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Development and Application of Different Methods for the Detection of *Toxoplasma gondii* in Water

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Two methods, centrifugation and flocculation, were evaluated to determine their efficiencies of recovery of *Toxoplasma gondii* oocysts from contaminated water samples. Demineralized and tap water replicates were inoculated with high numbers of sporulated or unsporulated *T. gondii* oocysts (1×10^5 and 1×10^4 oocysts). The strain, age, and concentration of the seeded oocysts were recorded. Oocysts were recovered either by centrifugation of the contaminated samples at various *g* values or by flocculation with two coagulants, $Fe_2(SO_4)_3$ and $Al_2(SO_4)_3$. The recovery rates were determined with the final pellets by phase-contrast microscopy. Sporulated oocysts were recovered more effectively by flocculation with $Al_2(SO_4)_3$ (96.5% ± 21.7%) than by flocculation with $Fe_2(SO_4)_3$ (93.1% ± 8.1%) or by centrifugation at 2,073 × *g* (82.5% ± 6.8%). For the unsporulated oocysts, flocculation with $Fe_2(SO_4)_3$ was more successful (100.3% ± 26.9%) than flocculation with $Al_2(SO_4)_3$ (90.4% ± 19.1%) or centrifugation at 2,565 × *g* (97.2% ± 12.5%). The infectivity of the sporulated oocysts recovered by centrifugation was confirmed by seroconversion of all inoculated mice 77 days postinfection. These data suggest that sporulated *Toxoplasma* oocysts purified by methods commonly used for waterborne pathogens retain their infectivity after mechanical treatment and are able to induce infections in mammals. This is the first step in developing a systematic approach for the detection of *Toxoplasma* oocysts in water.

Infections by the apicomplexan parasite *Toxoplasma gondii* are prevalent in humans and animals throughout the world. This ubiquitous parasite induces more severe illness in the intermediate hosts (mice, pigs, humans) than in the definitive host (cats) (8). However, only the definitive host releases oocysts into the environment through contaminated feces. The excreted oocysts are unsporulated and not immediately infective. Formation of infective sporozoites within sporulated oocysts requires 1 to 5 more days and occurs in the outside environment. Thus, ingestion of food or water contaminated with oocysts from infected cat feces is a common route for postnatal transmission of *T. gondii* in humans.

Ingestion of tissue cysts by consumption of infected meat is another significant source of human infection. Several foodborne outbreaks of toxoplasmosis have been reported worldwide (4–6, 13–15). These outbreaks were attributed mainly to consumption of raw or inadequately cooked meat or to ingestion of raw goat's milk (4, 15). Tissue cysts of *T. gondii* are killed by cooking and freezing (7), whereas oocysts are more resistant to such treatments and can survive for months in adverse environments.

The first reported waterborne toxoplasmosis outbreak was among United States Army soldiers in Panama in 1979. An investigation implicated drinking water obtained from a stream presumably contaminated with oocysts excreted by jungle cats as the source of the organism (2). When the largest documented waterborne outbreak of acute toxoplasmosis occurred in British Columbia, Canada, new significant data were added to the epidemiology of oocyst-transmitted *T. gondii* infections (3). The British Columbia outbreak was the first outbreak to be linked to municipal drinking water. Fecal contamination of a water reservoir by infected animals (cougars and domestic and feral cats) following extensive rainfall and runoff into the unfiltered water supply was the most probable cause. The largest toxoplasmosis outbreak recently reported in Brazil has also been attributed to contamination of an unfiltered water reservoir by suburban cats according to preliminary estimates (16). The occurrence of waterborne toxoplasmosis outbreaks, along with the fact that *T. gondii* oocysts show great resistance to various environmental influences, makes this worldwide parasite a potential threat for the safety of public water supplies.

The aim of the present study was to evaluate two concentration methods (centrifugation and flocculation) for the recovery of sporulated and unsporulated *Toxoplasma* oocysts from contaminated water samples. In the first method, oocysts were concentrated by centrifugation of the samples at different *g* values. In the second method, oocysts were sedimented and concentrated with the aid of two flocculants. The second method is widely used by large water treatment plants worldwide during the first steps in surface water treatment. The infectivity of the sporulated oocysts recovered by centrifugation was shown by performing a bioassay. Our final target is development and application of a method able to detect the environmentally resistant *Toxoplasma* oocysts in water, which could act as valuable diagnostic tool for the safe management of public water supplies.

