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Technical Article

Detection of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts in surface water destined for public supply in the state of Goiás, Brazil

Detecção de oocistos de Cryptosporidium spp. e cistos de Giardia spp. em mananciais superficiais destinados ao abastecimento público do estado de Goiás, Brasil

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ABSTRACT

The protozoa *Cryptosporidium* spp. and *Giardia* spp. are etiological agents responsible for the transmission of gastroenteritis, mainly due to the consumption of contaminated water. Their (oo)cysts are resistant to adverse environmental conditions, as well as to most conventional water treatment processes. Thus, the objective of this work was to evaluate the occurrence of (oo)cysts of these protozoans in surface water collected for human consumption in the state of Goiás, Brazil. Fifteen samples of raw water were collected to assess the occurrence of (oo)cysts of the protozoa using the Membrane Filtration method, in addition to turbidity and pH analyses. Recovery rates in tests with ultrapure water reached the USEPA (2012) criteria for *Giardia* (78.1% ± 0%) and for *Cryptosporidium* (60.6% ± 32.6%); however, recovery in raw water was lower due to turbidity. All samples (n = 15) were positive for *Cryptosporidium* spp. oocysts, with a maximum concentration of 250 oocysts/L, demonstrating that these protozoa are disseminated in the aquatic environment of the state of Goiás and pose a risk to public health. Due to the use of water sources for public consumption, it is recommended that public authorities and sanitation companies act to preserve and maintain water courses, carry out periodic monitoring of treatment plants that supply the Cerrado, Santana and São Manoel streams and improve existing treatment technologies. The results did not allow to infer whether animal load and grazing area promote an increase in contamination of the lotic aquatic systems.

Keywords: *Cryptosporidium*; *Giardia*; hydrographic basin; protozoa; surface water; treatment water

RESUMO

Os protozoários *Giardia* spp. e *Cryptosporidium* spp. são agentes etiológicos responsáveis pela transmissão de gastroenterites, principalmente por causa do consumo de água contaminada. Seus (oo)cistos são resistentes às condições ambientais adversas, bem como à maioria dos processos convencionais de tratamento da água. Assim, o objetivo deste trabalho foi avaliar a ocorrência de (oo)cistos desses protozoários em águas superficiais captadas para consumo humano no estado de Goiás, Brasil. Quinze amostras de água bruta foram coletadas para avaliar a ocorrência de (oo)cistos dos protozoários pelo método de Filtração por Membrana, além da análise de turbidez e pH. As taxas de recuperação nos testes com água ultrapura atingiram os critérios da USEPA (2012) para *Giardia* (78.1 ± 0%) e para *Cryptosporidium* (60.6 ± 32.6%), entretanto, em água bruta, a recuperação foi inferior devido à turbidez. Todas as amostras (n = 15) foram positivas para oocistos de *Cryptosporidium* spp., com valor máximo de 250 oocistos/L, demonstrando que esses protozoários se disseminam no meio aquático do estado de Goiás e geram risco à saúde pública. Devido à utilização de mananciais para consumo público, recomenda-se que as autoridades públicas e as empresas de saneamento atuem na preservação e manutenção dos cursos d'água, realizem o monitoramento periódico das plantas de tratamento que realizam adução no Córrego Cerrado, Córrego Santana e Córrego São Manoel e aprimorem as tecnologias de tratamento existente. Os resultados não permitiram inferir se a carga de animais e a área de pastagem promove o aumento da contaminação dos sistemas aquáticos lóticos.

Palavras-chave: *Cryptosporidium*; *Giardia*; bacia hidrográfica; protozoário; água superficial; tratamento de água.

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INTRODUCTION

Waterborne diseases are a relevant public health concern. Indeed, around 829,000 deaths from diarrhea worldwide each year (WHO, 2019) have been related to the use of water contaminated by microorganisms from untreated domestic sewage (CHENG *et al.*, 2009) or surface runoff from livestock farms (IMRE *et al.*, 2017).

Among the protozoa transmitted by water, the genera *Cryptosporidium* (4.0–6.0 µm in diameter) and *Giardia* (8–18 µm in length by 5–15 µm in width), stand out as being widely distributed in developed and developing countries (COTRUVO *et al.*, 2004).

These protozoa have been responsible for numerous waterborne outbreaks worldwide (KARANIS; KOURENTI; SMITH, 2007), since the oral ingestion of at least 10 cysts or a single oocyst can cause infection in susceptible individuals (KARANIS, 2011).

The outbreaks caused by these etiological agents are associated with severe diarrhea, and have been well documented (Table 1).

