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Short Communication

Cryptosporidium Species and *Giardia* Genotypes Detected in Surface Water Supply of Campinas, Southeast Brazil, by Molecular Methods

Franco RMB[1]*, Branco N[2], Amaro BCT[3], Neto RC[4], and Fiuza VRS[5]

[1]*Department of Animal Biology, University of Campinas (Unicamp), Brazil*
[2]*Department of Animal Biology, University of Campinas (Unicamp), Brazil*
[3]*Institute of Biology, University of Campinas (Unicamp), Brazil*
[4]*Laboratory of Microbiology, Society for Water Supply and Sanitation (SANASA), Brazil*
[5]*Department of Sanitation and Environment, University of Campinas (Unicamp), Brazil*

***Corresponding author**
Regina Maura Bueno Franco, Laboratory of Protozoology, Department of Animal Biology, Institute of Biology, Campinas State University, Rua Monteiro Lobato, n°255. CEP: 13 083 862, Campinas city, São Paulo state, Brazil, Email: mfranco@unicamp.br

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Abstract

Data about the presence of *Cryptosporidium* species and *Giardia* genotypes in water samples from Brazil are scarce. We investigate the occurrence of these pathogenic protozoa in raw water samples of Atibaia and Capivari Rivers which are the main water supplies of Campinas city (Southeast Brazil). These rivers show high degree of eutrophication. All samples were subjected to membrane filtration technique using 45°C heated elution solution followed by immunofluorescence and nested PCR. Recovery efficiencies were estimated using water aliquots spiked with Color Seed®. Average recovery efficiencies of spiked samples in Atibaia River were 18.0% ± 18.4 and 74.0% ± 22.1 for *Cryptosporidium* and *Giardia*, respectively. For Capivari River, recovery efficiencies were 29.7% ± 24.6 and 65.1% ± 33.0 for oocysts and cysts. *Cryptosporidium* oocysts were found in 42.8% samples from Atibaia River and in 85.7% samples from Capivari River, whereas *Giardia* cysts were present in 100% of samples in both rivers using immunofluorescence. Molecular analysis revealed the presence of C. *hominis* and C. *parvum* as well as G. *duodenalis* subgroup BIII in Atibaia River. All samples from Capivari River were PCR negative, probably due to inhibitors. These findings suggest anthroponotic and zoonotic contamination of Atibaia River. The occurrence of these pathogenic protozoa in both rivers highlight the potential risk for human and animal health. Close monitoring of water quality of these rivers is highly recommended.

ABBREVIATIONS

MF: Membrane Filtration Technique; IFA: Immunofluorescence Assay; DAPI: 4', 6-diamidino-2-Phenylindole Fluorescent Stain; DIC: Differential Interference Contrast Microscopy; SD: Standard Deviation; BSA: Bovine Serum Albumin; DMSO: Dimethylsulfoxide; LaCTAD: High Performance Technology Center Laboratory in Life Sciences/Campinas State University, Brazil; UT: Turbidity Unit

INTRODUCTION

Cryptosporidiosis is one of the most common food borne and waterborne diseases reported worldwide [1,2]. Self - limiting diarrhea is usually observed in immune competent people, whereas in young children, elderly and immune compromised people death can occur [3]. *Cryptosporidium* incidence is higher among children from developing countries [4]; in developed countries, adults are infected by ingestion of water or food contaminated with oocysts [5].

Thirty species and more than 40 genotypes are described in *Cryptosporidium* genus [6]. Recently, three new species were reported: *Cryptosporidium rubeyi* [7], *C. proliferans* [8] and *C. avium* [9]. However, not all species comprise risks to human health [6]. *C. hominis* and *C. parvum* are responsible for over 90% cases of infections in humans [10]. However, *C. parvum* has a

wider variety of hosts, including animals and is considered the most important zoonotic agent of cryptosporidiosis [11].

The greatest waterborne cryptosporidiosis outbreak occurred in Milwaukee, Wisconsin, EUA, in 1993, where over 400,000 people were reportedly infected [12]. Filter effluents reached a turbidity of 2.7 UT in few days signalizing a failure of treatment process. The largest waterborne outbreak ever reported in Europe occurred in Sweden [13] affecting 27,000 individuals.

Giardia duodenalis is a parasite of human and other mammals. Six species are described in the *Giardia* genera according to morphological characteristics and hosts infected [14]. It is currently accepted that *G. duodenalis* is a complex of eight distinct genetic groups (designated A-H). These groups are morphologically identical but differ in genomic mutations [15,16]. Humans are mainly infected by genetic groups A and B, which are divided into five sub - groups (named AI-III and BIII-BIV) [17].

