APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1981, p. 207-211
0099-2240/81/010207-05$02.00/0
Vol. 41, No. 1

Uptake and Survival of Enteric Viruses in the Blue Crab, *Callinectes sapidus*

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Uptake of poliovirus 1 by the blue crab, *Callinectes sapidus*, was measured to assess the likelihood of contamination by human enteric viruses. Virus was found in all parts of the crab within 2 h after the crab was placed in contaminated artificial seawater. The highest concentrations of virus were found in the hemolymph and digestive tract, but the meat also contained virus. The concentration of virus in the crabs was generally less than in the surrounding water. Changes in salinity did not substantially affect the rate of accumulation. An increase in temperature from 15 to 25°C increased the rates of both uptake and removal. Poliovirus survived up to 6 days in crabs at a temperature of 15°C and a salinity of 10 g/kg. When contaminated crabs were boiled, 99.9% of poliovirus 1, simian rotavirus SA11, and a natural isolate of echovirus 1 were inactivated within 8 min. These data demonstrate that viruses in crabs should not pose a serious health hazard if recommended cooking procedures are used.

Human enteric viruses are a health problem in oysters, clams, and mussels that are eaten raw or only partially cooked (5). These bivalve mollusks are filter feeders. While feeding, they can accumulate virus from large volumes of surrounding water. The concentration of viruses in bivalve mollusks can be many times higher than in the surrounding water (6, 7, 10, 15).

DiGirolamo and co-workers suggested that viruses in edible crabs may also present a health hazard (2, 4). They found that Pacific shore crabs accumulated poliovirus from artificially contaminated seawater and by feeding on virus-contaminated mussels (2). They also demonstrated the survival of coliphage T4 in edible West Coast crabs after boiling for 20 min (4).

Blue crabs, *Callinectes sapidus*, are often found in polluted water. Because of their mobility, even if caught in nonpolluted water they may have spent some time before that in polluted water.

The present study was designed to assess the relative health risk posed by human enteric viruses in blue crabs. This was done by measuring (i) the accumulation of human enteric virus in different parts of the crabs at various levels of temperature and salinity, (ii) the persistence of virus in live crabs at different water temperatures, and (iii) the cooking time required to inactivate viruses within crab tissue.

MATERIALS AND METHODS

Virus and virus assays. Plaque-purified stocks of poliovirus 1 (strain LSc), simian rotavirus SA11, and a natural isolate of echovirus 1 (V239) were used. SA11 served as a model for human rotavirus, which can cause severe diarrhea in children. Echovirus 1 (V239) was tested because previous studies indicated that it was relatively resistant to thermoinactivation (9). The enterovirus stocks were grown and assayed in BGM cells, a continuous monkey kidney cell line. Enterovirus assays were done by the plaque-forming unit (PFU) method as used in this laboratory (13). SA11 was grown and assayed in MA-104 cells by using a plaque assay technique developed in our laboratory (16).

Processing of samples. Samples of muscle and digestive tract were weighed and then homogenized in a 1:7 (wt/vol) amount of 0.05 M glycine containing 0.15 g of NaCl per liter at pH 9.5. This homogenate was centrifuged at 1,500 × *g* for 30 min. The supernatant fluid was withdrawn, treated with antibiotics, and assayed for virus. Hemolymph and seawater were assayed directly.

Accumulation and persistence experiments. Virus was added to the aquarium containing artificial seawater and crabs. Water was constantly circulated, but not filtered, during the course of the accumulation experiment. After appropriate time intervals, individual crabs were removed, dipped in a dilute sodium hypochlorite solution to disinfect the carapace, rinsed in distilled water, and placed in plastic sample bags. Hemolymph was collected from one swimming leg into an equal volume of sodium citrate solution (10 g/liter) to prevent clotting. The crabs were stored frozen at −20°C. The digestive tract was scraped out of each crab after thawing at 37°C for 2 h. Muscle tissue was obtained from the claws and from the swimming muscles of each crab, using care to avoid contamination from the digestive system.

