Concentrating *Toxoplasma gondii* and *Cyclospora cayetanensis* from surface water and drinking water by continuous separation channel centrifugation

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**Keywords**

**Abstract**

**Aims:** To evaluate the effectiveness of continuous separation channel centrifugation for concentrating *Toxoplasma gondii* and *Cyclospora cayetanensis* from drinking water and environmental waters.

**Methods and Results:** Ready-to-seed vials with known quantities of *T. gondii* and *C. cayetanensis* oocysts were prepared by flow cytometry. Oocysts were seeded at densities ranging from 1 to 1000 oocysts l⁻¹ into 10 to 100 l test volumes of finished drinking water, water with manipulated turbidity, and the source waters from nine drinking water utilities. Oocysts were recovered using continuous separation channel centrifugation and counted on membrane filters using epifluorescent microscopy. Recovery efficiencies of both parasites were ≥84% in 10 l volumes of drinking water. In source waters, recoveries ranged from 64% to 100%, with the lowest recoveries in the most turbid waters. Method precision was between 10% and 20% coefficient of variation.

**Conclusion:** *Toxoplasma gondii* and *C. cayetanensis* are effectively concentrated from various water matrices by continuous separation channel centrifugation.

**Significance and Impact of the Study:** Waterborne transmission of *T. gondii* and *C. cayetanensis* presents another challenge in producing clean drinking water and protecting public health. Detection of these parasites relies on effectively concentrating oocysts from ambient water, otherwise false negatives may result. Validation data specific to *T. gondii* and *C. cayetanensis* concentration methods are limited. Continuous separation channel centrifugation recovers oocysts with high efficiency and precision, the method attributes required to accurately assess the risk of waterborne transmission.

**Introduction**

*Toxoplasma gondii* and *Cyclospora cayetanensis* are two coccidian protozoan parasites that are increasingly recognized as having potential for waterborne transmission to humans. The environmental stage for both organisms is an oocyst, shed in the faeces of the infected host and capable of surviving in the environment for months or longer, more than enough time to be transported into a drinking water supply. The infectious dose is believed to be low for both parasites, suggesting that despite massive dilution in water there is still the possibility for people to be exposed and become infected. Similar to another coccidian, *Cryptosporidium* spp., the oocysts of *T. gondii* and *C. cayetanensis* are resistant to disinfection by chlorination. All these life history traits are favourable for waterborne transmission, presenting the drinking water profession with yet another set of emerging pathogens with which to contend.
Toxoplasma gondii has a ubiquitous host range, parasitizing marsupials, birds and mammals, but only species of the cat family (Felidae) are the definitive hosts in which it can complete the sexual stage of its lifecycle and be released into the environment as oocysts. Cats shed oocysts for 1–2 weeks at high concentrations, up to 100,000 oocysts g⁻¹ faeces (Wilson et al. 2003). Oocysts have a spherical to subspherical shape, 10 μm × 12 μm in diameter, and they are remarkably resistant to disinfectants (Dubey 2004; Wainwright et al. 2007). Treatment with 6% sodium hypochlorite for 24 h did not inactivate T. gondii oocysts (Dubey et al. 1970). Once released, oocysts require several days to sporulate and become infective, after which they can survive for months to years under moist conditions (Yilmaz and Hopkins 1972; Frenkel et al. 1975). Transmission to humans occurs by several routes, including consumption of undercooked meat containing the tissue stages, organ transplantation, congenital infection resulting from an acute primary infection to the mother during gestation, and ingestion of oocyst-contaminated food or water. This latter route is believed to result in the most severe infections in healthy adults (Dubey 2004). The most recent seroprevalence study in the United States showed the overall exposure rate to T. gondii in the age group 12–49 years was 15.8% (Jones et al. 2003). In some countries, seroprevalence may be as high as 85%, with higher prevalence in warmer regions (Wilson et al. 2003). Most new adult infections are asymptomatic; approximately 10% result in self-limiting flu-like symptoms or, especially among immunocompromised individuals, severe disease may result such as retinitis, myocarditis and encephalitis (Montoya and Liesenfeld 2004). Congenital infection is likely to result in visual or neural impairment, possibly even hydrocephaly or the death of the fetus (Dubey 2004).

