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Assessment of pathogenic protozoa in lentic and lotic compartments of a tropical reservoir impacted by cyanobacteria blooms in Brazil

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Abstract

Salto Grande, a reservoir located in São Paulo, Brazil, is mainly utilized for recreational and agricultural activities and is exposed to animal and anthropogenic impact. The goals of this study were to investigate the contamination by *Giardia* and *Cryptosporidium* in four sites at this reservoir, intermittently impacted with cyanobacteria blooms and to verify the correlation among bacteriological indicators of fecal contamination, physicochemical variables and the occurrence of both protozoa. A total of 48 water samples were examined from lotic and lentic sites of the reservoir. Protozoa were searched by using the membrane filtration technique and direct immunofluorescence assay. Immunomagnetic separation was performed in cyanobacteria positive water samples. The positivity for protozoa was 16.6% in lotic sites (Mini-Pantanal and Saltinho) with major fecal contribution. *Cryptosporidium* oocysts were detected in one sample from Saltinho (mean concentration of 170 oocysts/L); *Giardia* cysts were detected in two samples of site Mini-Pantanal (mean concentration of 27.1 cysts/L) and in 5 samples of site Saltinho – mean concentration of 65.4 cysts/L. Moderate correlations were found between *Giardia* cysts and thermotolerant coliforms (ρ=0.60), *Giardia* cysts and *Escherichia coli* (ρ= 0.67). Both protozoa were not detected at lentic environments where high concentrations of cyanobacteria were observed. Cross-reactions with protozoa monoclonal antibodies and *Microcystis* ensured difficulty for the diagnosis. These results denote the inadequacy of utilization of these sites for recreational and agricultural purposes posing a threat to public health. The monitoring of these protozoa and its association with environmental factors help elucidate its fate and transport in aquatic environments.

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Introduction

Eutrophication of lakes and reservoirs may occur in aquatic environments due to increasing levels of nutrient enrichment - nitrogen and phosphorus - related to rural runoff and discharge of untreated industrial or urban effluents (MacDowell and Hamilton, 2013). This phenomenon allied to an adequate exposure to light and stable conditions of the water column stimulate cyanobacteria blooms, excessive growth of macrophytes, changes in trophic structure (food chain) and reduction of dissolved oxygen in lentic bodies of water (Brookes *et al.*, 2004; Boqiang, 2009; Smith and Schindler, 2009; Martins, 2011; Yatigammana *et al.*, 2011; McDowell and Hamilton, 2013).

Cyanobacteria are photosynthetic microorganisms belonging to the Bacteria Domain and Cyanobacteria Phylum (Shih *et al.*, 2013). This group of organisms presents several species that produce harmful toxins, which may be released to the aquatic environment, posing a serious risk to animal and human health (Martins, 2011; Rowan *et al.*, 2012).

The cyanotoxins include neurotoxins, hepatotoxins and dermatotoxins. High levels of cyanotoxins in drinking water and recreational water may cause a wide range of symptoms in humans including fever, headaches, muscle and joint pain, blisters, stomach cramps, diarrhea, vomiting, mouth ulcers, allergic reactions and in some cases, can lead to death (USEPA, 2012). In the United States, 11 waterborne disease outbreaks caused by harmful algal blooms were reported during 2009-2010 linked to recreational activities among bathers of freshwater lakes (Hilborn *et al.*, 2014).

In Brazil, the Atibaia River belongs to the Piracicaba-Capivari-Jundiaí Basin and undergoes a deterioration process because it has been continuously exposed to domestic and industrial effluents originated from the metropolitan region of Campinas. Moreover, it forms the Salto Grande reservoir located in Americana city, São Paulo State. This reservoir is under the influence of the Atibaia River, agricultural activities and urban development and therefore its superficial water suffers the process of eutrophication (Zanata and Espíndola, 2002).

Considerable attention is now focused on waterborne parasitic protozoa because these pathogens have caused numerous outbreaks worldwide. In the last two decades, pathogenic protozoa caused 325 outbreaks (Karanis *et al.*,2007) and during the period of 2004 to 2010, 199 waterborne outbreaks were registered (Baldursson and Karanis, 2011).

