SYSTEMATIC REVIEW: THE IMPORTANCE OF WATER IN THE EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN BRAZIL*

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ABSTRACT

This is a review of the scientific literature on the presence and types of *Cryptosporidium* spp. in Brazilian water sources. We assess the importance of waterborne oocyst in the epidemiology of cryptosporidiosis in Brazil. The PubMed, ScienceDirect, and Scielo databases were searched using the following keywords: "*Cryptosporidium* and Waterborne", "*Cryptosporidium* and Water", "*Cryptosporidium* and Brazil", "*Cryptosporidium* and Environment", "*Cryptosporidium* and Brazil and Environmental", "*Cryptosporidium* and Brazil and waterborne and Environmental". We discuss original articles focused on waterborne cryptosporidiosis published in Portuguese or English and reporting research performed in Brazil. We excluded studies undertaken in countries other than Brazil and articles not directly relevant to waterborne cryptosporidiosis. Using the above listed search terms, a total of 149 articles were found in PubMed, of which 10 were met the inclusion criteria; in the ScienceDirect database, we found 306 articles all of which were excluded because they were foreign articles. In Scielo, 214 articles were found, of which we selected 8. Thus, 18 studies were analyzed. According to these publications, a large part of the Brazilian population is at risk of infection by this protozoan. We conclude that research on waterborne transmission of cryptosporidium should be intensified and should include regions of Brazil which are understudied. This research is particularly relevant to populations affected by AIDS or living in communities where AIDS is highly prevalent.

Keywords: *Cryptosporidium*, cryptosporidiosis, public health, environmental contamination, water contamination.

REVISÃO SISTEMÁTICA: A IMPORTÂNCIA DA ÁGUA NA EPIDEMIOLOGIA DA CRIPTOSPORIDIOSE NO BRASIL

RESUMO

Este trabalho é a compilação de publicações científicas que buscaram ativamente *Cryptosporidium* sp. em recursos hídricos brasileiros, e quais espécies foram mais encontradas, com o intuito de investigar qual é a importância da água na epidemiologia da criptosporidiose neste país. As bases de dados consultadas foram Pubmed, ScienceDirect e Scielo, por meio das palavras-chave: "*Cryptosporidium* and Waterborne", "*Cryptosporidium* and Water", "*Cryptosporidium* and Brazil", "*Cryptosporidium* and Environment", "*Cryptosporidium* and Brazil and Environmental", "*Cryptosporidium* and Brazil and waterborne and Environmental". A seleção foi realizada por critérios de inclusão, sendo estes:

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artigos originais sobre criptosporidiose por meio hídrico publicados em português ou inglês conduzidos no Brasil. Os seguintes critérios de exclusão foram adotados: trabalhos estrangeiros, revisões de literatura ou assuntos não pertinentes ao foco desta pesquisa. Na base de busca do Pubmed, foram encontrados 149 artigos, dos quais se selecionaram dez; na ScienceDirect 306 trabalhos foram identificados, sendo considerados inviáveis para revisão, pois são artigos estrangeiros; e na Scielo*,* 214 artigos, dos quais selecionaram-se oito. Desta forma, 18 estudos foram analisados. Há evidências de que uma grande parte da população brasileira esteja em risco de infecção por este protozoário e estudos acerca deste assunto deveriam ser intensificados e distribuídos de maneira mais uniforme a fim de beneficiar um maior número de brasileiros, especialmente em regiões onde as taxas de prevalência e de incidência de AIDS são mais elevadas.

Palavras-chave: *Cryptosporidium*, saúde pública, criptosporidiose, contaminação ambiental, contaminação hídrica.