About 280 million people worldwide are estimated to acquire *Giardia*sis each year and an incidence of 1.2 million cases are annually reported in the United States [18]. Waterborne *Giardia*sis outbreaks in humans are generally caused by contamination of water sources intended for human consumption [19]. In addition to gastrointestinal disorders, this flagellate protozoan can cause extra intestinal symptoms [20].

While there are other *Cryptosporidium* and *Giardia* transmission routes to humans, water is considered as the main route due to the low infectious dose, chlorine tolerance and zoonotic potential. These factors reinforces the need of knowing which species and genotypes occur in water sources in order to evaluate the risks for human and animal health; then, proper control measures can be outlined [21].

Data about the presence of *Cryptosporidium* species and *Giardia* genotypes in water samples from Brazil are scarce. In Paraná state, *C. parvum* oocysts were detected in raw water of Ribeirão Cafezal watershed [22]; *C. hominis* and *C. meleagridis* oocysts were found in superficial water from São Paulo state [23].

Campinas is the third most populous city in the state of São Paulo. This city is supplied by Atibaia and Capivari Rivers which belong to the basin of Piracicaba, Capivari and Jundiaí Rivers. The region where this basin is located is one of the most industrialized of São Paulo state [24]. Consequently, there is a high eutrophication degree in these rivers.

This study aimed to investigate the occurrence of *Cryptosporidium* species and *Giardia* genotypes in raw water samples from Atibaia and Capivari Rivers at Campinas city through immunofluorescence and molecular methods (nested-PCR), after membrane filtration (MF).

MATERIALS AND METHODS

All water samples 20 cm in depth were collected at the intake pipe of the Atibaia and Capivari Water Treatment Plants of Campinas city in new polystyrene bottles previously rinsed with Tween 80 0,1% solution. Samples were immediately transported to the laboratory in iceboxes at 4°C. Raw water samples were processed by the membrane filtration technique according Franco et al., (2001) [25] with the following modifications: organisms were eluted from filter through rinsing and scrapings of membrane using Tween 80 (0.1%) at 45°C and concentrated by 1500 x *g*/15 min. Cysts and oocysts were purified by immune magnetic separation using CGCombo Dynal® kit in accordance to USEPA Method 1623.1 [26] with dissociation by heat and use of 50µL of beads for initial incubation step.

Immunofluorescence assay (IFA) was performed following Cellabs® kit (Cellabs Pty. Ltd., Australia) instructions with minor modifications. Briefly, DAPI stain was applied on slide wells prior to monoclonal antibodies. Samples were microscopically examined at 200x and 400x magnification, using epifluorescence and DIC microscopy. Only organisms compliant with USEPA Method 1623.1 were considered, with internal morphological characteristics assessed by DIC microscopy.

In order to evaluate the water turbidity influence on MF technique using heated elution solution, replicates of water samples were also collected. In these samples Color Seed® (Biotechnology Frontiers, Australia) were seeded at a level of 100 ± 1 SD per water sample (1L). Further analyses were performed according to the methods previously described. Estimate number of organisms/L and recovery efficiency were calculated according Franco et al., 2012 [27]. Results were compared to acceptance criteria of USEPA Method 1623.1.

DNA extractions were performed using the DNeasy Blood & Tissue® kit (Qiagen, Hilden, Germany) or PowerSoil® kit (MoBio) for *Cryptosporidium*, and ZR Fungal/Bacterial DNA MiniPrep® kit for *Giardia* according to the availability of these kits in our laboratory. All reactions followed the manufacturer's instructions, using 300µL of the pellet obtained for each eluted water sample.

An 830 bp fragment of the 18S rRNA gene of *Cryptosporidium* was amplified by nested PCR using primers described by Xiao et al., 1999 [28] (Table 1). The mixture of first PCR reaction contained 1X PCR buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 2.5µL of BSA (bovine serum albumin - 0.1 g/10 mL), 2.5 U Taq, and 1 mM of each primer in a final reaction volume of 50µL [29]. The first PCR samples were subjected to initial denaturation at 94°C for 3min, 35 cycles of 94°C for 45s, 59°C for 45s and 78°C for 1 min. The final extension was at 72°C for 7 minutes. The second amplification reaction mix was identical to the first except for the $MgCl_2$ concentration (1.5 mM). Forty cycles of 94°C for 30s, 58°C for 90s and 72°C for 2 minutes, after initial denaturation at 94°C for 3 minutes and final extension at 72°C for 7 minutes [29].

