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Originals

DETECTION OF PROTOZOA AND COLIFORMS IN WATER FROM ALTERNATIVE SOURCES IN PETRÓPOLIS, RIO DE JANEIRO, BRAZIL

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Abstract

The aim of this study was to evaluate the contamination by total coliforms, fecal coliforms, *Escherichia coli*, and the protozoans *Giardia* sp. and *Cryptosporidium* sp. in fresh water of alternative sources of surface catchment of Petrópolis, Rio de Janeiro. Eighty-eight samples were collected for the parasitological survey and 44 for the microbiological one from 22 sources in the dry as well as in the rainy seasons. To the parasitological analysis, sedimentation and flotation techniques were used before the microscopic examination and Enzyme-Linked Immunosorbent Assay (ELISA) was performed in 88 samples. Direct Immunofluorescence Assay (DFA) was performed in 67 randomly selected samples due to the kit availability. For the microbiological analysis, in turn, the multiple-tube technique was used, being *E. coli* confirmed by Enterokit B®. Out of the samples, 11/88 (12.5%) presented positivity by microscopic examination, while 13/88 (14.8%) presented positivity for *Giardia* sp. antigens by ELISA and 2/67 (2.3%) by DFA. Microbiological analysis showed that 22/44 (50%) of the samples were contaminated by total coliforms, 9/44 (20.5%) by fecal coliforms and 8/44 (18.2%) by *E. coli*. There was detection of *E. coli* and/or *Giardia* sp. in 59% of the sources, which reveals the need for continuous monitoring, aiming to mitigate the risk of waterborne transmission of enteropathogens. ELISA seemed to be a useful technique for the evaluation of water contamination by *Giardia* sp., contributing to enhance the information about diagnosis in environmental samples.

Resumen

El objetivo de este estudio fue evaluar la contaminación por coliformes totales y fecales, *Escherichia coli*, y los protozoos *Giardia* sp. y *Cryptosporidium* sp. en el agua potable de fuentes alternativas de captación superficial de Petrópolis, Rio de Janeiro. Se recogieron 88 muestras para el estudio parasitológico y 44 para estudios microbiológicos de 22 fuentes, tanto en estaciones secas como lluviosas. Para el análisis parasitológico fueron utilizadas técnicas de sedimentación, flotación y enzimoinmunoensayo (ELISA) en 88 muestras. Se realizó un ensayo de inmunofluorescencia directa (DFA) a 67 de estas muestras seleccionadas al azar. Para el análisis microbiológico se utilizó la técnica de tubos múltiples, siendo la presencia de *E. coli* confirmada por Enterokit B®. Un 12,5% de las muestras (11/88) presentaron positividad en el examen microscópico, mientras que un 14,8% (13/88) fueron positivas para los antígenos de *Giardia* sp. por ELISA y 2/67 (2,3%) por DFA. El análisis microbiológico mostró que 22/44 (50%) de las muestras fueron contaminados por coliformes totales, 9/44 (20,5%) por coliformes fecales y 8/44 (18,2%) por *E. coli*. Hubo detección de *E. coli* y/o *Giardia* sp. en el 59% de las fuentes, lo que pone de manifiesto la necesidad de una vigilancia continua con objeto de reducir el riesgo de transmisión de enteropatógenos por el agua. ELISA resulto ser una técnica útil para la evaluación de la contaminación del agua por *Giardia* sp., lo que supone una clara mejora en el diagnóstico de muestras ambientales.

Keywords / Palabras clave

Protozoa / Protozoos, *Giardia*, coliforms / coliformes, *Escherichia*, drinking water / agua potable, water sources / fuentes de agua.

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1. Introduction

The water for human consumption may harbor several pathogenic microorganisms of enteric origin, such as viruses, bacteria and parasites [1, 2]. The main microorganisms used as quality indicators of the water earmarked for human consumption are the bacteria of the coliform group, especially *Escherichia coli* [3].

The parasites stand as an important cause for outbursts of waterborne epidemics. In this group, the protozoa *Cryptosporidium* sp. and *Giardia* sp. stand out owing to the resistance of their cysts, the adverse weather conditions and the capacity to determine infections with low infective dose [4].

Several reports about the presence of these parasites in water were made in different countries. [5] , analyzing 66 water samples of treatment stations of 14 U.S. states and a Canadian province found *Giardia* sp. or *Cryptosporidium* sp. in 97% of the samples. This study also made an association between the presence of protozoa with water quality indicators, finding a positive relationship for *Giardia* sp. and fecal coliforms.

In a study performed by Carmena.[6] in Northern Spain, in sources of raw and treated water, they compared the presence of cysts of *Giardia* sp. and the oocyst of *Cryptosporidium* sp. adopting microbiological, physicochemical and meteorological parameters. These authors reported a strong correlation between the presence of protozoa and *Escherichia coli*. When the weather parameters were compared, a higher detection of these protozoa was observed in the months of October and April, which is in keeping with the rainiest months in the region.

