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Detection of *Cryptosporidium* oocysts and *Giardia* cysts in swimming pool filter backwash water concentrates by flocculation and immunomagnetic separation

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The purpose of the present study was to evaluate techniques for detection of *Giardia* cysts and *Cryptosporidium* oocysts in swimming pool filter backwash water. Calcium carbonate flocculation was used for water samples concentration of 1 l filter backwash water samples. Immunomagnetic separation (IMS) was used for separation of cysts and oocysts from detrital material. Cysts and oocysts were then detected using direct immunofluorescence. ColorSeed C&G™ was used as an internal standard. Recoveries were examined at several processing points. Highest recoveries (23% *Cryptosporidium*, 18% *Giardia*) were obtained using lower filter backwash volumes, greater IMS volumes, and addition of Tween20™ to backwash samples prior to processing. The combination of $CaCO_3$ flocculation, IMS and direct immunofluorescence was found to be an effective tool for the detection and quantification of *Giardia* spp. and *Cryptosporidium* spp. in filter backwash water samples.

Keywords: *Giardia*; *Cryptosporidium*; swimming pool water; flocculation; immunomagnetic separation.

Introduction

The protozoan parasites *Giardia lamblia/duodenalis* and *Cryptosporidium parvum* are responsible for waterborne outbreaks of gastroenteritis via contaminated drinking (D'Antonio *et al*. 1985), and recreational waters (Bell *et al*. 1993; McAnulty *et al*. 1994; Mackenzie *et al*. 1995).

Giardia infects humans and other animals causing diarrhoea, weight loss, and abdominal pain. It is considered one of the most common causes of gastroenteritis worldwide (Thompson *et al*. 1993). *Cryptosporidium* spp. infect a wide range of vertebrates including humans and most domestic animals. They cause acute self-limiting, but frequently severe, diarrhoea in immunocompetent individuals, and chronic, life-threatening diseases in immunocompromised patients (Tzipori 1988).

The waterborne route of transmission of these parasites was recognized in the mid-1980s (D'Antonio *et al*. 1985), and waterborne outbreaks of cryptosporidiosis continue to occur (Call

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et al. 2001; Redlinger *et al*. 2002). A human ID_{50} for *C. parvum* as low as 132 oocysts has been reported (DuPont *et al*. 1995). Hence, a low level of environmental contamination with viable oocysts is enough to present a health risk. Chlorination kills a wide range of organisms not physically removed by typical drinking water treatment processes (flocculation, filtration, etc.). However, cysts and oocysts are resistant to chlorine concentrations routinely used for disinfection during water treatment (Slifko *et al*. 2000).

Recreational waters are also an important source of infection. (CDC 2000). It is therefore important to evaluate them for the presence and levels of *Giardia* and *Cryptosporidium*. Recently the poor bacterial and chemical quality of swimming pool water has been demonstrated in the West Bank of Palestine, as well as the necessity to improve the system for monitoring water quality since it is an important source of microorganisms, including pathogens as *Cryptosporidium* and *Giardia* (Al-Khatib and Salah 2003).

Numbers of *Giardia* cysts and *Cryptosporidium* oocysts present in water are generally low. In addition, levels of background detrital particulates may be high, particularly in untreated surface waters. Therefore, techniques employed for detection and quantification of *Giardia* and *Cryptosporidium* must have high degrees of both sensitivity and specificity.

Most currently used techniques for detection of *Giardia* and *Cryptosporidium* in water rely on concentration of large (10 l) volumes of water samples using one of several techniques (flat bed-, capsule-, or sponge-filtration, continuous flow centrifugation, flocculation, etc.).

The purpose of the present study was to evaluate and optimize a technique for detection of *Giardia* cysts and *Cryptosporidium* oocysts in swimming pool water through analysis of pool filter backwash water samples. Previous studies have shown that *Cryptosporidium* oocysts and *Giardia* cysts can be detected in treatment plant filter backwash water (Karanis *et al*. 1996; Karanis and Kimura 1998; Di Giovanni *et al*. 1999; Logan *et al*. 2001).