The occurrence of these outbreaks can be attributed to the high resistance of these organisms to the environment (KARANIS, 2011) and their high infectivity (THOMPSON, 2004).

Means of transmission begins with the ingestion of (oo)cysts via the fecal-oral route, through contact between people and between people and other animals; the ingestion of water contaminated by host feces (humans and other non-human animals), recreational activities, and contaminated surface water, in addition to eating contaminated food (RYAN; FAYER; XIAO, 2014; PLUTZER; KARANIS, 2016).

Several studies have reported the occurrence of these protozoa in surface water destined for public supply in Brazil (ALMEIDA *et al.*, 2015; DIAS *et al.*, 2008; FREITAS *et al.*, 2015; SATO *et al.*, 2013; TOLEDO *et al.*, 2017), indicating a risk for infection by gastrointestinal diseases to consumers and highlighting the need for special attention from public authorities.

Temporal and seasonal variation in the occurrence of these protozoa in the environment and in water supply systems justifies the importance of their monitoring since the absence of fecal indicators, such as *E. Coli*, does not guarantee the absence of *Cryptosporidium* spp. and *Giardia* spp., and, thus, are not considered standard indicators (HORMAN *et al.*, 2004).

Therefore, the objective of the present study was to analyze the occurrence of (oo)cysts of *Cryptosporidium* spp. and *Giardia* spp. in surface waters destined for public supply in the state of Goiás, Brazil.

Table 1 – Number of waterborne outbreaks caused by protozoan parasitic agents worldwide.

Year	Giardia spp.	Cryptosporidium spp.	Key reference
previous century–2004	132 outbreaks of Giardia duodenalis	165 outbreaks of Cryptosporidium parvum	Karanis, Kourenti and Smith (2007)
2004–2010	70 outbreaks of Giardia lamblia	120 outbreaks of Cryptosporidium spp.	Baldursson and Karanis (2011)
2011–2016	142 outbreaks of Giardia spp.	239 outbreaks of Cryptosporidium spp.	Efstratiou, Ongerth and Karanis (2017b)

METHODOLOGY

Investigated area

The study area encompasses 15 surface water sources for public water supply in the state of Goiás, as described in Table 2 and spatially distributed as shown in Figure 1. The state is located in the Center-West Region of Brazil and has a territorial extension of 340,106,492 km^2 bordering the states of Mato Grosso do Sul, Mato Grosso, Tocantins, Bahia, Minas Gerais and Distrito Federal.

Water sources were selected according to the occurrence of *Escherichia Coli* in raw water, with an annual geometric average greater than 1,000 most probable number (MPN)/100 mL, as established by Annex XX of Consolidation Ordinance No. 5 (BRASIL, 2017), in at least four years between 2009 and 2018. Monitoring raw water quality using only indicators (*E. Coli*) is not sufficient to assess the occurrence of specific pathogens (HORMAN *et al.*, 2004), and so the occurrence of pathogens in water courses must be assessed.

Table 2 – Coordinates of the sampling points.

Municipality	Superficial Water Source	Coordinates	
Adelândia	São Manoel Stream	16°24.387'S	50°11.267'W
Anicuns	Boa Esperança Stream	16°27.722'S	49°58.185'W
Barro Alto	Barro Alto Stream	14°57.647'S	48°55.452'W
Ceres	Das Almas River	15°18.679'S	49°35.373'W
Crixás	Forquilha Stream	14°33.388'S	49°58.334'W
Goianésia	Anda Só Stream	15°19.073'S	49°6.469'W
Jaraguá	Pari River	15°44.630'S	49°21.565'W
Morro Agudo de Goiás	Olho D'água Stream	15°18.746'S	50°3.300'W
Nova Glória	Jatobá Stream	15°8.806'S	49°34.110'W
Quirinópolis	Das Pedras Stream	18°27.084'S	50°28.643'W
Rubiataba	Novo River	15°10.416'S	49°48.112'W
Sanclerlândia	Cerrado Stream	16°12.942'S	50°18.640'W
São Luiz dos Montes Belos	Santana Stream	16°32.253'S	50°21.247'W
Uruanã	Uru River	15°30.638'S	49°40.742'W
Vianópolis	Dirceu Stream	16°44.418'S	48°31.022'W