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MATERIALS AND METHODS

Source of oocysts. Two strains (DX and AHC1) of sporulated and unsporulated *T. gondii* oocysts (provided by the Institute for Parasitology, Veterinary

School of Hanover) were used in this study. The oocysts were stored by 4°C until they were used.

Oocyst purification. Sporulated and unsporulated *T. gondii* oocysts were separately purified by using discontinuous sucrose gradients. The method adapted by Arrowood and Sterling (1) was used in these experiments, with several modifications. Sheather's sugar solution (500 g of sucrose, 6.5 g of phenol, 320 ml of H_2O) was diluted with 0.1 M phosphate-buffered saline (PBS) and supplemented with 1% Tween 80. Fifteen milliliters of solution A (Sheather's sugar solution-PBS, 1:4) was layered over 15 ml of solution B (Sheather's sugar solution-PBS, 1:2) in 50-ml sterile polypropylene centrifuge tubes. Oocyst suspensions were diluted with 2.5% $K_2Cr_2O_7$ (1:1), and 10 ml of each resulting suspension was layered over solution A. The tubes were centrifuged at 1,200 × *g* for 30 min (HERAEUS Cryofuge 6000i, 4°C, no brake). The resulting distinctive layers were separately transferred into new 50-ml tubes and washed with demineralized water by centrifugation at 1,000 × *g* for 10 min (4°C, no brake). The supernatants were discarded by using a vacuum pump (MULTIFIX MC 1000 PEC), and the resulting pellets (1 ml) were washed a second time as described above. The supernatants were discarded, and the final pellets (1 ml) were examined for the presence of oocysts by phase-contrast microscopy. Pellets resulting from the 30-min centrifugation were also treated by the same method and examined by phase-contrast microscopy. We observed that most of the oocysts were concentrated in the upper layers of the 30-min centrifugation preparations, while nearly all the dirt and organic matter were in the pellet. The oocyst concentration in each final pellet was determined by using a Neubauer hemocytometer (four independent counts were obtained for each sample). For the counting, oocysts were viewed with a phase-contrast microscope (Leitz Dialux 20 EB).

Recovery of sporulated and unsporulated oocysts from water samples. (i) Centrifugation. Portions (50 ml) of demineralized water replicates were each inoculated with 1×10^5 purified *Toxoplasma* oocysts. The tubes were vortexed and centrifuged at 1,040 × *g* to 2,889 × *g* for 10 min (4°C, no brake). The supernatants were discarded, and the resulting pellets (1 ml) were vortexed and examined for oocysts by phase-contrast microscopy. The numbers of recovered oocysts were determined with a Neubauer hemocytometer by averaging six independent counts. For these experiments, sporulated and unsporulated *Toxoplasma* oocysts of the DX strain of various ages (following extraction from cats) were used.

One-liter demineralized and tap water replicates were inoculated with 1×10^5 or 1×10^4 *Toxoplasma* oocysts. The contents of the 1-liter bags were thoroughly mixed and centrifuged for 10 min (HERAEUS Cryofuge 6000i, rotor no. 8165, 4°C, no brake) at a specific *g* value that was selected based on interpretation of the results obtained with the 50-ml water replicates. The supernatants were discarded, and the resulting pellets (200 ml) were divided and placed into four 50-ml tubes and recentrifuged under the same conditions. The supernatants were discarded, and the pellets from the same trial (four 1-ml pellets) were combined and centrifuged for the last time. The supernatants were discarded, the final pellets (1 ml) were examined by phase-contrast microscopy, and the numbers of recovered oocysts were determined with a Neubauer hemocytometer. The 1-liter bags, as well as the 50-ml tubes, were rinsed with 0.1% Tween 80 after every transfer and after every time that the pellets were combined. Sporulated and unsporulated *Toxoplasma* oocysts of two different strains (DX and AHC1) that were various ages were used for these experiments.