Microbiological contamination by total coliforms, fecal coliforms and evolutionary forms of parasites were also detected by Gomes [7] in the raw water of public utility of two cities in the State of São Paulo. Otherwise, Nishi [8] detected cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp. in raw and treated water of Ivaí Indian lands, in the State of Paraná showing the importance of studying water supplies.

The city of Petrópolis has several natural surface sources, which stand as an important water resource used by the population, supplementing or partially replacing the public supply. This practice is part of the population's routine, which refrains from using the water of the supply network on the allegation of flavor change. This city is located on the hydrogeological province of the Resende Basin, presenting fissured and mixed aquifers, on a relief of Serra do Mar [9], which causes the existence of several surface sources.

In the given context, the purpose of this study was to detect *Cryptosporidium* sp., *Giardia* sp. and coliforms in the fresh water of sources with surface catchment used for human consumption, aiming to contribute to broadening the existing information about epidemiology and methodology of environmental diagnosis.

2. Materials and Methods

2.1 Collection and Processing of the Samples

The study was carried out in the City of Petrópolis, Mountainous region of the State of Rio de Janeiro. Collections were made in 22 sources located in different neighborhoods. Most of the sources were tapped by the own dwellers to facilitate the collection and access by everyone, presenting, in some of them, reservoirs, and mostly a catchment point of very difficult access, located below the neighborhood or inside private properties. The location of the sources is widely known, and the microbiological control of some of them was seasonably performed by the City Epidemiological Department. Three of the sources were located next to slopes and vegetation (Sources: A, B and X), two next to urbanized slopes (Sources: E and C), 16 in urbanized locations (Sources: D, F, H, J, L, M, N, O, P, Q, R, S, T, V) and one next to a river and households with the movement of animals and one hen-house (Source U).

Collections were performed from August 2012 to July 2013, and two samples were made in each source, one in the dry season, and the other in the rainiest months. For the parasitological survey, 500 liters of water were filtered using commercial Aqualimp® filters having cartridges with 1 µm of porosity of the brand Cuno®, model DPPY-1 Micro-Wind II, for which 4 liters of water were collected using 2-liter PET (Polyethylene terephthalate) bottles to simulate the manner of collection by the population. For the microbiological survey, 200 ml of water were collected using sterile screw cap glass bottles. The cartridges and glass bottles were marked and stored in vacuum vessels. After the collections, the Nephelometric Turbidity Units (UNT) were measured using the Policontrol® turbidimeter of AP 2000 model.

In the laboratory, the water stored in the plastic bottles was transferred to a 2-liter glass for sedimentation for 24 hours, and the sediment was transferred to 250-ml glasses for sedimentation for more 24 hours. The cartridges were disassembled manually and flushed using a solution of 0,001% of neutral detergent. The flushing water was transferred to a 1-liter glass for sedimentation for 24 hours.

2.2 Parasitological and Immunological Survey

The sediments, from plastic bottles and cartridges, were aliquoted for centrifuge tubes for the performance of parasitological techniques: Ritchie [10] modified by Young [11] adapted by Cerqueira [12], Sheather [13] modified by Huber [14] and Faust [15]. For immunological survey, the samples were processed by ELISA (IVD® Research Inc., USA) and the DFA (MERIFLUOR® Meridian Diagnostics, Cincinnati, Ohio, USA). The immunological techniques were performed according manufacturer´s instructions. Due to the limits of samples, as we had only one kit for DFA, 67 samples were selected at random.

2.3 Microbiological survey

For the survey of coliforms, the multiple-tube technique was applied for ascertaining the most probable number according to APHA [16], and the confirmation of presence of *E. coli* was performed by biochemical tests: lactose fermentation, gas production from glucose, motility, growth in an environment containing citrate as the only carbon source, lysine decarboxylase, indole production, hydrogen sulphide production, hydrolysis of urea, tryptophan deaminase, and the use of ENTEROKIT B® (Probac do Brasil Batch EB070213) performed according to the manufacturer's recommendations.

2.4 Statistical analysis

The results about the occurrence of microbiological and parasitic contamination in the water samples were analyzed by the Fisher's exact test using the software IBM SPSS Statistics 20 (IBM®). Analyses showing a confidence interval higher than 95% ($P<0.05$) were considered significant.

3. Results

Out of the samples, 11/88 (12.5%) presented positivity on the application of the parasitological techniques, given that 7/88 (8%) occurred in the dry season and 4/88 (9.1%) in the rainy season, and there was no detection of *Giardia* sp. or *Cryptosporidium* sp. In the samples obtained by filtration, eight samples were positive, and in the samples obtained by PET bottles, 3 (Table 1).

The parasitological test detected free living nematode larvae in 10 samples, given that seven of them were obtained by filtration and the remaining three, from PET bottles; a hookworm egg was found in a sample obtained by filtration, unsporulated coccidia oocysts were found in two samples obtained by filtration, free living adult nematodes were found in one PET Bottle sample and evolutionary forms of arthropods were found in one of them, with different absolute numbers in the rainy and dry seasons (Table 2)

With ELISA, 13/88 (14.8%) of the samples presented positivity for the antigen of *Giardia* sp., given that 10/88 (11.4%) were in the rainy season and 3/88 (3.4%) in the dry season (P=0.0685). With DFA 2/67 (2.3%) of the samples were positive for *Giardia* sp., both in the rainy season, and one of them was also positive with ELISA (Table 3).

