

## Susceptibility of the Chesapeake Bay to Environmental Contamination with *Cryptosporidium parvum*

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Received February 2, 1999

### CRYPTOSPORIDIOSIS AS A PUBLIC HEALTH THREAT

*Cryptosporidium parvum*-associated cryptosporidiosis that causes acute diarrhea in immunocompetent humans or life-threatening illness in immunocompromised or immunosuppressed individuals has emerged as a global human health problem facilitated in its spread by zoonotic, waterborne, and foodborne transmission of the pathogen (Fayer *et al.*, 1997a; Graczyk, 1997; Graczyk *et al.*, 1997a). Of eight valid species infecting all vertebrate groups—*C. nesorum* (fish), *C. serpentis* (reptiles), *C. baileyi* and *C. meleagridis* (birds), and *C. felis*, *C. wairi*, *C. muris*, and *C. parvum* (mammals)—only one, *C. parvum*, infects humans (Fayer *et al.*, 1997a; Graczyk, 1997; Graczyk *et al.*, 1997a). *Cryptosporidium parvum* oocysts are small (3.5–6.0 µm) (Fayer *et al.*, 1997a), resistant to environmental stressors, and their infectivity is long lasting in aquatic environments (Fayer *et al.*, 1998; Robertson *et al.*, 1992; Rose *et al.*, 1997). *Cryptosporidium parvum* infects over 80 species of mammals, is highly prevalent in ruminants, and is readily cross-transmissible to humans (Fayer *et al.*, 1997a) but is noninfectious to other vertebrate groups (Graczyk *et al.*, 1996a). As few as 30 oocysts initiate infection (DuPont *et al.*, 1995), while over a billion oocysts can be excreted daily by an infected person or calf (Fayer *et al.*, 1997a; Graczyk, 1997; Graczyk *et al.*, 1997a). As a result, *Cryptospori-*

*dium* has caused massive waterborne epidemics worldwide, and has become recognized as the most important biological water contaminant in the United States (Rose *et al.*, 1997) (Table 1). Wastewater treatment plants and animal farms, in particular cattle farms, have been recognized as the most significant sources of environmental contamination with *C. parvum* (Fayer *et al.*, 1997a; Graczyk, 1997; Graczyk *et al.*, 1997a; Rose *et al.*, 1997).

*C. parvum* significantly contributes to the mortality of immunocompromised and immunosuppressed people due to lack of effective prophylaxis or therapy (Fayer *et al.*, 1997a). Two genotypes of *C. parvum* have been identified by molecular techniques (Sulaiman *et al.*, 1998). The “animal adapted” or “zoonotic” genotype has been shown to be transmissible among cattle, mice, and humans but may actually infect a greater number of mammalian species, whereas the “human adapted” genotype is thought to cycle within the human population (Sulaiman *et al.*, 1998). Both genotypes can produce life-threatening infections in people with impaired immune systems.

The largest outbreak of cryptosporidiosis occurred in 1993 in Milwaukee (WI) where approximately 403,000 people became sick (MacKenzie *et al.*, 1994) and eventually over 100 people died due to the pathogen that was contracted at that time (Rose, 1997). *Cryptosporidium* oocysts are continuously (as distinct from intermittently) prevalent in surface waters (Hansen and Ongerth, 1991; Rose *et al.*, 1997). Of surface water sites examined (Rose *et al.*, 1997), the prevalence of oocysts has been 100% with concentrations as high as 5800 oocysts per liter

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**TABLE 1**  
**Outbreaks of Waterborne Cryptosporidiosis in the United States**

Year	Location	Population infected (103)	Suspected cause(s)
1984	Braun Station, TX	2.00	Sewage-contaminated well
1987	Carrolton, GA	12.96	Water-treatment deficiencies
1988	Los Angeles, CA	—	Inoperative swimming-pool filters
1991	Pennsylvania	0.55	Water-treatment deficiencies
1992	Jackson County, CO	15.00	Water-treatment deficiencies
1992	Lane County, OR	—	Oocyst found in filter backwash water
1993	Madison, WI	—	Fecal accident in swimming pool
1993	Milwaukee, WI	403.00	Snow melt, flood, water-treatment deficiencies
1994	Clark County, NV	0.08	Fecal accident in swimming pool

Source. Modified from Graczyk *et al.*, 1997a.