Studies about the occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in environmental water samples of Brazil were initiated in the late 90's and since then has demonstrated the presence of oocysts and cysts in surface waters, groundwater, and effluents from heavily populated metropolitan regions and also in brackish surface waters from the Brazilian coast (Gamba *et al.*, 2000; Franco *et al.*, 2001; Hachich *et al.*, 2004; Araujo *et al.*, 2011; Souza *et al.*, 2012; Leal *et al.*, 2013).

The concomitance of cyanobacteria and protozoa such as *Giardia* and *Cryptosporidium* is one factor that adds greater complexity to the laboratory detection of oocysts and cysts in water samples. Among other factors, the cross-reaction between specific monoclonal antibodies anti-*Giardia* and anti-*Cryptosporidium* with algae, cyanobacteria and Ascomycota, can lead to false positive results when searching for these waterborne protozoa in complex environmental samples (Rodgers *et al.*, 1995; Shimizu *et al.*, 2012).

In West Central Africa, Ajeagah *et al.*, (2012) employed Envirocheck filtration followed by immunomagnetic separation and direct immunofluorescence to investigate the presence of protozoa in a eutrophic lake. These authors and Anceno *et al.*, (2007) pointed that very few studies have been carried out in developing countries. Given this background, it is interesting to investigate the occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in water from Salto Grande reservoir where

cyanobacteria blooms occur from a public health perspective.

The purposes of this research were: to verify the contamination by *Giardia* cysts and *Cryptosporidium* oocysts in four different sites of Salto Grande eutrophic reservoir (lentic and lotic environments); -perform the monitoring of cyanobacteria blooms and quantification of its cells density; - to evaluate water quality using bacteriological indicators of fecal contamination and physicochemical variables and verify if there is a correlation between the occurrence of cysts and oocysts in water samples with all variables mentioned above.

Materials and methods

Study area

The Salto Grande reservoir is formed by the Atibaia River and it is located in the city of Americana, São Paulo State, Brazil. This city is situated in the Metropolitan Region of Campinas, a highly urbanized area which possesses the largest economic development of São Paulo State. Salto Grande Reservoir has an area of 11.5 km², total water volume around 106 millions/m³ and average depth of 8 m (Martins *et al.*, 2011).

The construction of this reservoir occurred between 1940 and 1949 to satisfy the growing demand for energy resources due to industrialization and to regularize the flow of the Piracicaba River, which is also important for irrigation, fishing and recreational purpose. In the 70s and 80s it was considered a great local tourist attraction (Deberdt, 1997; Leite and Dornfeld, 2004; Fonseca, 2008).

Despite concentrating one of the largest textile industrial complex in Brazil, there is a resumption of agricultural activity such as the cultivation of sugar cane and orange advancing to urban areas and bodies of water in a disorderly way. Moreover, the left bank of Salto Grande reservoir is under influence of residential area, grazing and agriculture (Fonseca, 2008).

Sampling sites

Water samples were monthly collected from January 2008 to December 2008, in four different sites of the main compartments of the Salto Grande reservoir comprising a total of 48 samples.

The first site (lotic environment) is called "*Mini-Pantanal*" (MP) (S 22º45'6"; W 47º10'6"), and it is located in the upper reservoir being considered as a conservation area.

The second site (lotic environment) is called "*Saltinho*" (S) (S 22º44'6"; W 047º13'0"), and is characterized by siltation and the beginning of the impoundment (transition zone between river and reservoir).

The third site (lentic environment) is situated in a region known as "*Praia Azul*" (PA) (S 22º43'4"; W 047º14'2"), it is characterized by recreational activities, as well as grazing and sugar cane culture area. This site is located at the left bank of the reservoir.

