OCCURENCE OF CRYPTOSPORIDIUM SPP. IN TREATED WATER: A SYSTEMATIC REVIEW

Sônia de Fátima Oliveira Santos[12]
Hugo Delleon Silva[1, 2]
Ludimila Aparecida C. Wosnjuk[2]
Marco Tulio Antonio García-Zapata[1]

ABSTRACT

The transmission of waterborne cryptosporidiosis is a major cause of morbidity and mortality worldwide. Knowledge of *Cryptosporidium* cases and ways in which to detect the parasite can help with decision making surrounding an appropriate method that is sensitive and inexpensive, in order to identify the risk level of the pathogen's transmission. Thus, by means of a scientific literature review, the *Cryptosporidium* spp occurrence and the methodologies used for detecting it in treated water were verified. METHODS: Data was identified from a research carried out by PubMed, SciELO, Latin American and Caribbean Health Sciences (LILACS) and Sanitary Engineering and Environmental Sciences (REPIDISCA), using the following terms: *Cryptosporidium* and Tap water, *Cryptosporidium* and Drink water, *Cryptosporidium* and Treated water, *Cryptosporidium* and Water supply. Two independent researchers, initially, identified the list of titles and abstracts that were selected to be included or excluded. In the case of disagreement about data extraction between the two assessors, the differences were resolved by consensus or discussion with a third reviewer. Through this review, it was observed that *Cryptosporidium* spp. is present in treated water in several countries, including Brazil. Among the countries that stood out are Spain and Portugal, since in Brazil there is only a small amount of research published on the matter.
Keywords: *Cryptosporidium,* Tap water, Drink water, Treated water, Water supply

[1] Research Center on Emerging and Re-emerging Agents, Institute of Tropical Pathology and Public Health, Federal University of Goiás. Correspondence to: Marco Tulio Antonio García-Zapata, Research Center on Emerging and Re-emerging Agents, Department of Tropical Medicine and Dermatology - Institute of Tropical Pathology and Public Health, Federal University of Goiás, Caixa Postal 12911, Setor Leste Vila Nova, 74643-970, Goiânia‑GO, Brasil. E-mail: nupereme@gmail.com

[2] Genetic and Molecular Diagnostics Lab, Institute of Biological Sciences II, Federal University of Goiás.

INTRODUCTION

With the rapid industrial, agricultural and population growth in the past century, water, a mineral resource which had been viewed as an inexhaustible source, is becoming increasingly scarce in many regions of the world and is becoming increasingly contaminated, which further limits its use (Júnior 2004).

In Brazil, the concern about water quality led the Ministry of Health to issue the Ordinance No. 1,469 of 12/29/2000, republished by Decree 518/2004, which establishes procedures and responsibilities with respect to water quality control for human consumption and its potability standards. For example, ingestion of small amounts of *Cryptosporidium* spp. oocysts can cause infection (Tzípori and Ward 2002). According to Manzi and García-Zapata (2000), the most important intestinal coccidia that infect the human intestinal tract are *Cyclospora cayetanensis, Isospora belli* and, particularly, *Cryptosporidium* spp. This pathogen represents an important risk to public health (Cimerman et al. 1999), since one of its main routes of transmission is through contaminated water, either by surface water that may have been treated or not, contaminated delivery systems, or inadequate treating.

The largest outbreak of cryptosporidiosis that occurred worldwide was in 1993, in Milwaukee (USA). It affected approximately 403,000 people and culminated in 4,000 hospitalizations and approximately 100 deaths (Sodré and Franco 2001).

Cryptosporidium spp. is a protozoan from vertebrate that causes diarrhea in humans, in different parts of the world (Fayer et al. 2000). With the advent of molecular biology, it was possible to demonstrate that gender *C. parvum* comprises two genotypes: 1 or H - infectious only for humans (anthropogenetic), and 2 or C - infectous for cattle, humans and various animals, which confirms the zoonotic potential initially assigned to the parasite (Kosek et al. 2001).