(Rose *et al.*, 1997). Adverse weather conditions, such as heavy rains, snow melts, and floods wash oocysts from the land into the water, cause sewage overflow and increase urban and agricultural run-off, resulting in water contaminated with oocysts (Graczyk *et al.*, 1997a; Rose *et al.*, 1997).

#### MARYLAND SEA GRANT PROJECT

The Maryland Sea Grant Project brings experts on *Cryptosporidium* epidemiology, epizootiology, immunology, biology, and molecular biology, together with experts on oyster biology. The main focus of the project is to determine if Chesapeake Bay oysters (*Crassostrea virginica*) contain *C. parvum* oocysts potentially infectious to humans, and what environmental factors are associated with the contamination of oysters.

##### Objectives:

1. To select and methodically test through optimization and standardization the most efficient and easy to adopt techniques for the screening of Chesapeake Bay oysters for *C. parvum* contamination. The purpose is to: (a) improve the quality of the harvested oysters, (b) determine the infectivity to humans of the *C. parvum* oocysts recovered from oyster tissue, and (c) determine whether oysters can be used as bioindicators of water contamination by *C. parvum*.

2. To assess the role of environmental components, i.e., wastewater treatment facility discharges, cattle farm runoffs, and waterfowl presence, in contamination of the Chesapeake Bay with *C. parvum*. Also, to determine epizootiological and epidemiological interplay among these components in order to: (a) deter-

- mine the impact of water contamination on natural oyster populations, (b) determine the infectivity of the *C. parvum* oocysts recovered from water samples, and (c) provide guidelines for controlling, reducing, or preventing contamination of the Chesapeake Bay with *C. parvum*.

#### METHODOLOGY

##### Oyster Sampling and Processing

Thirty commercial-sized oysters were sampled by dredging from six Chesapeake Bay locations in May, August, and October (Fayer *et al.*, 1998). At the laboratory, the oyster shells were drilled and the hemolymph was collected (Fayer *et al.*, 1998). Hemocyte monolayers were prepared and processed using immunofluorescent antibody (IFA) available in the commercial MERIFLUOR test kit (Fayer *et al.*, 1998; Graczyk *et al.*, 1997b). The hemolymph from each of 6 oysters was pooled and centrifuged. After the supernatant was aspirated, the pellet was resuspended and the *Cryptosporidium* oocysts were removed and tested by mouse bioinfectivity assay (Fayer *et al.*, 1996; 1998) and polymerase chain reaction (PCR) (Jenkins and Fayer, 1995; Jenkins and Peterson, 1997; Jenkins *et al.*, 1997). Gill tissue from each oyster was excised and vortexed in saline solution (Fayer *et al.*, 1998). The tubes were centrifuged and the pellet was resuspended in saline and tested for *Cryptosporidium* oocysts by IFA (Fayer *et al.*, 1998). The resuspensions from each of 6 oysters were pooled and tested by the mouse bioinfectivity assay and PCR. Identification of presumptive *C. parvum* oocysts by IFA followed U.S. Environmental Protection Agency (EPA) recommendations issued for laboratories

approved for the Information Collection Rule (ICR) protozoa testing.

#### Mouse Bioinfectivity Assay

Mouse bioinfectivity assay is the most sensitive and specific technique for assessment of the infectivity of *C. parvum* oocyst isolates of public health importance such as bovine and human strains of the pathogen. This technique was used to detect mouse infection initiated by low numbers ( $>1.0 \times 10^2$ ) of *C. parvum* oocysts (Jenkins *et al.*, 1997). PCR detection of developmental stages of the pathogen in mouse tissues was specific to *C. parvum* (Jenkins and Peterson, 1997; Jenkins *et al.*, 1997). BALB/c mouse neonates were used for determination of infectivity of oocysts recovered from oysters and waterfowl fecal droppings (Fayer *et al.*, 1998; Graczyk *et al.*, 1997c, 1998a). The mice were euthanized 96 h postinfection and the ileum was extracted for PCR and histological analyses (Fayer *et al.*, 1998; Graczyk *et al.*, 1997c, 1998a).

#### Processing of Fecal Specimens

Waterfowl fecal droppings were collected at proximity of cattle farm runoffs and from cattle activity areas where waterfowl were abundant (Graczyk *et al.*, 1998a). Fecal droppings were processed according to

our protocols (Graczyk *et al.*, 1996c, 1998a). The method used for recovery of *Cryptosporidium* oocysts does not alter oocyst infectivity, and therefore the recovered oocysts can be subsequently subjected to the assessment of infectivity by the mouse bioinfectivity assay (Graczyk *et al.*, 1997c, 1998a).