Twenty-two samples were positive for total coliforms, out of which 9/44 (20.5%) were positive for fecal coliforms, and there was confirmation of the presence of *Escherichia coli* in 8 (18.2%) of them. The results connected to the positivity for total coliforms, fecal coliforms and their relationship with the season of collection are presented in Table 4.

Table 5 presents the flow rate of the 22 alternative sources of fresh water of surface catchment studied. Out of these, seven sources (A, E, F, G, L, T, V) presented an increase of flow rate during the rainy season, given that two of them presented association with cloudy and rainy weather conditions; seven (B, C, H, I, J, M, P) presented reduced flow rate in the rainy season, given that five collections were made in sunny weather. Eight sources (D, N, O Q, R, S, U, X) presented variation with time equal or lower than five minutes, which was considered irrelevant and, therefore, without variation between the rainy and dry season, and most of the collected in the rainy season from these sources were associated with rainy weather. There was no interference of the flow rate with the parasitological or microbiological positivity of the sources.

In four sources, the isolated presence of *E. coli* was observed in the rainy season and, in two sources, in the dry season. In two of them, there was positivity in both seasons. *Giardia* sp. was diagnosed in five sources in the rainy season and in two in the dry season, given that one presented positivity in both seasons. In two sources, both *E. coli* and *Giardia* sp. were detected, one of them being in the dry season, and one in the rainy season, with no significant difference between the collection seasons for none of the parameters (*E. coli* P=0.6640; *Giardia* sp. P=0.4121 and *E. coli* + *Giardia* sp. P=1).

The source A, in the rainy season, presented turbidity index of 2.9 UNT, with this being positive for total coliforms and *Giardia* sp. by ELISA and DFA. The other sources presented turbidity index below 0.5 UNT. Moreover, positivity for evolutionary forms of helminthes, protozoa and arthropods was observed in eight sources, and positivity for *Giardia* sp. by ELISA in seven sources, and for *Giardia* sp. by DFA, in one source, all having a turbidity index below 0.5 UNT (Table 6).

Out of the 22 sources studied, 13 (59.1%) presented positivity for *Giardia* sp. and/or *E. coli*. nine of them occurring in the rainy season, one in the dry season, and three in both seasons. In seven sources, there was positivity only for the detection of antigens of *Giardia* sp. by ELISA (Table 6).

Table 1. Parasitological analysis of fresh water samples from alternative sources in rainy and dry seasons of Petrópolis, Rio de Janeiro, Brazil, 2013.

Season	Type of collection	Total samples	Total of positive samples
Free Living Nematode Larva	Cartidge	22	5 (5.7%)
	PET Bottle	22	2 (2.3%)
Arthropods	Cartridge	22	3 (3.4%)
	PET Bottle	22	1 (1.1%)
TOTAL		88	11 (12.5%)

Table 2. Results of parasitological analysis detected in water samples from alternative sources in rainy and dry seasons, of Petrópolis, Rio de Janeiro, Brazil, 2013

Structures	Cartridge (n=44)		Pet (n=44)		TOTAL
	Dry	Rainy	Dry	Rainy	
Free Living Nematode Larva	6	2	2	0	10
Arthropods	0	1	0	0	1
Hookworm Egg	1	0	0	0	1
Unsporulated Coccidia Oocysts	2	0	0	0	2
Free Living Adult Nematode	0	0	0	1	1

Table 3. Results of *Giardia* sp. Enzyme-Linked Immunoassay (ELISA) and Direct Immunofluorescence Assay (DFA) in fresh water samples from alternative sources of Petrópolis, Rio de Janeiro, Brazil 2013

Season	Type of collection	Total no. of Samples	ELISA	Total no. of Samples	DFA
Dry	Cartridge	22	2 (2.3%)	16	0
	PET	22	1 (1.1%)	14	0
Rainy	Cartridge	22	5 (5.7%)	23	2(3%)
	PET	22	5 (5.7%)	14	0
TOTAL		88	13 (14.8%)	67*	2 (3%)

* samples for DFA were randomly selected due to kit availability

Table 4. Total and fecal coliforms, and *Escherichia coli* in fresh water samples from alternative sources of of Petrópolis, Rio de Janeiro, 2013

Season	Total no. of Samples	Total Coliforms	Fecal Coliforms	*Escherichia coli*
Dry	22	11/22 (50%)	3/22 (13.6%)	2/22 (9.1%)
Rainy	22	11/22 (50%)	6/22 (27.3%)	6/22 (27.3%)
TOTAL	44 (100%)	22/44 (50%)	9/44 (20.5%)	8/44 (18.2%)
P-Value		1	0.4566	0.2404

4. Discussion

Analysis of these results should must consider that the aim of the The positivity for evolutionary forms of parasites by microscope examination, such as nematode larvae and coccidian oocysts, in water samples of sources in the city of Petrópolis suggested that this environment is suitable for the development and survival of evolutionary forms of protozoa and helminthes, as stated by [17] These results demonstrate the contamination by human or other animal's fecal matter, as well as by natural biological agents of the location's microfauna.

