Expo Health (2016) 8:117–123
DOI 10.1007/s12403-015-0187-1

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ORIGINAL PAPER

Occurrence and Evaluation of Methodologies to Detect *Cryptosporidium* spp. in Treated Water in the Central-West Region of Brazil

Sônia F. O. Santos[1,2] · Hugo D. Silva[1,2] · Ludimila A. C. Wosnjuk[2] · Carlos E. Anunciação[2] · Elisângela P. Silveira-Lacerda[3] · Regina H. S. Peralta[4] · Flávia S. Cunha[4] · Tatiana Dela-Sávia Ferreira[1,2] · Marco T. A. García-Zapata[1]

Received: 18 September 2015 / Revised: 26 November 2015 / Accepted: 27 November 2015 / Published online: 29 December 2015
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Abstract *Cryptosporidium* spp. are an emerging pathogen responsible for a large number of diarrhea outbreaks in humans throughout the world. However, the occurrence of epidemic outbreaks caused by this agent in Brazil is poorly known and still needs more attention mainly in the Central-West Region of Brazil, where yet are not studied. Furthermore, there is a need for cheaper or faster methods for detecting *Cryptosporidium* spp. (given the cost of Envirocheck® filters and IMS kits). Thus, the implementation of standard techniques that enable the identification and quantification of this agent for further study of environmental samples is important. This study aimed at evaluating and comparing immunological techniques for detection of antigen and a real-time PCR for detection and differentiation of *Cryptosporidium* spp. in samples of treated water. Samples were collected directly from the taps at the entrance of residences and concentrated by a positively charged membrane filter. Oocysts of *Cryptosporidium* spp. were detected by direct immunofluorescence, ELISA and real-time PCR techniques, and the results were positive in 56.3 % (18/32), 28.1 % (9/32) and 50.0 % (16/32), respectively. The survey results showed for the first time the presence of *Cryptosporidium* spp. in treated water in the Central-West Region of Brazil. Although real-time PCR showed less positive, it is the one that enables the identification of the species and less expensive when processing a large number of samples. Probably, it would be better to use both techniques, due to their own virtues.

Keywords *Cryptosporidium* · ELISA · Immunofluorescence · Real-time PCR · Treated water

✉ Marco T. A. García-Zapata
nupereme@gmail.com

[1] Núcleo de Pesquisas em Agente Emergentes e Re-emegente, Instituto de Patologia e Saúde Pública, Universidade Federal de Goiás, Caixa Postal 12911, Setor Leste Vila Nova, 74643-970 Goiânia, Goiás State, Brazil

[2] Laboratório de Diagnóstico Genético e Molecular, Departamento de Bioquímica e Biologia Molecular, Instituto de Ciências Biológicas II, Universidade Federal de Goiás, 74001-970 Goiânia, Goiás State, Brazil

[3] Laboratório de Genética Molecular e Citogenética, Departamento de Genética, Instituto de Ciências Biológicas I, Universidade Federal de Goiás, 74001-970 Goiânia, Goiás State, Brazil

[4] Departamento de Patologia, Faculdade de Medicina, Universidade Federal Fluminense, RJ Rua Marquês do Paraná, 303, Prédio Principal, 4° andar, CEP, 24033-900 Niterói, Rio de Janeiro, Brazil

Introduction

Contamination of water resources due to sanitary conditions has been a risk factor for population health. Water plays an important role in the route of transmission of biological agents such as protozoa, bacteria and viruses, with emphasis on *Cryptosporidium* spp., causing diarrhea worldwide (Hlavasa et al. 2005; King et al. 2015). Even in countries with high standards of sanitation, river flooding can cause epidemics of gastroenteritis, when sewage systems overflow and pollute water ways used by humans (Gertler et al. 2015).

Cryptosporidium spp. are a genus of protozoa whose transmission occurs mainly through water, including treated water (Tzipori and Ward 2002; Sunderlanda et al. 2007; Xiao 2010).

The first description of the genus *Cryptosporidium* spp. and the identification of species of *Cryptosporidium muris* were made from samples obtained from gastric glands of

mice, in 1907 (Tyzzer 1907). In 1912, the second species was described through an observation of the intestine of laboratory mice and this was identified as *Cryptosporidium parvum* (Tyzzer 1912).