## RESULTS

#### Specificity of Detection of *C. parvum* Oocysts in the Oyster Tissue

The potential cross-reactivity of the combined *Cryptosporidium*/*Giardia* direct IFA of MERIFLUOR test was examined against tissues containing known developmental stages of 12 pathogens causing the principal infectious diseases in oysters (Graczyk *et al.*, 1998b). Only *Hexamita nelsoni* trophozoites produced a positive IFA reaction; however, the fluorescence intensity was considerably lower, and the fluorescein staining pattern was different than those of *Giardia* cysts (Table 2). The applicability of acid-fast stain (AFS) for screening of oysters for *Cryptosporidium* oocysts was low (Graczyk *et al.*, 1998b), and positive identification of *Cryptosporidium* oocysts cannot be accomplished based on the AFS (Graczyk *et al.*, 1998b). Presumptive IFA identification of the *Cryptosporidium* oocysts or *Giardia* cysts in the oyster tissue

TABLE 2  
Summary of Testing of Some Pathogens Causing Infectious Diseases of the Oysters

Disease/parasite	Pathogen stages	Infection site	Type of reaction	
			AFS <sup>a</sup>	IFA <sup>b</sup>
MSX disease/ <i>Haplosporidium nelsoni</i>	Plasmodia Spores	All tissues Digestive tubule epithelium	— +	— —
SSO disease/ <i>Haplosporidium costale</i>	Plasmodia Spores	All tissues All tissues except the epithelia	— +	— —
Dermo disease/ <i>Perkinsus marinus</i>	Zoospores Trophonts	All tissues All tissues	— —	— —
Juvenile oyster disease/unknown	Meronts Unidentified	All tissues Mantle	— —	— —
Miscellaneous protozoa				
<i>Nematopsis ostrearum</i>	Spores	All tissues	—	—
<i>N. prytherchi</i>	Spores	All tissues	—	—
<i>Martelia regringens</i>	Spores	All tissues	—	—
<i>Hexamita nelsoni</i>	Trophozoites	Intestine	—	+
<i>Bonamia</i> sp.	Spores	All tissues	—	—
<i>Gregarine</i> sp.	Oocysts	Intestinal epithelium	—	—
<i>Pseudoklossia</i> sp.	Oocysts	Kidney epithelium	—	—
Trematodiasis/ <i>Bucephalus cuculus</i>	Sporocysts Cercariae	Gonads, gills Digestive glands	— —	— —

Source. Modified from Graczyk *et al.*, 1998b.

<sup>a</sup>Acid-fast stain.

<sup>b</sup>Combination direct immunofluorescent monoclonal antibodies of the MERIFLUR™ *Cryptosporidium*/*Giardia* test kit.

**TABLE 3**  
**Oyster Collection Sites in the Chesapeake Bay Tributaries, Fecal Coliform Counts, and Number of Oysters Positive for *Cryptosporidium* Oocysts**

River	Proximity to outfall or cattle farm	Coliform counts <sup>a</sup>		No. (%) of oysters positive for <i>Cryptosporidium</i> oocysts	
		May-June	Aug-Sept	May-June	Aug-Sept
Choptank	Cambridge outfall	1.0	3.6	5 (17)	6 (20)
Severn	Annapolis	23.0	9.1	12 (40)	26 (87)
Miles	St. Michaels outfall	9.1	3.6	13 (43)	2 (7)
Wye <sup>b</sup>	Cattle farm	1.0	3.6	18 (60)	12 (40)
Potomac	Cattle farm drainage	3.6	1.0	12 (40)	21 (70)
Wicomico	Dairy farm drainage	3.6	43.0	11 (37)	4 (13)

Source. Modified from Fayer *et al.*, 1998.

<sup>a</sup>MPN/100 ml of seawater.

<sup>b</sup>Oysters containing infectious oocysts of *C. parvum*.

should fulfill three criteria: (a) bright-green fluorescence of the same intensity as *C. parvum* oocysts and *Giardia* cysts in the positive control, (b) correct size and shape of the fluorescein-stained objects, and (c) oocyst or cyst shell clearly visible (Graczyk *et al.*, 1998b).