REVISIÓN SISTEMÁTICA: LA IMPORTANCIA DEL AGUA EN LA CRIPTOSPORIDIOSIS EPIDEMIOLOGÍA EN BRASIL

RESUMEN

El presente trabajo es una recopilación de las publicaciones científicas que buscaban activamente *Cryptosporidium* sp. en los recursos hídricos de Brasil, y que las especies aisladas, con el fin de investigar lo que es la importancia del agua en la epidemiología de la criptosporidiosis en este país. Las bases de datos consultadas fueron PubMed, ScienceDirect y Scielo, a través de las palabras clave: "*Cryptosporidium* and Waterborne", "*Cryptosporidium* and Water", "*Cryptosporidium* and Brazil", "*Cryptosporidium* and Environment", "*Cryptosporidium* and Brazil and Environmental", "*Cryptosporidium* and Brazil and waterborne and Environmental". La selección se realizó mediante criterios de inclusión, a saber: artículos originales sobre el medio ambiente del agua de la criptosporidiosis publicados en portugués o inglés realizados en Brasil. Se adoptaron los siguientes criterios de exclusión: estudios extranjeros, revisiones bibliográficas o no pertinentes para el enfoque de estos temas de investigación. En la búsqueda de bases de datos Pubmed se encontraron 149 artículos, de los cuales se seleccionaron diez; ScienceDirect en 306 artículos fueron identificados para su revisión se consideraron inviable, ya que son artículos extranjeros y Scielo, 214 artículos, de los cuales se seleccionaron ocho. Por lo tanto, se analizaron 18 estudios. Hay evidencia de que una gran parte de la población está en riesgo de infección por este protozoo y estudios sobre este tema deben ser intensificados y extendidos de manera más uniforme con el fin de beneficiar a un mayor número de brasileños, especialmente en las regiones donde las tasas de prevalencia y la incidencia del SIDA son más frecuentes

Palabras clave: *Cryptosporidium*, salud pública, criptosporidiosis, contaminación ambiental, contaminación hídrica.

INTRODUCTION

The genus Cryptosporidium comprises obligate parasitic protozoa. These pathogens parasitize the epithelial cells of the gastrointestinal tract of their hosts (1), which include mammals, birds, reptiles and fish (2). To date, 30 *Cryptosporidium* species have been described, 12 infecting mammals. In addition 61 genotypes of uncertain taxonomic status have been identified based on host specificity and genotypic properties (3-5).

Morphology and antigenic properties cannot discriminate between different *Cryptosporidium* species because oocytes show little morphological variations. The presence of conserved antigens hinders species differentiation by immunological tests (1,6). Thus, molecular tools are essential in identifying which species are present in a host or in water and which species are most relevant to public health. Thus far, five species have been found to infect humans: *Cryptosporidium hominis*, *Cryptosporidium parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, and *Cryptosporidium canis* (7-10).

The presence of this parasite has been reported in humans. Historically, outbreaks of waterborne cryptosporidiosis were associated, in large part, to access of human beings to water reservoirs (11-13).

The first recorded outbreak caused by contaminated drinking water occurred in 1989 in Swindon and Oxfordshire in the United Kingdom, affecting about 5,000 people (14). In 1993, a larger outbreak afflicted 403,000 residents of Milwaukee in the United States (15). These outbreaks were linked to water as the main route of transmission. Contamination of drinking water with oocysts occurred even after treatment (16).

In 2004, cryptosporidiosis was included in the Neglected Diseases Initiative of the World Health Organization because of its close association with poor sanitation and low purchasing power (17). To assess the importance of water in the epidemiology of cryptosporidiosis in Brazil, here we summarize the scientific literature describing the presence and the species types of *Cryptosporidium* oocysts in Brazilian water sources.

METHODS

The PubMed (http://www.pubmed.org), Scielo (http://www.scielo.org), and ScienceDirect (http://www.sciencedirect.com) databases were searched in December 2012. The keywords used were “*Cryptosporidium* and Waterborne”, “*Cryptosporidium* and Water”, “*Cryptosporidium* and Brazil”, and “*Cryptosporidium* and Environment”. The term *Cryptosporidium* was used alone to quantify the sample universe in each database and to define the selection strategy of items. For databases with a sampling universe of <500 publications, a bibliographic triage was done by reading the title and abstract; when >500 publications were identified, a triage was performed directly by using the keywords “Cryptosporidium and Brazil and Waterborne and Environment”. Literature reviews, experiments conducted abroad, and articles not relevant to the purpose of this study were excluded.

RESULTS AND DISCUSSION

The keyword “*Cryptosporidium*” retrieved very different publication lists from three databases. We found 214 publications in Scielo, 10,378 in ScienceDirect, and 6,527 in PubMed (Table 1).

Applying the exclusion criteria for this systematic review, 8 scientific articles were found in Scielo and 10 articles in PubMed, with one article in common to these databases. No publication from ScienceDirect was included because all indexed studies were conducted in foreign regions, even if the word “Brazil” was included in the keywords searched. Therefore, in this review, we included 18 articles related to the importance of water in the epidemiology of cryptosporidiosis in Brazil. The oldest article in our list was published in 1993, with a new publication only 7 years later. In 1990’s, four papers were published compared with 14 in the next decade, equivalent to an increase of almost 350% in scientific productivity.

