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# Environmental Monitoring of Opportunistic Protozoa in Rivers and Lakes in the Neotropics Based on Yearly Monitoring

**Sônia F.O. Santos · Hugo D. Silva · Edson S. Souza Júnior · Carlos E. Anunciação · Elisângela P. Silveira-Lacerda · Cesar A.S.T. Vilanova-Costa · Marco T.A. Garcíazapata**

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**Abstract** The goal of this study was to standardise and use parasitological and molecular techniques in the analysis and seasonal monitoring of opportunistic protozoa in water from fluvial systems for human usage in the municipality of Goiânia, the capital of the state of Goiás, in the mid-western region of Brazil. We focused on *Cryptosporidium* sp. *Cyclospora cayetanensis*, *Isopora belli*, *Microsporidia* and *Giardia lamblia*. Water samples were collected monthly from February 2006 to January 2007 and concentrated using vacuum filtration and a positively charged membrane. Several methods were used to identify the different protozoa of interest. To detect coccidia (*Cryptosporidium* sp., *Isospora belli*, and *Cyclospora cayetanensis*), we used a Kinyoun hot-staining method and a modified Ziehl–Neelsen technique. Enteral microsporidia were detected by a hot-chromotrope technique while a MERIFLUOR® *Cryptosporidium* kit was used to confirm the presence of *Cryptosporidium* sp. Finally, we used PCR to detect *Cryptosporidium parvum/hominis*. Water is of vital importance to living beings; however, due to anthropic action, several microorganisms are disseminated into aquatic environments. Among them are opportunistic protozoa that infect mainly immunodepressed and immunosuppressed individuals, children and elderly people. These protozoa pose a significant health hazard. Nevertheless, the presence of these pathogens is underestimated because they are not considered during routine environmental monitoring. In our study, we were able to observe the presence of *Giardia* cysts, *Cryptosporidium* sp. and *Cryptosporidium parvum* oocysts in the bodies of water monitored in this research.

**Keywords** Environmental monitoring · Lakes · Neotropics · Opportunistic protozoa · River

S.F.O. Santos · H.D. Silva · E.S. Souza Júnior · C.E. Anunciação · E.P. Silveira-Lacerda · M.T.A. Garcíazapata (✉)
Núcleo de Pesquisas em Agentes Emergentes e Re-emergentes, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Caixa Postal 12911, Setor Leste Vila Nova, Goiânia, GO 74643-970, Brazil
e-mail: nupereme@gmail.com

C.A.S.T. Vilanova-Costa
Laboratório de Genética Molecular e Citogenética, Instituto de Ciências Biológicas I, Universidade Federal de Goiás, Campus Samambaia, Caixa Postal 131, Goiânia, GO 74001-970, Brazil

## Introduction

Water is a natural resource of vital importance to living beings. Due to anthropic action, several microorganisms are disseminated into aquatic environments. Opportunistic protozoa, such as coccidia (*Cryptosporidium parvum*, *Isospora belli*, and *Cyclospora* sp.), microsporidia, an emergent pathogen which infects the gastrointestinal tract (Gomes et al. 2002), and *Giardia lamblia* which causes diarrhea (States et al. 1997), can be spread through water.

In developing countries, over one billion people do not have access to clean, properly treated water, and approximately three billion people do not have access to adequate sanitary facilities (Kraszewski 2001). This lack of clean water is probably a consequence of increased environmental degradation, depletion of water resources, and constant contamination of bodies of water with wastewater and industrial effluents (Pedro and Germano 2001), which cause microorganisms from soil, faeces, decomposing organic matter, and other pollutant sources to spread into water.

The magnitude of the danger that enteric protozoa pose to public health should be emphasised because of their high

prevalence, cosmopolitan distribution, and deleterious effects on an individual's nutritional status and immune system. Although children are the most susceptible individuals to these pathogens, people from other age groups are also affected (Geldreich 1996), mainly in Neotropical areas.

