, Sawabe T, Vysotskii MV, Frolova GM, Mikhailov V, Christen R, Colwell RR. 2001. Retrieval of the species *Alteromonas tetraodonis* Simidu et al. 1990 as *Pseudoalteromonas tetraodonis* comb. nov. and emendation of description. Int J Syst Evol Microbiol. 51(3):1071–1078.

Karanis P, Kourent C, Smith H. 2007. Waterborne protozoan parasites: A worldwide review of outbreaks and lessons learnt. J Water Health. 5(1):1–38.

Karanis P, Sotiriadou I, Kartashev V, Kourenti C, Tsvetrova N, Stojanova K. 2006. Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. Environ Res. 102:260–271.

Mascarini LM, Donalísio MR. 2006. Giardíase e cryptosporidiose em crianças institucionalizadas em crèches no estado de São Paulo (Giadiasis and cyptosporidosis in children institucionalized at daycare centers in the state of São Paulo). Rev Sociedade Bras Medicina Tropical. 39(6):577–579.

Monis PT, Andrews RH, Mayrhofer G, Ey PL. 2003. Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. Infection, Genetics Evolut. 3:29–38.

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Muchiri JM, Ascolillo L, Mutwiri T, Ward HD, Naumova EN, Egorov AI, Cohen S, Else JG, Griffiths JK. 2009. Seasonality of *Cryptosporidium* oocists detection in surface waters of Meru, Kenya, as determined by two isolation methods followed by PCR. J Water Health. 7(1):67–75.

Prado MS, Strina A, Barreto ML, Oliveira-Assis AM, Paz LM, Cairncross S. 2003. Risks factors for infection with *Giardia duodenalis* in pre-school children in the city of Salvador, Brazil. Epidemiol Infect. 131:899–906.

Rose JB, Huffman DE, Gennaccaro A. 2002. Risk and control of waterborne cryptosporidiosis. FEMS Microbiol Rev. 26:113–123.

Rose JB, Haas CN, Regli S. 1991. Risk assessment and control of waterborne giardiasis. Am J Public Health. 81:709–713.

Rubinstein RY, Kroese DP. 2008. Simulation and the Monte Carlo Method. New York: John Wiley and Sons Inc.

Ryu H, Abbaszadegan M. 2008. Long-term study of *Cryptosporidium* and *Giardia* occurrence and quantitative microbial risk assessment in surface waters of Arizona in the USA. J Water Health. 6(2):263–273.

Soller JA, Eisenberg JNS. 2008. An evaluation of parsimony for microbial risk assessment models. Environmetrics. 19:61–78.

Soller JA. 2006. Use of microbial risk assessment to inform the national estimate of acute gastrointestinal illness attributable to microbes in drinking water. J Water Health. 4(Suppl. 2):165–186.

Solo-Gabriele HM, Ager AL Jr, Lindo JF, Dubón JM, Neumeister SM, Baum MK, Palmer CJ. 1998. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in water supplies of San Pedro Sula, Honduras. Rev Panam Salud Publica/Pan Am J Public Health. 4(6):398–400.

Souza SLP, Gennari SM, Richtzenhain LJ, Pena HFJ, Funada MR, Cortez A, Gregori F, Soares RM. 2007. Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from the state of São Paulo, Brazil, by sequence analysis of fragment of glutamate dehydrogenase (gdh) coding gene. Vet Parasitol. 149(3–4):258–64.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24:1596–1599.

Teixeira JC, Heller L, Barreto ML. 2007. *Giardia duodenalis* infectious: Risk factors for children living in sub-standard settlements in Brazil. Cad Saúde Pública. 23(6):1489–1493.

United States Environmental Protection Agency (US EPA). 1980. Office of Water. Washington DC 20460. Water quality criteria documents; availability. Federal Register, 45(231):79318–79379.

United States Environmental Protection Agency (US EPA). 1991. Office of Water. Washington DC 20460. Human health evaluation manual, Supplemental guidance: Standard default exposure factors, OSWER. Directive. 9285:6-03.

United States Environmental Protection Agency (US EPA). 2005. Office of Water. Washington DC 20460. Method 1623: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. EPA-815-R-05-002. Accessed 20 April 2006 from the website: URL: http://www.epa.gov/nerclcwww/1623ap01.pdf

World Health Organization (WHO). 2002. Guidelines for drinking-water quality. 2nd ed. Addendum Microbiological agents in drinking water. Geneva: WHO. p. 70.

World Health Organization (WHO). 2003. Emerging Issues in Water and Infectious Disease. Geneva: WHO.

World Health Organization (WHO). 2006. Preventing disease through healthy environments – towards an estimate of the environmental burden of disease. Executive summary.

World Health Organization and The United Nations Children's Fund (WHO/UNICEF). 2005. Water for life – make it happen. Accessed 1 August 2006 from the website: http://www.wssinfo.org/en/welcome.html

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