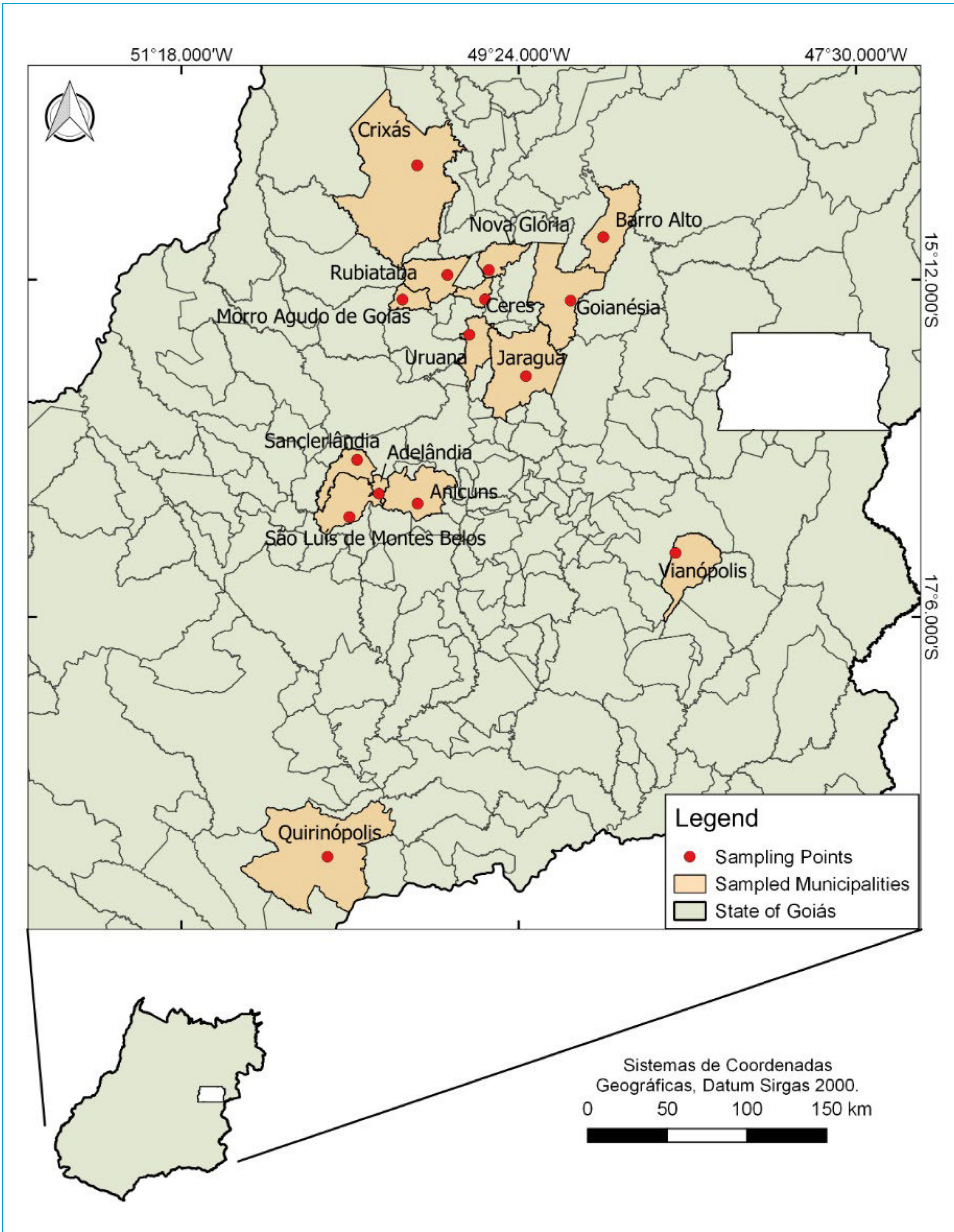

Figure 1 – Location of sampling points of water supply sources in the state of Goiás.

Sampling campaign

Parasitological analysis

The collections were made at the entrance of the treatment plants in August 2019, which is considered the dry period (May to September) in regions with a humid tropical climate, according to the classification of Köppen (1948). A sample of twenty liters of raw water was collected from each water source and stored in a polyethylene container that was previously cleaned with Tween 80 (0.1%).

The Membrane Filtration (MF) method was chosen due to the ease of implementation and the possibility of preserving (oo)cysts for future infectivity assays (MACIEL; SABOGAL-PAZ, 2016). Furthermore, the method has greater recovery efficiency than the cartridge filtration method (HSU *et al.*, 2001), and reduced cost of implantation and analysis than standard methods recommended by USEPA (2012).