Cryptosporidiosis is an important parasitic disease that can become a public health problem (Cimerman et al. 1999). The main modes of *Cryptosporidium* sp. transmission are frequently associated with contaminated water, either treated or non-treated superficial water, treated water contaminated along the distribution systems, or inappropriately treated water, usually treated only by a simple chlorination method (Solo-Gabriele and Neumeister 1996).

Among coccidia, *Isospora belli* and *Cyclospora* sp. are very important in terms of public health since they are pathogens frequently associated with gastroenteritis in immunodepressed patients (Meamar et al. 2007; Turk et al. 2004). Currently, given the increased number of elderly people and immunocompromised patients in the population worldwide, it is highly necessary to study the occurrence of these pathogens in recreational and treated water, important sources of transmission.

Giardiasis and cryptosporidiosis are also common infections of domestic and wild animals, which shed a large number of cysts and oocysts in the environment (Cacciò et al. 2003). Not only are *Giardia* cysts less resistant than *Cryptosporidium* oocysts (Sterling 1990), they have also been shown to survive in water for up to 2 months at temperatures as low as 8°C (Cacciò et al. 2003). In 1996, the American Environmental Protection Agency started a program to identify, standardise, and validate new methods for the detection of *Giardia* cysts and *Cryptosporidium* sp. oocysts in water environments. Programs to monitor these pathogens in water have been spontaneously carried out in some countries such as the United States and the United Kingdom (Clancy et al. 1999). Since this time, methods 1622 and 1623 (USEPA 1999) have been used as reference procedures in the United States (Clancy et al. 2003). Regarding microsporida, only the species *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are significant to public health. They are the most prevalent microsporidia identified in humans (Didier and Weiss 2006) and other mammals (Keeling and Fast 2002). The spores are the infective agent (Barbosa et al. 2009) because they are resistant to environmental conditions (Stine et al. 2005) and to the usual water treatment procedures (Hoffman et al. 2003). Consequently, transmission involves waterborne routes (Karanis et al. 2007).

Microsporida and coccidia are opportunistic pathogens, and the vast majority of studies report their presence in immunocompromised or HIV-positive patients (Didier et al. 2004; Franzen et al. 2004). It should be emphasised that the detection of cysts and oocysts in superficial water is a crucial component to control these pathogens. However, current methods are highly variable in terms of recovery efficiency of *Cryptosporidium* sp. oocysts and *Giardia* cysts (Hsu et al. 2001). Therefore, other methods are needed to guarantee that water potability achieves a higher degree of reliability.

The Brazilian Health Ministry recommends including methods that detect *Giardia* cysts and *Cryptosporidium* sp. oocysts in the attempt to reach a standard in which the water supplied to the population is free of these pathogens (Brasil 2004). However, in Brazil, routine monitoring of protozoa is not performed in bodies of water used for human consumption. The environmental research involving the detection of opportunistic protozoa such as *Giardia* and *Cryptosporidium* sp. has focused on southern and southeastern Brazil (Santos et al. 2004; Carvalho et al. 2006; Lallo et al. 2009). In the state of Goiás, located in the midwestern region of Brazil, no studies have indicated the occurrence of these protozoa in water.

The goal of this study was to standardise and use parasitological and molecular techniques for the analysis and seasonal monitoring of opportunistic protozoa in water from fluvial systems for human usage in the municipality of Goiânia, the capital of the state of Goiás, in the midwestern region of Brazil, focusing on *Cryptosporidium* sp., *Cyclospora cayetanensis*, *Isopora belli*, *Microsporidia* and *Giardia lamblia*.

