Association between the turbidity parameter and ELISA for monitoring of *Giardia lamblia*, *Cryptosporidium* sp. e *Entamoeba histolytica* in samples of fresh water

Associação entre o parâmetro de turbidez e o ELISA para monitoramento de *Giardia lamblia*, *Cryptosporidium* sp. e *Entamoeba histolytica* em amostras de água doce

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Abstract

This study aimed to evaluate the use of turbidity test and ELISA in monitoring the protozoa *Giardialamblia*, *Entamoeba histolytica*, *Cryptosporidium* sp, in water supply of the Guarani villages in Angra dos Reis (Sapukai) and Paraty (Rio Pequeno, Paraty Mirim, Araponga), RJ. It was collected 24 samples of surface water (raw water) and 24 reservoirs with chlorinated water. Turbidity was measured with portable turbidimeter field and laboratory testing was performed the immunosorbent essay for the test of antigens of *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium* sp. Out of the 17 positive samples in ELISA, 14 showed high turbidity, considering that three presented positive samples for Cryptosporidium sp., with low turbidity. The results indicate an association of the measurement of turbidity with the use of laboratory techniques in the study of parasites in water.

Key words: fresh water; parasites; turbidity of water; enzyme immunoassay.

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Resumo

Este estudo teve como objetivo avaliar a utilização do teste de turbidez e do ELISA no monitoramento dos protozoários *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* sp. em água de abastecimento de aldeias Guarani em Angra dos Reis (Sapukai) e Paraty (Rio Pequeno, Paraty Mirim, Araponga), RJ. Coletou-se 24 amostras de água dos mananciais superficiais (água bruta) e 24 de reservatórios com água clorada. Mensurou-se a turbidez com turbidimêtro portatil a campo e no laboratório realizou-se o ensaio imunoenzimático para pesquisa dos antígenos de *Giardia lamblia, Entamoeba histolytica* e *Cryptosporidium* sp. Das 17 amostras positivas, 14 apresentaram alta turbidez, mas destaca-se três amostras positivas para *Cryptosporidium* sp. com baixa turbidez. Indica-se associação da mensuração da turbidez junto com o uso de técnicas laboratoriais na pesquisa de parasitos em água.

Palavras-chave: água doce; parasitos; turbidez da água; ensaio imunoenzimático.

Introduction

The Ordinance no. 518/2004 of the Ministry of Health standardizes the parameters for the classification of potable water for human consumption, recommending the survey of cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp., in addition to determining that the turbidity of potable water must be lower than 0.5 NTU (Nephelometric Turbidity Unit) to ensure the elimination of the evolutionary forms of these protozoa with treatment processes (BRASIL, 2004). In Brazil, only a few laboratories perform survey of protozoa in water, claiming lack of standardization, complexity of techniques and high cost (LIMA; STAMFORD, 2003).

It is known that *Giardia lamblia* has widespread zoonotic potential, chiefly regarding types A and B. This protozoan may trigger gastroenteritis, mainly in children (MONIS et al., 2008). The *Entamoeba histolytica* may lead to serious clinical pictures of amoebic dysentery. This amoeboids has humans (PONCE-GORDO; MARTINEZ-DIAZ, 2010) and non-human primates (PEREIRA et al., 2010) as hosts. In its turn, *Cryptosporidium* sp. has several species of protozoa, some specific of animals and other zoonotic ones, such as *C. parvum*. Watery diarrhea is the main sign developed by this pathogen, and may become chronic depending on the host immune status (FAYER, 2010).

The ELISA (Enzyme-linked immunosorbent assay) has been used in fecal samples for survey of intestinal protozoa. The parasite antigens of *Cryptosporidium* sp. were detected by ELISA in fecal samples of children in the city of Blumenau, State of Santa Catarina (ANDRADE et al., 2008). In the city of Belém, State of Pará, antigens of *G. lamblia* were found in children's feces (MACHADO et al., 2001) and antigens of *E. histolytica,* in residents of the metropolitan region of Belém (SILVA et al., 2005).

The purpose of this study was to evaluate the use of turbidity test and ELISA for monitoring of *G. lamblia*, *E. histolytica* and *Cryptosporidium* sp. in samples of the water of the supply system of Guarani Indian Villages in the State of Rio de Janeiro.

Material and Methods

The survey was performed in four Guarani Indian Villages in the cities of Angra dos Reis (Sapukai Village) and Paraty (Araponga, Rio Pequeno and Paraty Mirim Villages) in the State of Rio de Janeiro. These villages have health and sanitation assistance of FUNASA (National Health Foundation) and water supply system using raw water collected in surface source and treatment with chlorination in polyethylene water reservoirs. Owing to its large territory, the Sapukai Indian Village is the only one to have three systems, which are known at the site as: Cachoeira, Cacique and Pupunha. The water treatment of Rio Pequeno is made with a sand and gravel filter. Sapukai Cacique, Cachoeira and Pupunha have slow sand filters. In their turn, the systems of Araponga and Paraty Mirim have no filters.