This reduced cost is due to the fact that most water treatment stations (WTS) already have the necessary equipment to perform the technique, which makes its implementation feasible in developing countries, and due to its wide use in research carried out in different regions of the world to evaluate the occurrence of these protozoa in water supply sources (BAUTISTA *et al.*, 2018; NISHI *et al.*, 2009; ALMEIDA *et al.*, 2015).

Prior to quantifying (oo)cysts of the protozoa in raw water, the analytical quality control of the method employed was tested in triplicate to verify its reliability and sensitivity regarding the recovery of (oo)cysts in ultrapure and *in natura* raw waters from the Santana stream, with turbidity of 8.20 Nephelometric Turbidity Unit (NTU). The samples were contaminated with 10 µL of protozoan inoculum from *Merifluor*® Kit (Meridian Bioscience Diagnostics, Cincinnati, USA), with the average concentration of protozoans being quantified in two 10 µL aliquots of the inoculum.

Recovery efficiency of (oo)cysts was determined according to Equation 1 and correlated with criteria established by USEPA (2012) [mean ± standard deviation (SD)]: 27 – 100 ± 39% for cysts and 38 – 100 ± 37% for oocysts in ultrapure water, and 8 – 100 ± 97% for cysts and 32 – 100 ± 46% for oocysts in raw water. Recovery rates above 100% were disregarded from the results, following Franco *et al.* (2012). The results were discussed in terms of descriptive statistics.

$$R = \frac{N}{T} x\, 100 \quad (1)$$

Where:
R = recovery efficiency (%);
N = number of recovered cysts and (oo)cysts;
T = number of inoculated cysts and (oo)cysts;

To evaluate the contamination of the method, 1.0 L of ultrapure water was analyzed in duplicate, with subsequent concentration of *in natura* raw water, whose results should indicate the absence of cysts/oocysts (CANTUSIO NETO, 2008).

Membrane Filtration was performed by the following steps:

- concentration of (oo)cysts of protozoa through filtration of 2.0 L of each sample through a mixed cellulose esters membrane (*Millipore*®), with a diameter of 47 mm and nominal porosity of 3.0 µm;
- elution to recover the (oo)cysts by scraping and washing the material retained on the membrane with elution solution Tween 80 (0.1%);
- centrifugation and concentration of the material resulting from the elution by double centrifugation process for 15 minutes with a rotational force of 1,500 × g, as recommended by USEPA (2012);
- slide preparation using the direct Immunofluorescence Assay (FA) technique with *Merifluor*® Kit, according to the manufacturer's guidelines for the simultaneous detection of oocysts and cysts, with aliquots of 10 µL for each slide well and three aliquots for each sample;
- reading of slides using a Zeiss Axio Imager M2 microscope for epifluorescence with 400X magnification, 450–490 nm excitation filter, and 520 nm barrier filter for detection and enumeration of (oo)cysts.

Fluorescence defined by bright apple-green- color was adopted to identify (oo)cysts: absence of pores or appendages; 8–18 µm in length and 5–15 µm in width, and oval shape for cysts of *Giardia* spp.; size of 4–6 µm in diameter, spherical shape and presence (not mandatory) of suture for *Cryptosporidium* spp. (CANTUSIO NETO, 2008; USEPA, 2012). The results were expressed as (oo)cysts/L according to Equation 2 described by Cantusio Neto and Franco (2004).

$$CP = \frac{\text{Number of (oo)cysts seen}}{\text{Volume of analyzed sediment }(\mu L)} x \frac{\text{Volume of obtained sediment }(\mu L)}{\text{Volume of filtered sample }(L)} \quad (2)$$

Where:
CP = concentration of protozoa [(oo)cysts/L].

Limit of Detection (LD) consists of the minimum number of recoverable organisms in an analyzed sample volume (ONGERTH, 2013), which varies according to the volume of the processed sample, which depends on the physical-chemical characteristics of the water, the final volume of the pellet, and the volume of aliquots. Calculation of LD follows Equation 3 (CANTUSIO NETO, 2008).

$$LD = \frac{1}{\text{Volume analyzed sediment }(\mu L)} x \frac{\text{Volume of obtained sediment }(\mu L)}{\text{Volume of filtered sample }(L)} \quad (3)$$

Analysis of physical parameters

The variables turbidity and pH were analyzed according to *Standard Methods* (APHA; AWWA; WEF, 2012), since they are part of the routine operational control of WTS.

Pasture area and animal density

Occupation data for the percentage of pasture area were obtained using ArcGIS software in conjunction with MapBiomas - Collection.3.0 database (PROJETO MAPBIOMAS, 2017), for the year 2017. Cattle load, which corresponds to the quotient between the number of cattle and the area of pasture in hectares, and the load of swine and horses as a function of the total area of the basin, was obtained from the *Censo Agropecuário* 2017 (IBGE, 2017).