Moreover, through several concentration and detection methodologies, many studies have been carried out; among these, optical microscopy by staining, such as resistant acid. However, it can present limiting factors, low sensitivity, as well as the size of oocysts (4-8μm), possibly being confused with stained organic matter. Thus, it is necessary to highlight the importance of a well-trained technician. In addition, it may present low sensitivity (Fahey 2003; Santos et al. 2010).

Molecular and immunological techniques presented an alternative to optical microscopy in the detection of *Cryptosporidium* spp oocysts. The enzyme immunosorbent assay (ELISA), which is an indirect test for the qualitative determination of *Cryptosporidium* antigen, has a

simple implementation and does not require direct observation (Ungar 1990). However, this technique has low sensitivity, which requires sample concentration for detection. Furthermore, used antibodies are not species-specific.

Direct immunofluorescence, which has high sensitivity and specificity, is associated with PCR variations that are species/specific (Jex et al. 2008) and has been employed in the detection of *Cryptosporidium* spp. on environmental samples. PCR variations have advantages because they are able to carry out typing on the *Cryptosporidium* spp. that is present in environmental samples.

The aim of this study was to conduct a systematic review by an analysis of the scientific literature regarding the occurrence of *Cryptosporidium* spp. in treated water and which methodologies were used for their detection. We expect a contribution to the knowledge of the presence of this pathogen, as well as define which methodologies have better performance, enabling better research and monitoring for the occurrence of this pathogen in the aquatic environment.

METHODOLOGY

Systematic searches were conducted for papers indexed in databases, in order to answer the following question: "What is the prevalence of *Cryptosporidium* spp. in treated water and what are the most common concentration and detection methodologies that are employed?" A systematic literature review was held on 01/05/2012, at 03:10 p.m., and a manual search was carried out on 02/27/2012, at 05:23 p.m. Studies were selected in the following databases: PubMed, SciELO, Latin American and Caribbean Health Sciences (LILACS) and REPIDISCA. For this, we used the following terms: *Cryptosporidium* and Tap water, *Cryptosporidium* and Drink water, *Cryptosporidium* and Treated water, *Cryptosporidium* and Water supply. Initially, two researchers identified the list of titles and abstracts that were selected to be included or excluded. In case of a disagreement surrounding data extraction between the two reviewers, differences were resolved by consensus or by discussion with a third reviewer. The selected papers were read in full, to confirm eligibility and extract data. Additional studies were carried out from a manual search, using references of retrieved papers. In case of a disagreement on the inclusion or exclusion of a study or on data extraction between the two reviewers, differences were also resolved by consensus or discussion with a third reviewer. From the included papers, the following information was extracted: author, title, year, place,

methodology, sample size and positivity. We excluded duplicates, papers without abstracts, case reports and editorials.

The inclusion criteria, which was set for the selection of the papers, were: papers published in Portuguese, English and Spanish; papers that portray the theme regarding the integrative review; and papers published and indexed on those databases, in the last two decades. The analysis of the selected studies, in relation to the research design, was based on Muñoz et al. (2002).

Both analysis and synthesis of data extracted from the papers were made descriptively, enabling to observe, count, describe and classify the data, in order to gather the knowledge produced on the theme explored in the review. The papers underwent the Relevance Test I (RTI) and then the Relevance Test II (RTII). (Table 1).

Table 1. Selected papers (21 papers included according to agreement of different researchers) with their respective data on positivity and diagnostic methods of *Cryptosporidium* spp., in treated water samples (n = 1.923).