Table 1. Number of publications about cryptosporidiosis retrieved in different databases according to the keyword(s) used.

Keywords	Number of publications by database		
	Scielo	**ScienceDirect**	**PubMed**
Cryptosporidium	214	10378	6527
Cryptosporidium AND Waterborne	9	2026	438
Cryptosporidium AND Water	50	6811	1656
Cryptosporidium AND Brazil	58	1244	149
Cryptosporidium AND Environmental	43	63	960

Among the 18 articles reviewed here, 6 (33.3%) were conducted exclusively in the region of Campinas, SP; 14 (77.8%) in the state of São Paulo; 1 (5.6%) in Minas Gerais; and 2 (11.1%) and 1 (5.6%) in the northeast and south of Brazil, respectively. This distribution seems to reflect that microbiological analysis of water was performed just in close proximity to the study institutions routinely. The University of Campinas (UNICAMP) and the University of São Paulo (USP) published the largest number of articles, representing 38.9% and 33.3% of the selected publications, respectively. The high percentage of work conducted in the state of São Paulo only benefits directly the local population, which is <17.6% of the national population (18).

This becomes even more critical considering that *Cryptosporidium* spp. were detected in water samples in 17 of 18 (94.4%) of the screened articles; however, only one study performed molecular characterization to assess if the oocysts were pathogenic to man.

Newman et al. (19) searched for water sources contaminated with *Cryptosporidium* in an urban slum in Fortaleza, in the state of Ceará, where cryptosporidiosis is common. Stool samples from 127 domestic animals and 18 local water sources were examined using acid fast staining and immunofluorescence techniques. Oocysts were detected in 10.2% of stool samples from domestic animals and in 22.2% of water sources. The authors concluded that the animals may be involved in water contamination, which could explain the high prevalence of the disease in this region.

Gamba et al. (20) sampled 10 deep wells and two septic tanks in the municipality of Itaquaquecetuba, São Paulo, a region with a high infant mortality. Convenience sampling was done, selecting critical locations and considering inadequate construction and/or proximity between a well and a septic tank. Oocysts were concentrated using the calcium carbonate flocculation method, and the Merifluor commercial kit (Meridian BioScience) was used to detect oocysts. Two of 10 wells and 2 of 2 septic tanks were positive for *Cryptosporidium* spp. The authors suggested a need for monitoring the water supplies of São Paulo.

Franco et al. (21) investigated water samples from the Atibaia River, Campinas, São Paulo, collected during three consecutive weeks. The membrane filtration technique was used to concentrate the oocysts, which were recovered by two different methods: membrane washing and scraping or dissolving it in acetone. All samples were positive. The authors recommend further work to determine the viability of these oocysts in order to assess their impact on public health.

Farias et al. (22) collected 24 effluent samples evenly distributed between the Edu Chaves Sewage Pumping Station and the Pirajussara creek in São Paulo between July and December 1998. Calcium carbonate flocculation and membrane filter dissolution were used to concentrate the oocysts. All samples were positive for *Cryptosporidium* spp., with a range of 80–912 and 65–760 oocysts/L in raw sewage and creek samples, respectively. The efficiencies of the oocyst concentration techniques did not differ, but the authors suggest the development of new methods aimed at minimizing losses of oocysts during the procedure.

Feitosa et al. (23) assessed the prevalence of *Cryptosporidium* spp. in stool samples from 459 newborn calves and in water and samples from the hutch floors of 33 dairy farms in the Araçatuba region, state of São Paulo. The prevalence of cryptosporidiosis in animals was 10.3% by enzyme-linked immunosorbent assay and 12.4% using the Sheather's flotation method. Water samples were negative, and two soil samples were positive. However, the analysis of environmental contamination may have underestimated the actual contamination owing to the method employed. The oocysts were concentrated exclusively by Sheather's method, unlike the techniques employed in other studies described in this review.