Phylogenetic studies were carried out by several groups of researchers (Tzipori and Griffiths 1998; Morgan and Thompson 1998; Xiao et al. 1998, 1999; Adl et al. 2005) in order to standardize the taxonomy of *Cryptosporidium*, but there are still disagreements about some species that cause serious health problems. Among these species, *C. hominis, C. parvum* are related to clinical events in the infected individuals and, occasionally, a few other species, such as *C. muris, C. suis, C. andersoni* and some different genotypes of *Cryptosporidium* coming from deer and monkeys (Xiao and Feng 2008), which differ significantly in their molecular sequences but, as yet, have not been ascribed species status (Plutzer and Karanis 2009). Literature also shows that domestic and wild mammals can be sources for human infection and water contamination (Feng et al. 2008).

Lake et al. (2007) showed that *C. parvum* and *C. hominis* differ in their epidemiology, being *C. hominis* mostly associated with areas with high socioeconomic status individuals, children under the age of four and urban areas, while *C. parvum* risk factors were rural areas and association with water reservoirs, watersheds and groundwater sources. Thus, it is important removal of *Cryptosporidium* and *Giardia* by treatment process.

Detection of *C. parvum* and *C. hominis* in environmental samples is a very important step to reduce and/or prevent the presence of these pathogens in drinking or recreation water. The presence of oocysts from these coccidia in the environment suggests that humans and animals can acquire the infection through different transmission routes (Xiao et al. 2001; Guy et al. 2003; Franco et al. 2012). These pathogens can be transmitted by ingesting contaminated food and/or water with infected animal or human feces (Gomes et al. 2004; Appelbee et al. 2005).

In 1984, the first cryptosporidiosis outbreak in Texas (USA) occurred and killed 79 people. Later there were others: one in 1987, in Georgia (USA), affecting 13,000 people; and the largest outbreak that infected 403,000 people in Milwaukee (USA), in the year of 1993, caused by the presence of *Cryptosporidium* in the icy water of the region. In 1996, reports showed that 8705 individuals were affected in Saitama (Japan), where *Cryptosporidium* was detected in treated and untreated water (Mark and John 1994; Mackenzie et al. 1994; Karanis et al. 2007).

The most employed technique used for staining fecal smears for the general detection of parasites in feces is those of staining, which can be used to detect other coccidia, such as *Isospora* and *Cyclospora*. However, this technique can present limiting factors, such as the size of oocysts (4–8 μm), which may be confused with colored organic matter, thus demonstrating the importance of a well-trained technician (Fahey 2003; Gonzáles-Ruiz and Bendall 1985). This technique was used in combination with morphometry for *Cryptosporidium* detection in untreated water by Santos et al. (2010).

Immunological methods may offer some advantages over optical microscopy for detection of *Cryptosporidium* spp. oocysts. For example, direct immunofluorescence technique (DIF), which uses monoclonal antibodies conjugated with fluorescein isothiocyanate, which recognize specific epitopes present on the oocysts' surface, has been widely used. This technique has high specificity (96–100 %) and sensitivity (98.5–100 %) in *Cryptosporidium* spp. oocysts detection on environmental samples (Jex et al. 2008), but it has the same disadvantage of leading to a large number of false negatives, depending on the number of oocysts in the sample.

The enzyme-linked immunosorbent assay (ELISA) is an indirect test used for qualitative determination of *Cryptosporidium* spp. antigens in feces. It is simple to perform and does not require direct observation (Ungar 1990; Anusz et al. 1990). In this technique, antibodies are used, which are not species-specific, being unable to distinguish between *Cryptosporidium* spp. species. Furthermore, it has low sensitivity and needs sample concentration.

Because of this, a variety of molecular techniques based on the polymerase chain reaction (PCR) has been developed for *Cryptosporidium* spp. detection in environmental samples (Soba et al. 2006; Soldan et al. 2006; Trotz-Williams et al. 2006; Santos et al. 2010).

Real-time PCR is a new technique that uses fluorescence to allow amplicon continuous monitoring throughout the reaction (Monis et al. 2005). It has been shown to be very useful in *Cryptosporidium* spp. detection and monitoring (Araújo et al. 2005; Carvalho-Almeida et al. 2005; Meireles et al. 2006; Francino et al. 2006; Huber et al. 2007; Souza et al. 2007; Thomaz et al. 2007; Volotão et al. 2007; Araújo et al. 2007; Gonçalves et al. 2006).

The aim of this study was to evaluate and compare immunological techniques of antigen detection and real-time PCR for the detection and differentiation of *Cryptosporidium* spp. in treated water samples of an area of the Central-West Region of Brazil.

