

Risk of Handling as a Route of Exposure to Infectious Waterborne *Cryptosporidium parvum* Oocysts via Atlantic Blue Crabs (*Callinectes sapidus*)[∇]

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Commercial Atlantic blue crabs (*Callinectes sapidus*) were exposed to 2.0×10^4 infectious waterborne oocysts of *Cryptosporidium parvum*. The study demonstrated that blue crabs can transfer *C. parvum* oocysts to persons involved in handling or preparing crabs and that they may contaminate other surfaces or products during storage.

Cryptosporidium parvum is a human enteric pathogen that can be transmitted very efficiently via the fecal-oral route (i.e., autoinfection and person-to-person) and indirectly via contact with contaminated water, including consumption and recreational activities (5). Edible crabs may take up and retain human-virulent bacterial contaminants (1, 10, 11, 13) and organic and inorganic pollutants from ambient water and sediments at levels posing risks to consumers (12). There is no published information on contamination of crabs with *Cryptosporidium*, a waterborne pathogen commonly reported from coastal waters (3, 6). However, our previous study demonstrated mechanical passage of *C. parvum* oocysts via handling of fish caught in urban watersheds to the hands of recreational anglers (16). The purposes of the present study were to determine if commercially harvested Atlantic blue crabs (*Callinectes sapidus*), which are widely consumed, can serve as a vehicle for infectious waterborne oocysts of *C. parvum* and if the handling of crabs collected from *Cryptosporidium*-contaminated water can result in oocyst transfer to the handler's hands.

A 120-liter-capacity marine tank was filled with 4 liters of artificial seawater of 12-ppt salinity (9) to which 2.0×10^4 *C. parvum* oocysts were added. The oocysts were tested and found to be infectious to neonatal BALB/c mice (6). *C. parvum* oocysts were eluted from 12 blue crabs (*C. sapidus*) purchased from a local market by sprinkling 0.5 liter of the eluting fluid (4) on the crabs' surfaces and then collecting all the fluid into a single plastic bottle. The crabs were alive and actively ventilating with their mouthpart appendages during the experiments. The crabs were left in the tank for 24 h, and then the oocysts were eluted from the individual surfaces of six randomly selected crabs as described above and the fluid was

collected into six corresponding plastic bottles. The oocysts were eluted collectively from the surfaces of the remaining crabs, and the fluid was collected into a single plastic bottle. The crabs were handled by a single person, and the hands of that person were washed in a plastic ziplock bag (16) containing 0.5 liter of eluting fluid (4). The tank water was collected into a plastic container, and the tank was washed with 1 liter of the eluting fluid (4), which was added to the container. The samples were processed by a cellulose acetate membrane filter dissolution method (4), and the recovered material was tested by combined fluorescence in situ hybridization and a direct immunofluorescent antibody assay for *C. parvum* (7–9).

C. parvum oocysts were detected in the eluting fluid from crabs after the exposure in contaminated water, in the hand wash sample, and in the tank water (Table 1). Overall, 74.8% of the oocysts from the original inoculum were recovered through testing (Table 1). The numbers of *C. parvum* oocysts recovered individually from six crabs varied from 8.0×10^2 to 3.1×10^2 , with a mean of 5.6×10^2 . The data presented in Table 1 indicate that (i) on average, a single crab carried on its external surfaces approximately 7.6×10^2 oocysts (i.e., approximately 3.8% of the original inoculum); (ii) all 12 crabs collectively accumulated 9.2×10^3 oocysts on their shells (i.e., 45.8% of the original inoculum); and (iii) approximately 10.4% of oocysts carried by the crabs ended up on the hands of a person who was handling these crabs during the experiment. The fraction of *C. parvum* oocysts retained by the crabs (45.8%, i.e., 9.2×10^3 oocysts) was significantly higher (chi-square test; $\chi^2 = 16.2$, $P < 0.001$) than the fraction of oocysts that remained in the water (29.0%, i.e., 5.8×10^3 oocysts) after the experiment (Table 1), thus demonstrating the uptake and retention effects.

The present study raises a serious question concerning the safety of handling blue crabs from waters contaminated with *Cryptosporidium*, such as from coastal regions receiving wastewater effluents and agricultural runoff, particularly from dairy

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TABLE 1. Results of elution of Atlantic blue crabs exposed to *Cryptosporidium parvum* oocysts^a

Collected fluid	No. of oocysts	% of inoculum
Crab eluting fluid		
Collective	4.9×10^3	24.5
Individual	3.3×10^3	16.5
Hand eluting fluid	9.5×10^2	4.8
Tank water	5.8×10^3	29.0

^a Twelve commercially harvested Atlantic blue crabs (*Callinectes sapidus*) were exposed for 24 h to 4 liters of artificial seawater of 12-ppt salinity inoculated with 2.0×10^4 infectious *Cryptosporidium parvum* oocysts. After the exposure, the oocysts were eluted from the crab shells (six crabs collectively and six individually), the hands of the person handling the crabs, and the tank water.

and beef cattle operations (3, 6). *Cryptosporidium*-contaminated edible crabs may not cause food-borne cryptosporidiosis via consumption, as the oocysts will most likely be inactivated by adequate steaming or cooking processes; however, handling such crabs will expose the persons involved in the handling and may also contaminate the areas where they are stored. Such epidemiological circumstances have been incriminated in *Vibrio cholerae* and *Vibrio parahaemolyticus* outbreaks caused by crabs destined for human consumption (14, 15, 17). Chesapeake Bay blue crabs, a major seafood item harvested from this region, have been shown to contain *V. parahaemolyticus* (2).