RESULTS AND DISCUSSION

Validation of the membrane filtration method

Results regarding the performance of the MF method with samples contaminated with *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts are provided in Table 3 for ultrapure water, and Table 4 for *in natura* raw water samples from the Santana stream. The absence of contamination in the analytical process is noteworthy, as no (oo)cysts were detected in the duplicated test.

The individual results in Table 3 show anomalous recovery efficiency values, with 156.3 and 121.3% for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts, respectively, which may be explained by possible agglomeration of organisms in the aliquot. Table 4 shows atypical results of 260.9 and 104.7% for *Giardia* spp. cysts, which may be explained by possible agglomeration of organisms in the aliquot due to the turbidity of samples, which requires more of membranes to filter samples and a greater final pellet volume.

Agglomerations can be caused by age, origin of organisms, storage time of the inoculum suspension (CANALE, 2014), and presence of algae, yeasts and debris that self-fluoresce and can affect the detection of (oo)cysts under the microscope by generating false positives (USEPA, 2012).

The average recovery rates in ultrapure water were 78.1 ± 0% for *Giardia* spp. cysts and 60.6 ± 32.6% for *Cryptosporidium* spp. oocysts (Table 3), as recommended by USEPA (2012).

Recovery in raw water was lower than that recommended by USEPA (2012), with values of 12.4 ± 8.8% for *Cryptosporidium* spp. oocysts (Table 4), due to the turbidity of the samples which, according to Efstratiou, Ongerth and Karanis (2017a), affects the detection performance for protozoa toward underreporting.

Particle is one of the limitations faced by the MF method in recovering (oo)cysts (FRANCO; BRANCO; LEAL, 2012; CANTUSIO NETO,

Table 3 – Initial Precision and recovery of *Giardia* spp. and *Cryptosporidium* spp. inoculated in ultrapure water, containing the inoculated (oo)cysts, recovery efficiency, mean, SD and CV.

Samples	Cysts of *Giardia* spp.			Oocysts of *Cryptosporidium* spp.		
	Number of inoculated cysts	Number of recovered cysts	Recovery efficiency (%)	Number of inoculated oocysts	Number of recovered oocysts	Recovery efficiency (%)
1	32.0	25.0	78.1	268.0	325.0	121.3
2	32.0	25.0	78.1	268.0	250.0	93.3
3	32.0	50.0	156.3	268.0	75.0	28.0
		Mean	78.1		Mean	60.6
		SD	0.0		SD	32.6
		CV	0.0		CV	53.8

SD: standard deviation; CV: coefficient of variation.

Table 4 – Continued precision and recovery of *Giardia* spp. and *Cryptosporidium* spp. inoculated in raw water *in natura* of Santana Stream with turbidity of 8.20 NTU, containing the inoculated (oo)cysts, recovery efficiency, mean, SD and CV.

Samples	Cysts of *Giardia* spp.			Oocysts of *Cryptosporidium* spp.		
	Number of inoculated cysts	Number of recovered cysts	Recovery efficiency (%)	Number of inoculated oocysts	Number of recovered oocysts	Recovery efficiency (%)
1	32.0	0.0	0.0	268.0	16.5	6.2
2	32.0	83.5	260.9	268.0	66.5	24.8
3	32.0	33.5	104.7	268.0	16.5	6.2
		Mean	-		Mean	12.4
		SD	-		SD	8.8
		CV	-		CV	71.1

SD: standard deviation; CV: coefficient of variation.

2008), since, under conditions of greater turbidity, a large number of membranes is required for filtration, which generates a greater volume of sediment destined for slide preparation. Using the MF method, Bautista *et al*. (2018) reported that the lowest means for detected (oo) cysts/L were in samples with turbidities of 554 and 800 NTU, during the rainy season, in Rímac stream of Peru.

Recovery outside the range recommended by USEPA (2012) for raw water was reported by Canale (2014) and Maciel (2006). The latter associated the method in question with immunomagnetic separation (IMS) and obtained mean recoveries of 31.5% cysts and 5.7% oocysts. Efstratiou, Ongerth and Karanis (2017a) reported that, in general, *Giardia* spp. cysts achieve greater recovery than *Cryptosporidium* spp. oocysts.