For *Giardia* DNA amplification, genetic markers SSU-rRNA and beta-giardin were addressed using the protocols described by Appelbee et al., (2003) [30] and Hopkins et al., (1997) [31], respectively. Primer sets of first and second reactions are described in Table (1). For primary amplification of SSU-rRNA genetic marker, a mix was prepared containing 1X PCR buffer; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 2 U Taq; 2.5 µL of dimethyl sulfoxide (DMSO) and 0.5 µM of each primer in a final volume of 50 µl. First PCR conditions were: initial denaturation of 96°C (2 min.), 35 cycles of 96°C for 45s, 58°C for 30s, 72°C for 45s, and final extension at 72°C for 4 min. For the second amplification

the PCR mixture and cycling conditions were identical to the first [29].

Amplification of a beta - giardin gene fragment was performed using 12.5 µL PCR Master Mix (Promega), 0.6 mM of each primer, 2 µL of BSA (0.1/10 mL) and 4.5 µL of nuclease - free water. Three micro liters of sample DNA were used in each reaction. The following amplification conditions were performed: initial denaturation step for 5 minutes at 94°C, followed by 40 cycles, each consisting of: 30s at 94°C, 30s at 65°C and 60s at 72°C, and final extension of 7 minutes at 72°C. In the nested reaction, an internal fragment of 511 bp was amplified [32,33]. The mixture was prepared with 13.5 µL PCR Master Mix (Promega), 0.6 mM of each primer, and 4.5 µL of nuclease - free water and 4 µL of sample DNA. Initial denaturation was performed at 98°C for 15 minutes followed by 40 cycles of 30 seconds at 95°C, 55°C for 30s, and 60s at 72 °C. The final extension was done at 72°C for 7 min.

For all samples, IFA-DAPI-DIC visualizations were performed first, followed by DNA extractions for the PCR assays. PCRs for each sample were repeated in triplicate in order to reduce false positive or negative results. All gels were stained with GelRed® (Biotium, Hayward, USA) for visualization.

All PCR positive samples were purified using ExoSAP-IT® and sequenced in LaCTAD Center of Campinas State University with same primers used in the second PCR reaction. The sequences of each strand were aligned and examined with Lasergene software (DNASTAR), and submitted to the Basic Local Alignment Search Tool (BLAST) analysis to identify similarities with the GenBank sequences.

RESULTS

Seven water samples from each river were collected between September 2014 and June 2015. Water turbidity from Atibaia River was higher (average: 46.1 UT, SD: 34.3, minimum value: 17.1, maximum value: 110) than Capivari River (average: 34.4 UT, SD: 19.7, minimum value: 16.8, maximum value: 74.0) during this research. Overall recovery of *Cryptosporidium* oocysts and *Giardia* cysts from Atibaia River averaged 18.0% ± 18.4 (SD) and 74.0% ± 22.1 (SD) respectively (Table 2). From Capivari River, overall oocysts and cysts recovery averaged 29.7% ± 24.6 (SD) and 65.1% ± 33.0 (SD), respectively (Table 2). *Cryptosporidium* recovery efficiency was lower than *Giardia* in both rivers. MF technique was less affected by turbidity considering oocyst recovery in Capivari river. A decline in the ability of this technique to detect *Cryptosporidium* was observed with the increase of turbidity of water samples from Atibaia River.

Cryptosporidium oocysts were present in 42.8% samples from Atibaia River and in 85.7% samples from Capivari River. *Cryptosporidium* oocysts were found in fewer numbers than *Giardia* cysts in Atibaia River (Table 2). *Cryptosporidium* averaged 2.4 ± 3.5 (SD) oocysts/L and was not detected in four months of this study in Atibaia River. In Capivari River, *Cryptosporidium* averaged 5.2 ± 5.6 (SD) oocysts/L, and was not detected in one month (Table 2).

PCR amplification of genomic DNA extracted from water samples of Atibaia River produced a band of the expected size of 830 bp for *Cryptosporidium* in three samples (3A, 4A, 5A) (Figure 1). These samples were sequenced but one positive sample could

Table 1: Primers sets used for PCR reactions targeting genetic markers 18S - rRNA (*Cryptosporidium*), SSU-rRNA and beta - giardin (*Giardia*) to analyze water samples from Atibaia and Capivari Rivers, Campinas city, Southeast Brazil.