Santos et al. (24) collected samples of activated sludge from the sewage treatment station in Campinas, São Paulo, for eight consecutive weeks. The samples were analyzed using two methods of oocyst concentration: clarification with ether and sucrose flotation, followed by the use of two commercial fluorescent monoclonal antibody kits to detect oocysts (Merifluor, Meridian BioScience, and Crypto/*Giardia*-Cel I. F. Test, CELLABS PTY LTD). *Cryptosporidium* oocysts were present in 5 of 8 (62.5%) samples, considering all the techniques used to concentrate and detect oocysts. The authors concluded that viability studies of the protozoan found are required before the activated sludge is reused in agriculture, since it could endanger public health.

Hachich et al. (25) assessed the presence of *Giardia* spp. and *Cryptosporidium* spp. in drinking water sources in the state of São Paulo, Brazil. A total of 278 water samples were collected during a period of 19 months from 28 locations in 10 watersheds. Oocysts were concentrated and visualized using calcium carbonate flocculation followed by direct immunofluorescence. The authors reported that 27% and 2.5% were contaminated with *Giardia* spp. and *Cryptosporidium*, respectively. The authors warned about the need to improve the quality of drinking water used in the state of São Paulo.

Iacovski et al. (26) investigated the presence of *Cryptosporidium* spp. by immunomagnetic separation (IMS) followed by immunofluorescence in sludge samples obtained from the Municipal Wastewater Treatment Plant in Florianopolis, in the state of Santa Catarina. Among the 27 samples, 20 (74.1%) were positive for coccidia. The method applied in this study was an effective multiple analysis tool for monitoring the presence of *Cryptosporidium* oocysts in sludge samples originating from urban wastewater. The authors warned about the risk of oocyst contamination of sludge intended for land application.

Neto et al. (27) investigated the efficiency of (oo)cyst removal by an activated sludge reactor and ultraviolet (UV) light in a wastewater treatment plant in Campinas, SP. Three sampled sites were evaluated for the study: influent, wastewater without UV treatment, and wastewater disinfected with UV. *Cryptosporidium* oocysts were found in two influent samples and in one effluent disinfected with UV radiation. The authors concluded that activated sludge treatment reduced cysts and oocysts by 98.9% and 99.7%, respectively, whereas the UV disinfection process was not completely effective way to inactivation against *Giardia* spp.

Rondello et al. (28) compared two methods for detecting *Giardia* spp. and *Cryptosporidium* spp. in samples of activated sludge from a sewage treatment plant in the city of Campinas, SP. A total of 22 samples were assessed for the presence of these protozoa after concentration by centrifugation, followed or not by purification (clarification with ether and sucrose flotation). The recovery rate of *Giardia* cysts was higher in the absence of purification. No significant differences between the two methods were found in the detection of *Cryptosporidium* oocysts, as only two samples were positive. The researchers concluded that centrifugation is the simplest and cheapest method for (oo)cyst concentration.

Dias et al. (29) examined samples from eight watersheds, raw sewage from two swine facilities and wastewater treatment effluent (WWTE) in the Ribeirão São Bartolomeu basin, municipality of Viçosa, MG. Quarterly samples were collected at each point for a year to investigate the prevalence of *Giardia* spp. and *Cryptosporidium* spp. and their possible seasonality. Oocysts were concentrated by calcium carbonate precipitation and detected by direct immunofluorescence. The geometric mean concentration of *Giardia* cysts and *Cryptosporidium* oocysts water sources was 3.9 cysts/L and 3.6 oocysts/L, respectively. There was a high concentration of *Giardia* spp. (geometric mean in the order of 10^4/L) but not *Cryptosporidium* spp. in the effluent of the WWTE. The mean values of (oo)cysts and rainfall collection of the quarter were well correlated (R^2 = 98.3%; P = 0.0087 for *Giardia* spp. and R^2 = 91.8%; p = 0.0421 for *Cryptosporidium* spp.), which indicates a seasonal pattern. The authors recommend the protection of watershed areas to prevent the transmission of waterborne parasitic diseases.