Materials and Methods

Place of Study and Sample Collection

The treated water samples were collected in previously sterilized bottles, with a volume of 5 L. Samples were collected directly from the taps, at the entrance of the residences, in four points of the city of Goiânia. A total of

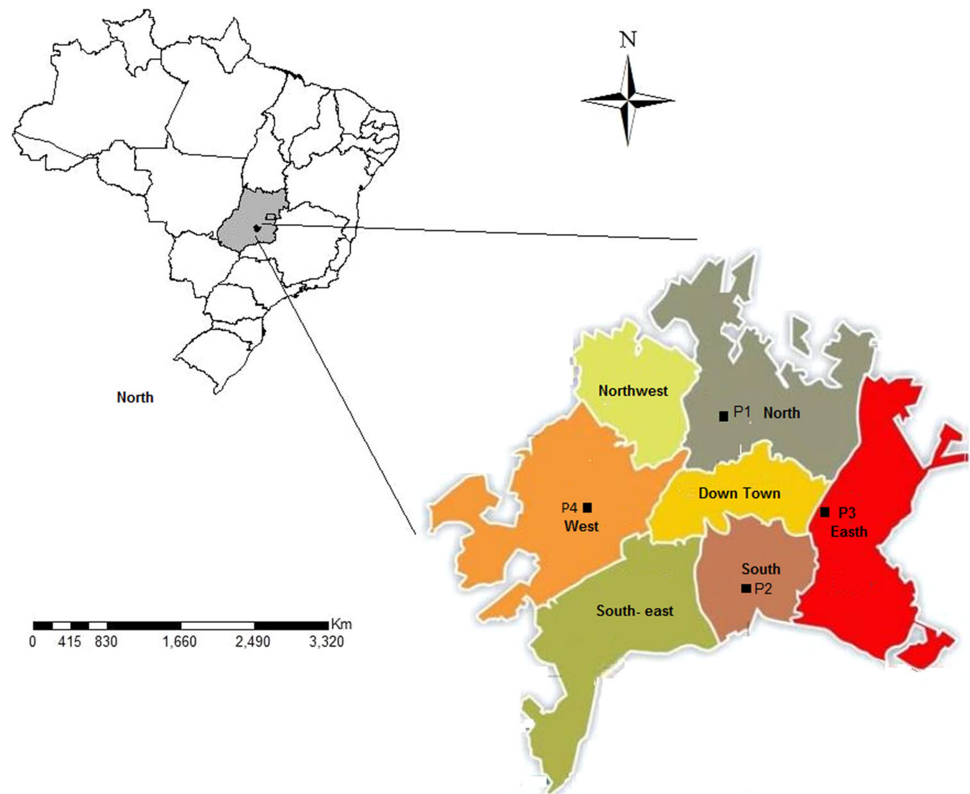

Fig. 1 First point was to the north (*P1*), located at latitude 16°35′07.82″S and longitude 49°16′50.28″O; south (*P2*), latitude 16°42′14.43″S and longitude 49°16′17.32″O; east (*P3*), with latitude 16°39′43.02″S and longitude 49°13′39.95″O; and west (*P4*), longitude 16°41′00.91″S and latitude 49°18′25.02″W

32 samples were collected during the second semester of 2013 (August–November). The first point was to the north, located at latitude 16°35′07.82″S and longitude 49°16′50.28″O; the second point was to the south, latitude 16°42′14.43″S and longitude 49°16′17.32″O; the third point was to the east, with latitude 16°39′43.02″S and longitude 49°13′39.95″O, and the fourth point was to the west, longitude 16°41′00.91″S and latitude 49°18′25.02″W (Fig. 1).

Sample Concentration

The samples were sent for analysis to the Protozoology Unit of the Institute of Tropical Pathology and Public Health (IPTSP/UFG) and the Laboratory of Molecular and Genetic Diagnosis (LDGM-ICB/UFG). Samples were concentrated through the 0.45-µm porosity nylon membrane technique, with 47 mm in diameter and positively charged, according to Silva et al. (2010). The concentrate was transferred to 1.5-mL microcentrifuge tubes and stored at −80 °C for later analysis (immunological and molecular) (Fig. 2).

Detection of *Cryptosporidium* spp. Antigen by DIF and ELISA

For the *Cryptosporidium* spp. investigation, the DIF technique was employed (kit MeriFluor® anti-*Cryptosporidium/Giardia*—Meridian Bioscience, Cincinnati, OH, USA) and, simultaneously, the quantification of oocysts was made (DIF quantitative) from 10 µL of the concentrate, according to the protocol developed by Palmateer et al. (1999).