(ii) Flocculation. (a) Ferric sulfate flocculation. One-liter demineralized water replicates (in 1-liter conical glass flasks) were inoculated with 1×10^5 or 1×10^4 *Toxoplasma* oocysts (strain DX or AHC1) of various ages. Two milliliters of an $Fe_2(SO_4)_3$ solution was added to each preparation (final concentration of Fe^{3+} in the sample, 5 mg/liter), and the pH was adjusted to 6.0 ± 0.05 with constant mild stirring. Samples were left overnight (approximately 24 h) at room temperature, so that precipitation of the floc could be completed. The supernatants were carefully discarded the next day by using a vacuum pump without disturbing the sediment. The resulted pellets (200 ml) were transferred into 50-ml sterile polypropylene centrifuge tubes and centrifuged at 2,000 × *g* for 10 min (4°C, no brake). The supernatants were discarded, and pellets from the same trial (four 1-ml pellets) were combined and recentrifuged. The supernatants were discarded, and the pellets (1 ml) were resuspended with 1 ml of lysis buffer (8.4 g of citric acid monohydrate, 17.64 g of trisodium citrate dihydrate, and H_2O up to 100 ml; pH 4.7). The pellets were left to settle with the lysis buffer for 1 h (with vortexing every 15 min). By the lysis procedure, big flocs were dissolved and the trapped oocysts were set free in the sample and became visible by microscopy. After the flocs settled, samples were washed further with demineralized water by centrifugation at 2,000 × *g* for 10 min (4°C, no brake). The supernatants were discarded, and the pellets (1 ml) were washed with demineralized water, as described above. After the supernatants were discarded, the final pellets (1 ml) were obtained and examined by phase-contrast microscopy for oocysts. The numbers of recovered oocysts were determined with a Neubauer hemocytometer.

(b) Aluminum sulfate flocculation. A total of 1×10^5 or 1×10^4 *Toxoplasma* oocysts (strain AHC1) were seeded into 1-liter demineralized water replicates. Two milliliters of an aluminum sulfate solution was added to each glass flask (final concentration of Al^{3+} in the sample, 16 mg/liter), and the pH was adjusted to 5.4 to 5.8. As described above for the ferric sulfate flocculation process, samples were left overnight and the supernatants were discarded the next day. The resulting pellets (200 ml) were treated exactly as the pellets in the ferric sulfate flocculation process were treated, and the same lysis buffer was used for resuspension of the sediments. After settlement for 1 h and washing of the samples twice, the final pellets (1 ml) were obtained and examined by phase-contrast microscopy, and the numbers of oocysts recovered were determined with a Neubauer hemocytometer.

Statistical analysis. Statistical analysis of the recovery results obtained in the centrifugation and flocculation experiments was carried out by using the average percentage of recovery, the standard deviation of the average percentage of recovery, the number of individual trials, the Kruskal-Wallis test, and the Mann-Whitney test ($\alpha = 0.05$).

Infectivity of recovered sporulated oocysts: bioassay. All mice used for the bioassay experiments were initially confirmed to be serologically negative for a previous *Toxoplasma* infection by using the indirect fluorescent antibody test (IFAT). To do this, blood samples were collected from the mice, and the sera were obtained by a previously described procedure (17). For the serological investigations, *T. gondii* tachyzoites of the BK strain were used as the antigen, and 24-well glass slides were prepared with this antigen (17) and stored at −80°C until they were used. For IFAT performance, slides were initially left at room temperature for 30 min to defrost them, fixed in 100% methanol for 10 min, and then washed with PBS (pH 7.4) for 10 min on a magnetic stirrer (medium speed). All test sera were titrated with PBS, starting at 1:5, and 5 μl of each dilution was added to each well. Positive and negative serum controls, as well as control without serum (PBS only), were included in every test slide. The slides were incubated at 37°C for 30 min in a humid chamber, rinsed carefully with PBS by using a spraying bottle, and washed again with PBS for 10 min with mild constant mixing. Rabbit anti-mouse immunoglobulin G (H+L) conjugated to fluorescein isothiocyanate was obtained commercially (Dianova 315-095-003), diluted with PBS, and stored in aliquots (7.5 μl) at −80°C. A fresh solution containing 125 μl of an Evans' blue stock solution (0.025 g of Evans' blue in 99.9 ml of PBS) and 375 μl of PBS was prepared and vortexed. Then 7.5 μl of this solution was removed, and a conjugate aliquot was thawed and pipetted gently into the solution (final conjugate working dilution, 1:64). Each well was filled with 5 μl of this solution, and slides were incubated at 37°C for 30 min in a humid chamber, rinsed, and washed with PBS as described above. Then each well was overlaid with 3 drops of mounting fluid (glycerine-PBS, 9:1), covered with a coverslip, and immediately examined by fluorescence microscopy. The results were interpreted and the IFAT titer was estimated as described by Tenter (17).