In experiments to measure the persistence of viruses in the crabs, the crabs were contaminated by letting them accumulate viruses from artificial seawater at 25°C for 4 h. The contaminated crabs were then placed

in an aquarium containing clean artificial seawater. Water was recirculated and filtered through activated charcoal during these experiments.

RESULTS

The accumulation of poliovirus in the hemolymph, digestive system, and meat of blue crabs was measured at four combinations of temperature and salinity. Temperatures of 15 and 25°C were tested since these are within the range of water temperatures normally found in Galveston Bay and provide a large enough difference to detect an effect due to temperature. Salinity of the water from which the crabs were taken in Galveston Bay was within the range of 0 to 30 g/kg. The results are shown in Fig. 1. The initial concentration of virus in the water ranged from 1×10^5 to 3×10^5 PFU/ml. The experiments at 25°C were terminated after 56 h, and those at 15°C were terminated after 70 h. The loss of virus titer in the water over the course of the experiment was 1.1 to 1.9 $\log_{10}$ at 15°C and 3.0 $\log_{10}$ at 25°C.

Virus was found in the hemolymph and digestive tract within 2 min after the addition of virus to the water and was detected in the meat after 2 h. Virus levels in the crabs rose rapidly in the first 8 h and then leveled off and decreased at a rate similar to that in the surrounding water. The concentration of virus in the crabs was consistently less than in the surrounding water, except at 25°C and a salinity of 10 g/kg. Under these conditions virus concentrations in the hemolymph and digestive tract were higher than in the water at three sampling times.

The highest concentrations of virus were generally found in the hemolymph and digestive tract of the crab. However, after 24 h of accumulation, the concentration in the meat approached, and in some cases exceeded, the concentrations in the hemolymph and digestive tract. Also, the average ratio of the concentration in the surrounding water to the concentration in the meat decreased from 320 after 2 h to 29 after 72 h of accumulation.

Increasing the temperature from 15 to 25°C increased the rate of accumulation of virus in the crabs. Also, comparison of the average concentration factors (PFU per gram of crab/PFU per milliliter of surrounding water) at ≥4 h after addition of virus showed that a greater proportion of the virus was accumulated by the crabs at 25°C than at 15°C. No substantial or consistent effect was observed when the salinity was increased from 10 to 30 g/kg.

Figure 2 shows the persistence of poliovirus in blue crabs when contaminated crabs were placed in clean water at a salinity of 10 g/kg and a temperature of either 15 or 25°C. At 15°C virus was still detectable up to 6 days in the hemolymph and digestive tract and up to 3 days in the meat. The loss of titer was 96.4% in the digestive tract, 98.2% in the hemolymph, and >98% in the meat in 6 days. This was slightly less than the loss of titer from seawater at 15°C (99% in 3 days; Fig. 1).

Loss of virus from the crabs was much faster at 25°C. After 20 h, no virus was detected in the meat or digestive tract.The hemolymph had lost 99.7% of its original titer in 20 h, and 1 PFU was recovered from the hemolymph of one crab after 44 h.

Comparison of the ratios of the virus in hemolymph and digestive tract to the virus in the meat showed that there was little difference in the rate of virus loss in these three portions of the crab. The ratios were variable, but again there was consistently less virus in the meat than in the hemolymph or digestive tract.

Finally, experiments were performed to determine the persistence of viruses in crabs when they are boiled. The swimming muscle (backfin lump meat) of each crab was injected with 0.1 ml of virus suspension. The swimming muscle was used since it is within the body of the crab and would be a likely place for viruses to survive boiling. A thermocouple was inserted into the opposite swimming muscle. Each crab was plunged into 5 liters of boiling water, and the container was covered loosely. At the appropriate time, the crab was removed and immediately placed on ice. The entire inoculated muscle was removed and assayed for virus as described above. Figure 3 shows the inactivation curves of the three viruses tested. There was no substantial difference in the inactivation rates of these three viruses in the crabs.

