Detection of *Giardia* and *Cryptosporidium* cysts/oocysts in watersheds and drinking water sources in Brazil urban areas

Maria Tereza Pepe Razzolini, Thaís Filomena da Silva Santos and Veridiana Karmann Bastos

ABSTRACT

The protozoan parasites *Giardia* and *Cryptosporidium* have been described as important waterborne disease pathogens, and are associated with severe gastrointestinal illnesses. The objective of this paper was to investigate the presence of *Giardia* cysts and *Cryptosporidium* oocysts in samples from watershed catchments and treated water sources. A total of 25 water samples were collected and examined according to the US EPA—Method 1623, 2005, consisting of 12 from drinking water and 13 from raw water. Positive samples from raw water for *Giardia* cysts and *Cryptosporidium* oocysts were 46.1 and 7.6%, respectively. In finished water, positive samples were 41.7% for *Giardia* cysts and 25.0% for *Cryptosporidium* oocysts. Concentrations of *Giardia* cysts found in raw water samples ranged from "not detected" to 3.4 cysts/L, whereas concentrations of *Cryptoporidium* oocysts ranged from "not detected" to 0.1 oocysts/L. In finished water, *Giardia* concentrations ranged from "not detected" to 0.06 cysts/L, and *Cryptosporidium,* from "not detected" to 0.01 oocysts/L. Concentrations of *Giardia* cysts and *Cryptosporidium* oocysts were not high in the samples analyzed. Nevertheless, the results of this study highlight the need to monitor these organisms in both raw and drinking water.

Key words | *Cryptosporidium*, drinking water, *Giardia*, sanitary quality, surface water

Maria Tereza Pepe Razzolini
(corresponding author)
Thaís Filomena da Silva Santos
Veridiana Karmann Bastos
School of Public Health, University of São Paulo,
Av. Dr Arnaldo, 715 - 1° andar,
01246-904, São Paulo SP,
Brazil
Tel.: +55 11 3061 7888
Fax: +55 11 3061 7732
E-mail: *razzolini@usp.br*

INTRODUCTION

Water-related diseases are a major cause of morbidity and mortality worldwide (WHO 2003) and bring not only economic consequences but also social costs. The protozoan parasites *Giardia* and *Cryptosporidium* have been described as important waterborne-disease pathogens, and are associated with severe gastrointestinal diseases. Karanis *et al.* (2007) conducted a study showing 325 water-associated outbreaks of parasitic protozoans reported worldwide and verified that *Giardia duodenalis* and *Cryptosporidium parvum* were responsible for the largest number of recorded outbreaks.

These protozoans can be detected in several water sources including wastewaters, surface waters, spring waters, recreational waters, ground waters and drinking waters (Solo-Gabriele *et al.* 1998; Franco *et al.* 2001; Rose *et al.* 2002; Hachich *et al.* 2004; Briancesco & Bonadonna 2005; Ryu & Abbaszadegan 2008; Muchiri *et al.* 2009).

An important issue related to the presence of *Giardia* and *Cryptosporidum* cysts/oocysts in water which should be taken into account refers to some of the characteristics of these protozoans, such as resistance to conventional methods of disinfection, high persistence in the environment and their low infectious dose. For these reasons, the presence of *Giardia* and *Cryptosporidium* cysts/oocysts in water sources represents a major public health concern.

In Brazil, some workers have studied the occurrence of these protozoans in water sources. The frequency of

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Cryptoporidium obtained by Franco *et al.* (2001) in water samples from a river was 60.8%. Hachich *et al.* (2004) reported the occurrence of *Giardia* and *Cryptosporidium* cysts/oocysts at 27 and 2.5%, respectively, in samples from watersheds. Another Brazilian study was conducted by Machado *et al.* (2005) in two different sites with the purpose of assessing the presence of *Giardia* and *Cryptosporidium* cysts/oocysts both in raw and treated waters. In the first site it was observed that 50% of raw water samples were positive for *Cryptosporidium,* and 100% for *Giardia,* whereas the presence of these parasites was not detected in treated water. The presence of *Cryptosporidium* oocysts and the frequency for *Giardia* cysts reached 18%. In the second site, *Cryptosporidium* oocysts were not detected in samples from raw water nor in treated water samples, whereas in raw water samples the frequency of *Giardia* cysts was 33%.