#### *Positivity of the Chesapeake Bay Oysters for Cryptosporidium Oocysts*

Eastern oysters were collected with a dredge or with hand tongs at each of six sites (30 oysters/site) within Maryland tributaries of the Chesapeake Bay (Table 3). Hemocytes and gill washing from all oysters were examined for the presence of *Cryptosporidium* and *Giardia* cysts by IFA. *Cryptosporidium* oocysts were detected in either hemocytes and gill washings of all oysters from all six sites. Pooled oyster hemocytes and gill washings were delivered by gastric intubation to neonatal mice to produce a bioassay for oocyst infectivity. The intestinal tissue of mice that received gill washings from oysters collected at a site near a large cattle farm and shoreline homes with septic tanks (Table 3) was positive for developmental stages of *C. parvum*.

#### *Cryptosporidium Oocysts in Fecal Droppings of Waterfowl in the Chesapeake Bay Area*

Aquatic birds have been implicated in the dispersal of human and animal pathogens but have never before been confirmed as mechanical vectors of *C. parvum* (Fayer *et al.*, 1997b; Graczyk *et al.*, 1996c, 1997c). Freshly deposited fecal droppings (total of 12.6 kg) of migratory Canada geese, *Branta canadensis*, collected during the fall of 1997 from nine locations near the Chesapeake Bay area (Table 4), were examined

for cystic stages of *Cryptosporidium* and *Giardia* (Graczyk *et al.*, 1998a). As determined by IFA analysis, *Cryptosporidium* sp. oocysts were found in goose feces at seven of nine sites, and feces from all nine sites contained *Giardia* sp. cysts (Graczyk *et al.*, 1998a). The concentration of *Cryptosporidium* sp. oocysts ranged from 67 to 686 per gram of the goose feces ( $x = 375$  oocysts/g); the concentration of *Giardia* sp. cysts ranged from 75 to 786 cysts/g ( $x = 405$  cysts/g) (Graczyk *et al.*, 1998a). *Cryptosporidium* sp. oocysts recovered from three sites were infectious for neonatal BALB/c mice (Graczyk *et al.*, 1998a). Utilizing polymerase chain reaction for *Cryptosporidium* TRAP C2 and beta-tubulin genes, these oocyst isolates were identified as "animal adapted" or "zoonotic" strain of *C. parvum* (Graczyk *et al.*, 1998a). Demonstration of the presence of infectious oocysts of *C. parvum* in the fecal droppings of free-ranging waterfowl clearly indicates that they can act as mechanical carriers of this waterborne human pathogen and can disseminate infectious *C. parvum* oocysts throughout terrestrial and aquatic environments (Graczyk *et al.*, 1998a).

#### CONCLUSIONS

The researchers involved in the Maryland Sea Grant Project feel it is important that proper information on *Cryptosporidium* and oysters be disseminated by professional media, to avoid sensationalism and misinterpretation which could impact the oyster industry.

As the oysters are often consumed raw, infectious *C. parvum* oocysts within oysters pose a potential public health problem. With the explosive emergence and global spread of cryptosporidiosis, and foodborne

TABLE 4

The Numbers and Concentrations of *Giardia* sp. Cysts, *Cryptosporidium* sp. Oocysts, and Infectious *Cryptosporidium parvum*<sup>a</sup> Oocysts Recovered from Fecal Droppings Collected in the Chesapeake Bay Area from Nine Flocks of Migratory Canada Geese (*Branta canadensis*)

Site of fecal dropping collection			<i>Cryptosporidium</i> oocysts		<i>Giardia</i> cysts	
Name	Latitude	Longitude	Total recovered (10 <sup>6</sup> )	Concentration (per g)	Total recovered (10 <sup>6</sup> )	Concentration (per g)
Grasonville <sup>b</sup>	38°56.886'	76°13.709'	0.12	67	0.31	173
Oxford 1 <sup>c</sup>	38°41.120'	76°09.654'	0	0	0.12	75
Oxford 2 <sup>c</sup>	38°41.052'	76°09.717'	0.31	341	0.54	593
Perrys Corner <sup>d</sup>	38°56.090'	76°11.523'	0	0	0.37	446
Bryatown <sup>d</sup>	38°56.692'	76°10.311'	0.37	276	0.51	381
Carmichael <sup>d</sup>	38°56.858'	76°08.226'	0.71	390	0.97	533
Wye Island 1 <sup>a,e</sup>	38°53.848'	76°08.952'	0.62	530	0.92	786
Wye Island 2 <sup>a,f</sup>	38°53.803'	76°09.360'	1.31	686	0.38	199
Wye Island 3 <sup>a,f</sup>	38°53.628'	76°09.774'	0.37	301	0.56	455

Source. Modified from Graczyk *et al.*, 1998a.

