High Hydrostatic Pressure Processing of Murine Norovirus 1–Contaminated Oysters Inhibits Oral Infection in STAT-1−/−–Deficient Female Mice

R. M. GOGAL, JR.,1,2*, R. KERR,1 D. H. KINGSLEY,2 L. A. GRANATA,4 T. LEROITH,1 S. D. HOLLIMAN,4 B. A. DANCHO,3 AND G. J. FLICK, JR.4

1Center for Molecular Medicine and Infectious Disease, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, and 4Food Science and Technology Department, Virginia Polytechnic and State University, Blacksburg, Virginia 24061; 2Department of Anatomy and Radiology, University of Georgia, Athens, Georgia 30602; and 3U.S. Department of Agriculture, Agricultural Research Service, Delaware State University, Dover, Delaware 19901, USA

ABSTRACT

We have previously demonstrated that high pressure processing (HPP) is effective in preventing in vitro replication of murine norovirus strain 1 (MNV-1), a human norovirus surrogate, in a monocytoid cell line following extraction from MNV-1–contaminated oysters. In the present study, the efficacy of HPP to prevent in vivo replication within mice fed HPP-treated MNV-1–seeded oyster extracts was evaluated. Oyster homogenate extracts seeded with MNV-1 were given 5-min, 400-MPa (58,016-psi) treatments and orally gavaged into immunodeficient (STAT-1−/−) female mice. Mice orally gavaged with HPP-treated MNV-1 showed significant (P ≤ 0.05) weight loss leading to enhanced morbidity, whereas those given 100 and 200 PFU of HPP-treated MNV-1 were comparable to uninfected controls. MNV-1 was detected, via real-time PCR, within the liver, spleen, and brain of all mice fed non–HPP-treated homogenate but was not detected in the tissues of mice fed HPP-treated homogenates or in uninfected control mice. Hepatocellular necrosis and lymphoid depletion in the spleen were observed in non–HPP-treated MNV-1 mice only. These results clearly show that HPP prevents MNV-1 infection in vivo and validates that viral inactivation by HPP in vitro is essentially equivalent to that in vivo. Further, the data suggest that HPP may be an effective food processing intervention for norovirus-contaminated shellfish and thus may decrease risk to both immunocompromised and immunocompetent individuals who consume shellfish.

Human noroviruses (HuNVs) are estimated to cause 23 million cases of acute gastrointestinal disease in the United States annually, representing approximately 50 to 67% of all foodborne illness episodes (24). These small, nonenveloped viruses are resistant to environmental degradation (27) and are highly infectious with one reverse transcriptase PCR (RT-PCR) detectable unit predicted to have a 50% probability of initiating an infection within susceptible persons (30). Noroviruses, as well as other enteric viruses, bioconcentrate within bivalve shellfish; therefore, when eaten raw or lightly cooked, these filter-feeding mollusks can serve as a vector for acquisition of norovirus. HuNV contamination of shellfish is not uncommon. For example, Cheng et al. (5), using RT-PCR–based detection methods, reported that 53 (10.5%) of 507 oyster samples from 11 countries were contaminated with HuNV. Norovirus is widely distributed within oyster meat. Wang et al. (32) detected the virus in the gills, stomach, digestive diverticula, and cilia of the mantle, with the heaviest accumulation in the gills and digestive glands (31, 32). Currently, there exist no animal models or cell culture systems that have proven suitable for HuNV isolation, propagation, or titering.

High hydrostatic pressure processing (HPP) has emerged as an intervention method to inactivate spoilage and pathogenic microorganisms in food (7–9, 22, 28). The advantages of this nonthermal process are rapid and uniform application and minimal alterations of taste, odor, appearance, nutritional composition, and texture of foods from their original, fresh state (11, 22). Based on these qualities, HPP is appealing as an alternative to thermal processing for high-value, high-risk seafood, such as raw molluscan shellfish. HPP is currently used commercially to facilitate shucking and to destroy or inactivate pathogenic *Vibrio* bacteria in oysters. Pressure treatments ranging from 250 to 300 MPa are sufficient to eliminate substantial populations of *Vibrio* spp. (2, 6, 21), and pressures as high as 400 MPa have been reported to result in oysters of acceptable taste (23).