PCR primers and Nucleotide Sequence	Fragment	References
Primary Amplification:		
Crypto F: 5'-TTCTAGAGCTAATACATGCG-3'	1025bp	Xiao et al., 1999 [28]
Crypto R: 5'-CCCATTTCCTTCGAAACAGGA-3'		
Secondary Amplification:		
AL1598: 5'-AAGGAGTAAGGAACAACCTCCA-3'	830bp	
AL3032:5'-GGAAGGGTTGTATTTATTAGATAAAG-3'		
Giardia (SSU rRNA):		
Primary Amplification:		
GiaF: 5'-AAGTGTGGTGCAGACGGACTC-3'	500 bp	Appelbee
Gia R: 5'-CTGCTGCCGTCCTTGGATGT-3'		
Secondary Amplification:		
RH11: 5'-CATCCGGTCGATCCTGCC-3'	300 bp	Hopkins et al.,1997 [31]
RH4: 5'-AGTCGAACCCTGATTCTCCGCCAGG-3'		
Giardia (beta-giardin):		
Primary Amplification:		
G7: 5'AAGCCCGACGACCTCACCCGCAGTGC-3'	753 bp	Cacciò et al., 2002 [32]
G759: 5'-GAGGCCGCCCTGGATCTTCGAGACGAC-3'		
Secondary Amplification:		
F: 5'-GAACGAACGAGATCGAGGTCCG–3'	511 bp	Lalle et al., 2005 [33]
R: 5'-CTCGACGAGCTTCGTGTT-3'		

Table 2: Number of *Cryptosporidium* oocysts/L and *Giardia* cysts/L detected in raw water samples of Atibaia and Capivari Rivers (Campinas, Southeast Brazil) and recovery efficiencies of membrane filtration technique (followed by direct immunofluorescence assay using monoclonal antibodies) of seeded samples using ColorSeed®.

Collect Sample	Cryptosporidium oocysts/L		Giardia cysts/L	
Number	Atibaia River	Capivari River	Atibaia River	Capivari River
I	0	1	125	21
II	9	4	56	
III	3	4	48	
IV	5	4	44	
V	0	7	67	104
VI	0	0	73	4
VII	0	17	43	36
Average ± SD	2.4 ± 3.5	5.2 ± 5.6	65.1 ± 28.7	27.7 ± 36.1
Recovery of Spiked Samples:				
Trial Number	Cryptosporidium oocysts/L		Giardia cysts/L	
	Atibaia River	Capivari River	Atibaia River	Capivari River
I	9	31	86	81
II	41	26	61	22
III	48	6	68	18
IV	11	67	74	82
V	7	7	100	106
VI	9	12	35	66
VII	1	59	94	81
Average ± SD	18.0 ± 18.4	29.7 ± 24.6	74.0 ± 22.1	65.1 ± 33.0

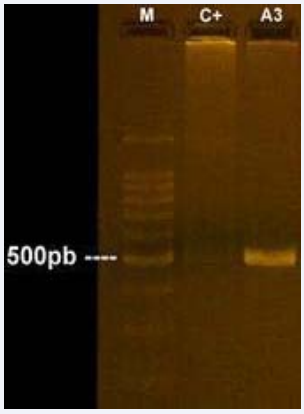

Figure 1 Nested-PCR results for the beta - giardin gene visualized on agarose gel after electrophoresis. The C+ (positive control) and sample A3 showed band of approximately 511 bp. M = molecular weight marker.

not be identified due to insufficient sequence quality (sample 3A). Sample 4A was identified as *C. parvum* and matched the HCTX8 isolate (97.9% of homology) described by Xiao et al., 1999 [28]. Sample 5A was identified as *C. hominis* and had 88.0% homology with *C. hominis* isolate by Araújo et al., 2013, in São Paulo city [23]. No DNA amplification was achieved from Capivari River samples.

Giardia cysts were present in 100% of samples in both water sources. A higher number of cysts was found in Atibaia River (average: 65.1 ± 28.7 (SD) cysts / L) than in Capivari River (average: 27.7 ± 36.1cysts / L) (Table 2).

Only one sample (A3) was PCR positive for *Giardia* in beta - giardin analysis (Figure 2). This sample had 99.8% homology with *G. duodenalis* sub - group BIII and matched the HC07 isolate described by Durigan et al., 2014 [16] with a different base at position 494 (a cytosine instead of thymine). All samples from Capivari River were PCR negative for *Cryptosporidium* and *Giardia*.

DISCUSSION

Concerns about water contamination by pathogenic protozoa has considerably increased during the past two decades in Brazil given the occurrence of two *Cyclospora cayetanensis* and one large toxoplasmosis waterborne outbreaks which affected 950 and 496 people, respectively [34,35].