<sup>a</sup>*Cryptosporidium* sp. oocysts identified by PCR and mouse bioassay as zoonotic (animal adapted) strain of *C. parvum*.

<sup>b</sup>Meadow near pond.

<sup>c</sup>Soybean stubble.

<sup>d</sup>Corn stubble.

<sup>e</sup>Standing soybeans.

<sup>f</sup>Standing corn/corn stubble.

*Cryptosporidium* infections associated with solid food items, oysters may be cited as possible pathogen reservoirs, mechanical vectors, or causes of foodborne cryptosporidiosis. However the presence of *Cryptosporidium* oocysts in the tissues of oysters harvested from the Chesapeake Bay should not yet be considered as a public health problem because: (1) the oocysts may represent one of seven other species of *Cryptosporidium* (Fayer *et al.*, 1997a; Graczyk, 1997; Graczyk *et al.*, 1996b) that are noninfectious to humans; (2) *Cryptosporidium parvum* oocysts detected in the oyster tissue may not be viable and therefore noninfectious to humans; and (3) the prevalence of oysters contaminated with *C. parvum*, and oocyst concentration in the oyster tissues, might be so low that *C. parvum* contamination does not represent a significant public health concern.

Our studies indicate that *C. parvum* oocysts can survive for relatively long periods in seawater at salinities and temperatures overlapping those in which Chesapeake oysters live (Fayer *et al.*, 1998). We demonstrated for the first time the presence of infectious oocysts of *C. parvum* in the oysters collected from the Chesapeake Bay (Fayer *et al.*, 1998), and that oysters can serve as mechanical vectors of this pathogen. However, it is important to emphasize that the oysters were selected from collection sites determined by their close proximity to possible sources of *C. parvum* contamination (Fayer *et al.*, 1998).

Oysters have been incriminated in foodborne epidemics of enteric diseases (Bean *et al.*, 1996). Oysters can remove from the water and retain in their tissues a variety of human waterborne pathogens, e.g., *Vibrio cholerae*, *V. vulnificus*, *Escherichia coli*, *Salmonella tallassee*, *Shigella* spp., and hepatitis A virus (Graczyk *et al.*, 1997b). *Vibrio*, *Pseudomonas*, *Achromobacter*, and *Cytophaga/Flavobacterium* have been found in the Chesapeake Bay oysters (Fayer *et al.*, 1998). Although no human cases of cryptosporidiosis have been linked to ingestion of raw shellfish, our studies indicate that the potential for such transmission exists (Fayer *et al.*, 1997c, 1998).

Because oysters are efficient filter-feeders, local populations of oysters in the Chesapeake Bay may efficiently decrease the concentration of waterborne oocysts of *Cryptosporidium* (Graczyk *et al.*, 1997b). As the oysters retain water-recovered oocysts in their tissue, they can be used as biological indicators of contamination of water with *Cryptosporidium* oocysts (Graczyk *et al.*, 1997b).

Our studies demonstrated that aquatic birds, i.e., Canada geese, are involved in the dispersal of *C. parvum* and can be considered as mechanical vectors of this pathogen (Graczyk *et al.*, 1998a). Canada geese are abundantly prevalent in the wild and constitute a major component of residential and migratory waterfowl in North America. Canada geese use agricultural areas, e.g., pastures and cattle grazing lands, for

feeding and resting, and these areas have been known to carry high loads of *Cryptosporidium* oocysts (Fayer *et al.*, 1997a; Graczyk *et al.*, 1997a, 1998a). The Eastern Shore of Maryland is located in the main migratory pathway of Canada geese and other waterfowl that fly along the east coast of the United States. This predominantly agricultural region with scattered cattle farms accumulates high numbers of migratory birds during fall and spring. Migratory Canada geese use cattle pastures for resting and feeding and birds were actually observed wandering behind the cattle and picking up undigested corn from their feces (Graczyk *et al.*, 1998a). Because these birds travel great distances, *Cryptosporidium* oocysts can be excreted miles away from where they were ingested (Graczyk *et al.*, 1998a). Such an example was provided in our studies with two different flocks of Canada geese, whose stop-over places were separated only by the state highway (Graczyk *et al.*, 1998a) (Table 4).