Cyclospora cayetanensis is an emerging pathogen whose lifecycle, host range, and environmental reservoirs are not very well understood. The organism has not been unequivocally identified in any host but human (Eberhard and Arrowood 2002). Oocysts are spherical, 8–10 μm in diameter, shed in low numbers during an infection compared to other coccidians, and based on limited data appear to be highly resistant to chlorination (Herwaldt 2000). The infectious dose is believed to be low (Sterling and Ortega 1999). So far, initiating infections in controlled trials in humans has been unsuccessful for unknown reasons (Alfano-Sobsey et al. 2004). In the United States and Canada there have been a large number of well-documented Cyclospora outbreaks, related primarily to the consumption of imported raspberries and fresh green produce (Herwaldt 2000; Hoang et al. 2005). In contrast, the rate of endemic infection in North America and Europe appears to be low as several studies have shown fewer than 0.5% of stool specimens are positive for Cyclospora oocysts (Herwaldt 2000; Ribes et al. 2004). High rates of endemic infections have been observed in Guatemala, Peru, and Nepal (Sterling and Ortega 1999), and Cyclospora oocysts have been detected in the wastewater from a primary oxidation pond in Lima, Peru, illustrating the potential for transmission via faecal contamination (Sturbaum et al. 1998). Symptoms of cyclosporiasis include prolonged watery diarrhoea, vomiting, abdominal cramping, fatigue, and significant weight loss, and the illness is particularly severe for immunocompromised individuals.

Both T. gondii and C. cayetanensis have been responsible for significant waterborne outbreaks. In 1995, 100 cases of acute toxoplasmosis were identified in residents of the Greater Victoria area of British Columbia, Canada who drank water from an area of the distribution system served by one reservoir (Bowie et al. 1997). Water treatment did not include filtration and disinfection was by chloramination. Oocyst contamination was suspected from domestic and feral cats and cougars living in the watershed. Waterborne T. gondii outbreaks have also been reported in Panama (Benenson et al. 1982), and viable T. gondii was demonstrated in water samples linked to an outbreak in Brazil (de Moura et al. 2006). Moreover, endemic toxoplasmosis in a large Brazilian city has been linked with drinking water from the municipal treatment plants when the water was not further filtered at home (Bahia-Oliveira et al. 2003).

The first reported waterborne outbreak of C. cayetanensis was at a Chicago hospital in 1990 (Huang et al. 1995). Consumption of tap water in the hospital dormitory was significantly associated with illness; water was gravity fed from two water storage tanks covered only in canvas and open to the environment, although a foodborne route for the outbreak has not been excluded (Herwaldt 2000). Cyclospora cayetanensis infections have been associated with drinking water in Nepal (Hoge et al. 1993; Rabold et al. 1994), Haiti (Lopez et al. 2003), and Egypt (el-Karmany et al. 2005). Amplifying and sequencing the 18S-rDNA gene, Dowd et al. (2003) unequivocally identified C. cayetanensis in several water sources supplying drinking water to the rural region surrounding the city of Guatemala, providing evidence, ipso facto, that waterborne transmission is possible.

Like many waterborne pathogens, T. gondii and C. cayetanensis may be present in water in very dilute concentrations, making it difficult to quantify accurately the number of oocysts. Such data are necessary for understanding the occurrence, distribution, and fate of oocysts in the environment, as well as being able to estimate the level of disease risk and evaluate the effectiveness of water treatment. The standard approach is to sample as large a
water volume as physically possible. Typically this is accomplished with a filter, although after sampling it can be a challenge to separate the oocysts from the filter media before the next analytical step in the detection process. Continuous separation channel centrifugation has been shown to be very effective for recovering Cryptosporidium spp., Giardia spp. and microsporidia from water samples (Borchardt and Spencer 2002; Hoffman et al. 2007; Assavasilavasukul et al. 2008). The objective of the present study was to evaluate the effectiveness of continuous separation channel centrifugation for recovering T. gondii and C. cayetanensis oocysts from surface water and finished drinking water.

Materials and methods

Centrifuge

All experiments were performed with an Amicus blood cell separator (Baxter Healthcare Corp., Round Lake, IL, USA) that was modified by the manufacturer to operate as a simple continuous centrifuge. The principle of continuous separation channel centrifugation has been described previously (Borchardt and Spencer 2002). In brief, the water to be concentrated is pumped into a flexible channel attached to a centrifuge rotor. To exit the channel the water must traverse its entire length while particulates within the water are subject to centrifugal force. The force is directed perpendicular and outwards relative to the direction of water flow so that the target micro-organisms and other particulates are retained against the outer channel wall. The Amicus separation channel is a sterile, disposable, flexible bag with two independent chambers partitioned into a serpentine flow path (Fig. 1). Two water samples can be concentrated simultaneously. There is no filter involved in the process.