Sanitation is still far from being available to everyone in Brazil whereas only 48.6% of the Brazilian population is served by sewage collection, and just 39% of sewage is treated (www.tratabrasil.org.br) [36]. Microbial load is a major concern regarding contamination of water supplies. Sato et al., (2013) [37] found high risks (> 1/10.000) for both *Cryptosporidium* and *Giardia* parasites in four metropolitan areas in São Paulo state,

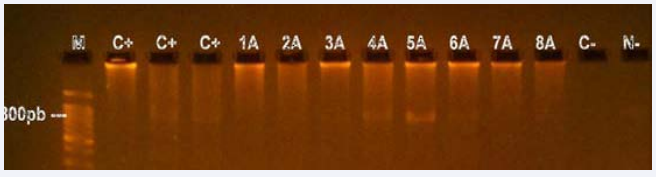

Figure 2 Results of nested-PCR for 18S r RNA visualized on agarose gel after electrophoresis. Two positive controls (C+) and samples 3A, 4A, 5A show band of approximately 830 bp. M = molecular weight marker; 1A to 8A = water samples, C- = negative control of first reaction, N- = negative control.

emphasizing the need for concerted efforts to maintain higher water quality in catchment points [38].

In order to determine the public health threat associated with two main water sources in Campinas, we applied molecular methods to the parasitological monitoring of *Cryptosporidium* and *Giardia*. Parallel processing of natural and spiked samples allowed us to evaluate the effect of water turbidity on performance of chosen method to analyze the occurrence of these protozoan parasites. This approach was important because water turbidity impacts negatively both membrane filtration method as well as PCR.

High turbidity of water matrix is the major factor for poor oocysts recovery from samples concentrated after filtration [39]. The recovery efficiency achieved for *Cryptosporidium* in water samples from Atibaia and Capivari Rivers did not meet the acceptance criteria established in USEPA Method 1623.1 (32%). According to Ongerth (2013) [40], the recovery of protozoa (especially *Cryptosporidium*) is inversely proportional to the sample turbidity. The recovery of *Giardia* is less affected by the matrix effect due to the larger size of the cysts and lower hydrophobic behavior at neutral pH [41] as required by IMS purification. However, samples turbidity has also a negative effect on IMS [42]. The impact of water quality on recovery is still relatively poorly understood [43]. Several groups of constituents may be implicated with the matrix effect such as iron, manganese, and other metals as pointed by Rosen et al., (2014) [43], which found that cations and metals were strongly and negatively correlated with protozoan recoveries.

Furthermore, water sampling in this study coincides with a prolonged drought ("seca") which affected São Paulo state during 2014/2015 years [44], impacting negatively the flow of these rivers and the dilution of contaminants discharged in these water bodies. In this scenario, it is not surprising that only three samples were IFA and PCR - positives for *Cryptosporidium* regarding samples collected in Atibaia River, and one for *Giardia* by PCR. From Capivari River, all samples IFA - positive by microscopy could not be amplified by nested PCR.

Pollution industrial loads, sand and clay extractions, and intense agricultural activity are registered in the banks of these rivers and its water may contain large amounts of silt, decaying organic material, humic acids and metals which are PCR inhibitors. Such inhibitors probably were not efficiently removed by the DNA extraction kits used in this study. Even the use of additives such as DMSO for *Giardia* and BSA for *Cryptosporidium* in our PCR reactions in order to reduce inhibitory effects and increase amplification efficiency, were not sufficient [45].

Both pathogenic protozoa were detected in Atibaia and Capivari Rivers. These findings corroborates with previous studies, where oocysts were found in minor concentrations than *Giardia* cysts [37,46,47].

Besides the three *Cryptosporidium* species reported in human (*C. hominis, C. parvum*, and *C. meleagridis*), the following species were described in animal fecal samples in Brazil: *C. bovis*, *C. canis*, *C. felis*, *C. baileyi*, *C. galli*, *C. meleagridis*, [48], *C. scrofarum* [49], *C. andersoni* [50-52], *C. varanii, C. serpentis* [53]; *C. ryanae* [54], *C. tyzzeri, C. muris* [55]*; C. ubiquitum, C. xiaoi* [56]. However, only *C. parvum*, *C. hominis* and *C. meleagridis* were described in water samples from São Paulo city [23]. *C. parvum* was recently found in Paraná state [22].