Sporulated *Toxoplasma* oocysts recovered by centrifugation were inoculated into 20 serologically negative mice (as determined by IFAT). The mice were divided into four groups. Each mouse in a group received 1×10^4 oocysts centrifuged at the same *g* value. Blood samples were obtained from the infected mice 21 and 77 days postinfection, the sera were extracted, and a serological investigation was performed (IFAT).

RESULTS

All results obtained in the recovery experiments are summarized in Tables 1 to 5.

Table 1 shows the results obtained from centrifugation of the 50-ml water replicates (1×10^5 oocysts; strain DX). Fresh sporulated oocysts (age, 2 months) gave highest mean recovery rates of 66% ± 3.2% ($n = 3$) at 1,983 × *g* (66% ± 3.2%; $n = 3$) and 66% ± 10.9% ($n = 3$) at 2,266 × *g*. Old sporulated oocysts (age, 10 months) gave a highest mean recovery rate of 82.5% ± 7.5% ($n = 3$) at 2,266 × *g*. Unsporulated oocysts (age, 7 to 8 months) gave a highest mean recovery rate of 89.4% ± 13% ($n = 3$) at 2,568 × *g*.

Tables 2 and 3 show the results obtained from centrifugation of the 1-liter water replicates.

Centrifugation of the sporulated oocysts at 2,073 × *g* gave

TABLE 1. Recovery of *T. gondii* oocysts by the centrifugation method

g value	% Recovery (avg ± SD)[a]		
	Sporulated, fresh oocysts (age, 2 months)	Sporulated, old oocysts (age, 10 months)	Unsporulated oocysts (age, 7 to 8 months)
1,040	46.0 ± 14.1 (3)[b]	52.1 ± 2.1 (3)	62.5 ± 9.3 (3)
1,247	59.3 ± 12.0 (4)	65.1 ± 11.0 (3)	68.7 ± 6.7 (3)
1,474	57.9 ± 19.3 (4)	73.8 ± 23.9 (3)	53.8 ± 17.7 (3)
1,719	63.2 ± 13.8 (4)	66.9 ± 26.8 (3)	73.8 ± 19.3 (3)
1,983	66.0 ± 3.2 (3)	80.6 ± 15.1 (3)	83.3 ± 5.7 (3)
2,266	66.0 ± 10.9 (3)	82.5 ± 7.5 (3)	59.9 ± 11.2 (3)
2,568	61.6 ± 10.5 (3)	62.5 ± 4.2 (3)	89.4 ± 13.0 (3)
2,889	ND[c]	ND	80.7 ± 27.9 (3)

[a] The organism used was strain DX, and the inoculum contained 1×10^5 oocysts in 50 ml of demineralized water.
[b] The numbers in parentheses are numbers of replicates.
[c] ND, not done.