To improve performance in recovery efficiency with raw water samples, a more voluminous aliquot can be added to each slide well, once that according to Maciel (2006), the variability of tests may be associated with low aliquot representativeness(10 μL) in relation to the final sediment volume (250–1,000 μL), mainly for techniques that do not use IMS.

In addition, a rotation energy greater than 1,500 × g (15 minutes) could be adopted, as verified by Clancy *et al.* (2000), who achieved best recovery performance for *Cryptosporidium* spp. with a gravitational force of 2,170 × g and a centrifugation time of 15 minutes.

Prevalence of (oo)cysts in surface water sources

Data on the occurrence of *E. Coli* in raw water (frequency and range), the percentage of pasture area and animal load in the catchment hydrographic basins are presented together, with the results of the physical and parasitological parameters, in Table 5. As it turns out, in the present scenario, (oo)cysts are distributed in the aquatic environment of the state of Goiás, suggesting a potential risk to the population's public health.

Table 5 – Percentage pasture area; densities of cattle, swine and horses; incidence of *E. Coli* from 2009 to 2018 (frequency and amplitude); and results of physical and parasitological parameters, as well as the LD.

Municipality	Superficial Water Source	Pasture area (%)	Cattle density (number/hectare)	Swine density (number/hectare)	Horse density (number/hectare)	Incidence of *E. Coli* ≥ 1000 MPN/100 mL	*E. Coli* (minimum - maximum)	Turbidity (NTU)	pH	*Giardia* (cysts/L)	*Cryptosporidium* (oocysts/L)	LD ((oo) cysts/L)
Adelândia	São Manoel Stream	71.60	1.78	0.066	0.022	6	211 - 3,890	6.80	7.89	66.7	**208.3**	8.3
Anicuns	Boa Esperança Stream	66.83	2.06	0.059	0.015	4	8 - 2,295	6.86	7.94	**100.0**	175.0	8.3
Barro Alto	Barro Alto Stream	40.90	0.77	0.020	0.009	7	974 - 4,685	7.47	7.71	< LD	58.3	8.3
Ceres	Das Almas River	52.23	1.36	0.105	0.033	5	157 - 6,432	4.84	7.84	58.3	66.7	8.3
Crixás	Forquilha Stream	34.73	0.96	0.020	0.011	6	48 - 2,727	2.11	7.8	16.7	62.5	4.2
Goianésia	Anda Só Stream	51.83	1.42	0.065	0.013	6	284 - 5,291	11.9	7.65	16.7	108.3	8.3
Jaraguá	Pari River	61.18	1.32	0.055	0.018	6	282 - 6,429	7.38	8.01	< LD	8.3	8.3
Morro Agudo de Goiás	Olho D'água Stream	67.05	1.57	0.038	0.024	8	630 - 4,770	16.3	7.43	**116.7**	**250.0**	16.7
Nova Glória	Jatobá Stream	24.16	1.88	0.053	0.012	5	473 - 9,289	2.44	7.27	8.3	16.7	4.2
Quirinópolis	Das Pedras Stream	45.59	1.38	0.018	0.008	5	39 - 2,003	5.98	7.85	**116.7**	133.3	8.3
Rubiataba	Novo River	61.11	1.57	0.052	0.019	5	498 - 6,125	4.99	7.8	50.0	66.7	8.3
Sanclerlândia	Cerrado Stream	**79.33**	1.63	0.050	0.022	7	337 - 10,089	7.19	7.88	58.3	**216.7**	8.3
São Luís de Montes Belos	Santana Stream	**78.46**	1.73	0.083	0.027	5	366 - 10,669	12.4	7.83	66.7	66.7	8.3
Uruana	Uru River	50.07	1.58	0.073	0.025	5	187 - 4,351	2.64	7.74	79.2	54.2	4.2
Vianópolis	Dirceu Stream	35.95	1.91	0.033	0.017	4	Absence - 4,133	5.93	7.74	58.3	108.3	8.3

LD: limit of detection; MPN: most probable number; NTU: Nephelometric turbidity unit.

According to Annex XX of Consolidation Ordinance No. 5 (BRASIL, 2017), the *E. Coli* microbiological parameter is an indicator of (oo)cysts of *Cryptosporidium* spp. and *Giardia* spp. in water resources; however, Grott *et al.* (2016) and Bastos *et al.* (2004) failed to find a significant correlation between the prevalence of these organisms when analyzing water from surface sources in the states of Santa Catarina and Minas Gerais, respectively.

According to Horman *et al.* (2004), the lack of statistical correlation can be partly associated with the survival time of each species in the aquatic environment, since *Cryptosporidium* spp. oocysts can survive for more than six months while cysts can survive for a maximum of two months (KARANIS; KOURENTI; SMITH, 2007).