The present finding of *C. hominis* and *C. parvum* in Atibaia River is very important given the multiple uses of waters from this river. In an event of treatment failure, *Cryptosporidium* is a primary threat to public health [40]. As observed in this research, more than one *Cryptosporidium* species are usually reported. This river is also used for crop irrigation which representing a considerable impact, as the Metropolitan Region Campinas has 17 municipalities with 808 small agricultural farms [57]. Regarding *Giardia*, the presence of sub - group BIII in Atibaia River suggests the risk of Zoonotic transmission [16]. It should be noted that genetic group B shows a higher prevalence (50%) than genetic group A (37%) [58] worldwide. By studying the risks of *Giardia*s is infections in most populous regions of São Paulo state, Razzolini et al., (2016) [38] found that the annual risk for *Giardia* exceeded the risks for *Cryptosporidium* by at least 1 Log.

Source tracking of fecal contamination through molecular methods is of primary relevance for detection of associated health risks.

CONCLUSION

The occurrence of *C. hominis*, *C. parvum*, and the genetic group BIII of *G. duodenalis* in Atibaia raw water samples highlights possible events of anthroponotic and zoonotic contaminations in this watercourse.

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REFERENCES

1. Karanis P, Kourenti C, Smith H. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J Water Health. 2007; 5: 1-38.
2. Baldursson S, Karanis P. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010. Water Res. 2011; 45: 6603-6614.
3. Chalmers RM, Davies AP. Mini review: clinical cryptosporidiosis. Exp Parasitol. 2010; 124: 138-146.
4. Kotllof KL, Nataro JP, Blackwelder WC, Nasrin D, Faraq TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013; 382: 209-222.
5. Fayer R. *Cryptosporidium*: a water-borne zoonotic parasite. Vet Parasitol. 2004; 126: 37-56.
6. Ryan U, Hijjawi N. New developments in *Cryptosporidium* research. Int J Parasitol. 2015; 45: 367-373.
7. Li X, Pereira MDGC, Larsen R, Xiao C, Phillips R, Striby K, et al. *Cryptosporidium rubeyi* n. sp. (Apicomplexa: Cryptosporidiidae) in multiple spermophilus ground squirrel species. Int J Parasitol: Parasites and Wildlife. 2015; 4: 343-350.

8. Kváč M, Havrdová N, Hlásková L, Daňková T, Kanděra J, Ježková J et al. *Cryptosporidium proliferans* n. sp. (Apicomplexa: Cryptosporidiidae): molecular and biological evidence of cryptic species within gastric *Cryptosporidium* of mammals. PLoS ONE. 2016; 11: 0147090.

9. Holubová N, Sak B, Horčičková M, Hlásková L, Květoňová D, Menchaca S, et al. *Cryptosporidium avium* n. sp. (Apicomplexa: Cryptosporiidae) in birds. Parasitol Res. 2016; 115: 2243-2251.

10. Rossle N, Latif B. Cryptosporidiosis as threatening health problem: a review. Asian Pac J Trop Biomed. 2013; 3: 916-924.

11. Cacciò SM, Putignani L. Epidemiology of human cryptosporidiosis. Chapter 2. *In*: Cacciò SM and Widmer G (eds.), *Cryptosporidium: parasite and disease*, Springer-verlag, 2014. Pages 43-79.

12. Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus S, Blair KA, Peterson De, et al. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the Public Water Supply. N Engl J Med. 1994; 331: 161-167.

13. Guzman-Herrador B, Carlander A, Ethelberg S, Blasio BF, Kuusi M, lund V, et al. Waterborne outbreaks in the Nordic Countries, 1998 to 2012. Euro Surveill. 2015; 20: 21160.

14. Thompson RCA; Monis PT. Taxonomy of *Giardia* species. Luján HD, Svard S, eds). *Giardia:* a model organism. Springer, 2011. 3-15.

15. Monis PT, Cacciò SM, Thompson RCA. Variation in *Giardia*: towards a taxonomic revision of the genus. Trends Parasitol. 2009; 25: 93-100.

16. Durigan M, Abreu AG, Zuchi MI, Franco RMB, de Souza AP. Genetic diversity of *Giardia duodenalis*: Multilocus genotyping reveals zoonotic potential between clinical and environmental sources in a metropolitan region of Brazil. PLoS ONE. 2014; 9: 115489.

17. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev. 2011; 24: 110-140.

18. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, et al. Food borne illness acquired in the United States - major pathogens. Emerg Infect Dis. 2011; 17: 7-15.

19. Zhang W, Zhang X, Wang R, Liu A, Shen Y, Ling H, et al. Genetic characterizations of *Giardia duodenalis* in sheep and goats in Heilongjiang Province, China and Possibility of zoonotic transmission. PLoS Negl Trop Dis. 2012; 6: 1826.