Using the ELISA and DFA, antigens or cyst of *Giardia* sp. were found, but *Cryptosporidium* sp. were not detected. These results are different from those found by [18], who detected only oocysts of *Cryptosporidium* sp. by DFA, without the presence of *Giardia* sp. A similar fact was observed by Gamba. [19]. In turn, Branco [20] detected both cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp., also adopting the DFA. The absence of *Cryptosporidium* sp., both by ELISA and DFA, may indicate that the parasite is not circulating in the studied area.

The USA Environmental Protection Agency (EPA) validated a method for simultaneous detection of *Cryptosporidium* and *Giardia* designated by Method 1623, using immunomagnetic separation system [22]. Even though it is a secure and reliable method, its intrinsic technical and methodological implications, does not exempt the need to research and develop cheaper and easier alternatives techniques to the implementation of routine analysis in laboratories responsible for the control of water quality supplied to the population.

Although the concentration of antigens/cysts in water is lower than in fecal sample, as they are diluted, the use of ELISA in environmental samples was proposed for monitoring by Barbosa [23], who observed a higher sensitivity in the detection of positive samples for the protozoa *Giardia duodenalis*, *Entamoeba histolytica*/*Entamoeba dispar* and *Cryptosporidium* sp. by ELISA than by microscopic examination. In this study, ELISA also presented higher sensitivity for the diagnosis of *Giardia* sp. In relation to the microscopic examination and DFA. In the literature, studies associating the two immunological techniques, in water samples, were not found.

In stool samples, [24] evaluating DFA and ELISA in the diagnosis of *Giardia* sp. detected positivity in 39/44 (88.6%) and 35/44 (79.5%) respectively, without statistically significant difference, [25] evaluating nine immunoassay kits, used DFA Meridan Meriflur *Cryptosporidium*/*Giardia* Kit® as a reference method. The authors recognize that a screening approach with immunoassay kits may be helpful when handling potencial outbreak situation, specially a waterborne outbreak. These procedures are also very useful when trying to confirm *Cryptosporidium* and *Giardia* infection [25].

In this study, ELISA showed to be a valid technique for the evaluation of the circulation of antigens of *Giardia* sp. in water. Thus, it should be noted that there is a possibility that the enzyme-linked immunosorbent assay shows false positive results upon the similarity of antigens of different parasites, and false negative results owing to the presence of nonspecific inhibitors, considering the sample complexity. On account of this, the priority should be the performance of studies to ascertain the correlation between the positivity of ELISA and the presence of unchanged and viable cysts, but in this study it wasn´t possible.

The source E, found next to a deforested and urbanized slope with poor sanitation conditions, showed contamination both by *Escherichia coli* and parasitic structures, such as unsporulated coccidia oocyst and free living nematode larvae, either in the rainy or in the dry season. The groundwater may present propensity to contamination by protozoa or other pathogenic microorganisms, to a lesser degree than surface waters, such as rivers and lakes, insomuch as the soil and rock layers may work as a natural barrier against the microorganisms [20]. According to Sá [26], the progressive and disorderly urbanization and the occupation of area unsuitable for dwelling, without infrastructure and basic sanitation, contribute to the jeopardy of water resources and environmental degradation. Nonetheless, the contamination of the water coming from springs and wells is strongly influenced

by the surface waters, and other contamination factors, such as the environment, wastewater, septic tanks, rain and animals' feces [5; 19; 8, 17].The contamination of the sources of Petrópolis may have derived from the movement of animals, as well as the presence of wastewater, associated in some sources with the presence of characteristic odor, without the visibility of wastewater ditches.

Rose [27] suggest that rainfall is one of the main factors of influence in the dispersion of parasites in the soil. In the study of Petrópolis, collections were made in the dry and rainy seasons. There was No influence on the positivity by the collection season no significant association between the collection season and positivity (P = 0.0685). With the parasitological techniques, there was positivity in the dry period, and with ELISA for the survey of *Giardia* sp., the frequency was higher in the rainy season. The higher contamination by parasites in the rainy season was observed by [28] when researching the circulation of oocysts of *Cryptosporidium* sp. and cysts of *Giardia* sp. in the city of Viçosa, Minas Gerais State.

In the 22 sources of Petrópolis, State of Rio de Janeiro, neither did the study observe interference of the rainy or dry season, in accordance with the region's weather description, with the flow rate of 500 liters of water, nor of the local weather conditions with the period of collections with this flow.

In contrast to the results obtained from sources of Petrópolis, Nishi [8], researching about the occurrence of cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp. in water of Ivaí Indian lands in the State of Paraná, attributed the contamination of the water samples to the strong rain before the collection. In turn, Machado [29] checked the occurrence of oocysts of *Cryptosporidium* sp. both in the dry season and in the rainy season.