Machado et al. (30) studied the occurrence of *Cryptosporidium* oocysts in raw surface water, from September 2003 to August 2004 in Recife, PE. The samples were analyzed for 12 months during periods of drought and normal rainfall. Water samples were collected in the catchments of the Tapacurá River, Capibaribe River in Tiúma, Capibaribe River in Castelo, and River Duas Unas. Samples from the inlet and outlet of the treatment system were stored in five-gallon containers. A total of five sites and 60 samples of raw water and 12 treated water samples were analyzed for the presence of oocysts. Samples were filtered with cellulose ester membranes under negative pressure to concentrate oocysts (31). To detect the oocysts, the authors used a direct immunofluorescence technique (Merifluor kit; Meridian Bioscience Diagnostics, Cincinnati, OH, USA) as recommended by the manufacturer, together with the dye 4′6′-diamidino-2-phenylindole (32). A mathematical formula was used to calculate the average number of oocysts per liter (33). Samples of raw and treated water were analyzed, and distilled water used as a negative control. Tests were carried out with two replicates for each sample type. *Cryptosporidium* oocysts were provided by the parasitology section of the Central State Laboratory, purified by flotation with a 1.2 g/mL saturated NaCl solution (34) and quantified according to the Kinyoun method and preserved in 10% formalin under refrigeration. Samples were inoculated according to the protocol of the USEPA Method 1622 (35). The treated water was negative for *Cryptosporidium* oocysts. However, raw water samples were positive by IFA/DAPI in 40% (2 of 5) of the locations and 5% (3 of 60) of the samples. There was a cattle pen near the pickup point of the Tapacurá River, was identified as possible source of oocysts (36). This was the first report of *Cryptosporidium* oocysts water sources in Pernambuco and the northeast of the country.

Razzolini et al. (37) detected the presence of *Giardia* cysts and *Cryptosporidium* oocysts in watersheds and drinking water sources in urban areas of Brazil. A total of 25 samples, 12 drinking water and 13 surface water, were examined (38). *Giardia* cysts were detected in 41.7% and *Cryptosporidium* oocysts in 25.0% drinking water samples. Positive samples from raw water for *Giardia* cysts and *Cryptosporidium* oocysts were 46.1 and 7.6%, respectively. The results of this study point to the need to monitor these organisms in drinking water and river basins.

Neto et al. (39) evaluated the presence of *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts in the water supply of Campinas, SP, which draws water from the Atibaia River. Protozoa were detected by immunofluorescence assay (IFA) and confirmed by differential interference contrast microscopy. *Cryptosporidium* oocysts were detected in 62.5% of samples with concentrations ranging from 15 to 60 oocysts/L. *Giardia* spp. cysts were detected in 87.5% of samples studied, at concentrations from 2.5 to 120 cysts/L. The authors considered the values high and associated high oocyst and cyst concentrations to the discharge of untreated sewage into the Atibaia River. Thus, they recommended the adoption of measures to protect sources of drinking water.

Santos et al. (40) collected 53 samples of raw sewage, 53 samples of treated sewage without disinfection by UV light (EFL), and 38 samples treated and disinfected by this technique (EFL + UV) from the Campinas Sewage Treatment Plant. The samples were concentrated by two methods: centrifugation-flotation or membrane filtration. *Cryptosporidium* oocysts were detected in 6.4% of raw sewage samples and 2.6% of UV treated effluent samples. No statistical differences were noted in the results between the concentration methods used.

Araújo et al. (41) surveyed 30 different locations in the state of São Paulo for the presence of waterborne *Cryptosporidium* oocysts. The polymerase chain reaction was used to amplify a portion of the 18S rRNA gene. DNA was extracted from water concentrate samples recovered by filtration *Cryptosporidium* DNA was detected in 30% of the samples. Sequencing of the 18S amplicons revealed the presence of *Cryptosporidium hominis* and *C. meleagridis*. Samples of recreational water were also positive for *Cryptosporidium* spp. *C. hominis* and *C. meleagridis* were identified in surface water samples. This was the first report of *C. hominis* in environmental samples in Brazil.

Branco et al. (42) investigated the occurrence of *Cryptosporidium* spp. and *Giardia* spp. in natural springs and residents of Campos do Jordão, SP. They evaluated 12 springs (six in urban areas and six in rural areas) bimonthly for a year, in three strategic occasions: dry season (June to September), the rainiest months (October to January), and the drier months (February to May). The samples were subjected to membrane filtration followed by elution and centrifugation. Water pellets were analyzed by IFA using monoclonal antibodies and visualized by phase contrast microscopy. At least one of these protozoa was detected in 25.0% (3 of 12) of the spring samples, with mean concentrations ranging from 0.2 to 0.3 oocysts/L and 0.07 to 0.1 cysts/L. Enteroparasites were detected in 91 of 185 stool specimens analyzed. *Cryptosporidium* spp. was the most prevalent parasite (8.1%), followed by *Giardia*. (5.9%). The authors concluded that the high prevalence of parasitic infections in specific populations favors transmission through contaminated soil and water, contributing to the maintenance of the parasite's life cycle. Thus, it would be necessary to adopt measures such as protecting the springs, installing a minimum health infrastructure, and providing primary education to the population, in order to control and prevent infections by these parasites.