20. Cantey PT, Roy S, Lee B, Cronquist A, Smith K, Liang J, et al. Study of nonoutbreak giardiasis: novel findings and implications for research. Amer J Med. 2011; 124: 1175.

21. Prystajecky N, Huck PM, Schreier H, Isaac-Renton JL. Assessing *Giardia* and *Cryptosporidium* spp. as a microbial source tracking tool for surface water: application in a mixed use watershed. Appl Environ Microbiol. 2014; 80: 2328-2338.

22. Almeida JC, Martins FDC, Ferreira Neto JM, Santos MM, Garcia JL, Navarro I, et al. Occurrence of *Cryptosporidium* spp. and *Giardia* spp. in a public water-treatment system, Paraná, Southern Brazil. Rev Bras Parasitol Vet. 2015; 24: 303-308.

23. Araújo RS, Dropa M, Fernandes LC, Carvalho TT, Sato MIZ, Soares RM, et al. Genotypic characterization of *Cryptosporidium hominis* from water samples in São Paulo, Brazil. Am J Trop Med Hyg. 2011; 85: 834-838.

24. Montagner CC, Jardim WF. Spatial and seasonal variations of pharmaceuticals and endocrine disruptors in the Atibaia River, São Paulo State (Brazil). J Braz Chem Soc. 2011; 22: 1452-1462.

25. Franco RMB, Rocha-Eberhardt R, Cantusio Neto R. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw water from the Atibaia River, Campinas, Brazil. Rev Inst Med trop, São Paulo. 2001; 43: 109-111.

26. USEPA Method 1623.1: *Cryptosporidium* and *Giardia* in water by Filtration/IMS/FA. EPA-R-12-001. Office of Water. Washington: US Environmental Protection Agency. 2012.

27. Franco RMB, Hachich EM, Sato MIZ, Naveira RML, Silva EC, Campos MM de C, et al. Performance evaluation of different methodologies for detection of *Cryptosporidium* spp. and *Giardia* spp. in water for human consumption to meet the demands of the Environmental Health Surveillance in Brazil. Epidemiol Serv Saúde, Brasília, 2012. 21: 233-242.

28. Xiao L, Escalante L, Yang C, Sulaiman I, Escalante AA, Montali RJ, et al. Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. Appl Environ Microbiol. 1999; 65: 1578-1583.

29. Santín M, Trout JM, Vecino JAC, Dubey JP, Fayer R. *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in cats from Bogotá (Colombia) and genotyping of isolates. Vet Parasitol. 2006; 141: 334-339.

30. Appelbee AJ, Frederick LM, Heitman TL, Olson ME. Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada. Vet Parasitol. 2003; 112: 289-294.

31. Hopkins RM, Meloni BP, Groth DM, Wetherall JD, Reynoldson JA, Thompson RC. Ribossomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered form humans and dogs living in the same locality. J Parasitol. 1997; 83: 44-51.

32. Cacciò SM, De Giacomo M, Pozio E. Sequence analysis of the *β*-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. Int J Parasitol. 2002; 32: 1023-1030.

33. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Cacciò SM. Genetic heterogeneity at the β-giardin locus among human and animals isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int J Parasitol. 2005; 35: 207-213.

34. De Moura L, Bahia-Oliveira LM, Wada MY, Jones JL, Tuboi SH, Carmo EH, et al. Waterborne toxoplasmosis, Brazil, from field to gene. Emerg Infect Dis. 2006; 12: 326-329.

35. Moura JE, PEnaforte C, Kerti R, Daufenbach LZ, Camargo NJ, Trevisan R et al. Surto de doença diarréica aguda por *Cyclospora cayetanensis*, Antonina, Paraná. Fundação Nacional da Saúde. Bol. Eletrônico Epidemiólogico. 2002; 3.

36. Ranking do Saneamento 2015. Access in: 07/06/2016.

37. Sato MI, Galvani AT, Padula JA, Naradocci AC, Lauretto M de S, Razzolini MT, et al. Assessing the infection risk of *Giardia* and *Cryptosporidium* in public drinking water delivered by surface water systems in São Paulo State, Brazil. Sci Total Environ. 2013; 442: 389-396.

38. Razzolini MTP, Lauretto MS, Hachich EM, Sato MIZ, Nardocci AC. *Giardia* and *Cryptosporidium* infection risk by simultaneous exposure to drinking water. Microbial Risk Analysis. 2016.

39. Kothavade RJ. Potential molecular tools for assessing the public health risks associated with waterborne *Cryptosporidium* oocysts. J Med Microbiol. 2012; 61: 1039-1051.