Materials and methods

Sources of oocyst and cyst suspensions

For the preliminary experiments, Wild-type (WT) *Cryptosporidium parvum* oocysts were used as control for recovery studies in distilled water. Oocysts were purified at Macaquarie University in Sydney, Australia from pooled faeces of naturally-infected neonatal calves. Faecal samples were centrifuged (2,000 × *g*, 10 min), washed in distilled water twice, resuspended in five volumes 1% (w/v) $NaHCO_3$, and extracted twice in one volume of ether, followed by centrifugation (2,000 × *g*, 10 min). Pellets were resuspended in water and filtered through a layer of pre-wetted non-adsorbent cotton wool. The eluate was then overlaid onto 10 volumes 55% (w/v) sucrose solution and centrifuged (2,000 × *g* for 20 min). Oocysts were collected from the sucrose interface and the floatation step repeated until no visible contaminating material was detected. Purified oocysts were surface-sterilized using ice cold 70% (v/v) ethanol for 30 min, washed once, and stored in phosphate buffered saline solution (PBS; 150 mmol NaCl, 15 mmol KH_2PO_4, 20 mmol Na_2HPO_4, 27 mmol l-1 KCl, pH 7.4) at 4°C. Stock suspensions of oocysts were diluted to a final concentration of 350.6 ± 11.9 oocysts/10 μl in PBS containing 0.05% (w/v) sodium azide and Tween 20™ 0.1% (v/v) and stored at 4°C. Oocysts were counted using haemacytometer chamber counts. Subsequently, ColorSeed C&G™ (Biotechnology Frontiers Pty Ltd., Sydney, Australia) was used as an internal standard for recovery studies in distilled water and swimming pool filter backwash water. ColorSeed C&G™ is a 5 ml test-tube containing 100 ± 2.5 of gamma-irradiated, inactivated, red-fluorescently-stained H3 strain *Giardia* cysts and Moredun and Iowa strain *Cryptospor-*

idium oocysts in 1 ml of saline solution. ColorSeed C&G™ *Cryptosporidium parvum* oocysts were isolated in sulphuric acid followed by sucrose density gradient centrifugation. ColorSeed C&G™ *Giardia lamblia* cysts were isolated using discontinuous $CsCl^2$ density gradient centrifugation. All cysts and oocysts doses were used for seeding experiments within 4 months of isolation.

Water matrices for assessing Cryptosporidium *oocyst and* Giardia *cyst recoveries*

Swimming pool filter backwash samples were collected from the Universidade Federal de Santa Catarina (UFSC) Olympic swimming pool between December 2000 and August 2001. The pool water is filtered using a mixed-medium (thin sand – 0.125 – 0.250 mm, medium sand – 0.25 – 0.50 mm, large sand – 0.5 – 1.0 mm, stones – 64 – 256 mm) conforming to the standard methods Brazilian Association (ABNT 1987). The filtration medium is replaced once per year, and was replaced in March, 2001 during this study. The filter operates 24 h per day and is backwashed every 15 days using 125,000 l ambient pool water. The mean number of bathers between each backwash was 3,026. All filter backwash samples used were tested for total and faecal coliforms using the multiple tube fermentation technique (APHA 1995).

Optimization of distilled water and swimming pool filter backwash water concentration procedures

