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Research Note

Cooking Mussels (*Mytilus galloprovincialis*) by Steam Does Not Destroy the Infectivity of *Cryptosporidium parvum*

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ABSTRACT

The consumption of shellfish has increased considerably worldwide, with an associated increase in foodborne illnesses. Among the bivalves, the mussels are usually cooked by steam, which constitutes a typical dish in several regions. In this article, we demonstrate that this preparation is not sufficient to destroy completely the infectivity of *Cryptosporidium parvum.* Oocysts recovered from experimentally contaminated mussels (*Mytilus galloprovincialis*) were infectious to neonatal mice after cooking. Although, to date, no official cases of cryptosporidiosis linked to shellfish consumption have been reported, we recommend that people with reduced immunity avoid this type of food because they are at high risk of being infected with *Cryptosporidium* spp. after eating raw or undercooked contaminated bivalves.

Cryptosporidium spp. are apicomplexan parasites that infect the luminal surface of the gastrointestinal-respiratory epithelium of a wide range of vertebrate hosts. Cryptosporidiosis is a frequent cause of diarrheal disease in humans. Several groups are particularly susceptible to infection, e.g., children younger than 2 years, elderly people, and immunocompromised patients, particularly people with AIDS (who may have the disease for life), with severe diarrhea and invasion of the pulmonary system contributing to death. *Cryptosporidium parvum* and *Cryptosporidium hominis* are responsible for most human cryptosporidial infections, but other *Cryptosporidium* spp. (*C. meleagridis, C. felis, C. canis, C. muris,* the cervine genotype, and pig genotype I) have also been found in humans. Humans can acquire *Cryptosporidium* infections through several routes of transmission, including person-to-person transmission, zoonotic transmission, and water and foodborne transmission *(5).* In a previous transmission study, DuPont et al. *(4)* showed that immunocompetent adult humans can be infected by as few as 30 oocysts and that 130 oocysts is the median infective dose. It has even been suggested that the illness can be caused by a single oocyst in immunocompromised persons *(14).*

The oocysts of *Cryptosporidium* spp. can enter estuaries and eventually coastal areas through runoff from agricultural, suburban, and urban land surfaces; wastewater discharges; and recreational activities *(6).* It has been demonstrated that bivalve molluscs can take up *Cryptosporidium* oocysts from artificially and naturally contaminated water *(11)* and retain them within their body tissues for at least 1 month, with infectivity for mice being maintained *(7).* On the other hand, an approximation of the parasite load of shellfish contaminated naturally indicated that each shellfish could transport more than 10^3 oocysts *(8).* Moreover, the standard depuration process applied to commercially harvested bivalve molluscs may not be sufficiently effective to ensure the safe consumption of this food with respect to *C. parvum* oocysts, which can retain their infectivity in shellfish *(10).*

Mussels are one of the most commonly consumed bivalve molluscs worldwide. This shellfish is usually cooked by steam, which constitutes a typical dish in several regions. Using a murine model, we assessed whether this common method of cooking could eliminate the infectivity of *C. parvum* oocysts retained in experimentally contaminated mussels (*Mytilus galloprovincialis*).

MATERIALS AND METHODS

Live mussels, *M. galloprovincialis,* of medium valve size (8.21 by 4.07 cm [±0.46 by 0.23]) that were packed and ready for human consumption were bought in a local supermarket. To check for the presence or absence of *Cryptosporidium* oocysts, eight randomly selected specimens were analyzed. The valves were forced to open, and the gills and gastrointestinal tracts were dissected with a sterile scalpel. These tissues were homogenized in 0.04 M phosphate-buffered saline (PBS), pH 7.2, with an Osterizer pulse-matic 16 homogenizer (Sunbeam-Oster Company, Inc., Milwaukee, Wis.). The resulting homogenate was passed through a sieve (mesh size, 150 to 45 μm), suspended in PBS: diethyl ether (2:1, vol/vol), and concentrated by centrifugation at 1,250 × *g* at 4°C for 5 min. A direct immunofluorescence antibody test was carried out according to the manufacturer's instructions (Merifluor *Cryptosporidium/Giardia,* Meridian Bioscience,

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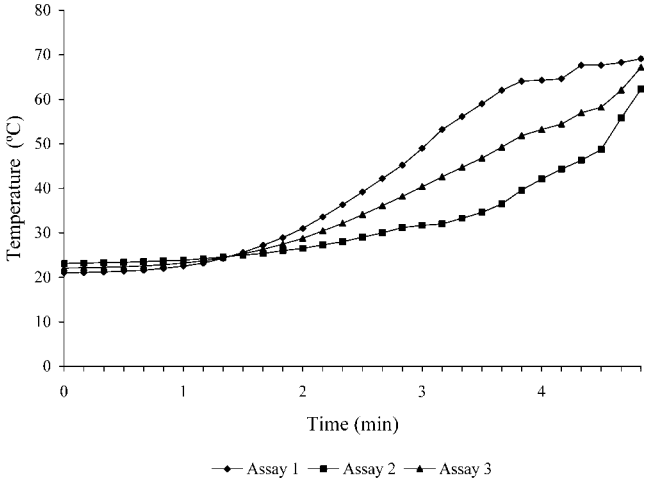

FIGURE 1. *Temperature of the mussel flesh reached during the process of cooking by steam.*

Inc., Cincinnati, Ohio) to rule out the presence of *Cryptosporidium* oocysts in the recovered concentrate. Moreover, the negative results obtained by the immunofluorescence antibody test were confirmed by PCR analysis on a fragment of the wall protein of the *Cryptosporidium* oocysts (COWP). The DNA extraction, PCR, and visualization of the results were performed as described previously *(9).*

Then, a total of 13 mussels from the same lot were placed in a tank that contained 40 liters of natural seawater at 15 ± 3°C with constant aeration. The tank was contaminated with 13×10^6 purified oocysts from an isolate of *Cryptosporidium,* less than 1 month old, collected from a naturally infected Friesian calf *(12).* A restriction fragment length polymorphism–PCR assay *(9)* identified the isolate as *C. parvum* (also previously designated *C. parvum* genotype II).