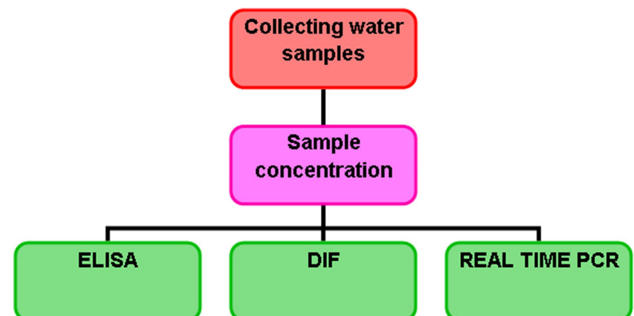

Fig. 2 Immunological and molecular methods used for the detection of *Cryptosporidium* spp. in treated water in the Central-West Region of Brazil

As a comparative measurement, ELISA assay (kit RIDASCREEN®—*Cryptosporidium*) was performed using 10 µL of the concentrate. The DIF and ELISA tests were performed according to manufacturer instructions.

Detection and Differentiation of *Cryptosporidium* spp. by Real-Time PCR

DNA extraction was performed by MagMAX™ commercial kit (Ambion) and following the manufacturer's

recommendations. The extracted DNA and *in natura* samples were sent for molecular diagnosis by real-time PCR at the Institute of Microbiology Paulo de Góes in the Federal University of Rio de Janeiro (UFRJ). When appropriate, a new DNA extraction was performed using the FastDNA® kit (MP Biomedicals).

The systematic approach to performing the real-time PCR assay consisted of a combination of a duplex assay for the detection of *Cryptosporidium* spp. and *C. parvum* and a singleplex one for the detection of *C. hominis* (Jothikumar et al. 2008). In the duplex assay, target sequences were amplified using a specific pair of primers, based on the gene sequence that encodes the small subunit of the ribosomal RNA, JVAF (5′-ATGACGGGTAACGGGGAAT-3′) and JVAR (5′-CCAATTACAAAACCAAAAAGTCC-3′), and genus-specific probe with fluorescent dye and suppressor JVAP18S (CY5—5′-CGCGCCTGCTGCCTTC CTTAGATG-3′—BHQ2). In this same assay, the primers and probe used for *C. parvum* were, respectively, JVAGF (5′-ACTTTTTGTTTGTTTTACGCCG-3′), JVAGR (5′-A ATGTGGTAGTTGCGGTTGAA-3′) and JVAGP2 (FAM—5′-ATTTATCTCTTCGTAGCGGCG-3′—BHQ1). In the singleplex assay, the primers used were JVAGF and JVAGR and the JVAGP1 probe (FAM—5′-ATTTATTAATTTATC TCTTACTTCGT-3′—BHQ3). The total volume of each reaction was 20 µL.

For each duplex reaction, we used 0.5 µL of the solution to 10 pmoL/µL of each primer, 1 µL of 2 pmoL/µL solution of each probe, 1 µL of ultrapure water and 10 µL of Platinum® Quantitative PCR SuperMix-UDG with ROX (Invitrogen). DNA samples were diluted 1:20 to avoid inhibition, and all reactions were run in triplicate, using at 5 µL of DNA and ultrapure water as nontarget control (NTC).

For the singleplex reaction, we used 0.5 µL of the solution to 10 pmoL/µL of each primer, 2 µL solution of 2 pmoL/µL probe, 1.2 µL of ultrapure water, 0.8 µL of $MgCl_2$, 10 µL of Platinum® Quantitative PCR SuperMix-UDG with ROX (Invitrogen) and 5 µL of purified DNA solution at dilution of 1:20.

At the DNA amplification, using the thermocycler 7500 System (Applied Biosystems), there was an initial activation step at 50 °C for 2 min and pre-denaturation at 95 °C for 2 min, followed by 45 cycles that included a denaturation step for 10 s at 94 °C, hybridization for 33 s at 55 °C and extension for 20 s at 72 °C (Jothikumar et al. 2008).

Microbiological Diagnosis

An aliquot of the sample collected (100 mL) was used to qualitative detection of total/fecal coliforms, in accordance with the kit Alfakit-Tecnobac recommendations, which has a minimum detection limit of 60 CFU/100 mL. These data were compared with that obtained from the detection of *Cryptosporidium* spp. oocysts using the techniques described above.

Statistical Analysis

The positivity results were presented by f (absolute frequency) and % (percentage). The Spearman correlation test was used to analyze the correlation between the used techniques, sensitivity and specificity values, positive and negative predictive values and concordance tests. We also calculated the cost/benefit for each technique. All analyses were fixed at 95 % confidence, considering $p < 0.05$.