Calcium carbonate flocculation was used for water samples concentration (Vesey *et al*. 1993). One litre and 10 l of distilled water samples were seeded, in triplicate, with 350.6 ± 11.9 *Cryptosporidium* oocysts in glass containers (Pyrex Schott bottles), and plastic containers, respectively. Method recoveries were determined by seeding oocysts at three different points in the concentration procedure (Fig. 1): point 1 – before $CaCO_3$ flocculation (water sample); point 2 – before centrifugation (after $CaCO_3$ dissolution in sulphamic acid) and point 3 – before immunomagnetic separation (the pellets resuspended in Bead Buffer™). Calcium carbonate flocculation technique utilizes $CaCl_2$ (1 M) and $NaHCO_3$ (1 M) to $CaCO_3$ flocculation, optimization studies were conducted using three different final concentrations (1, 2 and 3%) of $CaCl_2$ (1 M) and $NaHCO_3$ (1 M) in 1 l distilled water to establish whether that would improve recoveries. The pH was adjusted to 10 with 1 N NaOH and the precipitates were allowed to settle overnight at room temperature. The supernatant was carefully aspirated and discarded and the precipitates dissolved in 10% (w/v) sulphamic acid. Oocysts were further concentrated by centrifugation in a 50 ml tube at $5{,}000 \times g$ for 5 min in a swinging bucket centrifuge. The packed pellet volumes were measured and the pellets resuspended in 50 ml phosphate buffered saline (PBS 0.01 M, pH 7.2), centrifuged and the supernatant discarded. The neutralized pellets were resuspended in either 6 or 50 ml Bead Buffer™ (BTF, Inc.) prior to IMS. Subsequently, triplicate one-litre distilled water and swimming pool filter backwash samples were each seeded with ColorSeed CG™ and the same process performed. Three additional one-litre swimming pool filter backwash samples were pre-treated by addition of Tween20™ to a final concentration of 0.01% (v/v) before flocculation. Backwash samples were vigorously mixed by hand (inversion and shaking at least 25 times in 1.5 l Pyrex Schott bottles) after Tween20™ addition and allowed to stand at room temperature for 30 min. The supernatants were carefully aspirated, the debris discarded and flocculation performed on the supernatant.

Immunomagnetic separation

Biotechnology Frontiers Inc. (Sydney, Australia) Immunomagnetic separation (IMS) products were used to separate *Giardia* cysts and *Cryptosporidium* oocysts from detrital material after flocculation. The IMS was performed in three stages that included a pre-clear step to remove

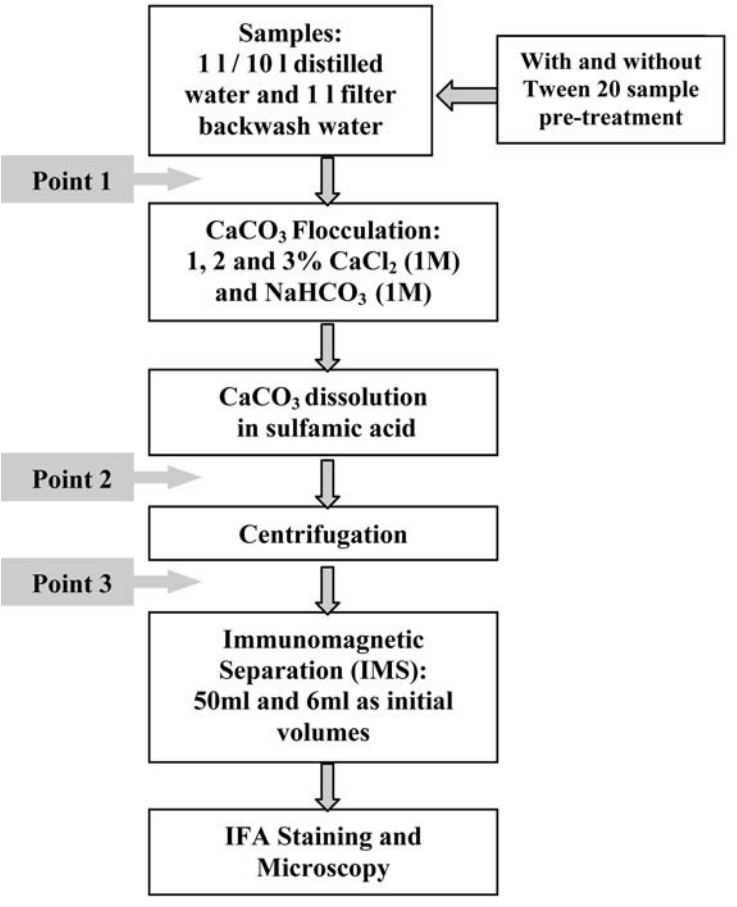

Fig 1. Flow diagram of the cysts and oocysts concentration process from water samples, showing three different control points where cysts and oocysts were seeded.

magnetic debris contained within the water sample; *Giardia* and *Cryptosporidium* capture from the sample; and beads-cysts and oocysts complexes dissociation.