There are four fundamental steps used in development of a formal quantitative public risk assessment associated with consumption of raw shellfish (Rose and Sobsey, 1993). These steps include hazard identification, dose-response determination, exposure assessment, and risk characterization (Rose and Sobsey, 1993). A public health risk assessment model has been developed for shellfish contaminated with the human enteric viruses (Rose and Sobsey, 1993). The risk assessment model for *Cryptosporidium* contamination of oysters has not been developed yet, although the data on hazard identification due to *C. parvum* and information on dose-response determination does exist (DuPont *et al.*, 1995). The monitoring data on contamination of Chesapeake Bay oysters with *C. parvum* gathered under the Maryland Sea Grant Project are expected to provide reliable information on the values of parameters that are necessary for determination of the exposure assessment and risk characterization of foodborne cryptosporidiosis due to consumption of raw oysters.

#### ACKNOWLEDGMENTS

We thank J. Michalski and J. Collier for assistance as skippers of the research vessel used for oyster collection, and J. Ferry and W. Benton for assistance in collecting the oysters. The assistance of K. Brohawn, Maryland Department of the Environment, in providing data on oyster bar location and fecal coliform counts is greatly appreciated. We acknowledge the Wye Island Natural Resources Management Area (Queenstown, MD) and the Wildfowl Trust of North America (Grasonville, MD) for facilitating collection of goose fecal droppings and K.N. Greenhawk (COLMAP, Oxford, MD) for providing geographical location data. We thank D. Howard for preparation of histological sections of oyster tissues, and C. Carpenter for technical assistance. The study was supported by the Maryland Sea Grant R/F-88.

#### REFERENCES

- Bean, N. H., Goulding, J. S., Lao, C., and Angulo, F. J. (1996). Surveillance for foodborne-disease outbreaks—United States, 1982–1992. *Morb. Mort. Wkl. Rep.* **45**, 1–66.
- DuPont, H. L., Chappel, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B., and Jakubowski, W. (1995). The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N. Engl. J. Med.* **332**, 855–859.
- Fayer, R., Graczyk, T. K., Cranfield, M. R., and Trout, J. M. (1996). Gaseous disinfection of *Cryptosporidium parvum* oocysts. *Appl. Environ. Microbiol.* **62**, 3908–3909.
- Fayer, R., Speer, C. A., and Dubey, J. P. (1997a). In “General biology of *Cryptosporidium* and cryptosporidiosis” (R. Fayer, Ed.), pp. 1–49. CRC Press, Boca Raton, FL.
- Fayer, R., Graczyk, T. K., Farley, C. A., Lewis, E. J., and Trout, J. M. (1997b). The potential role of waterfowl and oysters in the complex epidemiology of *Cryptosporidium parvum*. In “International Symposium on Waterborne *Cryptosporidium*” (R. R. Fricker, J. L. Clancy, and P. A. Rochelle, Eds.), pp. 153–158, American Water Work Association Press, Denver, CO.
- Fayer, R., Farley, C. A., Lewis, E. J., Trout, J. M., and Graczyk, T. K. (1997c). The potential role of the oyster *Crassostrea virginica* in the epidemiology of *Cryptosporidium parvum*. *Appl. Environ. Microbiol.* **63**, 2086–2088.
- Fayer, R., Graczyk, T. K., Lewis, E. J., Trout, J. M., and Farley, C. A. (1998). Survival of infectious *Cryptosporidium parvum* oocysts in seawater and Eastern oysters (*Crassostrea virginica*) in the Chesapeake Bay. *Appl. Environ. Microbiol.* **64**, 1070–1074.
- Graczyk, T. K. (1997). Epidemiology and epizootiology of *Cryptosporidium* infections. *Recent Res. Dev. Microbiol.* **1**, 13–23.
- Graczyk, T. K., Fayer, R., and Cranfield, M. R. (1996a). *Cryptosporidium parvum* is not transmissible to fish, amphibia, or reptiles. *J. Parasitol.* **82**, 748–751.
- Graczyk, T. K., Cranfield, M. R., and Fayer, R. (1996b). Evaluation of commercial enzyme immunoassay (EIA) and immunofluorescent antibody (IFA) tests kits for detection of *Cryptosporidium* oocysts other than *Cryptosporidium parvum*. *Am. J. Trop. Med. Hyg.* **53**, 274–279.
- Graczyk, T. K., Fayer, R., and Cranfield, M. R. (1997a). Zoonotic transmission of *Cryptosporidium parvum*: Implications for waterborne cryptosporidiosis. *Parasitol. Today* **13**, 348–351.
- Graczyk, T. K., Cranfield, M. R., Fayer, R., and Anderson, M. S. (1996c). Viability and infectivity of *Cryptosporidium parvum* oocysts are retained upon intestinal passage through a refractory avian host. *Appl. Environ. Microbiol.* **62**, 3234–3237.
- Graczyk, T. K., Fayer, R., Lewis, E. J., Farley, C. A., and Trout, J. M. (1997b). *In vitro* interactions between hemocytes of the Eastern oyster, *Crassostrea virginica* Gmelin, 1791 and *Cryptosporidium parvum* oocysts. *J. Parasitol.* **83**, 949–952.
- Graczyk, T. K., Cranfield, M. R., Fayer, R., Trout, J. M., and Goodale, H. J. (1997c). Infectivity of *Cryptosporidium parvum* oocysts is retained upon intestinal passage through a migratory waterfowl species (Canada goose, *Branta canadensis*). *Trop. Med. Int. Health* **2**, 341–347.
- Graczyk, T. K., Fayer, R., Trout, J. M., Lewis, E. J., Farley, C. A., Sulaiman, I., and Lal, A. (1998a). *Giardia* sp. and infectious *Cryptosporidium parvum* oocysts in the feces of migratory Canada geese (*Branta canadensis*). *Appl. Environ. Microbiol.* **64**, 2736–2738.
- Graczyk, T. K., Farley, C. A., Fayer, R., Lewis, E. J., and Trout, J. M. (1998b). Detection of *Cryptosporidium* oocysts and *Giardia* cysts in the tissue of Eastern oysters (*Crassostrea virginica*) carrying principal oyster infectious diseases. *J. Parasitol.* **84**, 1039–1042.