TABLE 3. Recovery of unsporulated *T. gondii* oocysts by the centrifugation method

Strain	Age (months)	No. of oocysts	Water (1 liter)	% Recovery (avg ± SD)[a]
DX	8	1×10^5	Demineralized	71.2 ± 16.0 (3)[b]
DX	8	1×10^4	Demineralized	43.8 ± 8.6 (3)
AHC1	7–8	1×10^5	Demineralized	80.7 ± 11.3 (3)
AHC1	7–8	1×10^4	Demineralized	55.6 ± 9.1 (3)
DX	10	1×10^5	Tap	82.5 ± 8.6 (3)
DX	10	1×10^4	Tap	41.4 ± 7.1 (3)
AHC1	6	1×10^5	Tap	97.2 ± 12.5 (3)
AHC1	6	1×10^4	Tap	77.2 ± 17.9 (3)
AHC1	21	1×10^4	Demineralized	93.2 ± 11.7 (3)

[a] Preparations were centrifuged at $2,565 \times g$.
[b] The numbers in parentheses are numbers of replicates.

highest mean recovery efficiencies of 82.5% ± 6.8% ($n = 3$) and 52.5% ± 11.5% ($n = 3$) for 1×10^5 and 1×10^4 oocysts, respectively (Table 2).

Centrifugation of the unsporulated oocysts at $2,565 \times g$ gave highest mean recovery rates of 97.2% ± 12.5% ($n = 3$) and 93.2% ± 11.7% ($n = 3$) for 1×10^5 and 1×10^4 oocysts, respectively (Table 3).

Table 4 shows the results obtained with the ferric sulfate flocculation process. Sporulated oocysts gave highest mean recovery efficiencies of 93.1% ± 8.1% ($n = 4$) and 70.4% ± 16.5% ($n = 5$) for 1×10^5 and 1×10^4 oocysts, respectively. For the unsporulated oocysts, the highest mean recovery rates were 100.3% ± 26.9% ($n = 6$) and 73.3% ± 11.0% ($n = 5$), respectively.

Table 5 shows the results obtained in the experiments in which aluminum sulfate was used as the flocculant. Sporulated oocysts gave highest mean recovery rates of 96.5% ± 21.7% ($n = 5$) and 41.5% ± 9.0% ($n = 5$) for 1×10^5 and 1×10^4 oocysts, respectively. For the same initial numbers, unsporulated oocysts gave highest mean recovery efficiencies of 90.4% ± 19.1% ($n = 5$) and 55.9% ± 13.9% ($n = 5$), respectively.

Statistical evaluation of the results. Ferric sulfate flocculation is more effective than centrifugation for recovery of 1×10^5 sporulated oocysts of the same age and strain (age, 4 months; strain DX; Kruskal-Wallis test; $\alpha = 0.05$). A similar statistical difference was not observed, however, for 1×10^4 sporulated oocysts that were either fresh (age, 4 months) or old (age, 21 months) and belonged either to strain DX or strain AHC1 (Kruskal-Wallis test; $\alpha = 0.05$). Both methods were less effective for recovery of 1×10^4 oocysts than for recovery of 1×10^5 sporulated oocysts of the same age and strain (age, 4 months; strain DX; Kruskal-Wallis test; $\alpha = 0.05$). However, the two methods had the same effectiveness for recovery of either 1×10^5 or 1×10^4 unsporulated oocysts of a specific age and strain (age, 7 to 8 months; strain DX; Kruskal-Wallis test; $\alpha = 0.05$). In addition, the effectiveness of ferric sulfate flocculation was not influenced by the age of the sporulated oocysts (ages, 4 and 11 months; strain DX; 1×10^5 oocysts; Kruskal-Wallis test; $\alpha = 0.05$).

The recovery efficiency of the centrifugation method was not affected by the type of water used for the recovery experiments, either for high numbers or for low numbers of sporulated oocysts (age, 7 to 8 months; strain AHC1; Kruskal-Wallis test; $\alpha = 0.05$). It was affected, however, by the age of the unsporulated oocysts (ages, 7 to 8 and 21 months; strain AHC1; 1×10^4 oocysts; Kruskal-Wallis test; $\alpha = 0.05$).