Briefly, the packed pellets obtained from the flocculation procedure were neutralized in 50 ml PBS, centrifuged and the packed pellets were resuspended in two different volumes of Bead Buffer™ (BTF Inc.) in order to discover whether or not the volume could affect the recoveries (6 ml polycarbonate conical tubes or 50 ml polycarbonate conical-bottomed Falcon™ centrifuge tubes). To each tube 200 μl of IMS Beads ™ (BTF Inc.) were added in order to remove magnetic debris (pre-clear step). Samples were mixed in an orbital rotator at room temperature for 30 min. Tubes were then placed in a magnetic-capture holder for 5 min with the edge of the magnet (not the face) flush against the side of the tube. The supernatants were carefully aspirated to clean tubes and the pellets were washed with Bead Buffer™ (BTF Inc.), and these washings transferred to the same supernatant tubes. To each supernatant tube 200 μl of IMS Beads™ (BTF Inc.) coupled with anti *Cryptosporidium* monoclonal antibody (mAb CRY104) and 200 μl of IMS Beads™ (BTF Inc.) coupled with anti-*Giardia* monoclonal antibody (mAb G203) were added to capture cysts and oocysts from the sample. Samples were

mixed in an orbital rotator at room temperature for 30 min. Tubes were then placed in a magnetic-capture holder for 5 min, and then rotated. The supernatants were carefully aspirated and discarded. The tubes were then removed from the magnetic holder and 1 ml of 0.1 M glycine (pH 2.2) was added and homogenized by vortex for 1 min and allowed to stand undisturbed for 5 min to dissociate bead-cyst and oocyst complexes. Tubes were placed in a magnetic-capture holder for 5 min and the supernatants were filtered through 13-mm-diameter, polycarbonate, 0.8 μm membrane filters (Nuclepore^{TM}, USA) on a plastic Swinnex^{TM} filter holder. This process was repeated twice. The membranes were then washed twice with 500 ml Mab Buffer^{TM}.

IFA staining and microscopy

Fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-*Cryptosporidium* and anti-*Giardia* mAbs (CRY104 and G203, respectively, 80–100 μl at 10 μg/ml each, BTF Inc.) in MabBuffer^{TM} (BTF Inc.) were added to each membrane and incubated for 5 min. The membranes were then washed three times with 500 μl Mab Buffer^{TM} (BTF Inc.). The membranes were placed on a glass microscope slide and 10 μl mounting medium (40 : 50 : 10 : 5, 150 mM PBS:glycerol : formalin : 5 M NaCl, 2% DABCO [Sigma]) was then added to the top of the membrane. A 22 × 22 mm cover slip was then applied and the edges sealed with nail polish and the slides were subsequently read.

An Olympus BX-FLA40 epifluorescence microscope equipped with a blue filter block (excitation 450–480 nm; emission 515 nm) was used for detection of FITC-labelled oocysts and cysts at 200 × magnification, and in order to confirm morphology and size, individual cysts/oocysts were examined at 400 × . A green filter block (excitation 545–580 nm; emission 619 nm) was used for examination and detection of red-labelled ColorSeed CG^{TM} at 400 × magnification. Cyst and oocyst % recoveries were calculated as follows:

$$\%\ \text{ColorSeed C\&G}^{TM}\ \text{recovery} = \frac{\text{oocysts} - \text{cysts red labelled detected} \times 100}{\text{ColorSeed C\&G}^{TM}\ \text{seed dose}}$$

The number of cysts and oocysts present in each sample was calculated as follows:

$$\text{Number of cysts/oocysts in sample} = \frac{\text{cysts/oocysts FITC-labelled only}}{\%\ \text{ColorSeed C\&G}^{TM}\ \text{recovery}}$$

Results

Optimization of distilled water concentration procedure

Oocyst recoveries are shown in Table 1. Recovery of oocysts seeded at points 1, 2 and 3 by using 10-litre samples were 0.7 ± 0.2; 9.2 ± 1.9 and 48.3 ± 6.4%, respectively, and by using 1-litre samples were 20.7 ± 3.1; 36 ± 1.3 and 53 ± 2.6, respectively. These results indicated that looses occurred at point 1 (during the flocculation process) rather than points 2 and 3.