Table 5 shows that all samples were positive for *Cryptosporidium* spp. oocysts and 86,66% (13/15) for *Giardia* spp. cysts. Detention rates have not been compared with other investigations (IMRE *et al.*, 2017) as, according to Hachich *et al.* (2004), lotic environments have a higher incidence of protozoa than lotic aquatic systems. Lopes *et al.* (2017) found maximum concentrations of 0.8 cysts/L and 0.4 oocysts/L in the Vargem das Flores reservoir, a lentic environment intended for supplying Belo Horizonte, Minas Gerais. The lower occurrence of protozoa is associated with removal factors, including sedimentation and inactivation by temperature, ultraviolet radiation (UV) and predation (BROOKES *et al.*, 2004).

In the Olho D'água stream, the maximum concentrations of *Cryptosporidium* spp. (250 oocysts/L) and *Giardia* spp. (116.7 cysts/L) were detected, along with eight occurrences of the *E. Coli* indicator above 1,000 MPN/100 mL, due to the predominance of pasture area (67.05%) and cattle load (1.57 per hectare). This source presents intense agricultural activities with a pig load in the range of 0.038 per hectare, the main host of the species *C. scrofarum* and *C. suis*, and a horse density of 0.024 per hectare, the host of *C. erinacei* (RYAN; FAYER; XIAO, 2014).

These concentrations were notably higher than the maximum concentration of 75 cysts/L and 19.2 oocysts/L reported for raw water from Corumbataí river in Piracicaba, São Paulo (CANALE, 2014) and the maximum concentration of 35.8 cysts/L in raw water from Atibaia river in Campinas, São Paulo (CANTUSIO NETO, 2008). Since these studies did not provide data on land use and occupation, it appears that the higher prevalence of protozoa in the basins studied here is related to characteristics of land use, especially pasture area, as the presence of cattle feces in the vicinity of the water courses promotes water contamination, especially in the rainy season and when the pasture is located in the floodplain (GRACZYK *et al.*, 2000).

A high occurrence of *Giardia* spp. (116.7 cysts/L) was also detected in the Pedras stream, with 45.59% of pasture area, five occurrences of *E. Coli* above the recommended level, and a cattle load of 1.38.

The predominance of pastures may indicate the extent of the source of contamination upstream of the catchment point (ADAMSKA, 2015). However, molecular characterization of the organisms is needed to confirm this relationship, with a focus on species infectious of humans, such as *C. parvum* and *hominis*(EFSTRATIOU; ONGERTH; KARANIS, 2017a).

These results corroborate the study by Almeida *et al.* (2015), which identified the presence of *Cryptosporidium parvum* and *Giardia duodenalis* in Ribeirão Cafezal in Londrina Paraná, and associated this finding with the existence of dairy cattle in the region, and that of Farizawati *et al.* (2005), which verified the contribution of cattle farms along the watercourse for contamination by protozoa, with high concentrations in the range of 1.5 to 3.9×10^5 (oo)cysts/L.

The risk associated with *C. parvum*, isolated from cattle, horses, and humans (ROSE; HUFFMAN; GENNACCARO, 2002), in diarrheal children hospitalized in Goiânia, state of Goiás, has been associated with environmental factors such as contact with surface water in the last 30 days and distance between residence and a watercourse (PEREIRA *et al.*, 2002).

The water sources Barro Alto stream and Pari river had *Giardia* spp. cyst concentrations below the LD and *Cryptosporidium* spp. at 58.3 and 8.3 oocysts/L, respectively. It should be noted that the hydrographic basin of the Pari river, with greater pasture area (61.18%) than that of Barro Alto stream (40.90%), six occurrences of *E. Coli* and high densities of cattle (1.32), swine (0.055) and horses (0.018) per hectare, had the lowest concentration of *Cryptosporidium* spp. of the studied surface water sources, although still with a risk to human health, since the infectious dose corresponds to at least one oocyst (KARANIS, 2011; STEINER; THIELMAN; GUERRANT, 1997).

Concentrations of 16.7 oocysts/L and 8,3 cysts/L were detected at the Jatobá stream, which had the smallest pasture area (24.16%), five loads of *E. Coli* higher than that established by the standard and low densities of cattle (1.88 per hectare). These results indicate that the characteristics of land occupation positively influenced the parasitological quality of raw water (DIAS *et al.*, 2008), due to the smaller area of pasture that was reflected in a smaller number of cattle in the region, since studies in other countries reported that a bovine is capable of releasing 50 to 3.9×10^5 (oo)cysts/g of feces (FARIZAWATI *et al.*, 2005).