Quadruplicate samples were collected over the period from February to October, 2010. In the end, there were 24 samples of raw water and 24 ones of treated water from six different systems, totaling eight samples of water for each system. The water from surface sources (raw water) was collected by means of the passage of 2000 liters in commercial Aqualimp® filters, having inside cartridges and coiled wire with 1 µm porosity of Cuno® DPPPY-1 Micro Wind II model. After filtration, the cartridges were packaged in properly marked plastic bags. Two liters of reservoir-bottom (treated water with clorine) sediment were collected with the aid of a 1' plastic hose through siphoning. This sediment was stored in 2 liter plastic bottles, previously cleaned and identified.

All collected material was stored refrigerated and sent to the Parasitology Laboratory of the Biomedical Institute (CMB) at Federal Fluminense University (UFF) and then to the Immunodiagnostic Laboratory of the Department of Biological Sciences (DCB), at the National School of Public Health (ENSP)/Fiocruz.

The filter cartridges were eluted by washing with 1 liter q.s.p. solution of neutral detergent at 0,001%, filtered, placed for sedimentation and washed until a clear supernatant was obtained. The samples, stored in 2 liter plastic bottles, were placed for sedimentation and washed until a clear supernatant was obtained. The sediment of all samples were aliquoted for Axygen® eppendorf microtubes, which were kept at a temperature of -18 °C, intended for performance of immunoassay.

ELISA was performed with all samples. The kits were purchased commercially: Wampole *Cryptosporidium* II test Techlab®, which surveys oocyst wall antigens of *Crypstosporidium* sp. Entamoeba Ridascreen®, which surveys specific adhesins of *Entamoeba histolytica* (N-acetyl-D-galactosamine inhibitor lectin), present on the parasite membrane. *Giardia* Ridascreen®, which surveys specific antigens of the cyst wall of *Giardia lamblia*. Before the performance of the enzyme immunoassay, a pre-dilution of 60 µL of the sample and 60 µL of the diluent provided by manufacturer was performed, and 100 µL of this solution was transferred to the duplicate test plate. Then, was followed the protocol recommended by the manufacturer. The reading was made in ELISA reader (Testline® ELx 800) with wavelength according to the manufacturer's technical standards.

In addition to the hydrological collection for research of protozoa, the turbidity was also measured at the same points (raw water and treated) that were

taken for research by ELISA. The evaluation was performed in the field as recommended by APHA (American Public Health Association, 1998) with a model 2100P Hach® portable turbidimeter and the results were recorded in NTU.

This research was part of the project entitled "Evaluation of the prevalence of geohelminthiasis, cutaneous leishmaniasis and leprosy among Guarani Indians in the State of Rio de Janeiro, Brazil" of the Department of Biological Sciences at the National School of Public Health, for arrangement of approval of the Ethics Committee of Sérgio Arouca School/CEP-ENSP No. 56/09, protocol: 0056. 0.031. 000 – 09, under the terms of Resolution 196/96, on assessment: May 6, 2009.

Results and Discussion

In the assessment of the results of turbidity, the samples considered within the standard for consumption were those presenting values lower than 0.5 NTU, the limit recommended by Ordinance no. 518/2004 of the Ministry of Health to ensure the non-existence of cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp.

A total of 24 samples of raw water collected, 18 showed turbidity above the limit recommended by laws (Table 1), and 11 out of the 24 treated water samples had high turbidity standards (Table 2). In all measurements performed, the raw and treated water samples of Paraty Mirim and rio Pequeno Villages had values above 0.5 NTU.

Table 1 - Results of turbidity and antigens of *Giardia lamblia*, *Cryptosporidium* sp. and *Entamoeba histolytica* detected in the raw water of the supply systems of Guarani indian Villages of Angra dos Reis and Paraty, RJ

		Guarani Indian Villages					
Collections	RW	Araponga	Rio Pequeno	Paraty Mirim	Sapukai Cachoeria	Sapukai Cacique	Sapukai Pupunha
1ª	NTU	0.9	0.9	6.1	0.82	2.59	1.04
	Gl	A	P	P	P	P	A
	Cr	A	A	P	A	A	A
	Eh	A	P	A	P	A	A
2ª	NTU	0.2	0.61	2.52	1.14	0.62	0.52
	Gl	A	A	A	A	A	A
	Cr	A	P	P	A	A	A
	Eh	A	A	A	A	A	A
3ª	NTU	0.15	1.69	2.2	0.79	0.31	0.26
	Gl	A	A	P	A	A	A
	Cr	A	A	A	A	A	A
	Eh	A	A	P	A	A	A
4ª	NTU	0.4	2.22	2.55	0.66	0.7	0.19
	Gl	A	A	P	A	A	A
	Cr	A	P	P	A	A	A
	Eh	A	A	A	A	A	A

Source: Barbosa, AS (by author). Detection of parasites served by water and soil Guarani villages in the municipalities of Angra dos Reis and Paraty in the state of Rio de Janeiro, Brazil. (Master's dissertation), Niterói, 2011.

Note: RW: Raw Water; NTU: Nephelometric Turbidity Unit; *Gl: Giardia lamblia*; *Cr: Cryptosporidium* sp.; *Eh: Entamoeba histolytica*; A: absence; P: presence.