Methodology	Reference	n(1)	n(2)	pos%(1)	pos%(2)	P
MF + IMS + PCR*	8/20	12/12	24	75.0/66.6	70.8	0.244
MF + OM*	1/21	14/32	46	57.0/53.0	54.3	0.209
IMS + IF*	10/19	175/37	212	46.3/65.0	49.5	0.061
UF + IMS + IF	2	26	26	35.0	34.6	-
MF + PCR**	4/13/21	18/27/32	77	44.4/3.7/53.0	33.7	0.008
IMS + PCR*	10/14/17	175/4/518	697	21.7/0.0/18.9	31.5	0.068
MF + IF**	4/5/7/9/11/12/14/15/18	18/58/21/21/15/12/17/12/52	226	11.0/8.33/76.0/0.0/0.0/0.0/10.2/ 0.0/ 32.7	18.6	< 0.001
MF + IMS + IF	3/6/16	33/284/240	557	0.0/28.8/0.0	14.7	-
FL + IF	5	58	58	6.65	6.1	-

n(1): number of samples per study; n(2): total number of samples per method; pos%(1): frequency of positive samples per study; pos%(2): frequency of positive samples within the method. Subtitles: MF - microfiltration (membrane filtration with porosity of micrometers); IMS - Immunomagnetic Separation, FL - Flocculation; UF - Ultrafiltration; - PCR - Polymerase Chain Reaction and all other variations (Nested-PCR, qPCR, etc.) , IF - Immunofluorecence (Direct or Indirect), OM - Optics Microscopy (stains); UF - Ultrafiltration. Fisher test* chi-squared test **

After selection, the papers were evaluated, independently, by double entry, using standardized forms and according to previously determined criteria for inclusion and exclusion contained in the Relevance Test I (RTI), which was applied only to the paper abstracts and the Relevance Test II (RTII), applied to the full paper, from the studies previously selected by the Relevance Test I (Silva et al. 2010).

The following definitions are relevant and are part of the criteria for inclusion and exclusion of selected papers: *Cryptosporidium* spp., any species, among them *C. parvum* (human), *C. hominis*, *C. meleagridis* (bird), *C. felis* (cat) and *C. canis* (dog), *C. parvum* (mammal; treated water: it is drinkable water that went through a treatment process and must meet the standards established by the ordinances of each country).

Systematic Review of the Literature was consisted of the following phases:

PHASE 1 - Preparation of the relevance test and descriptors definition

The relevance tests were defined according to data present in Table 1 and addressed issues relating to clarity and consistency with the defined objectives in this study, as well as the selection of the keywords *Cryptosporidium*, Tap water, Drink water, Treated water, Water supply and type of sample (treated water).

Table 1 - Application form for the Relevance Tests I and II. (Modified from Silva et al. (2010)).

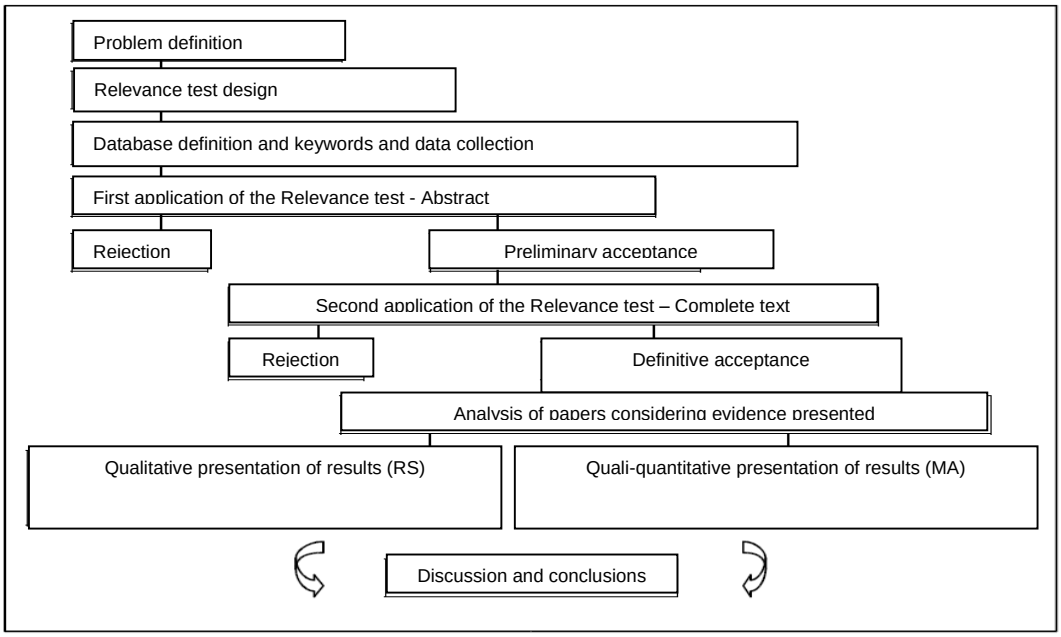
Application Form for the Relevance Test I		
Exclusion Criteria	**Yes**	**No**
1. Is the study about *Cryptosporidium* spp. detected in treated water?		
Exclusion Criteria	**Yes**	**No**
1. Does the study show *Cryptosporidium* spp. detection in untreated water?		
2. Was the paper written in a different language to that which is understood by the researchers (English, Spanish, Portuguese)?		
3. Are the selected studies a literature review/book chapter?		