The study detected 22/44 (50%) of the sources contaminated by coliforms, out of which, 13/44 (29.5%) of the samples were contaminated only by total coliforms. The positivity with total coliforms may also be connected to the presence of bacteria naturally found in the soil as part of the microbiota, and may have been carried by the continuous water flow. The absence of coliforms in 50% of the sources could be associated to the canalization of most of them by the community.

In the sources positive for total coliforms, 9/44 (20.4%) were positive for fecal coliforms too, and there was confirmation of the presence of *Escherichia coli* in 8/44 (18.2%). The levels of contamination for fecal coliforms range from 3NMP/100 mL to 150 NMP/100 mL, and there amounts are lower than those found by Almeida [21] in the water of stream Ribeirão dos Porcos, located in the city of Espírito Santo do Pinhal, State of São Paulo, which presented an average of 700 NMP/100 mL, with these amounts being outside the allowable limits.

Four sources of Petrópolis presented contamination by *E. coli* in the rainy season and two in the dry season. Gonzalez [30], in a study performed in a Mexican neighborhood, concluded that the presence of coliforms in the water sources studied and in the households presented a direct relationship with the occurrence of rains, associated by the authors with the dragging of excreta of men and other animals. Amaral [31] verified the hygienic/sanitary quality of the water for human consumption in rural properties in the State of São Paulo by means of microbiological survey. The authors observed that, during the rainy season, the count of the most probable number increased. This fact may also have happened in the alternative sources studied in Petrópolis.

In the sources of Petrópolis, one of them presented turbidity index of 0.5 UNT, and there was detection of *Giardia* sp. Medema [32] concluded that the kinetic of sedimentation of cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp. increased when adhered to substances present in the water. Differently, Le Chevalier [33] found cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp. even with low turbidity (0.19 UNT), similar to what was found in Petrópolis. The detection of parasitological positivity in samples with low turbidity strengthens the importance of the parasitological survey, regardless of the turbidity, as recommended by Ordinance No. 2914/2011-MS [3].

Thirteen (59.1%) of the sources presented positivity for *Giardia* sp and/or *E. coli*, with the detection of seven of them contaminated by *Giardia* sp. This result differs from that found by Carmena [6], who found a positive relationship between *Cryptosporidium* sp. and *Giardia* sp. with fecal coliforms in raw and treated water in the province of Álava, Spain. Gomes [7] found *E. coli* in all sources that presented parasitological contamination in sources of public utility in two cities of the State of São Paulo, as well as Lechavalier [33], who found a positive relationship between *Giardia* sp. and fecal coliforms. In turn, Gamba [9], researching the groundwater contamination in the city of São Paulo did not find association between *E. coli* and *Cryptosporidium* sp. the ordinance no. 2914 of the Health Ministry considers as standard of water potability the absence of *E. coli*, and the survey of protozoa is recommended only when the count of *E. coli* is high. Nonetheless, in the sources of Petrópolis, antigens and cysts of *Giardia* sp. were detected even with the absence of *E. coli*, which represents the risk to population health, since the presence of antigens/cysts of *Giardia* sp. indicates a contamination of fecal source.

The detection of contamination by *E.coli* and/or *Giardia* sp. in 59.1% of the 22 sources of fresh water of surface catchment in the city of Petrópolis, State of Rio de Janeiro, points to the need for continuous monitoring of the sources used by the community by the competent agencies, using parasitological and microbiological parameters, in order to minimize the risk of waterborne transmission of pathogens of fecal origin, as suggested by Porto [34].

The results of this study suggest that ELISA could be a good technique for the evaluation of water contamination by *Giardia* sp. contributing to enhance the information about diagnosis in environmental samples. For Public Health in Petrópolis, an effective monitoring will evaluate, in a more accurate manner, the microbiological and parasitological quality of the water from sources, identifying and indicating only the sources with water unsuitable for consumption, considering that nine of them presented no fecal contamination over the period studied and are, therefore, suitable for consumption. Similarly, the results of the continuous monitoring could support environmental education efforts, allowing the maintenance of this water wealth. These efforts would make possible the consumption of a quality product, along with the local culture strengthening, meeting the community demand for a colorless, odorless and tasteless product.

References

1. Grabow W. Waterborne diseases: update on water quality assessment and control. Water. S.A. 1996; 22 (2):193-202.

2. Franco RMB. Protozoários de veiculação hídrica: relevância em saúde pública. Rev Panam Infectol. 2007 Aug; 9(4):36-43.

3. Brasil - Ministério da Saúde. Portaria nº 2914 de 12 de dezembro de 2011. Estabelece os procedimentos e responsabilidades relativos ao controle e vigilância da qualidade da água para consumo humano e seu padrão de potabilidade, e dá outras providências.

4. Franco RMB, Branco N, Leal AG. Parasitologia Ambiental: Métodos de Concentração e detecção de *Cryptosporidium* spp. e *Giardia* spp. em amostras de água. Rev Pat Trop. 2012 Jun; 41 (2):119-135.