The purpose of this study was to investigate the presence of *Giardia* and *Cryptosporidium* cysts/oocysts in samples from watershed catchments and treated water delivered from a Water Treatment Plant in SE Brazil.

METHODS

Area characterization

Important watershed catchments were chosen in Brazil's Southeast regions comprising Water Resources Management Units (WRMU). This system of watershed catchment is responsible for delivering, after conventional treatment, drinking water for millions of people. It is enclosed in a metropolitan area which is undergoing a rapid population expansion, with subnormal urbanization and inadequate sanitation facilities provided that could directly impact on the quality of water. The area that encompasses this watershed has drinking water supply concerns; sanitation conditions in the area are poor, and include a lack of collection, treatment and sanitary disposal of wastewater and inadequate disposal of solid waste.

Sampling and analysis

Raw water and drinking water samples were collected on a monthly basis during an entire year (March 2008/2009). Sampling was performed according to the US EPA–Method 1623 (USEPA 2005). Ten liters of raw water for each sample site was collected utilizing sterile disposable vessels. A volume of 400 L of treated water was collected *in situ* and filtered through an IDDEX FiltaMax® filter with a controlled flow between 1 and 4 L/minute. The sample bottles and filter containing concentrated treated water were kept chilled during transportation and analyzed within a 24-hour period. The occurrence of rainfall within a 24-hour period of collection was recorded.

A total of 25 water samples, 12 from drinking water and 13 from raw water, were examined according to the US EPA Method 1623 (USEPA 2005). Afterwards, both kinds of samples were submitted to a 5-minute elution in a stomacher with 600 mL of phosphate-buffered saline (PBS) solution. This procedure was repeated twice. The eluate obtained was centrifuged at 2,500 rpm for 15 minutes until a pellet was obtained. The immunomagnetic separation (IMS) step was carried out with the use of the commercial kit Dynabeads GC-Combo (Dynal Biotech, UK) as described in Method 1623 (USEPA 2005). Following this step, samples were stained with fluorescent antibody A100FLR-1X (Waterborne, USA) according to manufacturer's instructions, and DAPI staining solution was applied. Finally, cysts and oocysts were enumerated with the use of an epifluorescence microscope (BX-60, Olympus Optical Co., Ltd, Tokyo, Japan). Enumeration of *Giardia* cysts and *Cryptosporidium* oocysts was obtained from FITC, DAPI and DIC examination. The results were expressed in cysts/oocysts/L. Both positive and negative controls were also prepared.

Initial precision and recovery

In order to measure the recovery rate using the method mentioned above, five independent spiking experiments were performed by filtering 10 liters of contaminated reagent grade water. Spiking suspensions with 100 *Giardia* cysts and 100 *Cryptosporidium* oocysts were used (EasySeed™, BTF Precise Microbiology, Australia). The analytical procedure was the same as described above. The recovery rate (R%) was calculated according

to the following equation:

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

The mean rate of recovery was calculated as the standard deviation (SD) and relative standard deviation (RSD) for *Giardia* and *Cryptosporidium*.

Quality control

In order to determine the interference of the matrix with the methods used, a volume of 10 L-sample from raw water was collected and contaminated by using spike suspensions with 100 cysts of *Giardia* and 100 oocysts of *Cryptosporidium* (EasySeed™, BTF Precise Microbiology, Australia). The analytical procedure was the same as described above. This procedure was performed only once, since the frequency recommended by EPA is 1 Matrix Spiked (MS) sample per 20 field samples; for raw water, 13 samples were collected. The recovery rate (R%) was calculated according to the following equation:

$$R = 100 \times [(\text{Nsp}/\text{Ns})/T]$$

where:

R = recovery rate
Nsp = number of cysts/oocysts counted on spiked sample
Ns = number of cysts/oocysts counted on unspiked sample
T = value of cysts/oocysts spiked.