The fourth site (lentic environment) is a region known as "*Iate Clube*" (IC) (S 22º43'1"; W 047º16'1"). This area presents smaller thickness of siltation since it is situated far from Atibaia River, the main source of sediments. This site is utilized for recreational purposes, such as Jet Ski and water skiing.

Water sampling

Samples of 5L of superficial water up to 15 cm of depth were harvested in polypropylene bottles previously rinsed with Tween 80 solution (0.1%) to avoid the adherence of protozoa to the bottles and immediately stored and transported under refrigerated conditions to the laboratory.

Parasitological examination of water

For parasitological analysis, water samples were submitted to membrane filtration (47mm - diameter sterile cellulose esters membranes) (Millipore®), with nominal porosity of 3 μm (Franco *et al.*, 2001). It was used more than one membrane when clogging of

pores membrane occurred according to turbidity of sample.

Afterwards, sample elution was performed by alternatively scraping the membrane with a smooth-edged plastic loop and rinsing it with 0.1% Tween 80 solution; the eluates were centrifuged twice (1050 x *g*; 15 min) and the pellets stored under refrigerated conditions.

Aliquots of all pellets were examined by staining with specific monoclonal antibodies anti-*Giardia* and anti-*Cryptosporidium* using Merifluor® kit - Meridian Bioscience Diagnostics, Cincinnatti, Ohio) and the vital dye DAPI (4', 6- diamidino-2-phenylindole) in accordance with Method 1623/2005 (USEPA, 2005). The slides were examined under epifluorescence microscopy (Nikon50i) with excitation filter 450 nm - 490 nm and a barrier filter of 520 nm (for fluorescein isothiocyanate, FITC). The typical morphology of both protozoa were confirmed using the incorporation of DAPI - ultraviolet light excitation filter of 355 nm and a barrier filter of 450 nm - and phase contrast microscopy to evaluate specific structures of both protozoa in accordance with criteria stipulated by the United States Environmental Protection Agency (USEPA, 2005).

Estimative of the number of cysts and oocysts per liter of water sample

The number of (oo)cysts per liter of water sample, was calculated by the equation:

$$F = \frac{A}{C} \times \frac{B}{D} \quad (1)$$

where:

F = number of (oo) cysts per liter

A = number of (oo)cysts found in raw water samples

B= volume of pellet (µL)

C= volume of aliquot analyzed in immunofluorescence well (µL)

D= filtered sample volume (L).

Cyanobacteria analysis

Samples of 1 liter of superficial water from four sites of the reservoir were collected in amber bottles, monthly. All water samples were submitted to the method of counting cyanobacteria cells using Utermöhl sedimentation chamber, an inverted microscope and a Whipple reticle to identify these organisms (CETESB, 2005). The criteria used to perform the immunomagnetic separation (IMS) in water samples with cyanobacteria was density value above 460,365 cells/mL (see "Purification of water samples by immunomagnetic separation").

Control test experiments

To assess the recovery efficiency of the methodology, the control experiments were performed in accordance with Branco *et al.*,(2012). Water samples from lotic sites (MP, S) and lentic sites (PA, IC) were spiked with both protozoa and the control tests were performed. The same control tests were also performed including the immunomagnetic separation step. In the experiments of positive water samples for cyanobacteria blooms, the IMS was applied to confirm the presence of the parasites.

Seeded samples were analyzed using the same filtration and visualization methods described above. The average inoculum concentration used when artificially contaminating the samples followed a predetermined order to magnitude: 10^3 for cysts and oocysts, enumerated previously by direct immunofluorescence assay. The recovery efficiency (R.E) for cysts and oocysts was calculated as follow:

$$RE(\%) = \frac{X-Y}{W} \times 100 \quad (2)$$

R.E: recovery efficiency

X= number of (oo) cysts per liter recovered from the positive control test

Y= number of (oo) cysts per liter found in raw water sample

W=number of (oo) cysts seeded.

Negative control experiments were conducted during the monitoring of natural occurrence of protozoa for all sampling sites analyzed and during the positive control experiments. The same filtration and visualization procedures were applied as described previously.