The possibility of increasing recovery efficiency was investigated using different concentrations of $NaHCO_3$ (1 M) and $CaCl_2$ (1 M) for $CaCO_3$ flocculation. Our results showed that $NaHCO_3$ and $CaCl_2$ concentrations above 1% increased the amount of precipitate decreasing the number of oocysts recovered. Recovery of oocysts seeded at point 1 using 1, 2 and 3% of $NaHCO_3$ and $CaCl_2$ were 20.3 ± 3.1; 10.7 ± 2.5 and 5.7 ± 1.5%, respectively, by using 1 litre samples (Table 2).

Initial recovery results were obtained using *Cryptosporidium* oocysts concentrate (350.6 ± 11.9) counted in a hemacytometer chamber as the seed dose. Subsequently, ColorSeed

Table 1. *Cryptosporidium* oocyst recoveries from l and 10 l distilled water sample, after seeding at three different points, by using 1% of $NaHCO_3$ (1 M) and $CaCl_2$ (1 M) method 350.6 ± 11.9 oocysts seed see Fig. 1

Seeding at process points	*Initial volume (10 l) Mean % recovery ± SD (n = 3)*	*Initial volume (1 l) Mean % recovery ± SD (n = 3)*
Point 1 – Initial water sample	0.7 ± 0.2	20.3 ± 3.1
Point 2 – before centrifugation	9.2 ± 1.9	36.0 ± 1.3
Point 3 – Before IMS	48.3 ± 6.4	53.0 ± 2.6

Table 2. Comparison of *Cryptosporidium* oocyst recoveries from 1 l distilled water samples by using different concentrations of $NaHCO_3$ (1 M) and $CaCl_2$ (1 M) for $CaCO_3$ flocculation and when 350.6 ± 11.9 oocysts were seeded

$NaHCO_3$ and $CaCl_2$ concentrations # (%)	*Mean % oocyst recovery (n = 3)*
1	20.3 ± 3.1
2	10.7 ± 2.5
3	5.7 ± 1.5

#Percentages represent final water sample concentrations.

C&G™ containing 100 ± 2.5 cysts/oocysts was used for seeding distilled water samples. Recoveries of ColorSeed CG™ oocysts and cysts seeded at point 1 using 1% of $NaHCO_3$ and $CaCl_2$ were respectively 20.7 ± 0.6 and 21.7 ± 2.5%; at point 2 were 44.3 ± 1.2 and 45.7 ± 1.5%; and at point 3 were 51.3 ± 2.1 and 49.0 ± 1.0%. These results are shown in Table 3.

The use of swimming pool filter backwash water allowed the utilization of 1-litre rather than 10-litre samples since the sand filtration itself performed a pre-concentration of the sample. The same technique used for distilled water concentration resulted in recoveries of 2.7 ± 1.2% for oocysts and 3.3 ± 1.5% for cysts when filter backwash water samples were used. The inclusion of a Tween20™ sample prewashing step and the utilization of a greater IMS volume (50 ml rather than 6 ml) resulted in recoveries of 23 ± 4.4% for oocysts, and 18.7 ± 2.1 for cysts when samples were seeded at point 1 (Table 4).

Recovery results using ColorSeed CG™ as internal control for filter backwash water samples obtained between December 2000 and August 2001 (Table 5) showed a great homogeneity (mean % of oocyst recovery = 21.7 ± 5.1, CV = 23%; mean % cyst recovery = 21.0 ± 3.5, CV = 17%). Cysts and oocysts were detected in backwash samples in December 2000 (two oocysts and three cysts per litre), and February 2001 (4.6 oocysts and 11 cysts per litre). These data were corrected using ColorSeed C&G™ recovery percentages as previous described in Materials and methods. In March 2001 the filtration medium was changed and no cysts and oocysts were found in subsequent analysis performed monthly.

All backwash water samples showed < 1 MPN total and fecal coliform/100 ml during the period of these experiments (between December 2000 and August 2001).