The mean recovery rate was calculated as the RSD for *Giardia* and *Cryptosporidium*.

RESULTS AND DISCUSSION

The recovery rate obtained by the method ($n = 5$) was 33.6%, SD was 5.1 and RSD was 15.2% for *Giardia* cysts; and 28.6% for *Cryptosporidium* oocysts; SD was 3.9 and RSD was 13.6%. According to the USEPA (2005), the initial precision and recovery criteria for *Giardia* and *Cryptosporidium* should range from 24 to 100%, and the maximum RSD for *Giardia* cysts should be 49%, and for *Cryptosporidium* oocysts, 55%. Data obtained from this study are in accordance with USEPA's recommendations.

As for spiked and unspiked water samples, the recovery rate obtained was 34% for *Giardia* and 30% for *Cryptosporidium*.

The rates relative to positive samples are shown in Figure 1. Raw water positive samples for *Giardia* cysts and *Cryptosporidium* oocysts were 46.1% and 7.6%, respectively. Concerning finished water samples, positive samples registered 41.7% for *Giardia* cysts and 25.0% for *Cryptosporidium* oocysts. In 16.7% of the drinking water samples analyzed only the occurrence of *Giardia* cysts was detected, while in raw water samples this rate registered 38.4%. Occurrence of *Giardia* cysts was higher than occurrence of *Cryptosporidium* oocysts on both water sources assessed.

A similar study was conducted by Quintero-Betancourt & Botero de Ledesma (2000) with the purpose of evaluating occurrence of *Giardia* cysts and *Cryptoporidium* oocysts

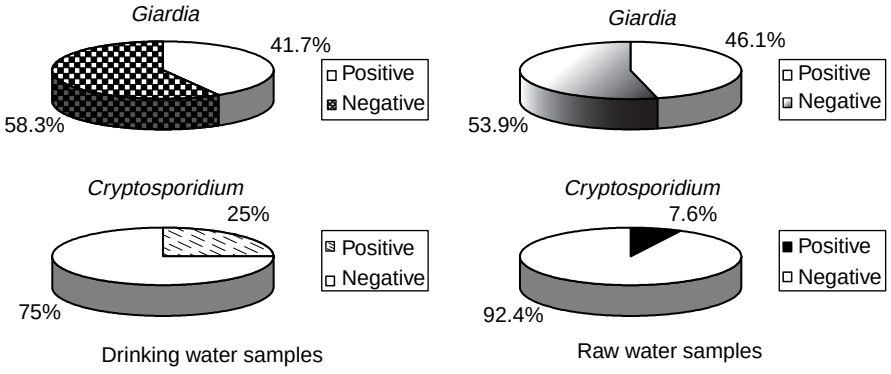

Figure 1 | Percentage of positive samples referring to *Giardia* cysts and *Cryptosporidium* oocysts present in raw and drinking water samples analyzed.

both in raw and finished water. The samples assessed came from a drinking water treatment plant in Maracaibo, Venezuela. The results showed that *Giardia* cysts occurred at a ratio of 20 and 11% in raw and finished water samples, respectively. *Cryptosporidium* oocysts were present in 40% of raw water samples and in 87.5% of finished water samples. On the other hand, Briancesco & Bonadonna (2005) did not find these parasite protozoans in the drinking water samples assessed.

Concentrations of *Giardia* and *Cryptosporidium* cysts/oocysts obtained in this study are summarized in Table 1, it is important to say that the method used to determine the concentrations of the parasites does not allow knowing about the viability and the infectious condition of these organisms. Concentration of *Giardia* cysts found in raw water samples ranged from "not detected" to 3.4 cysts/L, whereas concentration of *Cryptoporidium* oocysts ranged from "not detected" to 0.1 oocysts/L. In finished water, *Giardia* concentration ranged from "not detected" to 0.06 cysts/L, whereas *Cryptosporidium* concentration ranged from "not detected" to 0.01 oocysts/L.