5. Le Chevalier MW, Norton WD, Lee RG. *Giardia* and *Cryptosporidium* spp. in filtered drinking water supplies. Appl Environ Microbiol 1991; 57(9): 2617-2621.

6. Carmena D, Zigorraga C, Fernandez-Crespo JC, Ocio JA. Presence of *Giardia* cysts and *Cryptosporidium* oocysts in drinking water supplies in northern Spain. J Appl Microbiol. 2006; 102 (3): 619-629.

7. Gomes AHS, Pacheco MASR, Fonseca YSK, Cesar NPA, Dias HGG, Silva RP. Pesquisa de *Cryptosporidium* sp. em águas de fontes naturais e comparação com analise bacteriológica. Rev Inst Adolfo Lutz. 2002; 61(1): 59-63.

8. Nishi L, Bergamasco R, Ornelas MJ, Falavigna LM, Gomes ML, Mota LT et al. *Giardia* spp. and *Cryptosporidium* spp. in the Ivaí Indigenous Land, Brazil. Vector-Borne Zoonitic Dis. 2009; 9(5): 543-547.

9. Martins AM, Capucci E, Caetano LC, Cardoso G, Barreto ABC, Monsores AL et al. Hidrogeologia do Estado do Rio de Janeiro. Síntese do Estágio Atual do Conhecimento. Anais do XIV Congresso Brasileiro de Águas Subterrâneas, de 7 a 10 de novembro de 2006, em Curitiba (PR) (Internet) (cited 2006 november 10) avaliable from http://abas-rj.milo.kinghost.net/atuacao_tecnica/HidrogeologiaRJ.pdf

10. Ritchie LS. An ether sedimentation technique for routine stool examinations. Bull U S Army Med Dep. 1948; 8(4): 326.

11. Young KH. Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J Clin Microb 1979; 10 (6): 852-853.

12. Cerqueira F. L. Coprotest: Metodologia confiável para o exame parasitológico de fezes. NL diagnóstico. LAES/HAES. 1988 Mar; 9(51): 5-12.

13. Sheather AL. The detection of intestinal protozoa and mange parasites by a flotation. J Comp Pathol. 1923; 36(4): 266-275.

14. Huber F, Bomfim TC, Gomes RS. Comparação da eficiência da Técnica de Sedimentação pelo formaldeído-éter e da técnica de centrífugo-flutuação modificada detecção de cistos de *Giardia* sp. e oocistos de *Cryptosporidium* sp. em amostras fecais de bezerros. Rev Bras Parasitol. 2003; 12(2): 135-137.

15. Faust EC, D'antoni J.S, Odom V, Miller MJ, Peres C et al. Critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. I. Preliminary communication. Am J Trop Med. 1938; 18(2): 169-183.

16. APHA 1999. Standard Methods for the Examination of Water and Wastewater. (Internet) (Cited 2014 february 25) avaliable from: http://www.mwa.co.th/download /file_upload/SMWW_1000-3000. pdf

17. Barbosa AS, Uchôa CMA, Laurentino SM, Duarte AN, Conceição NF, Côrtes MBV et al. Avaliação parasitológica da água de abastecimento e do solo peridomiciliar de Aldeias Guarani. Rev Inst Adolfo Lutz. 2013; 72 (1): 72-80.

18. Franco RMB, Cantusio Neto R. Occurrence of Cryptosporidial Oocysts and *Giardia* Cysts in Bottled Mineral Water Commercialized in the City of Campinas, State of São Paulo, Brazil. Mem Inst. Oswaldo Cruz. 2002; 97(2): 205-207.

19. Gamba RC, Ciapina EMP, Espíndola SR, Pacheco A, Pellizari VH. Detection of *Cryptosporidium* sp. oocysts in groundwater for human consumption in Itaquaquecetuba city, São. Paulo-Brazil. Braz J Microbiol. 2000; 31(2): 151-53.

20. Branco N, Leal DAG, Franco RMB. Parasitological Survey of Natural Water Springs and Inhabitants of a Tourist City in Southeastern Brazil. Vector-Borne Zoonotic Dis. 2012; 12 (5) 410-417.

21. Almeida RMA, Hussar GJ, Peres MR, Junior ALF. Qualidade microbiológica do córrego "Ribeirão dos Porcos" no Município de Espírito Santo do Pinhal-SP. Espírito Santo do Pinhal. Eng Ambient. 2004; 1(1): 51-56.

22. EPA - Environmental Protection Agency. Office of Water. Method 1623: *Cryptosporidium* and *Giardia* in water by filtration/IMS/FA. EPA 815-R-05-002. US Environmental Protection Agency. Washington DC, EEUU. 2005

23. Barbosa AS, Uchôa ACM, Laurentino SV, Nascimento DA, Bandeira V M, Monteiro FAB et al. Comparative study of parasitological techniques and ELISA for analysis of environmental samples, RJ, Brazil. Mem. Inst. Oswaldo Cruz. 2012; 71 (1):90-96.