40. Ongerth JE. The concentration of *Cryptosporidium* and *Giardia* in water - The role and importance of recovery efficiency. Wat Res. 2013; 47: 2479-2488.

41. Hsu B-M, Huang C. Influence of ionic strength and pH on hydrophobicity and zeta potential of *Giardia* and *Cryptosporidium*. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2002; 201: 201-206.

42. Zarlenga DS, Trout JM. Concentrating, purifying and detecting waterborne parasites. Vet Parasitol. 2004; 126: 195-217.

43. Rosen JS, Bartrand T, Sobrinho J, Clancy JL, Del Rey Z, Richter A, et al. Matrix Effects on *Cryptosporidium* Oocyst Recovery. Project 4348. Water Research Foundation. 2014.

44. Cestesb, 2015. Qualidade das águas superficiais no Estado de São Paulo 2014. Cestesb, São Paulo, série Relatórios. CETESB, 2015. ISSN 0103-4103.

45. Pomp D, Medrano JF. Organic solvents as facilitators of polymerase chain reaction. Bio Techniques. 1991; 10: 58-59.

46. Carmena D, Aguinagalde X, Zigorraga C, Fernández-Crespo JC, Ocio JA. Presence of *Giardia* cysts and *Cryptosporidium* oocysts in drinking water supplies in Northern Spain. J Appl Microbiol. 2007; 102: 619-629.

47. Gallas-Lindemann C, Sotiriadou I, Plutzer J, Noack MJ, Mahmoudi MR, Karanis P. *Giardia* and *Cryptosporidium* spp. dissemination during wastewater treatment and comparative detection via immunofluorescence assay (IFA), nested polymerase Chain reaction (nested PCR) and loop mediated isothermal amplification. Acta Trop. 2016; 158: 43-51.

48. Meireles MV. *Cryptosporidium* infection in Brazil: implications for veterinary medicine and public health. Rev Bras Parasitol. 2010; 19: 197-204.

49. Fiuza VRS, Gallo SSM, Frazão-Teixeira E, Santín M, Fayer R, Oliveira FCR. *Cryptosporidium* Pig Genotype II diagnosed in pigs from the State of Rio De Janeiro, Brazil. J Parasitol. 2011; 97: 146-147.

50. Fiuza VRS, Almeida AJ, Frazão-Teixeira E, Santín M, Fayer R, Oliveira FCR. Occurrence of *Cryptosporidium andersoni* in Brazilian Cattle. J Parasitol. 2011; 97: 952-953.

51. Fiuza VRS, Cosendey RI, Frazão-Teixeira E, Santín M, Fayer R, Oliveira FCR. Molecular Characterization of *Cryptosporidium* in Brazilian Sheep. Vet Parasitol. 2011; 175: 360-362.

52. Couto MCN, Lima MF, Bomfim TCB. New *Cryptosporidium parvum* subtypes of IIa subfamily in dairy calves from Brazil. Acta Trop. 2014; 130: 117-122.

53. Da Silva DC, Paiva PRSO, Nakamura AA, Homem CG, De Souza MS, Greco KF, et al. The detection of *Cryptosporidium sepentis* in snake fecal samples by real-time PCR. Vet Parasitol. 2014; 204: 134-138.

54. Aquino MCC, Widmer G, Zucatto AS, Viol MA, Inácio SV, Nakamura AA, et al. First molecular characterization of *Cryptosporidium* spp. infecting buffalo calves in Brazil. J Eukaryot Microbiol. 2015; 62: 657-661.

55. Souza MS, Vieira BR, Riva HG, Homem CG, da Silva DC, Nakamura AA, et al. Occurrence of *Cryptosporidium* spp. in exotic animals raised as pets in Brazil. Arq Bras Med Vet Zootec. 2015; 67: 132-1326.

56. Zucatto AS, Aquino MCC, Inácio SV, Figueiredo RN, Pierucci JC, Perri SHV, et al. Molecular characterization of *Cryptosporidium* spp. in lambs in the South Central Region of the State of São Paulo. Arq Bras Med Vet Zootec. 2015; 67: 441-446.

57. Pereira MA. Occurrence of *Cryptosporidium* spp., *Giardia* spp. in vegetables of the metropolitan area of Campinas, SP: her relationship with the workers' community and with the agricultural area. PhD Thesis. Biology Institute, Campinas State University. Brazil, 2008.

58. Ryan U, Cacciò SM. Zoonotic potential of *Giardia*. Int J Parasitol. 2013; 43: 943-956.

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