The three viruses tested all showed at least a 3.3 $\log_{10}$ decrease, or 99.9% inactivation, after 8 min of boiling. Some virus was still detected after 16 min in two cases, but this represented a 4 $\log_{10}$ decrease for echovirus 1 and a 4.3 $\log_{10}$ decrease for the simian rotavirus SA11. The average internal temperature of the crabs was 70°C after 8 min of boiling and 94°C after 16 min.

DISCUSSION

These data can be used to assess the health hazard due to viruses in crabs grown in contaminated waters. It is useful to compare and contrast the situation of viruses in crabs with the situation of viruses in bivalve mollusks.

First, blue crabs did not concentrate virus from the water as do filter-feeding bivalve mollusks. This is probably because of the different feeding mechanisms of crustaceans and mollusks. The amount of virus in crabs came to an

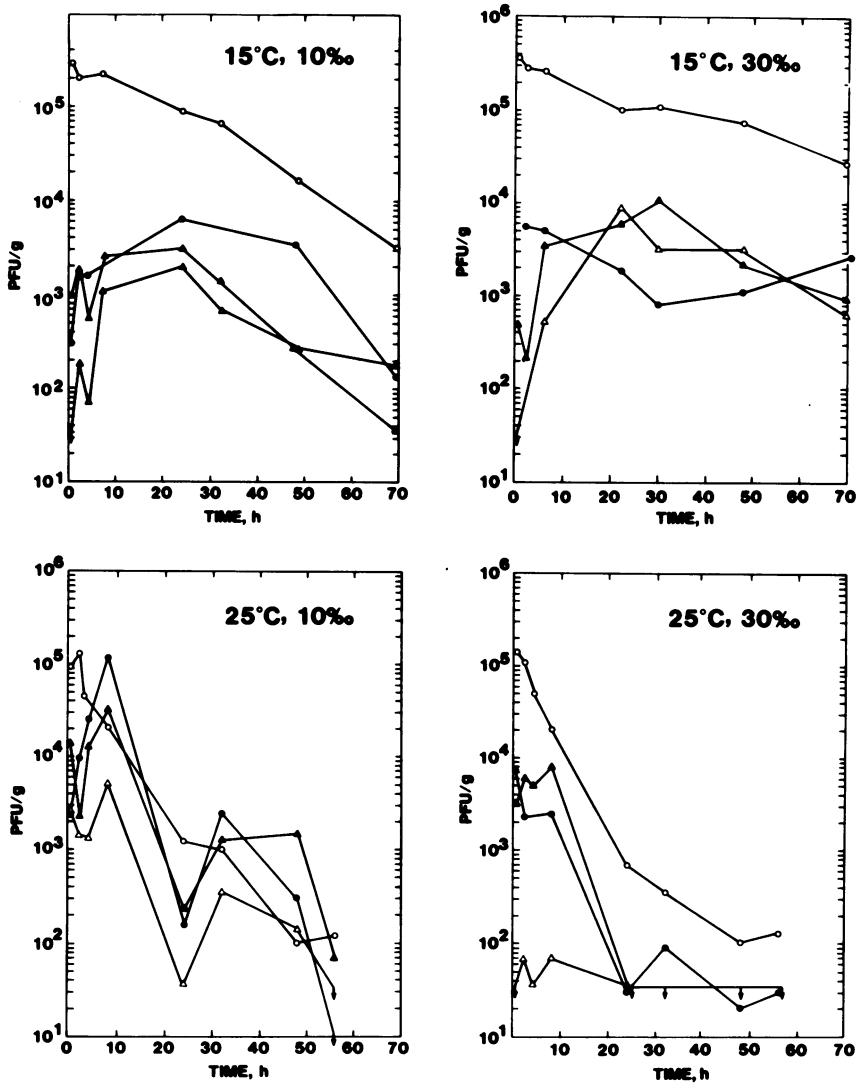

FIG. 1. *Accumulation of poliovirus 1 (LSc) in blue crabs. Crabs were held in 40 liters of artificial seawater at the indicated temperature and salinity. At time 0, virus was added to an initial concentration of 10^5 to 10^6 PFU/ml of water. Arrows indicate below detection limit; ‰ indicates salinity in grams per kilogram. Symbols: ○, water; ●, hemolymph; ▲, digestive tract; △, meat.*