Machado *et al.* (2005) found higher concentrations of these parasite protozoans in raw and treated water in two different water supply sources compared to our findings. The sites assessed were Itapecerica system and Pará system in Minas Gerais, Brazil. Concentration of *Giardia* cysts and *Cryptosporidium* oocysts in raw water samples from the Itapecerica system ranged from 2 to 250/10 L and from 0 to 3/10 L, respectively. Concerning treated water, concentrations ranged from 0 to 0.1/10 L for *Giardia* cysts; *Cryptosporidium* oocysts were not detected. In the Pará system, concentration of *Giardia* cysts ranged from 0 to 23/10 L; *Cryptosporidium* oocysts were not present, while in treated water neither *Giardia* cysts nor *Cryptosporidium* oocysts were detected. On a similar study, Carmena *et al.* (2007) assessed several water samples coming from raw and treated water sources, the latter undergoing distinct types of treatment, as follows: (a) conventional process (coagulation, flocculation, sedimentation, filtration and disinfection); and (b): simplified treatment (quick filtration and/or disinfection process only). *Giardia* and *Cryptosporidium* cysts/oocysts were not detected in finished water systems undergoing conventional treatment; after simplified treatment, concentration of *Giardia* cysts ranged from 0 to 184/100 L, whereas concentration of *Cryptosporidium* oocysts ranged from 0 to 25/100 L.

Table 1 | Concentration of *Giardia* and *Cryptosporidium* cysts/oocysts in raw and drinking water samples analyzed

Sample	Drinking water		Raw water	
Sampling Month	*Giardia* cysts /L	*Cryptosporidium* oocysts /L	*Giardia* Cysts/L	*Cryptosporidium* oocysts /L
Mar/08	0.0	0.0	0.0	0.0
Apr/08	0.0025	0.0050	0.0	0.0
May/08	0.0	0.0	0.0	0.0
Jun/08	0.0025	0.0025	0.100	0.0
Jul/08	0.0050	0.0	0.0	0.0
Aug/08	0.0050	0.0	0.100	0.0
Sep/08	0.0	0.0	0.100	0.100
Oct/08	NP	NP	3.4	0.0
Nov/08	0.0	0.0	0.0	0.0
Dec/08	0.0600	0.0100	0.400	0.0
Jan/09	0.0	0.0	0.300	0.0
Feb/09	0.0	0.0	0.0	0.0
Mar/09	0.0	0.0	0.0	0.0

NP = Not performed.

In this study, there was not found a seasonal distribution of cysts of *Giardia* and oocysts of *Cryptosporidium* in the sampled waters analyzed.

As it can be seen, occurrence of *Giardia* and *Cryptosporidium* cysts/oocysts in water sources is a public health concern. With this regard, the Brazilian Regulation issued by the Brazilian Ministry of Health, Portaria 518/2004 (Brasil 2004) recommends investigation of the presence of these organisms in drinking water; so far, no maximum value for these parameters has been established. Although it is not an obligation to be complied with by water producing companies, it can be considered a way to show the importance of controlling these organisms in water systems, in order to ensure water sanitary conditions and quality, so as to protect and promote the population's health.

CONCLUSIONS

Even though concentrations of *Giardia* cysts and *Cryptosporidium* oocysts were not high in the samples analyzed, the results brought by this study highlight the need to monitor the presence of these organisms both in raw and drinking water, so as to provide a basis for intervention measures and protect the consumer's health. One aspect that should be taken into consideration is the high volume of drinking water samples that would have to be collected and analyzed; no doubt, this is a hard task to be accomplished in large Water Treatment Plants. According to Briancesco & Bonadonna (2005), an alternative approach would be the establishment of a surrogate parameter. Another aspect to be taken into account would the protection of raw water supplies against sources of contamination, such as disposable waste and sewage discharges. These kinds of contaminators are responsible for carrying pathogenic organisms into water bodies, jeopardizing the quality of the water supplied and endangering human health, besides raising water treatment costs.

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