Results and Discussion

The methodologies used in this study enabled the observation of a high positivity for *Cryptosporidium*, demonstrating that these are useful tools in monitoring treated water, since the treatment processes are not able to completely eliminate the parasite (Pereira et al. 2008). Thus, *Cryptosporidium* monitoring in treated water could reduce the risks of contamination and outbreaks like those that occurred in developed countries. Therefore, researches with the goal of discovering new removal indicators of *Cryptosporidium* spp. oocysts have been carried out, examples of which are the use of polystyrene beads and algae (Le Chevallier and Norton 1992; Huck et al. 2002).

Table 1 Comparison of DIF and ELISA test results (positive and negative)

DIF	Elisa				Total	
	Positive		Negative			
	f	%	*f*	%	*f*	%
Positive	9	100.0	9	39.1	18	56.3
Negative	–	0.0	14	60.9	14	43.8
Total	9	28.1	23	71.9	32	100.0

Spearman correlation $p = 0.001$

Table 2 Comparison of DIF and PCR test results (positive and negative)

DIF	PCR				Total	
	Positive		Negative			
	f	%	*f*	%	*f*	%
Positive	9	60.0	9	52.9	18	56.3
Negative	6	40.0	8	47.1	14	43.8
Total	15	46.9	17	53.1	32	100.0

Spearman correlation $p = 0.699$

Table 3 Microbiological correlation indicators at the presence of *Cryptosporidium* spp. in relation to the employed techniques

Variables	Discordance (%)	Concordance (%)	Positive Conc. (%)	Negative Conc. (%)
PCR × coliform	31.25	68.75	37.50	31.25
ELISA × coliform	40.63	59.37	28.13	31.24
DIF × coliform	34.38	65.62	37.50	28.12

Positive Conc. Concordance between doubly positive results

Negative Conc. Concordance between doubly negative results

The standard method known to the detection of *Cryptosporidium* spp. in water is the method 1623 of the Environmental Protection Agency (EPA—USA), that used indirect immunofluorescence (DIF) as diagnostic. So, we used DIF in comparison with other tests, as ELISA and real-time PCR.

Of the 32 analyzed samples, 50.0 % (16/32) were positive for *Cryptosporidium* using real-time PCR technique, 28.1 % (9/32) using ELISA technique and 56.3 % (18/32) using the DIF technique.

In comparison between tests, it is observed in Table 1 that there is a correlation between the DIF and ELISA tests ($p = 0.001$), with 23 (71.9 %) concordant samples and 9 (28.1 %) discordant ones, for these techniques.

It can be observed in Table 2 that there is no correlation of results between PCR and DIF tests ($p = 0.699$), because there were 17 (53.1 %) concordant samples and 15 (46.9 %) discordant ones.

DIF and real-time PCR showed higher positivity than the enzyme-linked immunosorbent assay (ELISA). This result is due to the fact that ELISA, although standardized by researchers and technicians and used for oocysts control in water, has a disadvantage of being slower and having low sensitivity (Stancari 2013; Ungar 1990). Thus, the DIF has the advantage of high sensitivity and specificity, and it is simple to perform, being effective for the detection of *Cryptosporidium* spp. in treated water (Jex et al. 2008).

PCR detected the specific species *C. hominis*, reinforcing that correct identification of the protozoan species, especially in this water, is of great importance to assess the risk of human infection, suggesting contamination of source waters by human sewage (or contamination after water treatment depending on the integrity of the distribution system).

In samples in which PCR was negative and DIF was positive, interfering action on the DIF could also have occurred, such as organic and inorganic substances capable of binding to antibodies and promoting a false-positive result (Rodgers et al. 1995).

Although DIF is recommended as the gold standard, it has some limitations, such as the use of antibodies that are not species-specific. The variations of PCR have been developed and standardized to detect species of *Cryptosporidium* in water (Soba et al. 2006; Soldan et al. 2006; Trotz-Williams et al. 2006; Hashimoto et al. 2006, Keshavarz et al. 2009; Doi 2009; Pilai 2009).