equilibrium with the virus in the water at a level below that of the water. Therefore, unlike oysters and clams which may have concentrations of virus up to 1,000 times the concentration in the surrounding water (1, 7), crabs would not be expected to concentrate virus from water contaminated with very low levels of virus. They may, however, acquire virus by eating contaminated oysters or other organisms (3).

The highest concentrations of virus in crabs were found in the digestive tract and hemolymph. Because the muscle tissue of crabs is not isolated from the digestive tract and hemolymph, when the meat is removed it will almost surely be contaminated with hemolymph or material from the digestive tract or both. The method for removing the meat in this study was similar to the normal method of removing meat for consumption. Therefore, the amount of virus recovered from the meat indicates the relative amount that would be found in the edible portion of the crab. The location of the virus within the animal could be important for crabs since generally only the muscle tissue is eaten. Oysters

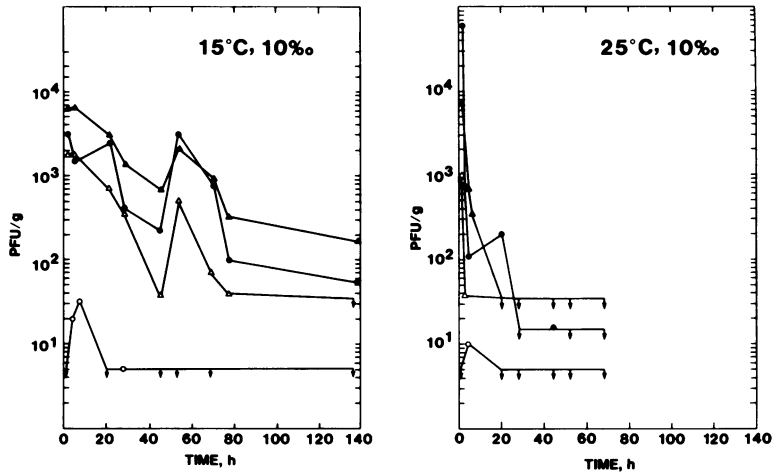

FIG. 2. *Persistence of poliovirus 1 (LSc) in blue crabs. Crabs were contaminated by exposure to artificial seawater containing 2 × 10^5 PFU of poliovirus per ml for 4 h. Contaminated crabs were then placed in clean artificial seawater at a salinity of 10 g/kg and the indicated temperature. Arrows indicate below detection limit; ‰ indicates salinity in grams per kilogram. Symbols: ○, water; ●, hemolymph; ▲, digestive tract; △, meat.*

also accumulate most of the virus in the digestive system (14), but since they are eaten whole the location is not as significant.

Virus was lost from crabs placed in clean water at a rate similar to the rates of inactivation reported for seawater at the same temperature (14), and a significant amount was retained for at least 6 days at 15°C. The crabs did not appear to depurate the virus in the manner that bivalve mollusks do (11, 15), although bivalves also may retain low numbers of virus for several weeks (1).

Temperature had a great effect on persistence of virus in crabs, and a similar effect has been reported for bivalves (1, 11, 12). Again, the difference between crabs and bivalves in elimination of virus, as in uptake, is probably due to different feeding mechanisms.