## Materials and Methods

### Study Area and Sampling

For a period of 12 months (from February 2006 to January 2007), water samples were collected monthly from six points in the city of Goiânia, resulting in a total of 72. Samples were collected from the following bodies of water: (a) Meia Ponte River—in this river, two sites were selected for sampling. The first site was located 2 km after the emission of wastewater treated by the municipal wastewater treatment plant of Goiânia, located at 16°37′40.94″S latitude and 49°16′13.41″W longitude ($MP^1$). The second site was located at 16°38′22.39″S latitude and 49°15′50.68″W longitude ($MP^2$); (b) João Leite River—In this river, two sites were selected for sampling. One site was located at 16°37′40.18″S latitude and 49°14′26.08″W longitude ($JL^1$), where this body of water reaches Goiânia, and the other site was located at 16°19′37.52″S latitude and 49°13′24.53″W longitude ($JL^2$), before Goiânia; (c) Vaca Brava Park Lake—This park encompasses an area of approximately 72,700 $m^2$, distributed among green areas, walking and jogging tracks, sports courts, playgrounds, and exercise facilities. The site selected for sampling is located at 16°42′31.18″S latitude and 49°16′15.67″W longitude (VB); (d) Bosque dos Buritis Park Lake—Bosque dos Buritis is an urban park encompassing an area of approximately 125 $m^2$ with three artificial lakes supplied by Buriti stream.

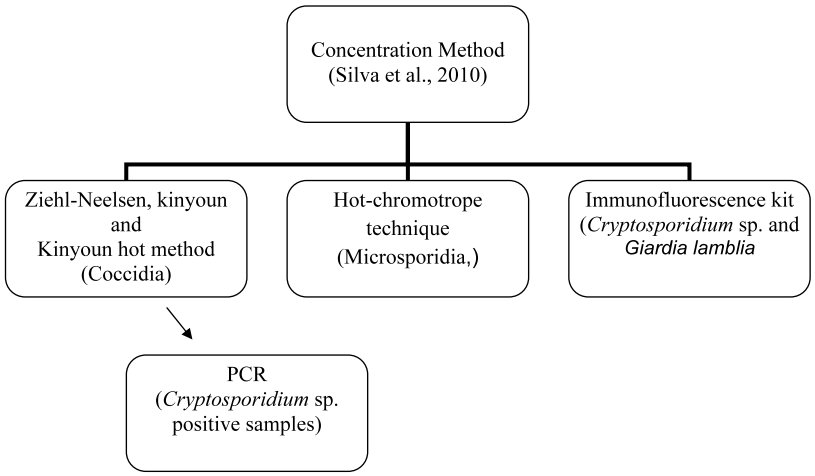


**Fig. 1** Methodology used for the detection of protozoa in water from Goiânia, Goiás, Brazil

The site selected for sampling is located at 16°40′58.51″S latitude and 49°15′38.35″W longitude (BB).

Each sample was collected in a clean 10 l polyethylene container from one point in the centre of the body of water ∼20 cm under the surface and sent within 2 h to the Genetics and Molecular Diagnostic Laboratory of the Federal University of Goiás.

The samples were concentrated by the adsorption-elution method described by Silva et al. (2010). Briefly, sample water was filtered under vacuum using qualitative filter paper to remove excess organic matter and immediately submitted to microfiltration using a positively charged nylon membrane. The material adsorbed to the membrane was eluted by vigorous manual agitation with 5 ml of TE low buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA) and 0.02% Tween-20. Figure 1 shows a flow chart of all methodologies used in our study.

## Parasitological Analysis

### *Staining Techniques*

Aliquots of 10 µl of concentrated material were smeared onto two series of two slides each using a modified Ziehl–Neelsen-stain technique (ZN) and the Kinyoun hot-staining method, fixed in 70% alcohol, and processed for specific detection of coccidia (*Cryptosporidium* sp., *Isospora belli*, and *Cyclospora cayetanensis*).

To detect enteral microsporidia, we used aliquots of 10 µl of concentrated material (onto two series) and the modified hot-chromotrope technique (HT) (Kokoskin et al. 1994).

All the slides were analysed in duplicate using a common optical microscope with an immersion 100X oil objective.