The study emphasizes the great potential for the spread of this pathogen via contamination of the crab storage and preparation areas and crab handlers. It also emphasizes a need for high hygiene standards to be maintained in facilities and restaurants that are cooking live crabs. Minor inattention to hygiene standards in handling edible crabs resulted in *V. parahaemolyticus* and *V. cholerae* outbreaks (14, 15, 17). After the harvest, blue crabs are usually stored alive for several days in a moist and low-temperature environment, which preserves the infectivity of potential *Cryptosporidium* oocysts. The present study indicates that handling and storage of edible crabs harvested from contaminated waters may represent an occupational health risk for cryptosporidiosis. Proper hand washing is effective in the removal of *C. parvum* oocysts (16).

Environmental pollution of coastal waters has shifted from a local problem to a global concern as agricultural and urban runoff and wastewater effluents intensify due to a steady growth of the human population, which, in turn, drives a higher demand for intensive seafood harvest and production (3). Approximately 60% of disease outbreaks and cases linked to seafood are due to unknown etiological agents with usually unexplained epidemiological circumstances (3). The present study demonstrated the potential of mechanical transmission of *C. parvum* oocysts from a common commercial seafood item such as blue crabs, which can then potentially result in an

enteric disease via contamination rather than the actual crab consumption.

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REFERENCES

- Faghri, M. A., C. L. Pennington, L. S. Cronholm, and R. M. Atlas. 1984. Bacteria associated with crabs from cold water with emphasis on the occurrence of potential human pathogens. *Appl. Environ. Microbiol.* **47**:1054–1061.
- Fishbein, M., J. Mehlman, and J. Pitcher. 1970. Isolation of *Vibrio parahaemolyticus* from the processed meat of Chesapeake Bay blue crabs. *Appl. Environ. Microbiol.* **20**:176–178.
- Graczyk, T. K., and K. J. Schwab. 2000. Foodborne infections vectored by molluscan shellfish. *Curr. Gastroenterol. Rep.* **2**:305–309.
- Graczyk, T. K., M. R. Cranfield, and R. Fayer. 1997. Recovery of waterborne oocysts of *Cryptosporidium parvum* from water samples by the membrane-filter dissolution method. *Parasitol. Res.* **83**:121–125.
- Graczyk, T. K., R. Fayer, and M. R. Cranfield. 1997. Zoonotic potential of *Cryptosporidium parvum*: implications for waterborne cryptosporidiosis. *Parasitol. Today* **13**:348–351.
- Graczyk, T. K., R. Fayer, M. C. Jenkins, J. M. Trout, J. Higgins, E. J. Lewis, and C. A. Farley. 2000. Susceptibility of the Chesapeake Bay to environmental contamination with *Cryptosporidium parvum*. *Environ. Res.* **82**:106–112.
- Graczyk, T. K., B. H. Grimes, R. Knight, A. J. DaSilva, N. J. Pieniazek, and D. A. Veal. 2003. Combined FISH and mAb detection of *Cryptosporidium parvum* and *Giardia lamblia* carried by synanthropic flies. *Am. J. Trop. Med. Hyg.* **68**:228–232.
- Graczyk, T. K., D. B. Conn, F. Lucy, D. Minchin, L. Tamang, L. N. S. Moura, and A. J. DaSilva. 2004. Human waterborne parasites in zebra mussels (*Dreissena polymorpha*) from the Shannon River drainage, Ireland. *Parasitol. Res.* **93**:389–391.
- Graczyk, T. K., A. S. Girouard, L. Tamang, S. P. Nappier, and K. J. Schwab. 2006. Recovery, bioaccumulation, and inactivation of human waterborne pathogens by the Chesapeake Bay nonnative oyster, *Crassostrea ariakensis*. *Appl. Environ. Microbiol.* **72**:3390–3395.
- Hauxhurst, J. D., M. I. Krichevsky, and R. M. Atlas. 1980. Numerical taxonomy of bacteria from the Gulf of Alaska. *J. Gen. Microbiol.* **120**:131–148.
- Hauxhurst, J. D., T. Kaneko, and R. M. Atlas. 1981. Characteristics of bacterial communities in the Gulf of Alaska. *Microb. Ecol.* **7**:167–182.
- Karouna-Renier, N., R. A. Snyder, J. G. Allison, M. G. Wagner, and K. R. Rao. 2007. Accumulation of organic and inorganic contaminants in shellfish collected in estuarine waters near Pensacola, Florida: contamination profiles and risk to human consumers. *Environ. Pollut.* **145**:474–488.
- Kloos, W. E. 1980. Coagulase-negative staphylococci. *Clin. Microbiol. Newsl.* **4**:75–79.
- Lowry, P. W., A. T. Pavia, L. M. McFarland, B. H. Peltier, T. J. Barrett, H. B. Bradford, J. M. Quan, J. Lynch, J. B. Mathison, and R. A. Gunn. 1989. Cholera in Louisiana. Widening spectrum of seafood vehicles. *Arch. Intern. Med.* **149**:2079–2084.
- Rabbani, G. H., and W. B. Greenough. 1999. Food as a vehicle of transmission of cholera. *J. Diarrhoeal Dis. Res.* **17**:1–9.
- Roberts, J. D., E. K. Silbergeld, and T. K. Graczyk. A probabilistic risk assessment of *Cryptosporidium* exposure among Baltimore urban anglers. *J. Toxicol. Environ. Health*, in press.
- Yamazaki, M., K. Inuzuka, M. Matsumoto, Y. Miwa, R. Hiramatsu, H. Matsui, K. Sakae, Y. Suzuki, and Y. Miyazaki. 2003. Epidemiological study of outbreaks and sporadic cases due to *Vibrio parahaemolyticus*—serotype O3:K6 in Aichi Prefecture, Japan, during 1988 and 2001. *Kansenshogaku Zasshi* **77**:1015–1023.