Table 3. *Cryptosporidium* oocyst and *Giardia* cyst recoveries from 1 l distilled water samples by using ColorSeed C&G^{TM} as an internal standard, after seeding at three points and when 1% of $NaHCO_3$ (1 M) and $CaCl_2$ (1 M) were used for $CaCO_3$ flocculation

Seeding at process points	*Mean % oocyst recovery* (n = 3)	*Mean % cyst recovery* (n = 3)
Point 1 – Initial water sample	20.7 ± 0.6	21.7 ± 2.5
Point 2 – Before centrifugation	44.3 ± 1.2	45.7 ± 1.5
Point 3 – Before IMS	51.3 ± 2.1	49.0 ± 1.0

Table 4. ColorSeed C&G^{TM} recoveries from treated and untreated swimming pool filter backwash water samples (with Tween 20 – prewashing step) by using two different IMS initial volumes

Backwash treatment#	*IMS volume (ml)*	*Packed pellet (ml)*	*Mean %* Cryptosporidium *spp. oocyst recovery* (n = 3)	*Mean %* Giardia *spp. cyst recovery* (n = 3)
(−)	6	3.0	0	0
(+)	6	0.5	6.0 ± 3.6	7.3 ± 0.6
(−)	50	3.3	2.7 ± 1.2	3.3 ± 1.5
(+)	50	0.6	23.0 ± 4.4	18.7 ± 2.1

#0.01 Tween 20^{TM} (final concentration).

Table 5. ColorSeed C&G^{TM} recoveries from backwash water samples collected from a brazilian olympic swimming pool located at UFSC* between December 2000 and August 2001 by using IMS

Period	*Packed pellet (ml)***	*Mean %* Cryptosporidium *spp. oocyst recovery* (n = 3)	*Mean %* Giardia *spp. cyst recovery* (n = 3)
December 2000	0.7 ± 0.2	23.0 ± 2.5	18.7 ± 1.2
January 2001	(−)	2.7 ± 1.5	2.3 ± 0.9
February 2001	0.4 ± 0.1	11.3 ± 3.4	14.3 ± 1.9
April 2001	0.4 ± 0.1	24.0 ± 1.0	25.0 ± 1.2
May 2001	0.7 ± 0.2	20.0 ± 1.5	21.7 ± 4.1
June 2001	0.5 ± 0.0	27.3 ± 2.2	22.3 ± 2.4
July 2001	0.5 ± 0.0	23.7 ± 1.8	20.7 ± 0.9
August 2001	0.7 ± 0.2	22.3 ± 2.4	24.3 ± 2.4

*UFSC = Universidade Federal de Santa Catarina, Florianópolis, SC.**Packed pellet obtained after precipitate dissolution with sulphamic acid and centrifugation.(−) It was not possible to measure the packed pellet.

Discussion

The calcium carbonate flocculation method developed by Vesey *et al.* (1993) and further evaluated by Shepherd and Wyn-Jones (1996), Skerrett and Holland (2000) and other authors has yielded recovery efficiencies greater than 50% when seeding with high levels (1,000) of

Cryptosporidium oocysts. The aim of this study was to evaluate this procedure coupled with the immunomagnetic separation technique and direct immunofluorescence for detection of *Giardia* and *Cryptosporidium* in swimming pool filter backwash water. Low seed doses of *Cryptosporidium* oocysts and *Giardia* cysts were used.

In initial trials, 350.6 ± 11.9 oocysts were seeded in 10 l of distilled water and this resulted in low oocyst recovery (0.67% ± 0.17%) (Table 1). These recovery data were significantly lower than those reported by previous investigators (Vesey *et al*. 1993; Shepherd and Wyn-Jones 1996; Skerrett and Holland 2000). This can be explained by a possible loss of oocysts, which are in low concentration, during repeated centrifugation in 50 ml tubes. After the flocculation procedure, several centrifugation steps were necessary in order to concentrate organisms recovered from the calcium carbonate precipitate dissolved in sulphamic acid. Optimization studies were conducted with a 1 l water sample as the initial volume and different concentrations of $NaHCO_3$ and $CaCl_2$ for $CaCO_3$ flocculation.