Sato et al. (43) assessed the risk of infection by *Giardia* spp. and *Cryptosporidium* spp., and the prevalence of these protozoa in the public water supply in four urbanized areas of São Paulo State, Brazil. The collection points were located in nine different watersheds of the eastern region of the state covering the Metropolitan Region of São Paulo (MASP), Campinas Metropolitan Region (CMR), Metropolitan South Coast (MRSC), and Paraíba Valley region (VPR), which together comprise 58% of the area of the state of São Paulo and 13% of the state's population. A total of 206 samples from 28 localities were examined for 24 months. Protozoa were detected by IMS-IFA. *Giardia* cysts were detected in 49.5% (102 of 206) of samples with concentrations ranging from 0.1 to 97 cysts/L, whereas *Cryptosporidium* oocysts were present in 9.2% (19 of 206) of samples with concentrations ranging from 0.1 to 6.0 oocysts/L. The highest frequency of positive samples and the highest concentrations of oocysts were observed in the metropolitan region of Campinas(44). The results indicated that infection risks by these protozoans are higher than what is considered acceptable by the World Health Organization (44). These observation highlight the need to implement safety measures for water intended for public consumption.

From 1980 to June 2011, a total of 608,230 cases of acquired immunodeficiency syndrome (AIDS) were reported in Brazil, with 343,095 (56.4%) in the southeast, 123,069 (20.2%) in the south, 78,686 (12.9%) in the northeast, 35,116 (5.8%) in the midwest region, and 28,248 (4.7%) in the northern region (45). Although the largest proportion of cases nationwide was reported in the southeastern region, we note an increase in the incidence in other Brazilian regions. Among the five regions of the country, from 1998 to 2010, there was a 30.9% decrease in the incidence in the southeast, which had 56.4% of the total accumulated cases in the country, and an increase in other regions. Of the 27 federative units, in 2010, eight of them had higher incidence rates than the national average (17.9 in 100,000 inhabitants): Amazonas (30.9), Roraima (35.7), and Pará (19.5) in the north; Espírito Santo (20.4) and Rio de Janeiro (28.2) in the southeast; and all southern states. Considering that cryptosporidiosis is more severe in immunosuppressed individuals and can be fatal, there is a clear need for decentralizing research on the importance of water in the epidemiology of cryptosporidiosis and to expanding research coverage to other populations at risk.

FINAL COMMENTS

Waterborne *Cryptosporidium* oocysts were found in almost all studies carried out in Brazil. However, molecular characterization efforts to verify the zoonotic or anthroponotic potential of circulating protozoans in Brazilian waters are scarce. Although the authors suggest the need to develop new techniques to concentrate oocysts, they do not usually differ in the work presented. Finally, there is evidence that a large part of the population is at risk of infection by this protozoan. Studies on this subject should be intensified and extended to understudied geographical areas. The results obtained can benefit many Brazilians, especially those in regions where the prevalence and incidence of AIDS is high.

REFERENCES

1. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int J Parasitol. 2000;30(12-13):1305-22.

2. Fayer R, Speer CA, Dubey JP. The general biology of *Cryptosporidium*. In: Fayer R. *Cryptosporidium* and Cryptosporidiosis. Boston: CRC Press; 1997. p.1- 42.

3. Carreno RA, Martins DS, Barta JR. *Cryptosporidium* is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences. Parasitol Res. 1999;85(11):899-904.

4. Plutzer J, Karanis P. Genetic polymorphism in *Cryptosporidium* species: an update. Vet Parasitol. 2009;165(3-4):187-99.

5. Smith HV, Nichols RAB. Cryptosporidium: detection in water and food. Exp Parasitol. 2010;124(1):61-79.

6. Meireles MV. *Cryptosporidium* e criptosporidiose uma análise crítica. In: 14º Congresso Brasileiro de Parasitologia Veterinária; 2006; Ribeirão Preto. Ribeirão Preto: Centro de Convenções de Ribeirão Preto; 2006. p.153-4.

7. Fayer R, Trout JM, Xiao L, Morgan UM, Lal AA, Dubey JP. *Cryptosporidium canis* n. sp. from domestic dogs. J Parasitol. 2001;87(6):1415-22.