Microscopy slides were positively stained by ZN and the HT as a standard for parasitological identification. The oocysts of *Cyclospora* sp. (8 to 10 µm) have morphotypes similar to *Cryptosporidium* sp. (3–6 µm), emphasising the need to measure the oocyte diameter to differentiate between the two species (Gonzáles-Ruiz and Bendall 1985). Morphometric analysis ($T$) of oocysts was done by measuring the oocyte diameter (model ERNST LEITZ, Wetzlar, Germany) with an optical microscope, taking into account the length ($C$) and width ($L$), and applying the formula $T = C/L$ (size) (Elliot et al. 1999; Sidião and Garcia-Zapata 2006).

### *Direct Immunofluorescence Assay Kit*

MERIFLUOR® immunofluorescence assay was prepared according to the manufacturer's instructions and one aliquot of each sample concentrate was tested to homologous monoclonal antibodies for the simultaneous detection of *Cryptosporidium* sp. and *Giardia lamblia*. Each sample was analysed in duplicate; however, due to a shortage of reagents, this technique was applied to 50% (36/72) of the samples taken at random for the confirmation of positive samples detected by parasitological methods. For all immunological assays, positive and negative controls (MERIFLUOR® Test kit) were used.

## Molecular Analysis

### *DNA Extraction*

A method modified from Boom et al. (1990) based on cation exchange was used simultaneously with the phenol-chloroform-isoamyl alcohol method (Sambrook and Russel 2001) to extract the genetic material, using aliquots of 20 and 500 µl of the material concentrated by filtration, respectively.

### *DNA Amplification*

DNA was detected using Nested-PCR, a variation of the polymerase chain reaction (PCR). Nested-PCR was applied only to samples that tested positive by parasitological methods for *Cryptosporidium* sp. and/or samples for which the parasitological results were inconclusive.

Three primer pairs were used: XIAF/XIAR (*Cryptosporidium* sp.), which flanked a region of approximately 1325 bp (Xiao et al. 1999); AWA995f/AWA1206R *(Cryptosporidium* sp.*),* which amplified a region of approximately 211 bp (Awad-el-Kariem et al. 1994); and LAX469F/LAX869R (*C. parvum/C. hominis),* which amplified a chromosomal region of approximately 451 bp (Laxer et al. 1991).

Conventional PCR was carried out using primers XIAF/XIAR. Two aliquots were taken from the resulting product, one for detection of protozoan genera via Nested-PCR, using primers AWA995f/AWA1206R, and the other for the detection of *C. parvum/C. hominis* using primers LAX469F/LAX869R.

PCR was performed using primers XIAF/XIAR and 28 µl extracted DNA in a final volume of 50 µl with the following reagents: 5.0 µl buffer 10X, 2.0 mM Mg, 200 µM dNTP (dATP, dCTP, dTTP, and dGTP), 0.5 µM of each primer, and 1.25 U Taq DNA polymerase. The reaction conditions were as follows: an initial denaturation step for 4 min followed by 35 cycles of a denaturation step at 94°C for 1 min, annealing at 55°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 7 min.

The AWA995f/AWA1206R primers were used to amplify 14 µl DNA of the product from the XIAF/XIAR primer amplification in a final volume of 25 µl with the following reagents: 2.5 µl buffer 10X, 1.5 mM Mg, 200 µM dNTP (dATP, dCTP, dTTP, and dGTP), 0.5 µM of each primer, and 1.25 U Taq DNA polymerase. The reaction conditions were as follows: an initial denaturation step for 7 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 3 min, and a final extension at 72°C for 7 min.

The LAX469F/LAX869R primers were used to amplify 14 µl DNA from the amplification by the XIAF/XIAR primers. The reaction was performed in a final volume of 25 µl with the following reagents: 2.5 µL buffer 10X, 2.0 mM Mg, 200 µM dNTP (dATP, dCTP, dTTP, and dGTP), 0.5 µM of each primer, and 1.25 U Taq DNA polymerase. The reaction conditions were as follows: an initial denaturation step for 7 min followed by 40 cycles of a denaturation step at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 7 min.