Aluminum sulfate flocculation was more effective for recovery of 1×10^5 *Toxoplasma* oocysts than for recovery of 1×10^4 *Toxoplasma* oocysts that were either sporulated or unsporulated and were 11 or 21 months old (Kruskal-Wallis Test; $\alpha = 0.05$). However, even for large numbers of oocysts (1×10^5 oocysts), this method seemed to be less effective for the older sporulated oocysts (age, 21 months) than for the younger sporulated oocysts (age, 11 months; Kruskal-Wallis test; $\alpha = 0.05$). In addition, 1×10^4 sporulated oocysts of the same age and strain (age, 21 months; strain AHC1) were more effectively

TABLE 2. Recovery of sporulated *T. gondii* oocysts by the centrifugation method

Strain	Age (months)	No. of oocysts	Water (1 liter)	% Recovery (avg ± SD)[a]
DX	4	1×10^5	Demineralized	66.0 ± 10.9 (3)[b]
DX	4	1×10^4	Demineralized	35.8 ± 7.6 (3)
AHC1	7–8	1×10^5	Demineralized	78.1 ± 18.5 (3)
AHC1	7–8	1×10^4	Demineralized	40.1 ± 10.7 (3)
AHC1	7–8	1×10^5	Tap	82.5 ± 6.8 (3)
AHC1	7–8	1×10^4	Tap	42.6 ± 7.6 (3)
AHC1	21	1×10^4	Demineralized	52.5 ± 11.5 (3)

[a] Preparations were centrifuged at $2,073 \times g$.
[b] The numbers in parentheses are numbers of replicates.

TABLE 4. Recovery of *T. gondii* oocysts by flocculation [$Fe_2(SO_4)_3$ method][a]

Strain	Oocyst type	Age (months)	No. of oocysts	% Recovery (avg ± SD)
DX	Sporulated	4	1×10^5	93.1 ± 8.1 (4)[b]
DX	Sporulated	4	1×10^4	35.9 ± 12.3 (5)
DX	Sporulated	11	1×10^5	74.4 ± 19.2 (4)
AHC1	Sporulated	21	1×10^4	70.4 ± 16.5 (5)
DX	Unsporulated	8	1×10^5	100.3 ± 26.9 (6)
DX	Unsporulated	8	1×10^4	60.8 ± 9.8 (5)
AHC1	Unsporulated	21	1×10^4	73.3 ± 11.0 (5)

[a] One liter of demineralized water was used in each experiment.
[b] The numbers in parentheses are numbers of replicates.

TABLE 5. Recovery of *T. gondii* AHC1 oocysts by flocculation [$Al_2(SO_4)_3$ method][a]

Oocyst type	Age (months)	No. of oocysts	% Recovery (avg ± SD)
Sporulated	11	1×10^5	96.5 ± 21.7 (5)[b]
Sporulated	11	1×10^4	41.5 ± 9.0 (5)
Sporulated	21	1×10^5	58.3 ± 11.8 (5)
Sporulated	21	1×10^4	37.0 ± 11.4 (5)
Unsporulated	11	1×10^5	68.8 ± 16.1 (5)
Unsporulated	11	1×10^4	29.9 ± 10.1 (5)
Unsporulated	21	1×10^5	90.4 ± 19.1 (5)
Unsporulated	21	1×10^4	55.9 ± 13.9 (5)

[a] One liter of demineralized water was used in each experiment.
[b] The numbers in parentheses are numbers of replicates.

recovered by using $Fe_2(SO_4)_3$ than by using $Al_2(SO_4)_3$ as flocculant (Kruskal-Wallis test; $\alpha = 0.05$).

Bioassay. The results obtained in the bioassay are shown in Table 6. Nineteen of 20 mice were found to be positive for *Toxoplasma* infection 21 days postinfection. All mice were positive 77 days postinfection.

DISCUSSION

All of the data presented here suggest that methods such as centrifugation or flocculation may provide sufficient recovery with large numbers of *Toxoplasma* oocysts. Centrifugation at higher *g* values ($\geq 2{,}000 \times g$) results in higher mean recovery rates. This was not statistically proved, however, by a comparison of the results obtained at $<2{,}000 \times g$ and at $\geq 2{,}000 \times g$ (Mann-Whitney test; $\alpha = 0.05$). On the other hand, the use of high numbers of oocysts in the experiments described here (1×10^5 and 1×10^4 oocysts) is a first and necessary step to examine the effectiveness of the method. As the number of the oocysts decreased (1×10^4 oocysts), lower mean recovery rates seemed to be obtained. This probably was due to the counting method which we used (Neubauer hemocytometer), which is more accurate for determining high numbers of parasites due to their heterogeneous distribution in samples. Thus, the high standard deviations obtained for some of our mean recovery results could be attributed to such deficiencies. Similar results have been obtained in other recovery studies performed with *Cryptosporidium* and *Giardia* (10).