Therefore, using 1 l samples, *Cryptosporidium* oocyst recovery increased from 0.7% ± 0.2 to 20.3 ± 3.1% (Table 1). Increases in $NaHCO_3$ 1 M and $CaCl_2$ 1 M concentrations increased the amount of $CaCO_3$ residue, without an increase in the number of oocysts sedimented, which resulted in the disadvantage of a volume increase during the centrifugation process. Results for $NaHCO_3$ and $CaCl_2$ concentration changes in the $CaCO_3$ flocculation procedure were similar to those reported by Vesey *et al*. (1993). Comparison of the ColorSeed C&G™ recoveries with those for the isolated *Cryptosporidium* oocysts used as a control, showed no significant difference for oocyst recovery when seeded at point 1 : 20.7% ± 0.6% and 20.3% ± 3.1%, respectively. However, ColorSeed C&G™ is red-labelled and can easily be used as an internal control. When cysts and oocysts were added directly to the sample concentrate and immediately processed by IMS (Point 3, Table 3), a mean recovery of 51.3% ± 2.1 ($n = 3$) was obtained. These results were similar to those obtained by Di Giovanni *et al*. (1999) who suggested that during the concentration procedure, the oocysts and cysts become associated with debris, a situation that hampers cyst and oocyst recovery. The recoveries obtained when the cysts and oocysts were seeded during the flocculation procedure (20.7% ± 0.6% oocyst and 21.7% ± 2.5% cyst recoveries) were significantly smaller than those obtained during the centrifugation procedure (44.3% ± 1.2% oocyst and 45.7 ± 1.5 cyst recoveries) and the IMS procedure (51.3% ± 2.1% oocyst and 49% ± 1% cyst recoveries).

Previous studies have shown that *Cryptosporidium* oocysts and *Giardia* cysts can be detected in treatment plant filter backwash water (Karanis *et al*. 1996, 1998; Di Giovanni *et al*. 1999). In this study, 1 l of swimming pool filter backwash water was used and processed using the same technique used to evaluated distilled water. Our recoveries (2.7 ± 1.2% for oocysts and 3.3 ± 1.5% for cysts – Table 4) were significantly lower than those reported by Di Giovanni *et al*. (1999) who obtained 25.4% oocyst recovery from treatment plant filter backwash water. The great debris volume present in samples hindered the immunomagnetic separation (IMS) procedure. Cysts and oocysts may become associated with debris hindering the specific binding of IMS beads to the cysts and oocysts. Increasing the initial sample volume for the IMS procedure coupled with a sample prewash with Tween20™ 0.01% (v/v) before the calcium carbonate flocculation process resulted in a significant increase in the cyst and oocyst recovery (from 2.7 ± 1.2% for cysts and 3.3 ± 1.5% for oocysts to 18.7 ± 2.1% and 23.4 ± 4.4%, respectively – Table 4). Karanis and Kimura (2002) compared calcium carbonate ($CaCO_3$), ferric sulphate ($FeSO_4$) and aluminum sulphate ($AlSO_4$) for the recovery of oocysts. The recovery data were 38.8%, 61.5% and 58.1%, respectively. The method for filter backwashes may be further optimized in the future by utilizing $FeSO_4$ or $AlSO_4$.

In December 2000 and February 2001 *Giardia* spp. and *Cryptosporidium* spp. were detected in the filter backwash. In contrast to this, no cysts and oocysts were found after the sand filter change in March 2001. However, this contamination may not have occurred during these specific months, since cysts and oocysts can be stable for long periods in the sand filter. Logan *et al*. (2001) demonstrated that sand filtration systems can be very effective in removing oocysts from water and wastewater even at very high oocyst influent concentrations. The fact that oocysts were entering the swimming pool emphasizes the role that effective water treatment plays in preventing illness in users. Cysts and oocysts that potentially pass by the filter could be inactivated by ozone (Corona-Vasquez *et al*. 2002) or ultraviolet (Abbaszadegan *et al*. 1997; Quian *et al*. 2004) application. Additional attention must be given to a greater control of the swimming pool users due to the presence of these parasites.

Conclusion

The combination of precipitation techniques with $CaCO_3$, centrifugation, immunomagnetic separation and direct immunofluorescence was shown to be an effective tool for the detection and quantification of *Giardia* spp. and *Cryptosporidium* spp. in swimming pool filter backwash water.

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