8. Cacciò SM, Pinter E, Fantini R, Mezzaroma I, Pozio E. Human infection with *Cryptosporidium felis*: case report and literature review. Emerg Infect Dis. 2002;8(1):85-6.

9. Thompson RCA, Palmer CS, O´Handley R. The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. Vet J. 2008;177(1):18-25.

10. Smith RP, Chalmers RM, Elwin K, Clifton-Hadley FA, Mueller-Doblies D, Watkins J, et al. Investigation of the role of companion animals in the zoonotic transmission of cryptosporidiosis. Zoonoses Public Health. 2009;56(1):24-33.

11. Cardona GA, Carabin H, Goñi P, Arriola L, Robinson G, Fernández-Crespo JC, et al. Identification and molecular characterization of *Cryptosporidium* and *Giardia* in children and cattle populations from the province of Álava, North of Spain. Sci Total Environ. 2011;412-413:101-8.

12. Hira KG, Mackay MR, Hempstead AD, Ahmed S, Karim MM, O'Connor RM, et al. Genetic diversity of *Cryptosporidium* spp. from Bangladeshi children. J Clin Microbiol. 2011;49(6):2307-10.

13. Lim YA, Iqbal A, Surin J, Sim BL, Jex AR, Nolan MJ, et al. First genetic classification of *Cryptosporidium* and *Giardia* from HIV/AIDS patients in Malaysia. Infect Genet Evol. 2011;11(5):968-74.

14. Richardson AJ, Frankenberg RA, Buck AC, Selkon JB, Colbourne JS, Parsons JW, et al. An outbreak of waterborne cryptosporidiosis in Swindon and Oxfordshire. Epidemiol Infect. 1991;107(3):485-95.

15. Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, 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(3):161-7.

16. Tzipori S, Giovani W. A hundred-year retrospective on cryptosporidiosis. Trends Parasitol. 2008;24(4):184-9.

17. Savioli L, Smith H., Thompson A. *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. Trends Parasitol. 2006;22(5):203-8.

18. Instituto Brasileiro de Geografia e Estatística [internet]. Rio de Janeiro: IBGE; 2012 [cited 2012 Jan 14]. Available from: www.ibge.gov.br.

19. Newman RD, Wuhib T, Lima AA, Guerrant RL, Sears CL. Environmental sources of *Cryptosporidium* in an urban slum in northeastern Brazil. Am J Trop Med Hyg. 1993;49(2):270-5.

20. Gamba RC, Ciapina EMP, Espíndola, RS, Pacheco A, Pellizari VH. Detection of *Cryptosporidium* sp. oocysts in groundwater for human consumption in Itaquaquecetuba City, S. Paulo-Brazil. Braz J Microbiol. 2000;31(2):151-3.

21. 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(2):109-11.

22. Farias EWC, Gamba RC, Pellizari VH. Detection of *Cryptosporidium* spp. oocysts in raw sewage and creek water in the city of São Paulo, Brazil. Braz J Microbiol. 2002;33(1):41-3.

23. Feitosa FLF, Shimamura GM, Roberto T, Meireles MV, Nunes CM, Ciarlini PC, et al. Prevalência de criptosporidiose em bezerros na região de Araçatuba, Estado de São Paulo, Brasil. Cienc Rural. 2004;34(1):189-93.

24. Santos LU, Bonatti TR, Cantusio-Neto R, Franco RMB. Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in activated sludge samples in Campinas, SP, Brazil. Rev Inst Med Trop São Paulo. 2004;46(6):309-13.

25. Hachich EM, Sato MI, Galvani AT, Menegon JR, Mucci JL. *Giardia* and *Cryptosporidium* in source waters of São Paulo State, Brazil. Water Sci Technol. 2004;50(1):239-45.

26. Iacovski RB, Barardi CR, Simões CM. Detection and enumeration of *Cryptosporidium* sp. oocysts in sewage sludge samples from the city of Florianópolis (Brazil) by using immunomagnetic separation combined with indirect immunofluorescence assay. Waste Manag Res. 2004;22(3):171-6.

27. Neto RC, Santos LU, Franco RM. Evaluation of activated sludge treatment and the efficiency of the disinfection of *Giardia* species cysts and *Cryptosporidium* oocysts by UV at a sludge treatment plant in Campinas, south-east Brazil. Water Sci Technol. 2006;54(3):89-94.