Use of appropriate flocculants was generally more successful than centrifugation for concentration of *Toxoplasma* oocysts, as the recovery efficiencies obtained by flocculation approached or even exceeded (for no more than three individual trials) 100%.

The choice of flocculants was based on previous studies conducted in our laboratory for concentration of *Cryptosporidium parvum* (12) and for detection of *Giardia* and *Cryptosporidium* in water samples (Karanis, unpublished data). In the first study, ferric sulfate, aluminum sulfate, and calcium carbonate were examined to determine their efficiencies of recovery of *C. parvum* oocysts from tap water. The results showed that ferric sulfate and aluminum sulfate were more effective than calcium carbonate (efficiencies of recovery, 61.5, 58.1, and 38.8%, respectively; initial concentration, 2.5×10^5 oocysts/liter). It was observed, however, that proper pH adjustment within the desired levels was the most crucial step for the formation of a floc and for successful outcome of the experiments. Thus, the high standard deviations observed for some of our mean recovery rates might be attributed to such difficulties, along with the deficiencies of the counting method.

As the bioassay results showed, sporulated oocysts retained their infectivity after concentration by centrifugation. Infectivity of oocysts depends on a variety of factors, such as origin and biological characteristics of the isolates, as well as the immune response of the host. In the present study, 1×10^4 recovered *T. gondii* oocysts of the DX strain were enough to induce an immunological response in mice. Mice are considered to be very susceptible to *Toxoplasma* infection, more than any other known secondary host. In addition, some environmental isolates of *T. gondii* have been found to be avirulent in mice but do cause an antibody response (at least some of the strains). Mice were used by Dubey et al. (9) and Isaac-Renton et al. (11) for detection and analysis of infectivity of *Toxoplasma* oocysts from drinking water samples, and this bioassay is considered the most reliable and sensitive method. Isaac-Renton et al. (11) tested a positive control mouse group in parallel with a group inoculated with drinking water samples suspected of being contaminated. The mice in the positive control group were inoculated with *Toxoplasma* oocysts at different concentrations. The minimum number of oocysts found to induce an infection was 2.5 oocysts, but this number did not induce infection in all mice (three of five mice) inoculated with the specific doses. In addition to serum investigation, tissue examination of experimental animals is often conducted to prove *Toxoplasma* infectivity. Both methods require infectivity experiments with animals kept over a relatively long time period until a definite positive or negative result can be obtained. Such procedures are expensive and time-consuming. We chose serology for our investigations because it was more practical

TABLE 6. Infectivity of *T. gondii* oocysts: bioassay results[a]

Sample	*g* value	Mouse	IFAT titer at:	
			21 days postinfection	77 days postinfection
1	1,040	1	160	>5,120
		2	160	2,560
		3	640	>5,120
		4	40	1,280
		5	20	2,560
2	1,719	6	320	1,280
		7	160	2,560
		8	40	>5,120
		9	40	1,280
		10	40	2,560
3	2,266	11	160	640
		12	80	1,280
		13	160	640
		14	Negative	1,280
		15	20	320
4	2,889	16	80	1,280
		17	80	640
		18	320	640
		19	640	1,280
		20	320	2,560

[a] The strain used was strain DX, and each mouse was inoculated with 1×10^4 4-month-old sporulated oocysts.

for the purposes of the present work and focused on the infectivity of oocysts after mechanical treatment.

In conclusion, we describe here one component of a method for detection of large numbers of *Toxoplasma* oocysts in environmental water. Although the results showed sufficient recovery rates for high oocyst numbers, further efforts are needed to develop and simplify the method, so that it can be adapted for lower numbers of oocysts as well.

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