At 24 h postcontamination, the mussels were removed, and the water of the tank was filtered with foam-compressed filters (Filta-Max, IDEXX Laboratories, Inc., Westbrook, Maine). The elution, washing, and concentration processes were carried out according to the manufacturer's instructions. The number of *C. parvum* oocysts present in the final water concentrate was quantified by the immunofluorescence antibody test. A total of 2.83×10^6 oocysts were detected in the 40 liters of water used to fill the tank.

The contaminated mussels were placed in a metal pan with water (0.5 to 1 cm in height), and the receptacle was heated until all the molluscs opened their valves. Immediately, the gills and gastrointestinal tracts were dissected and processed, and the number of *Cryptosporidium* oocysts in the recovered concentrate was quantified as described previously. A total of 6.0×10^4 oocysts were recovered (suspended in 3 ml of PBS). During the heating step, the temperature of the mussel flesh was monitored by the insertion of a beaded wire probe 3K1200 (OMEGA Engineering, Inc., Stamford, Conn.) into the center of the mussel (Fig. 1).

Aliquots of 100 μl of the recovered concentrate from the mussels (i.e., approximately 2.0×10^3 oocysts) were inoculated intragastrically into 26 neonatal CD-1 Swiss mice (2.5 to 3.0 g). As a control for infectivity, 20 neonatal mice received 2.5×10^4 oocysts of the original isolate of *C. parvum.* At 7 days postinoculation, the mice were killed, and the entire small and large intestines were removed and placed in 5 ml of 0.04 M PBS, pH 7.2. The intestines were homogenized with an Ultra-Turrax T8 homogenizer (IKA Werke Gmbh & Co. KG, Staufen, Germany). The number of oocysts present was counted in a modified Neubauer hemacytometer with malachite green as described previously *(3).*

TABLE 1. *Infectivity assay in neonatal mice of* Cryptosporidium parvum *oocysts recovered from experimentally contaminated mussels following a cooking process by steam*

Neonatal mice (*n*)	Dose (oocysts/100 μl)	Infected mice (%)	Infection intensity[a]
Control litters (20)	2.5×10^4	100	17.6 ± 6.7
Test litters (26)	2.0×10^3	50	16.1 ± 9.3

[a] Mean oocysts counted ± standard deviation $\times 10^5$ per homogenized intestinal tissue.

RESULTS AND DISCUSSION

The results obtained in this study, by a suckling murine model, demonstrate the inefficacy of a usual method of cooking mussels (steaming) for the total elimination of the infectivity of *C. parvum* oocysts previously taken up by mussels, *M. galloprovincialis.* Thus, of the 26 mice inoculated with aliquots of the concentrate recovered from the pool of gills and gastrointestinal tracts from the steam-cooked mussels, 13 were parasitized at 7 days postinoculation, with a median intensity of infection similar to that obtained in the control mice (Table 1).

This finding, which is of public health importance, should be considered carefully to avoid the sensationalism and misinterpretation that would affect not only the mussel industry but also the bivalve mollusc industry as a whole. It must be taken into account that, to date, no official cases of cryptosporidiosis linked to shellfish consumption have been reported, except for personal communications regarding the frequent occurrence of cases of self-limiting diarrhea associated with the consumption of raw oysters and clams *(11)* and one case of cryptosporidiosis in a patient with human immunodeficiency virus who ate raw oysters *(2).* Moreover, considering that the true prevalence of shellfish-vectored gastroenteritis is underestimated by as much as 20-fold *(1)* and that the mean incubation period of cryptosporidiosis is 7 days (range, 1 to 14 days) *(13),* it is difficult to establish epidemiological relationships between the disease and the source of infection.

Some authors have evaluated the effects of high temperature on the infectivity of *C. parvum* oocysts in water and milk, and it has been shown that the application of temperatures of 71.7°C for 5 s is sufficient to destroy the infectivity of *C. parvum* oocysts in these mediums; however, developmental stages of *C. parvum* were found in mice that received oocysts in water that had been subject to temperatures of 54.4, 59.9, and 67.5°C for 1 min and 59.7°C for 5 min *(5).* The infectivity of *C. parvum* oocysts present in mussels may be maintained because the steaming process does not reach the critical temperature long enough to inactivate the oocysts or because the mussel tissues have protective effects, favoring the survival of the oocysts retained inside *(7).*

Although further studies are necessary to establish an epidemiological relationship between the consumption of bivalves and outbreaks of cryptosporidiosis, we recommend

that people with reduced immunity avoid this type of cuisine, because they are at high risk of being infected with *Cryptosporidium* spp. after eating raw or undercooked contaminated bivalves. Furthermore, it is important that the responsible authorities take measures to improve wastewater disposal, runoff control, and farm manure management to achieve better water quality in the estuaries where the bivalve molluscs are harvested.

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