In all, 5.6 % of the samples were positive for bacterial indicators: 25 % (2/8) of samples were positive to the point P1, 75 % (6/8) for P2, 75 % (6/8) for P3 and 50 % (4/8) for P4. Correlation of microbiological indicators with the presence of *Cryptosporidium* spp. in analyzed samples is shown in Table 3. On the association of detection of *Cryptosporidium* spp. according to each used technique and the presence of fecal and/or total coliforms as indicators, it was observed that they are not correlated with the presence of *Cryptosporidium* spp. in the analyzed samples corroborating data (Ahmed et al. 2014). The largest discrepancy between the tests for the detection of *Cryptosporidium* and coliforms was observed with ELISA (40.63 %), and the higher concordance was observed between the results of real-time PCR, with 68.75 % agreement. The coliform presence in treated water is of concern, because it indicated a high percentage of samples outside the standards recommended by the Ordinance MS (Ordinance No. 2,914), which could expose consumers of this water to health problem risks.

Conclusions

There was no statistically significant difference between the techniques analyzed (DIF or RT-PCR) for the detection of *Cryptosporidium* spp. in environmental samples. From the methodology applied in this study, it was found that treated water that supplies the city of Goiânia (four regions) is contaminated with *Cryptosporidium* spp., denoting the importance of implementing techniques for protozoa detection, in the assessment of water quality for human consumption.

References

Ahmed W, Brandes H, Gyawali P, Sidhu JPS, Toze S (2014) Opportunistic pathogens in roof-captured rainwater samples, determined using quantitative PCR. Article Water Res 53:361–369

Adl SM, Simpson AG, Farmer MA, Andersen RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup O, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor MF (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J Eukaryot Microbiol 52(5):399–451

Anusz KZ, Mason PH, Riggs MW, Perryman LE (1990) Detection of *Cryptosporidium parvum* oocysts in bovine feces by monoclonal antibody capture enzyme-linked immunosor assay. J Clin Microbiol 28:2770–2774

Appelbee AJ, Thompson RCA, Olson ME (2005) *Giardia* and *Cryptosporidium* in mammalian wildlife—current status and future needs. Trends Parasitol 21:370–376

Araújo RS, Carvalho-Almeida TT, Matté GR, Rojas MVR, Pereira A, Matté MH (2005) Detecção de oocistos de *Cryptosporidium* spp. em amostras de água salobra. Bol Inst Adolfo Lutz 15:31–32

Araújo AJUS, Gomes AHS, Almeida ME, Kanamura HY (2007) Detecção de *Cryptosporidium* meleagridis em amostras fecais de pacientes HIV positivos no Brasil. Rev Panam Infectol 9:38–40

Carvalho-Almeida TT, Casimiro AM, Matte GR, Matte MH (2005) Na improved method for extracting *Cryptosporidium* spp. DNA from preserved faeces and potential application for cryptosporidiosis surveillance. Rev Bras Vig Sanit 1:208–231

Doi Y (2009) *Cryptosporidium* Pig genotype II in immunocompetent man. Emerg Infect Dis 15(6):982–983

Fahey T (2003) Cryptosporidiosis. Infect Dis Update 10(2):75–80

Feng Y, Ortega Y, Cama V, Terrell J, Xiao L (2008) High intragenotypic diversity of *Giardia duodenalis* in dairy cattle on three farms. Parasitol Res 103:87–92

Francino O, Altet L, Sanchez-Robert E, Rodriguez A, Solano-Gallego L, Alberola J, Ferrer L, Sanchez A, Roura X (2006) Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis. Vet Parasitol 137:214–221

Franco, RMB Elayse MH, Maria Ines ZS, Rita Maria LM, Eduardo de CS, Marcela MCC, Romeu CN, Daniel AC, Nilson B, Diego AGL (2012) Avaliação da performance de metodologias de detecção de *Cryptosporidium* spp. e *Giardia* spp. em água destinada ao consumo humano, para o atendimento às demandas da Vigilância em Saúde Ambiental no Brasil. Epidemiol. Serv. Saúde [online] 21(2), 233–242. ISSN 1679-4974

Gertler M, Dürr M, Renner P, Poppert S, Askar M, Breidenbach J, Frank C, Preußel K, Schielke A, Werber D, Chalmers R, Robinson G, Feuerpfeil I, Tannich E, Gröger C, Stark K, Wilking H (2015) Outbreak of *Cryptosporidium hominis* following river flooding in the city of Halle (Saale), Germany, August 2013. BMC Infect Dis 15:88

Gomes AHS, Kanamura HE, Almeida ME, Araújo AJUS (2004) Detecção de *Cryptosporidium* em amostras fecais por técnica de Nested – PCR e comparação com métodos imunológicos e parasitológicos. Revista Instituto Adolfo Lutz 2(63):255–261