Milli-Q water aliquots were included as a negative control, and *Cryptosporidium* sp. DNA was used as a positive control for all amplification reactions. Amplification products were separated on a 1.5% agarose gel (UltraPureTM Agarose, Invitrogen) by electrophoresis in 1X Tris-borate-EDTA buffer (TBE) and observed by ethidium bromide staining. Fragments were compared to a commercially available size standard (100 bp DNA ladder, Invitrogen, Brazil). Samples containing 211-bp and 451-bp bands were considered positive.

### Meteorological Data

The meteorological data (monthly environmental temperature, and relative humidity) were collected by the Division of Rural Engineering, School of Agronomy of the Universidade Federal de Goiás during the period of collects.

### Statistical Analyses

The results obtained in this study were analysed using the software Microsoft Office Excel 2007. Statistical analyses were performed using the logistic regression analysis to infer the contribution of environmental variables such as humidity and temperature for the detection of protozoa. Statistical significance level was set at $p < 0.05$ using the Statistical Package for the Social Sciences (SPSS) version 10.0.

## Results

Among the 72 samples processed, 8.33% (6/72) tested positive for the protozoa of interest.

Using the MERIFLUOR® direct immunofluorescence assay kit, six samples tested positive for protozoa (four for *Giardia* and two for *Cryptosporidium* sp.). Two of these samples were collected at $JL^2$ in September and November, one at $JL^1$ in August, two at $MP^1$ in July, and one at VB in September (Tables 1 and 2).

Using the Kinyoun hot-staining method and the hot-chromotrope method to detect coccidia, no samples tested positive. Table 1 shows the results of each test carried out for the six sampling sites.

Using the modified Ziehl–Neelsen stain technique, 2.7% (2/72) samples tested positive for coccidia. The presence of *Cryptosporidium* sp. was detected in two samples and confirmed by the MERIFLUOR® direct immunofluorescence assay kit.

We confirmed that the samples belonged to the genus *Cryptosporidium* sp. using primers AWA995f/AWA1206R, and showed that the sample collected in July contained *C. parvum* or *C. hominis* using primers LAX469F/LAX869R, since the latter detect the region conserved for these two species.

Microscopy was used to differentiate the two types of *Cryptosporidium* oocysts detected by Nested-PCR. Figure 2 shows a *Cryptosporidium* sp. oocyst and a *Cryptosporidium parvum* oocyst. Positive samples for *Isospora belli*, *Cyclospora cayetanensis*, and *Microsporidia* were not detected by any of the parasitologic methods applied.

The average temperature at the time that protozoa were detected was 26.8°C, while in the period when no pathogen was detected, it was 25.6°C. The logistic regression analysis for temperature revealed $p = 0.262$ and OR = 1.227 (Table 3). Average relative humidity in the period of protozoa

**Table 1** Results from the six sampling sites and the methods used to analyse the twelve samples from each site, a total of 72 samples

| Sampling site | Method | | | |
|---|---|---|---|---|
| | Ziehl–Neelsen | Kinyoun | Hot-chromotrope | MERIFLUOR® |
| MP[1] | *C. parvum*[a] | Negative | Negative | *Giardia lamblia* |
| MP[2] | Negative | Negative | Negative | Negative |
| JL[1] | Negative | Negative | Negative | *Giardia lamblia* |
| JL[2] | Negative | Negative | Negative | *Giardia lamblia*[b] |
| VB | *Cryptosporidium* sp.[a] | Negative | Negative | Negative |
| BB | Negative | Negative | Negative | Negative |