Among the basins studied, the highest cattle load (2.06) was observed for Boa Esperança stream, with an occurrence of 100 and 175 oocysts/L. It is noteworthy that this contamination is intensified in developing countries and rural areas due to untreated human and animal waste (HELLER *et al.*, 2004).

The results did not allow to infer whether the animal load in the hydrographic basin generated increased concentrations of protozoa. According to Bagley *et al.* (1998), water sources that receive contributions of cattle feces and sewage discharge as a result of land use activities have 10 to 100 times higher concentrations of (oo)cysts. However, in the present study, the Barro Alto stream basin, which had the lowest cattle load

(0.77), had an occurrence of 58.3 oocysts/L. This effect may be related to soil management, reducing the carrying of (oo)cysts (CHUAH *et al.*, 2016), and the presence of uninfected cattle in the catchment basins with lower prevalence of protozoa, which depends on the water source given to the animals and their age, since young animals are more frequently contaminated due to having less immunity (DIAS *et al.*, 2008).

The presence of protozoa in supply sources (Figure 1) in the state of Goiás represents a potential risk to consumer health if the water is not treated properly. It is recommended that sanitation authorities and companies dedicate themselves to the implementation of new treatment techniques, the improvement of existing techniques and the continuous monitoring of water courses (FREITAS *et al.*, 2010).

Sanitation companies must analyze the (oo)cysts in fresh water as established in Annex XX of Consolidation Ordinance No. 5 (BRASIL, 2017), since the monitoring of raw water is an essential tool to control and monitor treated water (HELLER *et al.*, 2004), in addition to preventing outbreaks of gastrointestinal diseases, negative impacts on public health and ensuring public health security, as the water resource is destined for different uses (LEE *et al.*, 2013).

In addition, seasonal monitoring aims to characterize the aquatic environment, since bacteria in the coliform group are not good indicators of the prevalence of protozoa (HORMAN *et al.*, 2004) and due to the underestimation of risk of infection caused by punctual samples (HELLER *et al.*, 2006), as performed in the present study.

The range of variation in the physical parameters of turbidity and pH were 2.11–16.30 NTU and 7.27–8.01, respectively. This range of turbidity values is in concordance with the drought period, as found by Vasco *et al.* (2011), who reported that the increased flow of Poxim river in the state of Sergipe, due to the rainy period, directly interferes with turbidity.

Evaluating existing studies on the theme revealed that the present study is the first to report the occurrence of *Giardia* spp. and *Cryptosporidium* spp. in water sources intended for public water supply in the state of Goiás. Therefore, there is a need for basic sanitation companies to establish routines for monitoring these protozoa, in order to prevent outbreaks of gastrointestinal diseases, as well as urgent action for the preservation and maintenance of the respective water courses.

CONCLUSION

From the results obtained in the present study, it can be concluded that:

- The precision and initial recovery tests reached USEPA (2012) criteria for *Giardia* (78.1 ± 0%) and *Cryptosporidium* (60.6 ± 32.6%);
- The precision and continuous recovery tests with *in natura* raw water did not present any sample in accordance with the international standard. This was due to the turbidity of samples, which requires more of membranes to filter samples and a greater final pellet volume;
- The Olho D'água stream located in Morro Agudo de Goiás, had maximum concentrations of 250 oocysts/L for *Cryptosporidium* spp. and 116.67 cysts/L for *Giardia* spp. These values can be associated with the predominance of livestock activities in the hydrographic basin. However, the punctual sampling campaign did not allow to infer whether the animal load in the hydrographic basin generates increased concentrations of protozoa;
- The Jabotá stream, with a low predominance of pasture area, presented less contamination of the watercourse by protozoa *Cryptosporidium* spp. and *Giardia* spp.;
- Cysts and oocysts were detected in the state's aquatic environment at concentrations ranging from < LD to 116.67 cysts/L and between 8.33 and 250 oocysts/L, which represents a potential risk to consumer health considering that the intake of at least 10 cysts of *Giardia* spp. and one oocysts of *Cryptosporidium* spp. can initiate the infectious process. Therefore, periodic monitoring of water sources by service delegates is recommended in order to improve existing technologies of treatment plants to ensure sanitary security;
- It is recommended that treatment plants that obtain water from Cerrado, Santana, and São Manoel streams monitor the occurrence of protozoa, as the predominance of pasture indicates a risk for cryptosporidiosis outbreaks.

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