24. Dogruman Al F, Kustimur S, Ozekinci T, Balaban N, Ilhan MN. The use of Enzyme Linked Immunosorbent Assay and Direct Fluorescente Antibody (DFA) methods for diagnosis of *Giardia* intestinalis. Turk Parazitol Derg. 2006; 30 (4):275-278.

25. Garcia LS, Shimizy RY. Evaluation of nine immunoassay kits (Enzyme immunoassay and direct flourescence) for detection of *Giardia lamblia* and *Crysptosporidium parvum* in human fecal specimens. J Clin Microbiol. 1997; 35(6):1526-1529.

26. Sá LLC, Jesus IM, Santos ECO, Vale ER, Loureiro ECB, Sá EV. Qualidade microbiológica da água para consume humano em duas áreas contempladas com intervenções de saneamento – Belém do Pará, Brasil. Epidemio. Serv Saúde. 2005; 14(3): 171-180.

27. Rose JB, Huffman DE, Gennaccaro A. Risk and control of waterborne cryptosporidiosis. Microbiol Rev. 2002; 26(2): 113-123.

28. Heller L, Bastos RKX, Vieira MBC. Bevilacqua PD, Brito LLA. Oocistos de *Cryptosporidium* e cistos de *Giardia*.

Circulação no ambiente e riscos á saúde humana. Epidemiol Serv.Saúde. 2004; 13(2): 79- 92.

29. Machado ECL, Stamford TL, Machado EHL, Soares DS, Albuquerque MNL. Ocorrência de oocistos de *Cryptosporidium* spp. Em águas superficiais na região metropolitana de Recife-PE. Arq BrasVet Zootec. 2009; 61(6): 1459-1462.

30. Gonzalez RG, Taylor ML, Alfaro G. Estudio Bacteriológico Del água de consumo em uma comunidad mexicana. Bol Sanit Panama. 1982; 93(2): 127-141.

31. Amaral LA, Filho AN, Junior ODR. Água de consumo humano como fator de risco à saúde em propriedades rurais. Rev Saúde Públ. 2003; 37: 510-514.

32. Medema G, Schets JF, Teunis MPFM, Haavelar AH. Sedimentation of Free and Attached *Cryptosporidium* Oocyts and *Giardia* Cysts in Water. Appl Environ Microbiol. 1998; 64: (11) 4460-4466.

33. Le Chevalier MW, Norton WD, Lee RG. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. Appl Environ Microbiol 1991; 57(9): 2610-2616.

34. Porto MAL, Oliveira AM, Fai AEC, Stamford TLM. Coliformes em água de abastecimento de lojas fast- food da Região Metropolitana de Recife (PE, Brasil). Ciênc. Saúde Colet. 2011; 16(5): 2653-2656.

Table 5. Weather conditions, flow rate time and positivity to microbiological or parasitogical tests of alternative sources of surface catchment of Petrópolis, Rio de Janeiro, Brazil.

Source	Season	Time	Collection month/year	Flow rate time	Positivity
A	Dry	Cloudy	08/2012	1h 40 min.	TC
	Rainy	Sunny	10/2012	53 min.	TC < cont.
B	Dry	Rainy	08/2012	5h 30min.	Neg
	Rainy	Sun	10/2012	7h	Neg
C	Dry	Sunny	08/2012	5h 48min.	Neg.
	Rainy	Sunny	01/2012	.8h 33min.	Neg.
D	Dry	Sunny	08/2012	24min.	Neg.
	Rainy	Sunny	11/2012	22min.	TC, FC, EC
E	Dry	Sunny	09/2012	5h 50min.	P, TC, FC, EC
	Rainy	Sunny	12/2012	50 min.	P, TC, FC < cont, EC
F	Dry	Sunny	09/2012	52 min.	TC, FC
	Rainy	Sunny	12/2012	33.3 min.	Neg.
G	Dry	Sunny	06/2013	1h 35min.	P, TC
	Rainy	Sunny	11/2012	1h 11min.	TC < cont.
H	Dry	Sunny	06/2013	24 min.	P
	Rainy	Sunny	11/2012	35 min.	Neg.
I	Dry	Sunny	06/2013	30 min.	Neg.
	Rainy	Sunny	12/2012	1h 18min.	Neg.
J	Dry	Sunny	06/2013	44 min.	TC
	Rainy	Sunny	12/2012	2h 25min.	TC > cont.
L	Dry	Sunny	06/2013	3h	Neg.
	Rainy	Sunny	12/2012	1h 17min.	Neg.
M	Dry	Sunny	06/2013	17 min.	P, TC
	Rainy	Sunny	01/2013	28 min.	TC > cont,, FC, EC
N	Dry	Sunny	06/2013	44min.	TC
	Rainy	Rainy	03/2013	43min.	TC > cont,, FC, EC
O	Dry	Sunny	06/2013	48min.	P
	Rainy	Rainy	03/2013	47min	TC
P	Dry	Sunny	06/2013	15min.	Neg.
	Rainy	Rainy	03/2013	23min.	P, TC, FC, EC
Q	Dry	Sunny	06/2013	2h 32min.	Neg.
	Rainy	Rainy	03/2013	2h 27min.	Neg.
R	Dry	Sunny	07/2013	26min.	Neg.
	Rainy	Rainy*	04/2013	25min.	Neg.
S	Dry	Sunny	07/2013	47min.	P, TC, FC, EC
	Rainy	Rainy*	04/2013	50 min.	P, TC < cont,, FC, EC
T	Dry	Sunny	06/2013	2h 30min.	TC
	Rainy	Cloudy	04/2013	1h 40min.	Neg.