Purification of water samples by immunomagnetic separation

The water samples found to be positive for cyanobacteria blooms were subjected to immunomagnetic separation process. Briefly, the samples were purified using Dynabeads Combo® GC – Dynal kit following the manufacturer's instructions. Samples with a final pellet volume of 500 µL were incubated with paramagnetic beads coated with specific capture monoclonal antibodies anti-*Giardia* and anti-*Cryptosporidium*. After a serial separation steps using magnetic particle concentrator and washings, the complex parasite-beads were dissociated using 0.1 N HCl and neutralized once using 1N NaOH. The resulting pellet was subjected to direct immunofluorescence assay using the Merifluor® kit as mentioned above for the detection of protozoa.

Bacteriological and physicochemical analysis of water samples

Superficial water (250 mL) corresponding to each sampling sites was collected for bacteriological analysis in sterile polypropylene bottles with 1.8% sodium thiosulfate and 0.3 mL of EDTA . For physicochemical analysis, one liter of superficial water was harvested in amber glass bottles.

To determine the concentration of thermotolerant coliforms and *Escherichia coli* (*E. coli*), the multiple tubes technique was employed and the concentration of bacteria expressed by the most probable number (MPN) in accordance with the procedures established by Standard Methods for the Evaluation of Water and Wastewater (APHA, 2005).

For the analysis of turbidity, color and pH, turbidimeter (HACH®) - DR2500 (HACH®) and pHmeter (Digimed®) were used respectively. The water temperature was measured with mercury thermometer Incoterm®, immediately after the collection.

Statistical analysis

Chi-square test was used to verify the presence of the association among the seasons and the occurrence of protozoa from the studied sites. The correlation among bacteriological, physicochemical variable and protozoa occurrence in each water sampling sites was assessed by Pearson's Correlation Coefficient with $\alpha = 0.05$. All statistical analysis was performed with SAS Inc. (2009) for Windows (Version 9.1. Cary NC, 1028p).

Results

Occurrence of pathogenic protozoa in superficial waters from the Salto Grande reservoir

Considering all water samples analyzed during the monitoring period, the positivity for pathogenic protozoa was 16.6 %. *Giardia* cysts were detected in two water samples (June and August / 2008) from site MP with concentrations ranging from 102 to 224 cysts/L (average concentration of 27.1 cysts/L). In site S, the prevalence of *Giardia* was 41.6 % (n = 5 samples) (January, March, June, July and August / 2008); the average concentration of cysts per liter was 65.4 (ranging from 122 to 203 cysts/L.) At the same site, *Cryptosporidium* oocysts were detected in 8.7 % of water samples with a concentration of 170 oocysts/L (May / 2008) (Figure 1).

It was not possible to detect both protozoa at lentic sites (PA and IC) where high densities of cyanobacteria blooms occurred, making the visualization of protozoa more laboriously due to cross-reaction with fluorescent monoclonal antibodies. Indeed, the purification process of immunomagnetic separation was performed in these raw samples and cysts or oocysts were not observed.

Recovery efficiency of the methodology applied to water samples from lotic sites MP, S and lentic sites PA and IC

In lotic sites, the recovery efficiency values for *Cryptosporidium* and *Giardia* were 6.8% and 21.2%, respectively. When submitted to IMS, the recovery efficiency for *Cryptosporidium* attained 3.7% and 69.4% for *Giardia*. In lentic sites PA and IC, the recovery values for *Cryptosporidium* and *Giardia* were 59.7% and 65.6% respectively. When submitted

to IMS, the recovery efficiency for *Cryptosporidium* was 46.2% and >100% for *Giardia* cysts (Table 1).

Bacteriological indicators of fecal contamination

At site MP, the average concentration of bacteriological indicators of fecal contamination was higher in comparison to the other sites; high concentration of fecal contamination was also evidenced at site S (Table 2). The lowest concentration of fecal bacteria was detected at lentic environments (Table 2).