Application Form for the Relevance Test II		
Inclusion Criteria	**Yes**	**No**
1. Is the study objective related to the issue that reviewers are analyzing?		
2. Is it treated water?		
3. Is the study about *Cryptosporidium* spp. detection in treated water?		
4. Did the study use a combination of concentration and detection methodologies?		

Exclusion Criteria	Yes	No
1. Was the study performed outside the period (the last two decades)?		
3. Does the study have insufficient data for analysis?		
4. Is the volume of water not mentioned?		

Figure 1 shows the study design flowchart according to Muñoz et al. (2002).

Figure 1 - Flowchart outlining the study design, in which the papers that did not fit the tests were summarily excluded (Muñoz et al. 2002).



PHASE 2 - Selection and analysis of papers

After the Relevance Test I, the confidence interval (CI) was calculated among researchers (Polit et al. 2004), which was obtained by dividing the number of accepted papers by the two researchers, independently, by that same number plus the number of papers accepted in disagree by the two researchers, expressing the value as a percentage. It was considered acceptable a CI ≥ 80% (Silva et al. 2010). The Relevance Test II was applied when the full reading of the papers was complete.

Only those papers that mentioned both concentration and detection methods used were included. Papers that commented on either one or the other were excluded from this study, which means that priority was given to selecting papers with better methodological quality.

RESULTS

No studies were found on REPIDISCA's Database. Table 2 shows the number of studies defined by the Relevance Tests I and II, in other databases. From 66 studies selected on the Relevance Test II, 44 papers were excluded, because they were repeated between the bases. Finally, at the stage of data collection, a paper was excluded. The concordance index between reviewers was 90.1%.

Table 2 - Databases with the application of their Relevance Tests I and II (RTI and RTII) are included.

DATABASES	DATE	TIME (Brazil)	No. Abstracts	R.T. I	R.T. II	Included (%)
PubMed	01/05/2012	03:10 p.m.	583	108	62	18 (81.8)
SciELO	01/05/2012	04:32 p.m.	28	3	2	2 (9.09)
LILACS	01/05/2012	05:02 p.m.	38	7	1	1 (4.54)
REPDISCA	01/05/2012	05:23 p.m.	0	0	0	0 (0.0)
Manual search	02/27/2012	03:32 p.m.	1	1	1	1 (4.54)
Total			650	119	66	22

Table 3 shows the locations where the studies were found, the year and the number of samples. Moreover, it shows the 21 papers selected by combining the respective concentration and detection methods, as well as the results of positive samples. As a paper can have more than one entry, all 66 studies were analyzed. Of these, 21 studies that detected *Cryptosporidium* spp. in treated water were selected, and nine different combinations of concentration and *Cryptosporidium* spp. detection methodologies were found. Among the selected studies, the associated methodologies that had greater positivity were Microfiltration (MF) + Immunomagnetic Separation (IMS) + Polymerase Chain Reaction (PCR) with 70.8% positivity, followed by MF + Optical Microscopy (OM) with 54.3% positivity. The least significant method for diagnosing *Cryptosporidium* spp. in treated water was FL + IF, with no positive sample. MF + IF method was the most used for concentration and detection, with nine studies (18.6%).