Table 5. Weather conditions, flow rate time and positivity to microbiological or parasitogical tests of alternative sources of surface catchment of Petrópolis, Rio de Janeiro, Brazil.

Source	Season	Time	Collection month/year	Flow rate time	Positivity
U	Dry	Sunny	06/2013	26min and 23s.	P, TC
	Rainy	Rainy*	04/2013	27min.	P, TC < cont.
V	Dry	Sunny	06/2013	2h 18min.	TC
	Rainy	Rainy*	04/2013	57min.	Neg.
X	Dry	Sunny	07/2013	45min.	Neg.
	Rainy	Rainy*	04/2013	40min.	Neg.

* Rain with great intensity in the week preceding the collection. P – Parasites, TC – total coliforms, FC – fecal coliforms, EC – *Escherichia coli*, Neg. – negative, <cont. – count reduction, >cont – count increase

Table 6. Result of the analysis of samples from 22 alternative sources of surface catchment of Petrópolis, Rio de Janeiro, in the rainy and dry seasons for evaluation of turbidity, presence of total coliforms, fecal coliforms, *Escherichia coli* and parasitic contamination, 2013.

Source	Season	Turbidity	Parasitol	ELISA	DFA	TC	FC	*E. coli*
Giardia ***sp***	TC	FC	*E.coli*	N	N	15	N	N
	Rainy	2.93	N	P	P	9.2	N	N
B	Dry	0.02	N	N	N	N	N	N
	Rainy	0.02	N	P	N	N	N	N
C	Dry	0.02	N	P	N	N	N	N
	Rainy	0.02	N	N	N	N	N	N
D	Dry	0.02	N	P	N	N	N	N
	Rainy	0.02	P	P	N	3.6	3.6	3.6
E	Dry	0.312	P	N	N	150	150	3.6
	Rainy	0.32	N	N	P	150	7.4	P
F	Dry	0.338	N	N	N	93	93	N
	Rainy	0.06	N	P	N	N	N	N
G	Dry	0.02	P	N	N	43	N	N
	Rainy	0.02	N	P	N	23	N	N
H	Dry	0.02	P	N	N	N	N	N
	Rainy	0.15	N	P	N	N	N	N
I	Dry	0.02	N	N	N	N	N	N
	Rainy	0.02	N	P	N	N	N	N
J	Dry	0.92	N	N	N	3.6	N	N
	Rainy	0.02	N	N	N	23	N	N
L	Dry	0.02	N	N	N	N	N	N
	Rainy	0.02	N	N	N	N	N	N
M	Dry	0.02	P	N	N	11	N	N
	Rainy	0.058	N	N	N	1100	28	11
N	Dry	0.126	N	N	N	9.2	N	N
	Rainy	0.18	N	N	N	43	9.2	9.2
O	Dry	0.142	P	N	N	N	N	N
	Rainy	0.02	N	N	N	3.6	N	N

Table 6. Result of the analysis of samples from 22 alternative sources of surface catchment of Petrópolis, Rio de Janeiro, in the rainy and dry seasons for evaluation of turbidity, presence of total coliforms, fecal coliforms, *Escherichia coli* and parasitic contamination, 2013.

Source	Season	Turbidity	Parasitol	ELISA	DFA	TC	FC	*E. coli*
P	Dry	0.02	N	N	N	N	N	N
	Rainy	0.248	P	N	N	3.6	3.6	3.6
Q	Dry	0.02	N	N	N	N	N	N
	Rainy	0.02	N	N	N	N	N	N
R	Dry	0.02	N	N	N	N	N	N
	Rainy	0.02	N	N	N	N	N	N
S	Dry	0.05	P	N	N	290	160	64
	Rainy	0.335	P	N	N	36	3	3
T	Dry	0.488	N	N	N	15	N	N
	Rainy	0.02	N	N	N	N	N	N
U	Dry	0.02	P	N	N	93	N	N
	Rainy	0.02	P	N	N	43	N	N
V	Dry	0.02	N	N	N	7.4	N	N
	Rainy	0.02	N	N	N	N	N	N
X	Dry	0.02	N	N	N	N	N	N
	Rainy	0.02	N	N	N	N	N	N

Parasitol: Parasitic Examination; DFA: Direct Immunofluorescence; TC: Total Coliforms (NMP/100 ml); FC: Fecal Coliforms (NMP/100 ml); *E.coli*: *Escherichia coli* (NMP/100 ml); P: Positive, N: Negative. Improper Turbidity ≥ 0.5 UNT