Gonçalves EM, Da Silva AJ, Eduardo MB, Uemura IH, Moura IN, Castilho VL, Corbett CE (2006) Multilocus genotyping of *Cryptosporidium hominis* associated with diarrhea outbreak in a day care unit in São Paulo. Clinics 61:119–126

Gonzáles-Ruiz A, Bendall R (1985) Size matters: the use of the ocular micrometer in diagnostic parasitology. Parasitol Today 11:83–85

Guy RA, Payment P, Krull UJ, Horgen PA (2003) Real-time PCR for quantification of *Giardia* and *Cryptosporidium* in environmental water samples and sewage. Appl Environ Microbiol 69(9): 5178–5185

Hashimoto A, Sugimoto H, Morita S, Hirata T (2006) Genotyping of single *Cryptosporidium* oocysts in sewage by semi-nested PCR and direct sequencing. Water Res 40:2527–2532

Hlavasa MC, Watson JC, Beach MJ (2005) Cryptosporidiosis surveillance—United States 1999–2002. MMWR Surveill Summ 54:1–8

Huber F, Da Silva S, Bomfim TC, Teixeira KR, Bello AR (2007) Genotypic characterization and phylogenetic analysis of *Cryptosporidium* spp. from domestic animals in Brazil. Vet Parasitol 150:65–74

Huck PM, Coffey BM, Emelko MB, Maurizio DD (2002) Effects of filter operation on *Cryptosporidium* removal. J Am Water Works Assoc 94(6):97–111

Jex AR, Smith HV, Monis PT, Campbell BE, Gasser RB (2008) *Cryptosporidium*—biotechnological advances in the detection, diagnosis and analysis of genetic variation. Biotechnol Adv 26:304–317

Jothikumar N, Silva AJ, Moura I, Qvarnstrom Y, Hill VR (2008) Detection and differentiation of *Cryptosporidium hominis* and *Cryptosporidium parvum* by dual TaqMan assays. J Med Microbiol 57:1099–1105

Karanis P, Kourenti C, Smith H (2007) Waterborne transmission of protozoan parasites: a world wide review of outbreaks and lessons learnt. J Wat Health 5:1–38

Keshavarz A, Haghighi A, Athari A, Kazemi B, Abadi A, Mojarad EN (2009) Prevalence and molecular characterization of bovine *Cryptosporidium* in Qazvin province, Iran. Vet Parasitol 160(3–4):316–318

King B, Fanok S, Phillips R, Swaffer B, Monis P (2015) Integrated *Cryptosporidium* assay to determine oocyst density, infectivity, and genotype for risk assessment of source and reuse water. Appl Environ Microbiol 81:3471–3481. doi:10.1128/AEM.00163-15

Lake IR, Nichols G, Bentham G, Harrison FC, Hunter PR, Kovats SR (2007) Cryptosporidiosis decline after regulation, England and Wales, 1989–2005. Emerg Infect Dis 13:623–625

Le Chevallier MW, Norton WD (1992) Examining relationships between particle counts and Giardia, *Cryptosporidium* and turbidity. J Am Water Works Assoc 84(12):54–60

Mackenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blaie KA, Peterson DE, Kazmierrczak JJ, Addiss DG, Fox KR, Rose JB, Davis JP (1994) A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. New Engl J Med Boston 3:161–167

Mark AG, John B (1994) Incidence of respiratory cryptosporidiosis in Georgia Broilers: 1987–92. Avian Dis 38(2):358–360

Meireles MV, Soares RM, Dos Santos MM, Gennari SM (2006) Biological studies and molecular characterization of a *Cryptosporidium* isolate from ostriches (*Struthio camelus*). J Parasitol 92:623–626

Monis PT, Giglio S, Keegan AR, Andrew Thompson RC (2005) Emerging technologies for the detection and genetic characterization of protozoan parasites. Trends Parasitol 21:340–346

Morgan UM, Thompson RCA (1998) PCR detection of *Cryptosporidium*, the way forward? Parasitol Today 14:241–245

Palmateer G, Manz D, Jurkovic A, Mclnnis R, Unger S, Kwan KK, Dutka BJ (1999) Toxicant and parasite challenge of manz intermittent slow sand filter. Environ Toxicol 14:217–225

Pereira JT, Costa A, Silva MBO, Schuchard W, Osaki SC, Castro EA, Paulino RC, Thomaz-Soccol V (2008) Comparing the efficacy of chlorine, chlorine dioxide, and ozone in the inactivation of *Cryptosporidium parvum* in water from Paraná State, Southern Brazil. Appl Biochem Biotechnol 151:464–473