Centrifugation procedure

Before centrifuging a water sample, the tubing and separation channel were primed with sterile 0.05% Tween 80 (Sigma, St Louis, MO, USA). Since the Amicus bag has two independent separation channels, usually two water samples were concentrated in a single centrifugation run. Water was pulled through the separation channels using two peristaltic pumps built into the centrifuge; the flow rate was 150 ml min⁻¹. Supernatant left the chambers via output lines and discarded. Rotor speed was set at 1640 rev min⁻¹, which generated a relative centrifugal force of approx. 900 g.

When centrifugation was finished, the channel bags were removed from the rotor and the input and output lines were clamped. The contents were vigorously massaged and the concentrate drained into a sterile beaker through the output line. The bags were rinsed three times with 20–30 ml of sterile 0.01% Tween 80 through the input line with a syringe. The tubing was clamped and the bag was massaged vigorously for 1 min and the contents drained into the beaker. The final volume was 120–135 ml. The channel bags are used only once so there is no possibility of pathogen carryover.

Preparation of oocysts for seeding

Toxoplasma gondii oocysts were purified from the faeces of infected cats reared in a laboratory colony. Oocysts were purified by sedimentation and flotation on sucrose solution, sporulated by vigorous aeration, and stored in 2% H₂SO₄ at 4°C (Speer et al. 1998). C. cayetanensis oocysts were purified from human faeces that had been preserved with 2.5% K₂Cr₂O₇. The oocysts were purified by continuous-flow sucrose flotation (Vetterling 1969) followed by 1 g Percoll velocity sedimentation (Sauch 1984). Oocysts were stored in 0.01% Tween 20 at room temperature in the dark.

Flow cytometry was used to prepare ready-to-seed vials with known quantities of Toxoplasma and Cyclospora oocysts. Oocysts of T. gondii were inactivated by heating to 80°C for 10 min prior to sorting. The oocysts were sorted with a FACS VantageSE (Becton Dickinson, Palo Alto, CA, USA) equipped with CloneCyt sorting software. Isoton II was the sheath fluid (Coulter Corp., Hialeah, FL, USA). A primary gate was drawn around...
the oocysts using forward scatter (FSC) and side scatter (SSC). Secondary gating was by FSC and fluorescence channel 5 (FL5) (illumination 351–364 nm, band pass filter 424/44 nm), detecting the characteristic light blue autofluorescence of Toxoplasma and Cyclospora oocysts illuminated by UV light. For T. gondii the settings were FSC and SSC in linear mode, FSC threshold at 98 V, SSC at 345 V, and FL5 in log mode at 599 V. For C. cayetanensis the settings were FSC and SSC in linear mode, FSC threshold at 435 V, SSC at 367 V, and FL5 in log mode at 635 V. Oocysts were sorted, using the secondary gate, into siliconized 1·5 ml flip top vials containing 1 ml 2% H2SO4 (T. gondii) or 1 ml reagent water (C. cayetanensis). Flow cytometry counts were confirmed by direct microscopic counts of several vials from the batch of vials prepared on the same date. Only sporulated T. gondii oocysts were sorted, however, sorted C. cayetanensis oocysts were a mix of sporulated and unsporulated.

All seed quantities were prepared at the flow cytometry facility of the National Exposure Research Laboratory, U.S. Environmental Protection Agency, and shipped overnight to Marshfield Clinic Research Foundation for the concentration and recovery experiments. Shipments were timed to arrive just prior to a scheduled set of recovery experiments. The oocysts were stored at 4°C until seeded.

Minutes before seeding, oocysts were formalin-fixed (10% final concentration) in the vial to protect the operator from infection during the experiment. The entire vial contents were then poured into the test water and rinsed four times with 1 ml 0·05% Tween 80 and one rinse with the test water. For most of the recovery experiments, Toxoplasma and Cyclospora were seeded into separate test volumes and centrifuged at different times, meaning the reported recovery efficiencies for the two organisms are independent. In one set of experiments, recovery from utility source waters, Toxoplasma and Cyclospora oocysts were combined and seeded into a single volume of test water.