[a]Confirmation by PCR and MERIFLUOR®
[b]Two positive samples
MP[1]: Meia Ponte River, at 16°37′40.94″S latitude and 49°16′13.41″W longitude
MP[2]: Meia Ponte River at 16°38′22.39″S latitude and 49°15′50.68″W longitude
JL[1]: João Leite River, at 16°37′40.18″S latitude and 49°14′26.08″W longitude
JL[2]: João Leite River, at 16°19′37.52″S latitude and 49°13′24.53″W longitude
VB: Vaca Brava Park Lake, at 16°42′31.18″S latitude and 49°16′15.67″W longitude
BB: Bosque dos Buritis Lake, at 16°40′58.51″S latitude and 49°15′38.35″W longitude

**Table 2** General distribution of samples in the six sites according to the presence of protozoa, from February 2006 to January 2007

| Protozoa | Sampling site | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MP[1] | | MP[2] | | JL[1] | | JL[2] | | VB | | BB | |
| | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % |
| Negative | 12 | 100.0 | 10 | 83.4 | 11 | 91.7 | 10 | 83.3 | 11 | 91.7 | 12 | 100.0 |
| *Cryptosporidium* sp. | 0 | 0.0 | 0 | 83.4 | 0 | 0.0 | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 |
| *Cryptosporidium parvum* | 0 | 0.0 | 1 | 8.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| *Giardia lamblia* | 0 | 0.0 | 1 | 8.3 | 1 | 8.3 | 2 | 16.7 | 0 | 0.0 | 0 | 0.0 |
| Total | 12 | 100.0 | 12 | 100.0 | 12 | 100.0 | 12 | 100.0 | 12 | 100.0 | 12 | 100.0 |

MP[1]: Meia Ponte River, at 16°37′40.94″S latitude and 49°16′13.41″W longitude
MP[2]: Meia Ponte River at 16°38′22.39″S latitude and 49°15′50.68″W longitude
JL[1]: João Leite River, at 16°37′40.18″S latitude and 49°14′26.08″W longitude
JL[2]: João Leite River, at 16°19′37.52″S latitude and 49°13′24.53″W longitude
VB: Vaca Brava Park Lake, at 16°42′31.18″S latitude and 49°16′15.67″W longitude
BB: Bosque dos Buritis Lake, at 16°40′58.51″S latitude and 49°15′38.35″W longitude

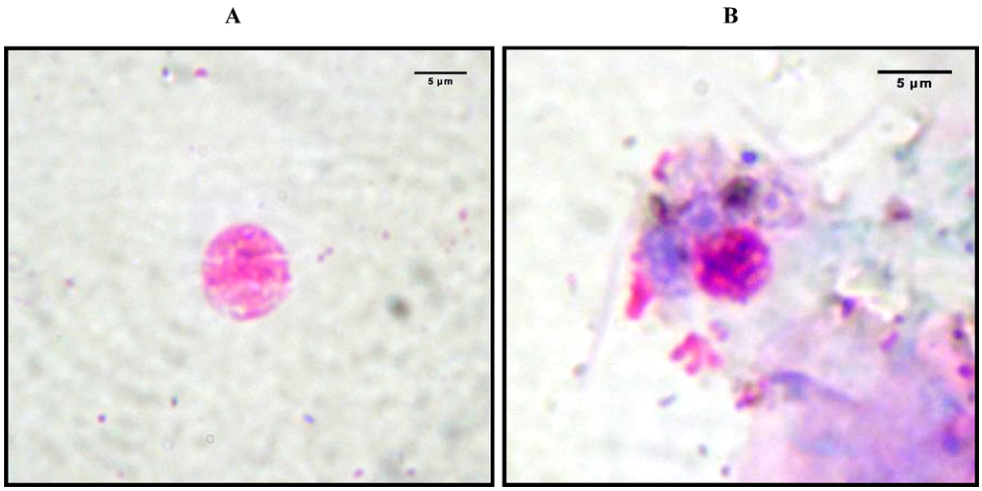


**Fig. 2** *Cryptosporidium* oocysts stained by the modified Ziehl–Neelsen technique and confirmed by the MERIFLUOR® direct immunofluorescence assay kit and PCR (magnified 100X). (**A**): *Cryptosporidium* sp. oocyst; (**B**): *Cryptosporidium parvum* oocyst