Table 1. Recovery efficiency averages of *Giardia* spp. and *Cryptosporidium* spp. in control tests of lotic sites (MP, S) and lentic sites (PA and IC) from Salto Grande reservoir, using membrane filtration, immunomagnetic separation (IMS) and visualization of the protozoa with monoclonal antibodies (Merifluor®).

Recovery efficiency (%)		
Lotic site (n=2)		
Spike dose	With IMS	Without IMS
Oocysts	C	C
8.4×10^3	3.7	6.8
Cysts	G	G
1.2×10^3	69.4	21.2
Lentic site (n=2)		
Spike dose	With IMS	Without IMS
Oocysts	C	C
3.8×10^3	46.2	59.7
Cysts	G	G
2.3×10^3	> 100	65.6

Physicochemical variables

The superficial water of lotic site (MP) showed the highest values of color and turbidity in comparison to the other sites (Table 2). The pH median values were found to be around neutral in all sites and the lowest average temperature of the superficial waters was found at site MP (Table 2).

Table 2. Values of bacteriological and physicochemical variables referring to the superficial raw water of sampling sites MP, S, PA e IC at Salto Grande Reservoir, city of Americana, São Paulo.

Arithmetic average*± SD
Range (Minimum value- Maximum value)

Sampling sites	Coliforms (MPN/100mL)		Cyanobacteria density (cells/mL)	Apparent Color (mg Pt/L)	Turbidity (NTU)	pH (Median)	Temperature (°C)
	Thermotolerants	*E.coli*					
MP	$2.0 \times 10^4 \pm 3.5 \times 10^4$ (9.0×10^2-1.3×10^5)	$7.0 \times 10^3 \pm 8.5 \times 10^3$ (9.0×10^2-3.0×10^4)	NA	368.10 ± 315.09 (106-993)	53.21 ± 52.92 (8.60-165)	7.13 ± 0.19 (6.93-7.50)	23.58 ± 3.77 (19-30)
S	$1.2 \times 10^4 \pm 1.6 \times 10^4$ (4.0×10^2-5.0×10^4)	$4.9 \times 10^3 \pm 7.7 \times 10^3$ (1.4×10^2-2.6×10^4)	NA	349.70± 236.20 (145 -858)	43.16 ± 30.17 (12.10-96.60)	7.14 ± 0.12 (6.90-7.35)	23.95 ± 3.67 (19.5-30)
PA	$5.3 \times 10^2 \pm 14.4 \times 10^2$ (<2-5.0×10^3)	$2.9 \times 10^1 \pm 38.2$ (<2-1.3×10^2)	527,029 ± 756,138 (28,823 – 2,313,718)	285.90 ± 410.41 (70-1570)	46.91 ± 59.94 (6.2-232)	7.30 ± 1.04 (7.0-9.67)	24.54 ± 3.20 (20-29)
IC	$1.6 \times 10^1 \pm 27.45$ (<2.0-7.0×10^1)	6.3 ± 11.87 (<2.0-4.0×10^1)	460,365 ± 653,478 (100,592 – 1,296)	148.60 ± 122.88 (40-445)	30.34 ± 28.01 (5.43-94.80)	7.30 ± 1.04 (7.0-9.67)	24.66 ± 3.24 (20-29)

NA: Not analyzed

SD: Standard Deviation

Statistical analysis

For sites MP and S, the statistical analysis revealed that the frequency of *Giardia* cysts in water was associated with seasons (Chi-Square test; p=0.0032). Analysis for *Cryptosporidium* was not performed because of its lower frequency. Moderate correlations were verified between *Giardia* cysts and thermotolerant coliforms (ρ=0.60, p=0.0386), and *Giardia* cysts and *Escherichia coli* (ρ=0.67, p=0.0152) at site S.

Discussion

Man-made reservoirs are constantly facing changes in its natural structure since they tend to suffer a gradual silting process which leads to the formation of shallow waters and deposit of sediments, and habitats potentially colonisable by flora and fauna (mainly birds) (Pegoraro, 2004).