Oocyst enumeration after concentration

Centrifuge concentrates were filtered through a 3·0 µm absolute porosity, blackened, polycarbonate 25 mm filter (Poretics Corp., Livermore, CA, USA). When tap water sources were centrifuged, the entire concentrate could be passed through one filter and all recovered oocysts enumerated. However, concentrates from turbid water sources had to be filtered in four to 10 aliquots of 0·5–30 ml volumes. The fraction of concentrate analysed from turbid water sources ranged from 4% to 100%.

The percentage recovery was calculated using eqn. (1):

\[ R = \frac{100(C/F)}{V} \]  

where \( R \) is the percentage recovery, \( C \) the number oocysts counted in centrifuge concentrate, \( F \) the fraction of concentrate analysed and \( V \) is the number oocysts in FACS sorted vial. All reported means are arithmetic.
Results

Recovery of oocysts from tap water

Toxoplasma gondii and Cyclospora cayetanensis oocysts were recovered from 10 l volumes of municipal tap water at a flow rate of 150 ml min\(^{-1}\) and seeded at densities of 1, 10, and 100 oocysts l\(^{-1}\). Both parasites were recovered with high efficiency regardless of oocyst density (Table 1). Among ten independent recovery experiments at each oocyst density, the coefficient of variation was ≤13.2\% for T. gondii and ≤13.0\% for C. cayetanensis.

Recovery of oocysts from tap water with manipulated turbidity

Both Toxoplasma and Cyclospora oocysts were recovered at lower efficiencies when the water had a turbidity of 20 NTU compared to water with a turbidity of 2 NTU (Table 2). At 20 NTU the Tennessee River sediment in the centrifuge concentrate was observed to mask some of the oocysts when filtered, likely reducing the overall oocyst count. Despite lower oocyst recovery at the higher turbidity level, method precision was similar to that measured in tap water, 17.1\% coefficient of variation for T. gondii and 13.3\% for C. cayetanensis.

Recovery of oocysts from large volumes of tap water

Toxoplasma and Cyclospora oocysts were each seeded five times independently at a final concentration of 10 oocysts l\(^{-1}\) into 100 l of finished tap water. Recoveries, although lower than with oocysts seeded into 10 l, were still greater than 60\% (Table 3).

Blinded recovery of oocysts

On several occasions during the study, the analyst performing the recovery experiments was blinded to the type (Toxoplasma or Cyclospora) and seeding density of oocysts. In the first set of such experiments, Toxoplasma oocysts were seeded into two 10 l volumes of tap water. The analyst correctly identified the oocysts as Toxoplasma and, in the first 10 l volume, counted 964 oocysts of 1000 seeded (96.4\% recovery). In the second 10 l volume 100 oocysts were seeded and the analyst recovered 95 (95\% recovery).

Table 1 Recovery of T. gondii and C. cayetanensis in 10 l of tap water using continuous separation channel centrifugation at a flow rate of 150 ml min\(^{-1}\)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of independent trials</th>
<th>Mean recovery (%)</th>
<th>Standard deviation (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. gondii</td>
<td>1</td>
<td>93.0</td>
<td>12.3</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>98.1</td>
<td>6.7</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.5</td>
<td>4.1</td>
<td>4.2</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>1</td>
<td>89.1</td>
<td>11.6</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>93.4</td>
<td>8.2</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>84.2</td>
<td>7.6</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Table 2 Recovery of T. gondii and C. cayetanensis in 10 l of tap water with manipulated turbidity using continuous separation channel centrifugation at a flow rate of 150 ml min\(^{-1}\)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of independent trials</th>
<th>Mean recovery (%)</th>
<th>Standard deviation (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. gondii</td>
<td>10</td>
<td>91.8</td>
<td>8.4</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>58.8</td>
<td>10.0</td>
<td>17.1</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>10</td>
<td>91.9</td>
<td>12.4</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>47.2</td>
<td>6.3</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 3 Recovery of T. gondii and C. cayetanensis in 100 l of tap water using continuous separation channel centrifugation at a flow rate of 150 ml min\(^{-1}\)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of independent trials</th>
<th>Mean recovery (%)</th>
<th>Standard deviation (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. gondii</td>
<td>10</td>
<td>72.8</td>
<td>14.6</td>
<td>20.1</td>
</tr>
<tr>
<td>C. cayetanensis</td>
<td>10</td>
<td>62.9</td>
<td>19.8</td>
<td>31.4</td>
</tr>
</tbody>
</table>
Toxoplasma was also seeded, blinded to the analyst, into two 10 l volumes of tap water with the turbidity increased to 20 NTU. In one volume the analyst counted 935 oocysts of 1000 seeded (93.5% recovery), and in the second volume he counted 86 oocysts of 100 seeded (86% recovery).

