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 experiment. 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

<table>
<thead>
<tr>
<th>Collected fluid</th>
<th>No. of oocysts</th>
<th>% of inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab eluting fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collective</td>
<td>$4.9 \times 10^3$</td>
<td>24.5</td>
</tr>
<tr>
<td>Individual</td>
<td>$3.3 \times 10^3$</td>
<td>16.5</td>
</tr>
<tr>
<td>Hand eluting fluid</td>
<td>$9.5 \times 10^2$</td>
<td>4.8</td>
</tr>
<tr>
<td>Tank water</td>
<td>$5.8 \times 10^3$</td>
<td>29.0</td>
</tr>
</tbody>
</table>

* 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 $2.0 \times 10^8$ 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