This scenery is similar to lotic sites MP and S. In the first site, there were possible multiple sources of pathogenic protozoa, such as cattle, birds and the influence of sewage from Atibaia River. It was not possible tracking the source contamination of *Giardia* using TPI and β giardin genes, because DNA amplification from positive water samples failed (data not showed).

Table 3. Seasonal frequencies of *Giardia* spp. from water sampling sites (MP and S) at Salto Grande Reservoir, Americana city, State of São Paulo (January to December 2008).

Sites	Winter	Autumn	Summer	χ^2 (DF= 2)	*P* value (α< 0.05)
MP	10.734	4.634	0		
S	5.093	4.997	9.836		
Total	15.828	9.631	9.836	11.462	0.0032

DF: Degrees of freedom.

Both waterborne pathogenic protozoa were detected at site S. This event can be attributed to the influence of shallow waters at this site, and solid particle from the sewage of farms that is dumped directly into reservoir. In addition, the presence of fecal deposit of cattle, and other animals such as dogs and pigs surrounding this site may have contributed to the positivity of protozoa. Castro-Hermida *et al.*, (2009) attributed the occurrence of cysts and oocysts in superficial water in Tambre River Basin, Spain, to the presence of cattle. Hsu *et al.*, (1999) found both protozoa in raw superficial water samples -next to the areas of rearing pigs- from River Basin Kau-Ping (Taiwan).

The occurrence of both protozoa at lotic sites MP and S might be also associated with rainfall events prior to collection of water samples (January, March, May, June and August), and associated with seasons - Chi-Square test showing significant difference in the Summer, Autumn and Winter (Table 3).

Keeley and Faulkner, (2008) observed a correlation between *Cryptosporidium* oocysts and rainfall in Lake Texoma, Texas (USA). In Brazil, Rose *et al.*, (2002) reported that *Cryptosporidium* was the common cause of diarrhea in AIDS patients and children, showing a distinct seasonality that was associated with the water transmission due to rainfall events.

When considered the lentic sites PA and IC, both parasites were not detected in superficial waters; the sedimentation rate of pathogens is favoured in lentic aquatic environments (Brookes *et al.*, 2004) and thus may explain the negative results for both protozoa at those sites. In these waters, variable concentrations of metals (Ni, Mn, Zn, Pb, Cd, Cu, and Cr) were documented in the Salto Grande Reservoir sediment (CETESB, 2009). These metals have a positive charge which interacts with negative surface charge of *Cryptosporidium* promoting sedimentation of the protozoan in the reservoir. Also, cyanobacteria were found in these sites making visualization of protozoa

more laboriously, because these bacteria exhibit cross-reaction with monoclonal antibodies of protozoa (Figure 1). Rodgers *et al.*, (1995) reported that algae and cyanobacteria showed varying degrees of fluorescence, since these organisms present molecules or antigenic proteins similar to *Giardia* and *Cryptosporidium*, allowing the antibody binding and consequently the cross-reactivity.

In the present study, *Microcystis* sp. was the main specie of cyanobacteria which influenced the diagnosis (Figure 1). Qualitatively, the immunomagnetic separation was an important tool for correct confirmation of these protozoa.

In relation to recovery efficiency, when compared with the performance criteria established by USEPA, (2005) (13% to 111% for *Cryptosporidium* and 15% to 118% for *Giardia*), the recovery of *Giardia* reached the established standards in the great majority of control experiments (Table 1). However, the anomalous value of *Giardia* in lentic sites (PA and IC) may be attributed to the use of well slide counting method for inoculum enumeration (Bellamy, 2004). For *Cryptosporidium,* the recovery rate did not reach the performance criteria in control experiments of lotic sites (MP and S). In lentic sites, the recovery efficiency of *Cryptosporidium* oocysts was higher when the IMS process was not employed (Table 1).