In the final set of experiments in which the analyst was blinded, oocysts were seeded into one 10 l volume of St. Claire River water (2.6 NTU), the source for the Algonac, MI, USA drinking water utility, and two 10 l volumes of source water entering the Marston treatment plant of Denver, CO, USA (0.6 NTU). Both source waters were negative for indigenous Toxoplasma and Cyclospora. For the St. Claire River water, 954 Toxoplasma oocysts were sorted by the flow cytometer and seeded and the analyst recovered 76%. Seeding one 10 l volume of Denver water simultaneously with Toxoplasma (40 oocysts) and Cyclospora (50 oocysts), the analyst recovered 48% and 20%, respectively. The second 10 l volume of Denver water was seeded, unknown to the analyst, with a blank containing no oocysts. The analyst reported zero Toxoplasma and Cyclospora recovered, indicating there were no false positives.

Recovery of oocysts from utility source waters
Source waters from nine municipal utilities were seeded simultaneously with 1000 Toxoplasma oocysts and 1000 Cyclospora oocysts into 10 l volumes (final concentration = 100 oocysts l⁻¹). Recovery of Toxoplasma oocysts ranged from 68.5% to 100%, with the lowest recovery from Mississippi River water, which had the highest turbidity, 33.6 NTU (Fig. 2). Cyclospora oocysts were recovered with efficiencies from 63.5% to 97%, the lowest recovery again from Mississippi River water. All source waters were negative for indigenous Toxoplasma and Cyclospora oocysts.

Discussion
This study has demonstrated that continuous separation channel centrifugation is an excellent method for recovering T. gondii and C. cayetanensis oocysts from water. In 100 l finished drinking water seeded with only 10 oocysts l⁻¹ of either parasite, the centrifuge was able to recover 60% or greater of the oocysts. Recovery efficiencies of T. gondii and C. cayetanensis from 10 l tap water volumes were 293% and 84%, respectively. Method precision, as measured by the coefficient of variation of 10 independent recovery experiments using the same water matrix, was between 10% and 20%. Seeding oocysts into naturally occurring surface waters from nine different lakes and rivers in the United States, recovery efficiencies for both parasites were greater than 60%. Recovery efficiencies were lower from the water sources with the highest turbidities, probably because particulates masked the oocysts on the membrane filters, preventing them from being counted. The standard deviation of the mean oocyst count in centrifuge concentrates from some surface water sources was large (Fig. 2), indicating it would be prudent to determine the number of counted aliquots necessary for a stable estimate of the mean before beginning routine testing.

Detecting waterborne pathogens can be considered a three-step process. Concentration of the large-volume water sample is the first step, followed with purification, if necessary, using such methods as immunomagnetic separation (Dumètre and Dardé 2007) or density gradient centrifugation. The final step is detection and a variety of widely accepted methods are available including microscopy, in vivo or in vitro culture, flow cytometry, and nucleic acid amplification. State-of-the-art nucleic acid
amplification methods have been published for detecting T. gondii and C. cayetanensis in water (Schwab and McDevitt 2003; Shields and Olson 2003; Kourenti and Karanis 2006; Lalonde and Gajadhar 2008; Sotiriadou and Karanis 2008). However, no matter how elegant, sensitive, and specific a detection method might be, if the target pathogen is not efficiently concentrated in the first step, all subsequent procedures may be for naught.