Table 3. Selected papers according to author, place of occurrence, detection and concentration methodology association and the number of samples and positivity.

REFERENCE/YEAR	PLACE	METHODS	(N)
Luna et al. 2002	Chile	MF – OM	14
Hashimoto 2002	Japan	UF – IMS – IF	26
Briancesco et al. 2005.	Rome	MF – IMS – IF	33 – All negative
Lemos et al. 2005	Portugal	MF – IF – PCR	18
Karanis et al. 2006	Russia and Bulgaria	MF – FL – IF	58=12/46
Carmena et al. 2007	Spain	FI – IMS – IF	284
Cermeño et al. 2008	Venezuela	FI – IF	21
Castro-Hermida et al. 2008	Spain	FI – IMS – PCR	12
Vernile et al. 2009	Portugal	FI – IFA	21 – All negative
Lobo ML et al. 2009.	Portugal	IMS – IF – PCR	175
Nishi et al. 2009	Brazil-PR	FI – IF	15
Machado et al. 2009	Brazil-RE	FI – IF	12 – All negative
Plutzer et al. 2010	Hungary	MF – PCR	27
Almeida et al. 2010	Portugal	FI – IMS – PCR – IF	17
Razzolini et al. 2010	Brazil-SP	IF – IF	12
Lee et al. 2010	Korea	IF –IMS -IF	240 – All negative
Nichols et al. 2010	Scotland	IMS – PCR	112
Castro-Hermida et al. 2010	Spain	FI – IFA	52
Brasseur et al. 2011	Haiti	IMS – IF	37
Feng et al. 2011	Spain	FI – IMS – PCR	30
Castro-Hermida et al. 2011	Luxemburg	FI – OM – PCR	12

The same table (3) shows that the results of four studies were negative for *Cryptosporidium* spp. presence.

DISCUSSION

In this study, the concordance rate was 90.1 %. It was acceptable up to CI ≥ 80%. This number increases the likelihood that search results were understood as credible. This study showed that the definition between concentration and detection methodologies association (MF + IMS + PCR) for diagnosis of *Cryptosporidium* spp. were the ones that were more sensitive and with higher positivity. Castro-Hermida (2008) obtained a detection of 12/75.0%. Although it was conducted with a small number of samples, it was possible to observe the efficiency of the techniques used, concluding that the basic and essential requirements to ensure safe drinking water, besides of the choice of high sensitive and specific methods, is to develop appropriate monitoring measures.

Even using these same techniques, Feng et al. (2011) found 66.6% positivity. These authors suggest that there is a need for regular monitoring of this pathogen on the

water, something currently impractical due to the cost of the method 1623. Moreover, they claim that data generated by this method is not very useful in assessing contamination risk by this pathogen. Both studies showed an association between these methodologies. In fact, there was a statistically significant association p = 0.244 between these methodologies.

Among the methodologies that have been most used, the MF + IF showed the largest number of studies, with a total of nine related ones. Carmeno et al. (2008) and Lemos et al. (2005) concluded that the filtration method is effective and that the techniques allow simultaneous viewing with high sensitivity. MF + IF and MF + PCR methods were significant, i.e., these methods showed a great variation of positive samples among the various selected studies. In some studies, the frequency of positivity was 76%; in others, it was 0.0%. That is, the use of this test should be better evaluated.

It is known that the compared samples are not identical and each one has its own special features, which might have contributed to this discrepancy in the result. However, the other analyzed samples that used other methods showed no statistically significant difference, i.e., the technique was carried out in the same way and these tests deserve special attention. In any case, many tests deserve reassessment, because of their small number of samples.

We found that among the screening tests, IF and PCR methods are more routinely used, with high sensitivity and specificity. In addition, PCR can detect species.

Through this review, it was possible to observe that *Cryptosporidium* spp. is present in treated water in different countries, including Brazil. The studies are recent. Among the countries which stood out are Spain and Portugal, since in Brazil there are still few studies on the topic.

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