SHORT COMMUNICATION

Occurrence of Cryptosporidial Oocysts and *Giardia* Cysts in Bottled Mineral Water Commercialized in the City of Campinas, State of São Paulo, Brazil

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The consumption of bottled mineral water has significantly increased in Brazil so that it is in the interest of public health to determine the parasitological and microbiological status of some brands of Brazilian mineral water available in the town of Campinas, São Paulo, Brazil. For this purpose, detection of protozoa by direct immunofluorescence technique and microbiological parameters were determined for each specimen after membrane filtration. Giardia *cysts were not present while cryptosporidial oocysts were detected in two samples. The counts of protozoa varied from 0.2 to 0.5 oocysts/l. The detected level of* Pseudomonas aeruginosa *and heterotrophic bacteria reflected the level of organic enrichment of the water.*

Key words: bottled mineral water - protozoa detection - microbiological parameters - Campinas - Brazil

Water is essential for life but may also become an important vehicle of many parasitic and infectious diseases (with agents of transmission such as bacteria, virus and protozoa), as well as of an increasing frequency of chronic diseases.

Once an emerging pathogen, *Cryptosporidium* is now firmly established as a widespread cause of enteric disease in humans and other animals. Cryptosporidiosis can be severe especially in the very young, the aged, the nutritionally deficient, and life-threatening in those whose immune systems may be compromised by disease or therapeutic agents. In Aids patients, *Cryptosporidium* contributes to death and no drug therapy has been found to be effective for this infection (Fayer et al. 2000). Transmission of oocysts excreted from infected hosts occurs either by direct host-to-host contact or indirectly by ingestion of contaminated water or food.

Giardia is a flagellated protozoan that causes clinically significant intestinal disease in humans as well as in livestock (Meyer 1990). Due to its increasing role in outbreaks of diarrhea among children attending day-care centers (Thompson 1994, Franco 1996) *Giardia* is now referred to as a re-emerging infectious agent (Thompson 2000). In this type of host *G. duodenalis* promoted a variety of symptoms such as diarrhea, weight loss, abdominal cramps, and growth stunt. This pathogen shares similar epidemiological features with *Cryptosporidium*, and giardiasis along with cryptosporidiosis represent one of the major public health concerns regarding water utilities today (Thompson 2000).

Since these protozoa were registered in ground and raw water (Gamba et al. 2000, Franco et al. 2001) in the State of São Paulo, Brazil, the detection of *Cryptosporidium* and *Giardia* in mineral water from natural sources provides an interesting area of research because the consumption of bottled mineral water has registered great increase in the last decade, with the State of São Paulo as the principal producer (Eiroa et al. 1996, Abinam). In addition, the presence of microbiological indicators marked the need for cysts and oocysts detection due to the possibility of contamination (Ministério da Saúde 2000).

The present work was carried out to verify the parasitological (*Cryptosporidium, Giardia)* and microbiological status of some Brazilian mineral water brands commercially available in the city of Campinas, São Paulo, Brazil.

Duplicate samples of 13 bottled brands of non-gaseous mineral waters (20 l; exception of L12-vol. 1.5 l) were submitted to filtration (4 l/min) in 45-mm-diameter-sterile cellulose acetate membranes (Schleicher & Schuell, Brazil), with nominal porosity of 3 µm. Afterwards, the filters were transported in refrigerated plastic containers to the Protozoology Laboratory, Parasitology Department, Campinas State University and were cut in two parts. Samples elution was performed by (i) alternately scraping the membrane with a smooth-edged plastic loop and rinsing it with 0.1% Tween 80 elution solution (RM method) (Shepherd & Wynn-Jones 1996) and (ii) the acetone-dissolution (ADM method) (Graczyk et al. 1997), respectively. The pellets were obtained by centrifugation (15 min; 600 x g) and diluted to 1 ml with 0.22 mm water filter system (MilliQ, Millipore, Brazil). One or more aliquots (5 µl) of these pellets were examined by direct immunofluorescence

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(Merifluor kits; Meridian Diagnostics, Cincinnati, Ohio) and simultaneously by phase contrast microscopy. The Zeiss Axiolab epifluorescent microscope with a 450-490 nm excitation filter and a 520 nm barrier filter was used to read the reaction.

Microbiological parameters were determined for each specimen (*Escherichia coli* or thermotolerant (fecal) coliforms, *Enterococcus* and *Pseudomonas aeruginosa)* according to methodology recommended by Standards Methods for the Examination of Water and Wastewater (APHA 1995) and by the Brazilian legislation for mineral waters (Resolution RDC no. 54).

A control trial was performed to evaluate the recovery efficiency of the methods employed by placing a known number of seed oocysts (from a suspension of oocysts obtained from fecal samples of HIV-infected patients, University Hospital, State University of Campinas – "CH isolate"), and cysts/oocysts (Easy-Seed; Biotechnology Frontiers, Australia) in a similar volume of mineral water previously filtered in 1.2 µm membrane. A small volume (2 l) was also submitted to membrane filtration; this assay was conducted twice for "CH isolate" and, finally, all pellets resulting (experimental and control samples) were numerated again prior to microscopy analysis (blinded assay).