28. Rondello BT, Bueno FRM, Neto RC. Comparison of two methodologies for detection of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts in activated sludge samples from a sewage treatment plant in the city of Campinas, São Paulo State, Brazil. J Water Health. 2007;5(4):609-14.

29. Dias GMF, Bevilacqua PD, Bastos RKX, Oliveira AA, Campos GMM. *Giardia* spp. e *Cryptosporidium* spp. em água de manancial superficial de abastecimento contaminada por dejetos humano e animal. Arq Bras Med Vet Zootec. 2008;60(6):1291-300.

30. Machado ECL, Stamford TLM, Machado EHL, Soares DS, Albuquerque MNL. Ocorrência de oocistos de *Cryptosporidium* spp. em águas superficiais na região metropolitana de Recife-PE. Arq Bras Med Vet Zootec. 2009;61(6):1459-62.

31. Aldom JE, Chagla AH. Recovery of *Cryptosporidium* spp. oocysts from water by a membrane filter dissolution method. Lett Appl Microbiol.1995;20(3):186-7.

32. Smith HV, Campbell BM, Paton CA, Nichols RA. Significance of enhanced morphological detection of *Cryptosporidium* sp. oocysts in water concentrates determined by using 4',6'-diamidino-2-phenylindole and immunofluorescence microscopy. Appl Environ Microbiol. 2002;68(1):5198-201.

33. Cantusio-Neto R, Franco RMB. Ocorrência de oocistos de *Cryptosporidium* spp. e cistos de *Giardia* spp. em diferentes pontos do processo de tratamento de água, em Campinas, São Paulo, Brasil. Hig Aliment. 2004;18(118):52-9.

34. Machado ECL. Ocorrência de oocistos de Cryptosporidium spp. em águas superficiais na região metropolitana de Recife/PE [tese]. Recife: Universidade Federal de Pernambuco; 2006.

35. United States Environmental Protection Agency. Method 1622: *Cryptosporidium* in water by filtration/IMS/FA. Washington, DC: Environmental Protection Agency/Office of Water; 2001. 51p. (EPA-821-R-99-061).

36. Fraser RH, Barten PK, Pinney DAK. Predicting stream pathogen loading from livestock using a geographical information system-based delivery model. J Environ Qual. 1998;27:935-45.

37. Razzolini MT, da Silva TF, Bastos VK. Detection of *Giardia* and *Cryptosporidium* cysts/oocysts in watersheds and drinking water sources in Brazil urban areas. J Water Health. 2010;8(2):399-404.

38. United States Environmental Protection Agency. Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. Washington, DC: Environmental Protection Agency/Office of Water; 2005. 68p. (EPA-821-R-99-061).

39. Neto RC, dos Santos LU, Sato MI, Franco R.M. *Cryptosporidium* spp. and *Giardia* spp. in surface water supply of Campinas, southeast Brazil. Water Sci Technol. 2010;62(1):217-22.

40. Santos LU, Cantusio-Neto R, Franco RMB, Guimarães JR. Detecção de oocistos de *Cryptosporidium* spp. e cistos de *Giardia* spp. em amostras de esgoto bruto ou tratado: avaliação crítica dos métodos. Eng Sanit Ambient. 2011;16(2):115-20.

41. Araújo RS, Dropa M, Fernandes LN, Carvalho TT, Sato MI, Soares RM, et al. Genotypic characterization of *Cryptosporidium hominis* from water samples in São Paulo, Brazil. Am J Trop Med Hyg. 2011;85(5):834-8.

42. Branco N, Leal DA, Franco RM. A parasitological survey of natural water springs and in habitants of a tourist city in southeastern Brazil. Vector Borne Zoonotic Dis. 2012;12(5):410-7.

43. Sato MI, Galvani AT, Padula JA, Nardocci AC, Lauretto MS, Razzolini MT, et al. Assessing the infection risk of *Giardia* and *Cryptosporidium* in public drinking water delivered by surface water systems in Sao Paulo State, Brazil. Sci Total Environ. 2013;442:389-96.

44. World Health Organization. Guidelines for drinking-water quality. 4th ed. Geneva: World Health Organization; 2011.

45. Ministério da Saúde (BR). Boletim Epidemiológico AIDS e DST. Brasília: Ministério da Saúde; 2012.

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