Despite the importance of this step, few studies have systematically evaluated methods for concentrating T. gondii from water, and as far as we are aware, no studies have evaluated methods for C. cayetanensis. Filtration has been used several times for investigating waterborne T. gondii and C. cayetanensis (Zuckerman and Tzipori 2006). EPA Method 1623 (U.S. Environmental Protection Agency 2005), which is approved for U.S. EPA Method 1623, was used (2004) for concentrating Cryptosporidium and Giardia (U.S. Environmental Protection Agency 2005), is thought to work for other protozoan pathogens. How well the filters worked in the aforementioned studies is unknown because recovery controls were not reported. Continuous flow centrifugation is also a recognized mechanism for water sample concentration of Giardia spp. and Cryptosporidium spp. cysts and oocysts (U.S. Environmental Protection Agency 2005). The Envirochek filter (Pall Corp., East Hills, NY, USA), which is approved for U.S. EPA Method 1623, was evaluated by Villena et al. (2004) for concentrating T. gondii oocysts. Recovery data are reported as the proportion of samples detected as oocyst positives by polymerase chain reaction (PCR) or mouse bioassay and thus are not directly comparable to the percentage recovery data reported in the present study. Detection by PCR was more sensitive than the mouse bioassay so the only the more favourable detection endpoint is discussed here. PCR inhibition was assessed and corrected as needed. Filtering 10 drinking water samples, each 100 l in volume and seeded with 10 oocysts l⁻¹, oocysts were detected by PCR in five samples. In 40 l volumes of raw source water seeded with 1000 oocysts l⁻¹, the detection rate after filtration and PCR was 50%, and at a seeding density of 100 oocysts l⁻¹, it was only 20%. This latter seeding density was the same as the one used for the nine source waters tested in the present study. A continuous flow centrifugation device, which uses a disposable bowl rather than a flexible channel, has also been approved for use in EPA Method 1623 (U.S. Environmental Protection Agency 2005) and the performance of this technique has been reported (Zuckerman and Tzipori 2006).

The other methods that have been used to concentrate T. gondii and C. cayetanensis in outbreak and field investigations are flocculation (el-Karamany et al. 2005) and bulk (i.e. non-continuous) centrifugation (Huang et al. 1995; de Moura et al. 2006). Kourenti et al. (2003) compared bulk centrifugation with flocculation by Al₂(SO₄)₃ or Fe₂(SO₄)₃ for recovering sporulated or unsporulated T. gondii oocysts. The water matrices were demineralized water or tap water and the test volumes were 50 ml or 1 l; the seeding densities into these volumes were 1 × 10⁴ or 1 × 10⁵ oocysts. The bulk centrifugation method required three separate centrifugation steps to reduce the 1 l samples to 1 ml. Recovery efficiencies for sporulated oocysts ranged from 40% to 83% with this method. Flocculation with Al₂(SO₄)₃ of 1 l with 1 × 10⁵ sporulated oocysts yielded the highest recovery, 97%, although with 1 × 10⁴ unsporulated oocysts recovery was lower, 30%. The authors also tested the effect of centrifugation on oocyst infectivity and found that at 2889 g, the highest g force tested, infectivity was not diminished as measured by mouse bioassay. The continuous separation channel centrifuge used in the present study generates a force of only 900 g, which had been shown previously not to affect Cryptosporidium excystation (Borchardt and Spencer 2002).

Besides high recovery efficiencies, continuous separation channel centrifugation has several other advantages. Because there are no filters, clogging is not an issue, and there are no losses of the target pathogen associated with the difficulty of disentangling them from the filter media. All micro-organisms that are bacteria size or larger are concentrated, and thus one concentrate can be used to detect multiple pathogens. The g-force of continuous separation channel centrifugation is too low to concentrate viruses. All dissolved substances pass through the centrifuge, but on the other hand, all particulates are retained, which may necessitate a second purification step such as immunomagnetic separation, depending on the turbidity of the water and the type of pathogen detection method employed after the centrifugation step. Continuous separation channel centrifugation is amenable to any sample volume, from a 10-l grab sample to tens of thousands of litres when the centrifuge is configured to sample continuously non-stop from a water body or pipe. The benefits of such a time-integrated sampling scheme using continuous centrifugation have been demonstrated in the production train of a pilot drinking water treatment plant (Assavasilavasukul et al. 2008). The primary limitation of the method is the availability of centrifuges. The machines used in this and previous studies (Borchardt and Spencer 2002; Hoffman et al. 2007; Assavasilavasukul et al. 2008) have been modified blood cell separators, which are expensive and not typically found in a microbiology laboratory. Data for the method’s proof-of-principle is considerable, but until more centrifuges...
are available, the full set of advantages the method has to offer will not be realized.

To the list of human pathogens transmitted by water are now added *T. gondii* and *C. cayetanensis*. Several outbreaks of each parasite have been attributable to water and it is conceivable there is some level of endemic toxoplasmosis and cyclosporiasis that is also waterborne. In April 2006 the U.S. EPA sponsored a workshop on large volume sampling for waterborne pathogens that highlighted the need for robust methods for concentrating pathogens from environmental waters and drinking water if we are to understand the disease risk presented by these organisms (U.S. Environmental Protection Agency 2008). Continuous separation channel centrifugation is one such promising method.

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