- Hansen, J. S., and Ongerth, J. E. (1991). Effect of time and watershed characteristic on the concentration of *Cryptosporidium* oocysts in river water. *Appl. Environ. Microbiol.* **57**, 2790–2795.
- Jenkins, M. C., and Fayer, R. (1995). Cloning and expression of cDNA encoding an antigenic *Cryptosporidium parvum* protein. *Mol. Biochem. Parasitol.* **71**, 199–152.
- Jenkins, M. C., and Peterson, K. (1997). Molecular biology of *Cryptosporidium*. In “General Biology of *Cryptosporidium* and Cryptosporidiosis” (R. Fayer, Ed.), pp. 225–232. CRC Press, Boca Raton, FL.
- Jenkins, M. C., Trout, J., and Fayer, R. (1997). A semi-quantitative method for measuring *Cryptosporidium parvum* infection using polymerase chain reaction. *J. Microbiol. Methods* **28**, 99–107.
- MacKenzie, W., Neil, M., Hoxie, M., Proctor, M., Gradus, M., Blair, K., Peterson, D., Kazmierczak, J., Addidd, D., Fox, K., Rose, J., and Davis, J. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N. Engl. J. Med.* **331**, 161–167.
- Robertson, L. J., Campbell, A. T., and Smith, H. V. (1992). Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Appl. Environ. Microbiol.* **58**, 3494–3500.
- Rose, J. B. (1997). Environmental ecology of *Cryptosporidium* and public health implications. *Annu. Rev. Public Health* **18**, 135–161.
- Rose, J. B., and Sobsey, M. D. (1993). Quantitative risk assessment for viral contamination of shellfish and coastal waters. *J. Food Protect.* **56**, 93–96.
- Rose, J. B., Lisle, J. T., and LeChevallier, M. (1997). Waterborne cryptosporidiosis: Incidence, outbreaks, and treatment strategies. In “General Biology of *Cryptosporidium* and cryptosporidiosis” (R. Fayer, Ed.), pp. 93–109. CRC Press, Boca Raton, FL.
- Sulaiman, I. M., Xiao, L., Yang, C., Escalante, L., More A., Beard, A. C., Arrowood, M. J., and Lal. A. (1998). Differentiating human from animal isolates of *Cryptosporidium parvum*. *Emerg. Infect. Dis.* **4**, 681–685.