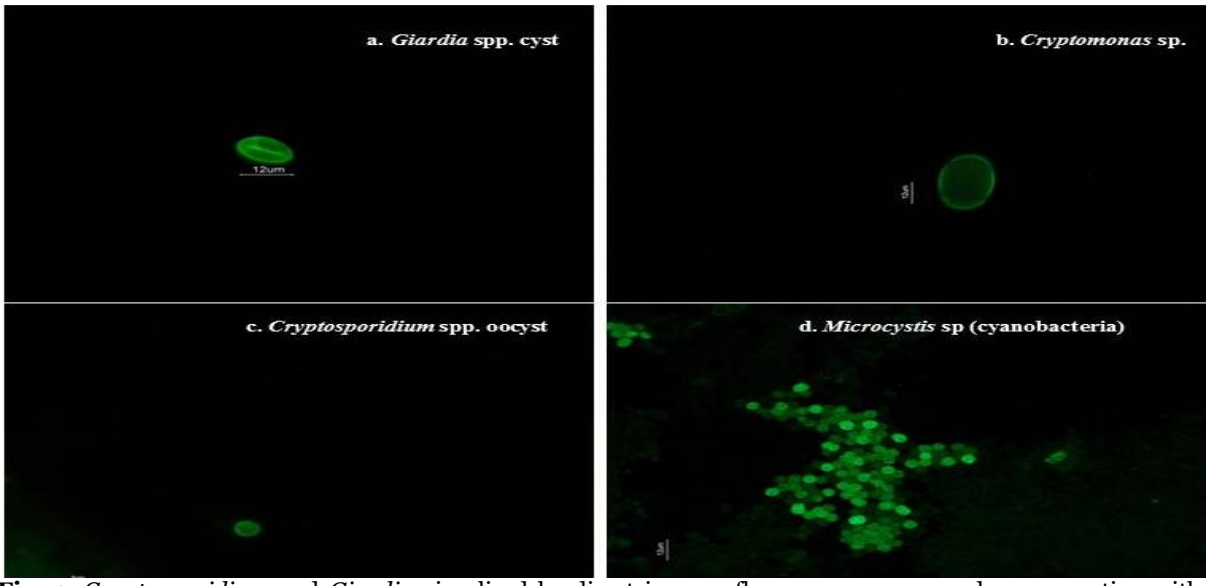

Fig. 1. *Cryptosporidium* and *Giardia* visualized by direct immunofluorescence assay and cross-reaction with cyanobacteria at Salto Grande Reservior (a, b, c, d-40 x).

The method used for the recovery of protozoa in water samples may be affected by many variables, including the raw water matrix composition: its organic and inorganic content. The presence of particles of clay, sand, metal and pH of the water sample also affects the recovery rate (McElroy *et al.*, 2001; Kuhn *et al.*, 2002; McCuin and Clancy, 2003). Studies have revealed that one of the critical factors that affect the efficiency of immunomagnetic separation is the volume of sediment whose increase may negatively affect the efficiency of capture and isolation of parasites through the sample (Quintero-Betancourt *et al.*, 2003; USEPA, 2005). The impact of pH on oocysts recovery in immunomagnetic separation may also occur in alkaline waters of lentic sites PA and IC (Table 2), due to the photosynthesis of cyanobacteria and macrophytes. The pH interferes on the superficial wall cell and influence on the stabilization of antigen-antibody bindings (Cook *et al.*, 2006). In neutral and acid pH, there are best recoveries of protozoa (Hsu and Huang, 2002). Affinity paratopes can bind oocyst epitopes less effectively in adverse conditions (e.g., high divalent cation concentration) (Cook *et al.*, 2006). In sediment

of sites PA and IC, were documented divalent cations such as Ni, Mn, Zn, Pb, Cd, Cu, and Cr (Cetesb, 2009). Therefore, the detection of cysts and oocysts in these lentic sites, may have been influenced by these factors.

The site MP receives domestic sewage discharge from Atibaia River, contributing to the high levels of fecal contamination observed in the present study. These data are concordant with those found by CETESB, (2009) (Company of Technology of Environmental Sanitation) that recorded average concentrations of fecal coliform (7.0 x10^4 MPN/100ml) near the site MP, above the limits specified by Federal Legislation resolution CONAMA (National Environmental Council, Nº 430) (Brasil, 2011). The same situation occurred with site (S) influenced by runoff and the presence of domestic animals.