In the association between the nephelometric turbidity rate and antigens detected by ELISA, we found that all water samples positive for antigens of *G. lamblia* and *E. histolytica* presented turbidity above 0.5 NTU both in raw water (Table 1) and in the treated water (Table 2). Out of the 17 positive samples, 14 showed high turbidity, in addition to three positive for *Cryptosporidium* sp. with low turbidity in three samples of treated water (Table 2). Paraty Mirim Village presented a greater number of positive samples for *G. lamblia* and *Cryptosporidium* sp. and Rio Pequeno Village presented the largest number of positive samples for *E. histolytica*.

Table 2 -Results of turbidity and antigens of *Giardia lamblia*, *Cryptosporidium* sp. and *Entamoeba histolytica* detected in the treated water of the supply systems of Guarani Indian Villages of Angra dos Reis and Paraty, RJ

		Guarani Indian Villages					
Collections	TW	Araponga	Rio Pequeno	Paraty Mirim	Sapukai Cachoeria	Sapukai Cacique	Sapukai Pupunha
1ª	NTU	0.47	0.7	4.45	0.4	0.50	0.99
	Gl	A	P	A	A	A	A
	Cr	A	A	A	P	A	A
	Eh	A	P	A	A	A	A
2ª	NTU	0.19	0.52	0.87	0.44	0.3	0.52
	Gl	A	A	A	A	A	A
	Cr	P	A	A	A	A	A
	Eh	A	P	A	A	A	A
3ª	NTU	0.14	1.6	1.5	0.12	0.24	0.26
	Gl	A	A	P	A	A	A
	Cr	A	A	A	A	A	A
	Eh	A	A	P	A	A	A
4ª	NTU	0.2	1.82	0.66	0.55	0.43	0.19
	Gl	A	A	P	A	A	A
	Cr	A	P	P	A	A	P
	Eh	A	A	A	A	A	A

Source: Barbosa, AS (by author). Detection of parasites served by water and soil Guarani villages in the municipalities of Angra dos Reis and Paraty in the state of Rio de Janeiro, Brazil. (Master's dissertation), Niterói, 2011.

Note: TW: Treated Water; NTU: Nephelometric Turbidity Unit; Gl: Giardia lamblia; Cr: Cryptosporidium sp.; Eh: Entamoeba histolytica; A: absence; P: presence.

Most samples positive for antigens of *G. lamblia*, *E. histolytica* and *Cryptosporidium* sp. presented turbidity above 0.5 NTU. These results are in keeping with Franco et al. (2001), which isolated cysts of *Giardia* sp. and oocysts of *Crypstosporidium* spp. in the water of River Atibaia in Campinas, State of São Paulo, and also with Machado et al. (2009), which detected oocysts of *Cryptosporidium* spp. in surface waters with high turbidity at the Metropolitan Region of Recife, State of Pernambuco. Despite the fact that the survey of the cyst of *E. histolytica* is not recommended by the Ordinance 518/2004 of the Ministry of Health, the turbidity is also believed to reveal the presence of said cyst.

Antigens of *Cryptosporidium* sp. were detected in treated water samples with turbidity below 0.5 NTU, although Ordinance 518/2004 of the Ministry of

Health establishes that this degree of turbidity was related to the lower possibility of existence of this protozoan. These results are in keeping with Lechevallier and Norton (1992), who compare the isolation of cysts of *Giardia* sp. and oocysts of *Cryptosporidium* sp. with the turbidity in water treatment stations in Ohio, in the United States, and Canada. These authors concluded that the cysts of protozoa may occur when the turbidity is found in a value of 0.19 NTU, so that the effective guarantee of security would be achieved with the turbidity of 0.1 NTU, and not 0.5 NTU, as recommended by the Ordinance 518/2004 of the Ministry of Health.

In the water supply systems of Paraty Mirim and rio Pequeno Villages, the measurement of turbidity in raw water have always been greater than 0.5 NTU, resulting in high turbidity in treated water, and possibly favoring the increase of positivity of the evolutionary forms of protozoa detected by means of ELISA. According to Lechevallier et al. (1991), the number of cysts and oocysts of protozoa in treated water is directly related to the original raw water, as it is known that the treatment adopted by potable water supply systems are not effective for eliminating these evolutionary forms.

The water treatment systems of rio Pequeno and Paraty Mirim Villages have been found to be insufficient to reduce the amount of organic and inorganic contamination. Thus, the turbidity of water in these sites presented values above the limit recommended by the Brazilian laws. This increase in organic and inorganic compounds may have allowed the detection of antigens of protozoa.

Conclusion

Despite the importance of the physical and chemical parameter of turbidity, this must not be used singly for monitoring the parasites in water, insofar as the low turbidity may reveal the presence of protozoa. Therefore, the association of this parameter with parasitological laboratory techniques and the reduction of the turbidity limits recommended by the Ordinance 518/2004 of the Ministry of Health are recommended to really guarantee the absence of evolutionary forms of parasites in the supplied water.

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