**Table 3** Mean and standard deviation of temperature and relative humidity according to the presence of protozoa in the bodies of water sampled in Goiânia (logistic regression analysis)

| Protozoa | *n* | Mean | Standard deviation | *p* | OR |
|---|---|---|---|---|---|
| | | | Temperature | | |
| Negative | 66 | 25.6 | 2.5 | | |
| Positive | 6 | 26.8 | 1.5 | 0.262 | 1.227 |
| | | | Relative humidity | | |
| Negative | 66 | 56.3 | 16.0 | | |
| Positive | 6 | 42.3 | 14.6 | 0.060 | 0.944 |

occurrence was 42.3%, whereas in the period when these pathogens were not detected, it was 56.3%. This difference is not significant because the logistic regression analysis for relative humidity resulted in $p = 0.060$ and OR = 0.944 (Table 3).

## Discussion

During monitoring, sites $MP^1$, $JL^1$, $JL^2$, and VB presented one or more positive samples for the protozoan under study. Also, we could observe people collecting water for human consumption, bathing, washing clothes, and even fishing in these sites. This fact is highly worrying because various waterborne diseases that are related not only to opportunistic protozoa but also to several other biological agents can be transmitted through these contaminated bodies of water (Corso et al. 2003).

We detected low recovery efficiency of opportunistic protozoa cysts and/or oocysts, which might be related to environmental influence and physical-chemical factors, such as water pH and turbidity (Vesey et al. 1993; Zuckerman et al. 1999). The influence of physical-chemical factors on sampling has been reported by other researchers (Fricker and Crabb 1998; McCuin and Clancy 2003). Adverse environmental factors have been proved to alter the morphology of cysts and oocysts (Ongerth and Stibbs 1987), thus justifying the low percentage of positive samples in this study using parasitological methods. According to Fricker and Crabb (1998), other factors might have influence as well, such as the concentration of *Cryptosporidium* sp. oocysts, which is based almost exclusively on particle size. Furthermore, large capacity water purification, especially in samples from rivers where the volume of leakage is accentuated, may have contributed to the low rate of positive samples. Moreover, the points monitored had no active contamination, and purified water may have contributed to it.

As for the lakes, a low positive result was expected because these bodies of water are not the final destination of the raw, non-treated sewage of Goiânia.

Despite the low frequency of positive findings, the data are crucial to public health because these pathogens can affect the health of the population that frequent the lakes or that use river water for use and consumption.

It appears that there is little information about the climatic influences on the detection of opportunistic protozoa. Temperature has been considered a factor for protozoa and autochthonous microorganism survival in rivers (Medema and Schijven 2001). In our study, the influence of temperature and relative humidity on the occurrence of protozoa was not statistically significant. Machado et al. (2009), by performing environmental monitoring of *Cryptosporidium* sp. in water, found a greater rate of positive results during the rainy season.

The state of Goiás has two well-defined seasons: a rainy season (October to March) and a dry season (April to the beginning of September), with low precipitation.

Temperature and relative humidity registers were employed to correlate the presence of protozoan to these two seasons and therefore generate data that may be used in studies to trace eco-epidemiologic routes of transmission and eventually propose suitable prophylactic measures against these pathogens. However, in our study, we did not observe a seasonal trend of protozoa occurrence, perhaps due to the small number of positive samples.

The methods used in the present study are in accordance with those recommended for concentration and detection of microorganisms by the *Standard Methods for the Examination of Water and Wastewater* (Clesceri et al. 2005). They are easily applied, do not pose a great risk to the technician, and are low-cost techniques, which can be employed by technicians trained to monitor water for human consumption.