Pilai DR (2009) *Cryptosporidium* spp. Rabbit genotype, a newly identified human pathogen. Emerg Infect Dis 15(5):829–830

Plutzer J, Karanis P (2009) Genetic polymorphism in *Cryptosporidium*: an update. Vet Parasitol 165:187–199

Rodgers MR, Flanigan DJ, Jakubowsk W (1995) Identification of algae which interfere with the detection of Giardia cysts and

Cryptosporidium oocysts and a method for alleviating this interference. Appl Environ Microbiol 61(10):3759–3763

Santos SFO, Silva HD, Souza-Junior ES, Anunciação CE, Silveira-Lacerda EP, Vilanova-Costa CAST, Garcia-Zapata MTA (2010) Environmental monitoring of opportunistic protozoa in rivers and lakes in the neotropics based on yearly monitoring. Water Quality Exposure Health 2:1–8

Silva HD, Wosnjuk LAC, Santos SFO, Vilanova-Costa CAST, Pereira FC, Silveira-Lacerda EP, García zapata MTA, Anunciação CE (2010) Molecular detection of adenoviruses in lakes and Rivers of Goiânia, Goiás, Brazil. Food Environ Virol 2:35–40

Soba B, Petrovec M, Mioc V, Logar J (2006) Molecular characterization of *Cryptosporidium* isolates from humans in Slovenia. Clin Microbiol Infect 12:918–921

Soldan OCP, Vasquez FV, Varas G, Cordo GP, Soto JRV, Sanches-Moreno M, Gonzales IR, Lombardo MJR (2006) Intestinal parasitism in Peruvian children and molecular characterization of *Cryptosporidium* species. Parasitol Res 98:576–581

Souza SL, Gennari SM, Richtzenhain LJ, Pena HF, Funada R, Cortez A, Gregori F, Soares RM (2007) Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from the state of São Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (GDH) coding gene. Vet Parasitol 149:258–264

Stancari RCA (2013) Determinação da sensibilidade do teste de ELISA para pesquisa de *Cryptosporidium* spp. e Giardia spp. em amostras de águas brutas. Rev Inst Adolfo Lutz. São Paulo 72(3):234–238

Sunderlanda D, Graczyk TK, Tamanga L, Breyssea PN (2007) Impact of bathers on levels of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts in recreational beach waters. Water Res 41:3483–3489

Thomaz A, Meireles MV, Soares RM, Pena HF, Gennari SM (2007) Molecular identification of *Cryptosporidium* spp. from fecal samples of felines, canines and bovines in the state of São Paulo, Brazil. Vet Parasitol 150:291–296

Trotz-Williams LA, Martin DS, Gatei W, Cama V, Peregrine AS, Martin SW, Nydam DV, Jamieson F, Xiao L (2006) Genotype and subtype analyses of *Cryptosporidium* isolates from dairy calves and humans in Ontario. Parasitol Res 99:346–352

Tyzzer EE (1907) A sporozoan found in the peptic glands of the common mouse. Proc Soc Exp Biol Med 5:12–13

Tyzzer EE (1912) *Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. Arch Protistenkd 26:394–412

Tzipori S, Griffiths JK (1998) Natural history and biology of *Cryptosporidium parvum*. Adv Parasitol 40:5–36

Tzipori S, Ward H (2002) Cryptosporidiosis: biology, pathogenesis and disease. Microbes Infect 4:1047–1058

Ungar B (1990) Enzyme-linked immunoassay for detection of *Cryptosporidium* antigens in fecal specimens. J Clin Micro 28(11):2491–2495

Volotão AC, Costa-Macedo LM, Haddad FS, Brandão A, Peralta JM, Fernandes O (2007) Genotyping of *Giardia duodenalis* from human and animal samples from Brazil using beta-giardin gene: a phylogenetic analysis. Acta Trop 102:10–19

Xiao L (2010) Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol 124:80–89

Xiao L, Feng Y (2008) Zoonotic cryptosporidiosis. FEMS Immunol Med Microbiol 52:309–323

Xiao L, Sulaiman I, Fayer R, Lal AA (1998) Species and strain-specific typing of *Cryptosporidium* parasites in clinical and environmental samples. Memórias do Instituto Oswaldo Cruz 93(5):687–692

Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA (1999) Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Microbiol 65:3386–3391

Xiao L, Singh A, Limor J, Graczyk TK, Gradus S, Lal A (2001) Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and wastewater. Appl Environ Microbiol 67(3):1097–1101