In this survey, 13 bottled brands of non-gaseous mineral waters were analyzed; these labels are among the most popular in supermarkets in the city of Campinas. *Cryptosporidium* oocysts were detected in two water labels (L6 and L7) but *Giardia* cysts were not present in the water brands included in this study, in spite of the method employed (RM or ADM; Table I). It is noteworthy that L6 showed positiveness on two occasions. The counts of protozoa varied from 0.2 oocyst/l to 0.5 oocyst/l. The detected oocysts are in conformity with standard fluorescence detection criteria (Connel et al. 2000) and contrast-phase microscopy carried out simultaneously at magnification of 400 and 1,000x; when possible sutures and other morphologic features compatible with *Cryptosporidium* were observed in these forms. The results of the control trial are shown in Table II.

As regards microbiological parameters, three samples (L8, L10, L11) (Table I) presented positiveness for *P. aeruginosa* and consequently did not comply with the standard potability values foreseen in the existing Brazilian resolution, although the same resolution recommends collection of significant samples before they are accepted. High densities of heterotrophic bacteria in all analyzed samples (Table I) point out to the level of organic enrichment of the water.

Concerning the efficiency of recovery of cysts and oocysts (Table II), the RM method was better than the ADM technique, as had already been shown in a previ-

TABLE I

Results of *Cryptosporidium* oocyst and microbiological analysis [a] of some brands of Brazilian mineral water commercially available in the city of Campinas, São Paulo, Brazil

Samples	Microbiological analysis					*Cryptosporidium* oocysts[a]
(brand/sample)	C_T	C_{TERM}	$CPBH_{ufc/ml}$	*P. a.*	*E.*	
L1.(S1)	A	A	> 6500	A	A	A
L1.(S2)	A	A	>6500	A	A	A
L2.(S1)	A	A	980	A	A	A
L2.(S2)	A	A	100	A	A	A
L3.(S1)	A	A	>6500	A	A	A
L3.(S2)	A	A	>6500	A	A	A
L4.(S1)	A	A	>6500	A	A	A
L4.(S2)	A	A	300	A	A	A
L5.(S1)	A	A	>6500	A	A	A
L5.(S2)	A	A	>6500	A	A	A
L6.(S1)	A	A	800	A	A	P
L6.(S2)	A	A	>6500	A	A	P
L7.(S1)	A	A	100	A	A	A
L7.(S2)	A	A	10	A	A	P
L8.(S1)	A	A	>6500	16	A	A
L8.(S2)	A	A	650	A	A	A
L9.(S1)	A	A	30	A	A	A
L9.(S2)	A	A	10	A	A	A
L10.(S1)	A	A	>6500	A	A	A
L10.(S2)	A	A	>6500	23	A	A
L11.(S1)	A	A	>6500	A	A	A
L11.(S2)	A	A	>6500	>23	A	A
L12.(S1)	A	A	10	A	A	A
L12.(S2)	A	A	120	A	A	A
L13.(S1)	A	A	>6500	A	A	A
L13.(S2)	A	A	>6500	A	A	A

a: *Giardia* were not detect in the analyzed water; C_T: total coliforms (NMP/100 ml); C_{TERM}: *Escherichia coli* or thermotolerant (fecal) coliforms (NMP/100 ml); $CPBH_{ufc/ml}$: heterotrophic bacteria; *P.a.: Pseudomonas aeruginosa*; *E: Enterococcus*; A: absence; P: presence

TABLE II

Efficiency rates (%) of recovery of cryptosporidial oocysts and *Giardia* cysts in control-trial

Elution method	Samples inoculated with		
	Cryptosporidium (CH) [a]	Easy-seed [b]	
		Cryptosporidium	*Giardia*
ADM	30.0 - 36.0	16	4
RM	90.0 - 91.8	20	91.8

a: CH isolate"; two experiments; number of seed oocysts: 16/l; *b:* Easy-seed (Biotechnology Frontiers, Australia), according to manucfaturer instructions; one experiment; number of seed oocysts: 99 ± 2/2 l; number of seed cysts: 98 ± 1.6/2 l; ADM: acetone-dissolution membrane; RM: rinsing membrane

ous study (Franco et al. 2001). The ADM procedure has the disadvantage of inducing the hardening of the pellet containing oocysts or cysts after the centrifugation step.

The findings obtained in this study are very important since recently published surveys reported that people drank bottled water because they believed it meant "drinking water of better quality" and "safe" (Anadu & Harding 2000) and this may be not the case. The level of oocysts detected in this survey is sufficient to cause infection; the ID_{50} for human volunteers ranged from 9 to 1,042 oocysts, depending on the isolate (Fayer et al. 2000) and because various genotypes were recently identified causing human infection as *C. muris* (Katsumata et al. 2001), *C. meleagridis* and *C. felis/C. "dog"* (Xiao et al. 2001, Pedraza-Díaz et al. 2001) it is more prudent to consider any oocyst present in water for human consumption as offering risk of cryptosporidiosis, principally for HIV/Aids subjects, when the use of molecular tools is not possible.

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