Finally, the most important consideration of health hazards associated with viruses in crabs is the effect of normal cooking procedures on the viruses. Viruses in crab muscle were rapidly inactivated when the crabs were boiled (Fig. 3). Boiling for 8 min inactivated more than 99.9% of the viruses tested. Commercially, crabs are cooked by steam under pressure or by boiling. Boiling for at least 8 min is used to maximize the yield of crab meat. If boiling times much less than this are used, a substantial proportion of the virus may survive. Also, if the water used for cooking is not fully boiling, the internal temperature of the crab will rise more slowly, and more virus will survive. The most probable danger from viruses in blue crabs would come to individuals who eat live crabs cooked for an insufficient time (e.g., until they turned red) or cooked

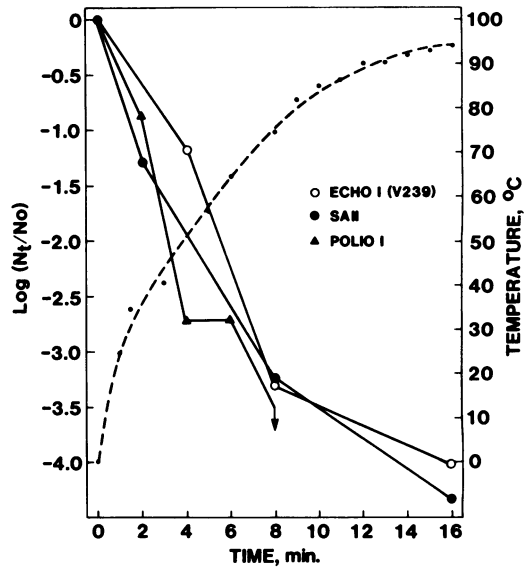

FIG. 3. *Thermoinactivation of viruses in blue crabs during boiling. One swimming muscle of each crab was inoculated with virus to give an initial concentration of about 10^5 PFU/g. The dashed line represents the average internal temperatures of the crabs. Arrow indicates below detection level. Log N_t/N_o = log of the (virus concentration at the time of sampling/virus concentration at 0 h).*

in water that is not boiling. Since oysters are often eaten raw or not fully cooked, they have been implicated in the transmission of viral disease (5).

Concern about the potential hazard due to viruses in cooked shellfish and crabs has been

generated by two reports from DiGirolamo and co-workers in 1970 (2) and 1972 (4) in which they reported survival of bacteriophage in oysters and in West Coast shore crabs after cooking. They reported survival of up to 13% of the initial virus after frying contaminated oysters for 8 min (2). The calculated values for percent survival do not agree with the reduction in PFU which they reported. The percent survival based on the reported values of 1.2×10^4 PFU/g initially and 1.7×10^1 PFU/g after frying for 8 min is 0.14%. The calculated percentages of survival for stewing, baking, and steaming also differ from the reported reduction in PFU by 1 or 2 orders of magnitude. In the other study, DiGirolamo et al. (4) found that bacteriophage T4 in West Coast crabs survived boiling for up to 20 min. They reported 2.5% survival, but again this was miscalculated based on the reported PFU per gram before and after cooking, which indicates a total survival of 1%. The present study shows that most of the virus in crab muscle can be inactivated by proper processing (i.e., boiling for 12 min or longer). However, it is possible that a small number of virions or their infectious ribonucleic acid could survive this treatment if the crab were heavily contaminated (8).

In summary, crabs that are living in contaminated water can be expected to accumulate some virus either from the water or from feeding on contaminated shellfish. Once the virus is in the crab, it may be retained for several days depending on the water temperature. Normal processing of blue crabs by boiling for 12 min or longer appears to be adequate for inactivating the amount of virus that one might expect in naturally contaminated crabs. The differences between crabs and bivalve mollusks mentioned above suggest that crabs present a lesser health hazard than do bivalves from a microbiological viewpoint. Similar evaluations which take into account accumulation, persistence, feeding habits, and the effect of normal processing can be made with other seafood organisms. The relative risks of growing these organisms in and harvesting them from potentially contaminated waters may then be estimated.

ACKNOWLEDGMENTS

This work is a result of a research program sponsored in part by the Texas A&M University Sea Grant College Program, supported by the National Oceanic and Atmospheric Administration Office of Sea Grant, Department of Commerce, under grant 04-7-158-44105.

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