In sites PA and IC, the process of sedimentation may have contributed to the reduction of fecal bacteria, keeping them in the sediment whose organic matter serves as a nutrient source for these organisms. The low levels of contamination found at these sites may be also associated with its location, e.g., far from the Atibaia River. Unlike, the bacteriological indicators of fecal contamination, mainly *Escherichia coli* exhibit higher concentrations in the adjacent areas to the intrusion of rivers (Brookes *et al.*, 2005), fact observed at lotic sites in Salto Grande Reservoir.

Lower concentrations of coliforms may also be attributed to the presence of macrophytes because like wetlands, they reduce or remove contaminants, pathogens and organic or inorganic matter from the water (Kivaisi, 2001). These plants transport oxygen from the aerial organs to the roots, promoting the oxidation of organic matter and mainly fecal coliforms (Valentim, 1999; Diniz *et al.*, 2005).

Chemical discharges that occurred in the water body may also have contributed to the decay of these coliforms. Fish mortality was observed in Salto Grande reservoir in January 2008, probably due to the same reason. Moreover, diffuse pollution points such as sewage and agricultural runoff caused eutrophication of water in sites PA and IC, favoring the large blooms of cyanobacteria and macrophytes observed at those sites.

The high density of cyanobacterial cells per mL found at sites PA and IC (Table 2) might be considered as a risk for recreational activities at those sites. In accordance with the World Health Organization (WHO), the density of 20,000 cells/mL and 100,000 cells/mL are a guideline for mild or moderate health alert in recreational waters, associated with risk for a short and long term illnesses, respectively (WHO, 1999, 2003).

Regarding physicochemical variables, the turbidity in MP is characterized to the significant input of organic matter from the contribution basin (Atibaia River), and it is related to the sediment particle size characterization: sandy, silt and clay (CETESB, 2009). According to CETESB (2009), it should be noted that the concentration of phosphorus (1,690 μg/ g) verified in the sediment of this site indicates that this contribution predominantly arises from the Atibaia river.

In relation to site S, there is a great amount of suspended matter mainly particles of soil and feces of wild animals that are abundant at this lotic environment, generating high values of turbidity.

The occurrence of *Giardia* cysts was correlated with thermotolerant coliforms and *E. coli* at site S. *Giardia* is frequently found in bodies of water impacted with sewage discharge and raw wastewater that present high levels of fecal indicators bacteria (Lane and Lloyd, 2002; Cacciò *et al.*, 2003). In the present survey, *Escherichia coli* may have been originated from the Atibaia River or from domestic animals such as dogs, pigs and cattle on site S.

Carmena *et al.*, (2007) also reported positive correlations between *Giardia* and *Escherichia coli* in water samples from rivers and reservoirs. Although *Giardia* and *E. coli* are eliminated and introduced by

feces in bodies of water, its survival rates and ecological characteristics are different, indicating that no single indicator organism can predict the presence of all enteric pathogens (Hörman *et al.*, 2004; Keeley and Faulkner, 2008).

Therefore, the occurrence of *Giardia* and *Cryptosporidium*, the high concentration of *Escherichia coli* and the presence of cyanobacteria that produce cyanotoxins, denote the inadequacy of utilization of these sites for fishing and recreational purposes posing a threat to public health.

It should be emphasized that the knowledge concerning the ecology, survival rates and environmental distribution of these waterborne protozoa, such as in huge reservoirs influenced by different biotic and abiotic factors which composes different micro and macro habitats is complex.

In summary, Salto Grande Reservoir may act as a natural barrier to microorganisms, because the reservoir retains organic matter and particles from the Atibaia River, positively contributing to improve the water quality of the Piracicaba River that supplies the Americana city. The monitoring of these protozoa through the environment and its association with a wide variety of variables help elucidate its fate and transport in aquatic environments.

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