As a mitigation strategy for nonenveloped viruses, HPP has had variable results. Kingsley et al. (19) reported that 5-min ambient temperature (23°C) treatments of 275 and 460 MPa were sufficient to inactivate 7 log PFU virus stocks of feline calicivirus and hepatitis A virus (HAV),
respectively. However, poliovirus was not inactivated at pressures of 600 MPa for 1 h (34). Kingsley et al. (15) reported that room temperature HPP treatments ranging from 400 to 600 MPa held for 5 min could result in a 3.4- to 7.6-log reduction for coxsackie A9 virus, with the same pressure treatments resulting in a 1.3- to 4.6-log reduction of human parechovirus. More recently, inactivation of HAV within food products, such as oysters (3, 14), green onions, and strawberry puree (17) has been demonstrated. Subsequent investigation of matrix composition and conditions that influence HPP efficiency indicate that increasing salt and sugar content reduces HPP efficiency for virus inactivation (13, 14). Lower temperatures (5°C) dramatically increase HPP efficiency for feline calcivirus (4) but reduce HPP’s effectiveness for HAV (16). Enhanced inactivation of HAV was observed in lower pH buffers (14).

Murine norovirus (MNV) was originally isolated from an immunodeficient mouse colony (12) and replicates well in vitro (35). Due to the close phylogenetic relationship of MNV and HuNVs, the former is now commonly used as a HuNV surrogate. For example, Nappier et al. (26) used MNV-1 as a research surrogate to evaluate potential differences in the bioconcentration, retention, and depuration of enteric viruses between two species of oysters, Crassostrea virginica and Crassostrea ariakensis. Previous HPP work with MNV-1 demonstrated that a 5-min, 450-MPa treatment at 23°C was sufficient to inactivate 6.85 log PFU (18). Low temperature (5°C) enhanced inactivation with a 5-min, 350-MPa treatment being sufficient to inactivate 5.56 log PFU, while the identical treatment at 30°C resulted in only a 1.15-log PFU reduction (18). This in vitro infection assay demonstrated that MNV-1 can be inactivated from MNV-1-contaminated oysters after simulated natural virus uptake (18). In the present study, we evaluated whether HPP treatment of MNV within extracts of oyster homogenates could render the virus noninfectious to immunocompromised mice in vivo, to determine if assays for inactivation by HPP of MNV-1 in vitro and in vivo give comparable results. The pathology of the liver, spleen, and brain associated with orally infected mice is also described.

MATERIALS AND METHODS

MNV-1. MNV strain 1 (MNV-1) was graciously provided by Dr. Herbert W. Virgin IV, Washington University, St. Louis, MO. The virus was propagated in RAW 264.7 (American Type Culture Collection [ATCC], Manassas, VA) cells cultured in Dulbecco’s minimal essential medium (Mediatech, Manassas, VA) containing 10% fetal bovine serum (Atlanta Biologicals, Atlanta, GA) and 1% HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid) buffer (Mediatech) as previously described (18). Following 72 h of incubation (37°C, 5% CO2), the medium-virus suspension was recovered, frozen at −80°C, and shipped on dry ice in 15-ml conical tubes to the Center for Molecular Medicine and Infectious Disease at the Virginia-Maryland Regional College of Veterinary Medicine. Blacksburg, VA, and immediately stored at −80°C.

Mice. Twenty-four STAT-1−/−-deficient female mice (6 weeks old) were obtained from Taconic Inc. (Albany, NY) and maintained in microisolator chambers (Charles River Labs, North Franklin, CT) in the Phase IV research facility of the Virginia-Maryland Regional College of Veterinary Medicine. Mice were fed an autoclaved commercial pellet diet and provided sterile water ad libitum. All animals were housed at 22°C, with humidity of 40 to 60%, and a 14:10 h light-dark cycle, in a strictly clean environment (10, 20). All experimental animals were cared for and maintained in accordance with the Virginia Tech Animal Care Committee guidelines.

Plaque-forming assay. Murine RAW 267.4 cells (ATCC) were grown to confluence in T-75 culture flasks (37°C, 5% CO2) in 30 ml of complete Dulbecco’s minimal essential medium containing 10% fetal bovine serum and 1% HEPES buffer as previously described. After 48 to 72 h of culture, the flasks were decanted and fresh medium was added. Cells were then scraped from each flask and resuspended in media, and 1-ml aliquots were added to each well of a six-well culture plate. These plates were then incubated until confluence under the same culture conditions. Prior to use, the remaining medium was aspirated and discarded. Six suspensions of precultured MNV-1 virus stock were prepared in 1:10 serial dilutions in phosphate-buffered saline (PBS) without calcium and magnesium (Mediatech). To duplicate wells of the six-well plates containing the confluent murine RAW 267.4 cells, 0.5-ml aliquots of the MNV-1 viral dilutions were added and incubated on a rocker (65 rpm) for 90 min (37°C, 5% CO2). After incubation, the supernatant was recovered, frozen at −80°C, and thawed. Neutral red (Sigma, St. Louis, MO) was added to each well at a concentration of 10 mg/ml. After 2 h, residual stain was aspirated and the plates were inverted to visualize plaques. Results are reported as PFU per milliliter of virus stock.

MNV-1 oyster homogenate. Eighteen Eastern oysters, