RECOVERY OF WATERBORNE *CRYPTOSPORIDIUM PARVUM* BY FRESHWATER CLAMS IN MISSISSIPPI

Lora Rickard Ballweber*, Carla Siefker*, Eric D. Dibble, Weifan Gao***
***College of Veterinary Medicine**
and
****Department of Wildlife and Fisheries**
Mississippi State University, Mississippi State, Mississippi

INTRODUCTION

Parasites of the genus *Cryptosporidium* are protozoans that inhabit the gastrointestinal or respiratory tract of birds and the gastrointestinal tract of mammals, reptiles, and fish. Eight species of *Cryptosporidium* are currently recognized (Fayer et al., 1997), although recent genetic analyses suggest more species probably exist (Morgan et al., 1998, 1999). *Cryptosporidium parvum*, which lives in the small intestine, is transmitted by the fecal-oral route via the oocyst. Depending on the immune status of the host, ingestion of oocysts results in either a severe, chronic or a self-limiting diarrheal disease in both humans and other mammals (Fayer et al., 1997). In humans, individuals at greatest risk from infection are the young, the elderly, and those that are otherwise immunocompromised or immunosuppressed. In animals, the young are considered to be the group most at risk. Consequently, cryptosporidiosis is considered to be primarily a disease of animals within the first 1-2 months of life. There is currently no effective treatment for infection with this parasite and, if supportive therapy is not instituted, infections can be fatal.

Of the species of *Cryptosporidium* recognized to date, only *C. parvum* was thought capable of infecting humans. However, identification of this parasite has been hampered by the fact that all species are morphologically similar. Traditional methods used to identify these parasites have rested on minor differences in oocyst size and whether the parasite can experimentally infect a specific type of animal. It is impossible to conduct infectivity studies with every isolate, yet, it is important to differentiate zoonotic isolates from those that are not. Recent advances in genetic techniques have been applied to this problem and have indicated that at least two types of *C. parvum* exist - one that apparently cycles among humans and a second type that cycles back and forth between humans and animals (Peng et al., 1997). Furthermore, genetic data suggests that at least one other species of *Cryptosporidium* - *C. felis* - and a third, as yet unidentified species of *Cryptosporidium* from dogs, may also be able to infect humans (Pieniazek et al., 1999).

As stated, the oocysts is the stage responsible for transmission of *Cryptosporidium* spp. Oocysts are unusually resistant to natural stresses and many man-made chemical disinfectants. This, coupled with their small size (3.5-5.5 µm) and low sedimentation rate (0.5 µm/sec) enhances transmission of the parasite. Waterborne outbreaks of cryptosporidiosis have been documented and contributes to the recognition of *C. parvum* as the most important newly recognized contaminant in drinking water worldwide. Rivers, lakes, springs, and ground water have all been implicated as sources of infection (see Rose et al., 1997; Smith and Rose, 1998).

Surveys of surface waters throughout the US have shown oocyst prevalences of 6-100% and oocyst concentrations of 0.003-240 oocysts per liter of water (Graczyk et al., 1997a; Rose et al., 1997). Water contamination is, in part, thought to occur as a result of adverse weather conditions (heavy rains, snow melts, floods) which wash oocysts from land areas into surface waters or cause sewage overflow.

The relative contribution of mammalian feces or human excreta to the pollution of the environment is currently unknown. Difficulties encountered

identifying the source of contamination stems, in part, from lack of information regarding both the true role of sewage overflow, agricultural runoff, and wild/feral mammals as possible nonpoint sources of *C. parvum* and other zoonotic species of *Cryptosporidium*. Complicating the picture even further is the recent discovery that waterfowl, which do not become infected with *C. parvum*, can pass undamaged oocysts through the digestive tract (Graczyk et al., 1996; 1997b) that retain their ability to cause infections in mammals. This potential ability by birds to transport oocysts far from the original source could mean contamination of protected watersheds.

Current US EPA water quality monitoring procedures for detecting and enumerating oocysts in surface water are incapable of distinguishing between isolates of *C. parvum* or between the other zoonotic and non-zoonotic species that could be present as well. In addition, these procedures are also incapable of determining when oocysts enter the system. Consequently, current methods are minimally effective as monitoring tools and in epidemiological investigations. Development of a more sensitive biomonitoring tool is needed. Freshwater bivalves have characteristics making them excellent candidates for biomonitoring (McMahon, 1991). They can develop extremely dense populations, have high filtration rates, are collectible year-round, and can be held in field enclosures. In addition, because adults of large species tend to remain in place, they are subjected to conditions representative of the monitored environment. As such, examination of these bivalves can provide evidence of contamination not currently available through random testing of water (see Graczyk et al., 1999).

Experimental studies have demonstrated that mussels, clams, and oysters are capable of filtering and retaining oocysts of *C. parvum* (Fayer et al., 1997b; Graczyk et al., 1997c, 1997d, 1998; Tamburrini and Pozio, 1999). Furthermore, even though *C. parvum* has never been reported in Chesapeake Bay water (see Graczyk et al., 1999), oocysts have been detected in Eastern oysters (*Crassostrea virginica*) and Bent mussels (*Ischadium recurvum*) collected from several sites in the Bay (Fayer et al., 1998, 1999; Graczyk et al., 1999). This latter observation verifies the utility of bivalves as biomonitors for this parasite.

To date, the only freshwater bivalve that has been studied is the introduced Asian freshwater clam (*Corbicula fluminea*) (Graczyk et al., 1997c, 1998). This clam, unlike many others, exhibits unique downstream dispersal behavior associated with reproductive periods (McMahon, 1991). Although the clam has a wide geographic range making direct comparisons across drainage systems possible, the dispersal behavior limits its usefulness in epidemiological investigations. Consequently, other bivalves must be examined in order to target more appropriate species for further investigation. Therefore, the objectives of the present pilot study were to: 1) test the ability of various freshwater bivalves found in Mississippi to filter and retain waterborne oocysts of *C. parvum*; and 2) identify appropriate species for further study based, in part, on ease of collection, filtering ability, and ease of laboratory maintenance.

MATERIALS AND METHODS

Field Collection of Bivalves

Bivalves were collected in July 1999 at 12 sites from within a 3 mile reach of the Upper Pearl River in Leake Co., Mississippi. A small boat and motor was used to access the sites, and each site was selected based on stability of substrate, water flow, and water depth. Habitat and water quality (DO, pH, temperature, current velocity, substrate, depth) were measured at each site. Five quadrants (2 m^2) were randomly located within each site for collecting specimens; quadrant locations were determined by water depth (<1 m) (Miller and Payne, 1988), and accessibility and efficiency of sampling. The smaller sized bivalves were sampled in each quadrant by thoroughly raking and sifting the substrate, and larger specimens were collected by hand. Once the specimens were collected, they were immediately wrapped in damp towels, placed on ice in coolers and transported that day to the laboratory at Mississippi State University. Once in the laboratory, specimens were identified to species and held in separate, interconnected aquaria systems for 2-4 weeks prior to use in the exposure experiments. Appropriate water flows (0.2-0.5 ft/sec) and temperatures (28-30 C) similar to environmental conditions were monitored and maintained with use

of a small 1/6 hp pumping system. Water was continuously filtered through a box filter, and DO and pH were closely monitored each day.

Source of Oocysts

Oocysts, propagated in calves, were purchased from a commercial source (Pat Mason, Troy, Idaho 83871 USA). Oocysts were stored at about 4 C and were approximately 3 months old at the time of the study.

Exposure of Bivalves to *C. parvum* Oocysts

Although several species of bivalves were used in the experiments (Table 1), 6 were the focus of the study. These were *Quadrula refulgens*, *Lampsilis teres*, *Lampsilis claibornesis*, *Fusconata cerina*, *Villosa lienosa*, and *Plectomerus dombeyanus*. Two sets of experiments were conducted using identical protocols. Ninety-seven bivalves were used in Experiment 1 and 40 in Experiment 2. Bivalves were first separated by species and placed in 1 of 4 (Experiment 1) or 3 (Experiment 2) aquaria (38 L). Each aquaria was filled with dechlorinated drinking water and equipped with a filter, air stone, and heater. The system was allowed to stabilize at approximately 25 C prior to the addition of bivalves on Day 0. Temperature was closely monitored each day and the clarity of the water subjectively evaluated. Any dead specimens were removed. Hemolymph, gill washings, and stomach contents from representative controls were processed on Day 0 and examined using an immunofluorescent staining technique to detect the presence of oocysts. On Day 1, 24 hours after bivalves were added to the aquaria, the filters were removed and 1×10^6 oocysts per bivalve were added to each aquaria. On Day 2 (24 hours post-exposure), 250 ml water samples were removed from each aquaria, oocysts concentrated by centrifugation and enumerated. On Day 4 (96 hours post-exposure), all bivalves were removed from the aquaria and hemolymph, gill washings and stomach contents processed and examined as for controls.

Collection and Processing of Samples

The general procedure for necropsy of bivalves and collection of samples was as follows: the anterior and posterior adductor muscles were cut by a knife or scalpel and the shell opened. Hemolymph (up to 6 ml) was aspirated from blood sinuses using an 18 or 20 ga needle attached to a 3 or 5 cc syringe. Hemolymph was immediately transferred to 1.5 ml microfuge tubes and held on ice until centrifugation at 4500 x *g* for 5 minutes. The supernatant was removed, pellets resuspended in 250 µl ELISA-PBS and 50 µl placed in 1 well of a 3 well glass microscope slide.

To obtain gill washings, the gills were removed and placed in 5 or 10 ml ELISA-PBS in 50 ml centrifuge tubes. Tubes were vortexed for approximately 10 secs and then the gills removed from each tube. The stomachs were then isolated, cut open longitudinally and rinsed in 10 ml ELISA-PBS in plastic petri dishes. The washings were then transferred to a 15 ml centrifuge tube. Both the gill washings and stomach contents were then centrifuged at approximately 3300 x *g* for 10 min. The supernatant was removed, the pellets resuspended in an equal volume ELISA-PBS and transferred to individual microfuge tubes. They were then centrifuged at 4500 x *g* for 5 minutes. The supernatant was removed, pellets resuspended in 250 µl ELISA-PBS, and 50 µl placed in 1 well of a 3 well glass microscope slide.

Slides were allowed to air dry overnight. They were then processed using a commercially available immunofluorescent test kit in accordance with manufacturer's instructions (MeriFluor, Meridian Diagnostics, Cincinnati, Ohio). Slides were examined and scored as positive if at least 1 fluorescing round body, approximately 5 µm in diameter, with a distinctive peripheral staining pattern was observed.

RESULTS

General aquaria conditions

Temperatures of each aquarium generally fluctuated between 24 and 26 C for both sets of experiments, although a spike to 28.5 C was experienced on Day 0 in Aquarium B in Experiment 2. This elevated temperature returned to normal after about 2 hours.

The water in aquaria C1 and C2 in Experiment 1 and in aquarium B in Experiment 2 remained clear for the duration of the study. For Experiment 1, the

clarity of water in aquarium A diminished on Day 1 and steadily worsened through Day 4. Severe algal growth was present on the sides of the tank. In contrast, water in aquarium B remained clear until Day 4, the day of necropsy. For Experiment 2, the water clarity in aquaria A and C diminished on Day 2; however, the clarity never diminished to the degree observed in aquarium A in the first experiment nor was algal growth noted.

Mortality

All together, a total of 120 bivalves (87.6%) survived to the end of the experiments. In Experiment 1, 86 of the 97 bivalves (88.7%) survived while 34 of the 40 bivalves (85%) survived in Experiment 2. The percent mortalities exhibited in each aquaria varied from 0 to 23.5% (Table 2). Most mortalities occurred on Days 3 or 4 of the experiment (Table 2).

The combined mortalities that were experienced during the 2 sets of experiments varied among the species from 0 to 30% (Table 3). Of those species having a total of 10 or more specimens present, only 2 had no mortality - *L. claibornesis* and *P. dombeyanus*.

Removal of waterborne *C. parvum* oocysts

All mortalities noted above occurred after water samples were taken from each aquaria to be examined for the presence of oocysts. Although the number of oocysts present in each aquarium at 24 hours post-exposure varied, >99% of the total number of oocysts added to each tank were removed within this short period of time (Table 4).

Controls examined consisted of *L. teres* (4), *P. dombeyanus* (5), *Q. refulgens* (4), *F. cerina* (1), and *V. lienosa* (2). Oocysts were not detected in hemolymph, gill washings or stomach contents from any of the controls.

Stomach and gill samples were obtained from all 120 bivalves necropsied at the end of the experiments. Hemolymph samples were obtained from all but 1 *L. claibornesis* in Experiment 2. Of the 33 hemolymph samples initially examined, only 1 had oocysts present. Oocysts were also detected in the stomach contents of this bivalve as well. Because of the low number of positive hemolymph samples, results presented in this report are for the combined results for the gill and stomach samples only.

Of the 10 species of bivalves used in this study, oocysts of *C. parvum* were detected in 9 (Table 5). *Tritogonia verrucosa* was the only species in which oocysts were not detected; however, only 3 specimens of this species were utilized.

DISCUSSION

The first objective for the present study was to test the ability of several species of freshwater bivalves found in Mississippi to filter and retain waterborne oocysts of *C. parvum*. First, the ability to remove oocysts within the first 24 hours of exposure in a closed system was examined. Greater than 99% of the oocysts added to the aquaria were removed from the water within 24 hours. Although all aquaria contained 2 or more species of bivalve, a single species predominated in most (Table 1). Consequently, it would appear that *Q refulgens*, *L. teres*, *P. dombeyanus* and possibly *F. cerina* are all highly efficient at filtering *C. parvum* oocysts from the water. This greatly extends the work of previous authors (Graczyk et al., 1997c, 1998) and indicates a wide variety freshwater bivalves could be used as biomonitors for *C. parvum* contamination.

Second, the ability to retain oocysts was determined by examining bivalves 72 hours post-exposure. Based on detection of oocysts in stomach contents and gill washings, at least 9 species of bivalves will filter and retain oocysts under experimental conditions. This further supports the concept that freshwater bivalves can be biomonitors for cryptosporidial contamination.

The second objective of the present study was to evaluate the ease of laboratory maintenance of the bivalves and identify species for further study. Relative water clarity and mortality were used as indicators of ease of maintenance during experiments. Based on clear water and no mortality (see Table 2), aquaria C1 and C2 (*P. dombeyanus*/*T. verrucosa*) in Experiment 1 were the easiest to maintain. Aquarium B in Experiment 2 also maintained relatively clear water; however, mortality in this tank was 16.7% even with low stocking density (Table 2). Interestingly, the

aquarium experiencing the worst loss of water clarity, (A - Experiment 1) was not the one in which the highest mortality occurred (B - Experiment 1) (Table 2).

Another factor in choice of species for further study is their availability in the field. Although 10 species were collected from the 3 mile reach of the Pearl River, 3 species (*Q. refulgens*, *L. teres*, and *P. dombeyanus*) accounted for two-thirds of the specimens collected. These 3 were also the only species which had >20 specimens available at the end of the experiments for necropsy (Table 5). Of these, oocysts were detected in 55.6% of *P. dombeyanus* and 50% of *L. teres*. Only 20% of *Q. refulgens* had detectable oocysts. In terms of mortality, *P. dombeyanus* had no mortality while *L. teres* had the highest mortality (Table 3). The relatively high mortality exhibited by *L. teres* may be a result of overstocking the aquaria and could possibly be reduced if fewer specimens are added to the tanks. Therefore, based on numbers available, mortality (ease of laboratory maintenance), and ability to detect oocysts 72 hours post-exposure, *P. dombeyanus* merits further study. *Lampsilis teres* may also merit further study if mortality can be decreased.

Of the remaining species which had over 10 specimens available for use in the experiments, oocysts were detected in 38.5% and 42.9% of *V. lienosa* and *F. cerina*, respectively and only 16.7% of *L. claibornesis* (Table 5). Mortality among these 3 species was least for *L. claibornesis* and greatest for *F. cerina* (Table 3). Therefore, based on the same parameters as above, it would appear that *V. lienosa* may warrant further study.

ACKNOWLEDGMENTS

The authors thank Mr. David Zitnik, Ms. Racheal Rogers, Mr. Scott Woodruff (Department of Wildlife and Fisheries) and Ms. Carolyn L. Slocumb (College of Veterinary Medicine) for field and laboratory assistance, Mr. Steven George (Waterways Experiment Station) for his assistance in species identification, and Dr. Andrew Miller (Waterways Experiment Station) for his help in designing the field sampling protocol. This study was funded in part by the Multidisciplinary In-House Funding Program, College of Veterinary Medicine, Mississippi State University.

LITERATURE CITED

Fayer, R., C. A. Speer, and J. P. Dubey. 1997a. The general biology of *Cryptosporidium*. In: *Cryptosporidium* and cryptosporidiosis. Boca Raton, FL: CRC Press, pp. 1-41.

Fayer, R., C. A. Farley, E. J. Lewis, J. M. Trout, and T. K. Graczyk. 1997b. Potential role of the Eastern Oyster, *Crass ostrea virginica*, in the epidemiology of *Cryptosporidium parvum*. Applied and Environmental Microbiology. 63: 2086-2088.

Fayer, R., T. K. Graczyk, E. J. Lewis, J. M. Trout, and C. A. Farley. 1998. Survival of infectious *Cryptosporidium parvum* oocysts in seawater and Eastern oysters (*Crass ostrea virginica*) in the Chesapeake Bay. Applied and Environmental Microbiology. 64: 1070-1974.

Fayer, R., E. J. Lewis, J. M. Trout, T. K. Graczyk, M. C. Jenkins, J. Higgins, L. Xiao, and A. A. Lal. 1999. *Cryptosporidium parvum* in oysters from commercial harvesting sites in the Chesapeake Bay. Emerging Infectious diseases. 5: 706-710.

Graczyk, T. K., M. R. Cranfield, R. Fayer, and M. S. Anderson. 1996. Viability and infectivity of *Cryptosporidium parvum* oocysts are retained upon intestinal passage through a refractory avian host. Applied and Environmental Microbiology. 62: 3234-3237.

Graczyk, T. K., R. Fayer, and M. R. Cranfield. 1997a. Zoonotic transmission of *Cryptosporidium parvum*: implications for water-borne cryptosporidiosis. Parasitology Today. 13: 348-351.

Graczyk, T. K. M. R. Cranfield, R. Fayer, J. Trout, and H. J. Goodale. 1997b. Infectivity of *Cryptosporidium parvum* oocysts is retained upon intestinal passage through a migratory water-fowl species (Canada goose, *Branta canadensis*). Tropical Medicine and International Health. 2:341-347.

Graczyk, T. K., R. Fayer, M. R. Cranfield, and D. B.

Conn. 1997c. In vitro interactions of Asian freshwater clam (*Corbicula fluminea* hemocytes and *Cryptosporidium parvum* oocysts. Applied and Environmental Microbiology. 63: 2910-2912.

Graczyk, T. K., R. Fayer, E. J. Lewis, C. A. Farley, and J. M. Trout. 1997d. In vitro interactions between hemocytes of the Eastern oyster, *Crass ostrea virginica* Gmelin, 1791 and *Cryptosporidium parvum* oocysts. Journal of Parasitology. 83: 949-952.

Graczyk, T. K., R. Fayer, M. R. Cranfield, and D. B. Conn. 1998. Recovery of waterborne *Cryptosporidium parvum* oocysts by freshwater benthic clams (*Corbicula fluminea*). Applied and Environmental Microbiology. 64: 427-430.

Graczyk, T. K., R. Fayer, E. J. Lewis, J. M. Trout, and C. A. Farley. 1999. *Cryptosporidium* oocysts in Bent mussels (*Ischadium recurvum*) in the Chesapeake Bay. Parasitology Research. 85: 518-521.

McMahon, R. R. 1991. Mollusca: Bivalvia. In: Ecology and classification of North American freshwater invertebrates, ed. Thorp, J. H., and A. P. Covich, pp 315-401. San Diego, CA: Academic Press/Harcourt Brace Jovanovich.

Miller, A. C., and B. S. Payne. 1988. The need for quantitative sampling to characterize demography and density of freshwater mussel communities. American Malacology Bulletin. 6: 49-54.

Morgan, U. M., K. D. Sargent, P. Deplazes. D. A. Forbes, F. Spano, H. Hertzberg, A. Elliot, and R. C. A. Thompson. 1998. Molecular characterization of *Cryptosporidium* from various hosts. Parasitology. 117: 31-37.

Morgan, U. M., J. R. Buddle, A. Armson, A. Elliot, and R. C. A. Thompson. 1999. Molecular and biological characterization of *Cryptosporidium* in pigs. Aust Vet J. 77:44-47.

Peng, M. M., L. Xiao, A. R. Freeman, M. J. Arrowood, A. A. Escalante, A. C. Weltman, C. S. L. Ong, W. R. MacKenzie, A. A. Lal, and C. B. Beard. 1997. Genetic polymorphism among *Cryptosporidium parvum* isolates: evidence of two distinct human transmission cycles. Emerging Infectious Diseases 3: 567-573.

Pieniazek, N. J. F. J. Bornay-Llinares, S. B. Slemenda, A. J. da Silva, I. C. S. Moura, M. J. Arrowood, O. Ditrich and D. G. Addiss. 1999. New *Cryptosporidium* genotypes in HIV-infected persons. Emerging Infectious Diseases. 5: 444-449.

Rose, J. B., J. T. Lisle, and M. LeChevallier. 1997. Waterborne cryptosporidiosis: incidence, outbreaks, and treatment strategies. In: *Cryptosporidium* and cryptosporidiosis. Boca Raton, FL: CRC Press, pp. 92-109. Boca Raton, FL: CRC Press.

Smith, H. V., and J. B. Rose. 1998. Waterborne cryptosporidiosis: current status. Parasitology Today. 14: 14-22.

Tamburrini, A., and E. Pozio. 1999. Long-term survival of *Cryptosporidium parvum* oocysts in seawater and in experimentally infected mussels (*Mytilus galloprovincialis*). International Journal for Parasitology. 29: 711-715.

Table 1. Species, size and total number of bivalves added to each tank on Day 0.

Aquarium	Species	No. added	Range in length (mm)
Experiment 1			
A	*Lampsilis teres*	1	87
A	*Quadrula refulgens*	32	33-69
	Total for Aquarium A	33	-
B	*Lampsilis claibornesis*	5	68-93
B	*Lampsilis teres*	28	60-103
	Quadrula refulgens	1	NM*
	Total for Aquarium B	34	-
C1	*Plectomerus dombeyanus*	13	80-132
	Tritogonia verrucosa	2	159-176
C2	*Plectomerus dombeyanus*	14	96-124
	Tritogonia verrucosa	1	137
	Total for Aquaria C1 and C2	30	-
Experiment 2			
A	*Fusconata cerina*	10	45-80
A	*Ablema plicata*	1	57
A	*Glebula rotunda*	1	32
A	*Quadrula refulgens*	1	66
	Total for Aquarium A	13	-
B	*Lampsilis claibornesis*	7	81-93
B	*Villosa lienosa*	5	68-74
	Total for Aquarium B	12	-
C	*Potamilus purputatus*	5	81-119
C	*Villosa lienosa*	10	42-99
	Total for Aquarium C	15	-

*NM = not measured

Table 2. Mortality of bivalves by aquarium in Experiments 1 and 2.

Aquarium	Total no. added	No. dead	% Mortality	Species (No. dead on day of study)
Experiment 1				
A	33	3	10.0	*Q. refulgens* (2 on Day 2, 1 on Day 3)
B	34	8	23.5	*Q. refulgens* (1 on Day 4); *L. teres* (1 on Day 3, 6 on Day 4)
C1	15	0	0	-
C2	15	0	0	-
Experiment 2				
A	13	3	23.1	*F. cerina* (1 on Day 3, 2 on Day 4)
B	12	2	16.7	*V. lienosa* (2 on Day 4)
C	15	1	6.7	*P. purputatus* (1 on Day 3)

Table 3. Combined mortality from Experiments 1 and 2 for each species of bivalve.

				Day of study*		
Species	Total no. used	No. dead	% Mortality	2	3	4
Quadrula refulgens	34	4	11.8	2**	1	1
Lampsilis teres	29	7	24.1	0	1	6
Lampsilis claibornesis	12	0	0	0	0	0
Plectomerus dombeyanus	27	0	0	0	0	0
Tritogonia verrucosa	3	0	0	0	0	0
Villosa lienosa	15	2	13.3	0	0	2
Fusconata cerina	10	3	30.0	0	1	2
Ablema plicata	1	0	0	0	0	0
Glebula rotunda	1	0	0	0	0	0
Potamilus purputatus	5	1	20.0	0	1	0

*Day of study on which the mortality occurred. Day 0 is defined as the day bivalves were added to the aquaria.
**Number of each species that died on Day 2, 3, or 4 of the study.

Table 4. Removal of waterborne oocysts by bivalves kept in 38 L aquaria.

Aquarium	No. of oocysts added	No. of oocysts after 24 hours	% removal
Experiment 1			
A	33×10^6	1.2×10^3	>99
B	34×10^6	1.32×10^4	>99
C1	15×10^6	4.8×10^3	>99
C2	15×10^6	1.2×10^3	>99
Total C	30×10^6	6.0×10^3	>99
Experiment 2			
A	13×10^6	1.2×10^3	>99
B	12×10^6	1.2×10^3	>99
C	15×10^6	1.2×10^3	>99

Table 5. Number of bivalves in which oocysts of *C. parvum* were detected 72 hours post-exposure.

Species	Total no. examined	No. positive	% positive
Quadrula refulgens	30	6	20.0
Lampsilis teres	22	11	50.0
Lampsilis claibornesis	12	2	16.7
Plectomerus dombeyanus	27	15	55.6
Tritogonia verrucosa	3	0	0.0
Villosa lienosa	13	5	38.5
Fusconata cerina	7	3	42.9
Ablema plicata	1	1	100
Glebula rotunda	1	1	100
Potamilus purputatus	4	2	50.0