We applied the filtration methodology as recommended by Silva et al. (2010). Vigorous manual shaking does not cause any damage to the filter membrane because it is made of nylon, a very resistant material. Furthermore, the protozoan cell walls are also resistant, and since the shaking is manual, it does not pose any risk to parasite integrity.

PCR and IFA have the disadvantage of being costly for use in routine environmental monitoring, especially in the Neotropical regions, which lack infrastructure and qualified manpower. Nevertheless, an increasingly growing number of studies have used molecular and immunological methods to detect protozoa in water in Neotropical countries such as Brazil (Franco et al. 2001; Santos et al. 2004; Machado et al. 2009).

In England, Hall and Croll (1997) evaluated the performance of rapid gravity filters using turbidity measurements and particle counts in filtered water as parameters for monitoring and controlling *Cryptosporidium* sp. oocysts as an indicator microorganism, a method similar to the one used in this study.

During this monitoring, it was expected to find *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts because they

possess a rigid cystic membrane that protects them from environmental adversities and allows them to survive over a long time.

The *in vitro* amplification of DNA fragments from *Cryptosporidium* sp. granted more specificity and sensibility than the parasitological methods. Nevertheless, amplification was only possible using Nested-PCR primers (AWA995f/AWA1206R and LAX469F/LAX869R).

Nested-PCR has the advantage of concentrating a smaller quantity of PCR inhibitors (Rigotto et al. 2005). In environmental samples, there are several Taq DNA polymerase inhibitors, such as faecal haemoglobin and phenolic compounds. It was possible to obtain satisfactory amplification with the two methods of DNA extraction applied. Furthermore, these methods are quick and low-cost, although close attention should be paid to the phenol/chloroform method, as it is toxic and corrosive.

Using primers for genus (XIAF/XI and AWA995f/AWA1206R) and posteriorly for species (LAX469F/LAX869R), it was only possible to differentiate *Cryptosporidium* detected at site V up to genus. Nevertheless, the sample collected at site MP[1] was positive for *C. parvum*/*C. hominis*. As *C. parvum* and *C. hominis* oocysts have an average diameter of 5 and 4 μm, respectively, using an optical microscope we concluded that the oocyst found belongs to *C. parvum* because it presents a 5-μm diameter. Molecular detection was applied exclusively for *Cryptosporidium*, because, among the protozoan under study, they are more likely to contaminate humans and therefore are highly important for public health programs. Moreover, it is important to differentiate the species of these pathogens since *C. parvum* contamination of water may have bovine and human origin, whereas *C. hominis* contamination is strictly human. The accurate differentiation between these two species may contribute to a better understanding of their eco-epidemiologic routes of transmission in the bodies of water we monitored during this study, so that more efficient public health programs can be developed.

We emphasise that the methodologies developed can be used for screening and specific detection of protozoa in water for public supply in Neotropical areas. These methods represent a major impact on public health because detection of these pathogens can provide improvements in public sanitation policy, ensuring better quality water for the population.

## Conclusions

- The rivers and lakes of Goiânia are contaminated with opportunistic protozoa (*Cryptosporidium* sp. and *Giardia lamblia*).
- Standardisation and application of parasitological and molecular techniques in the analysis and seasonal monitoring of opportunistic protozoa were successfully carried out for environmental samples.
- During seasonal monitoring of opportunistic protozoa, with emphasis on coccidia (*Cryptosporidium* sp., *Isospora belli*, and *Cyclospora cayetanensis*), it was possible to detect *Cryptosporidium parvum* and *Cryptosporidium* sp. using PCR and Nested-PCR, respectively.
- The parasitological and molecular techniques applied are quick, low-cost, and employable in laboratories that monitor the microbiological quality of water for human consumption.
- The detection of these pathogens will allow better definition of health policies and public sanitation for other protozoa diseases, including the ones potentially transmitted by water and even those of unknown routes.

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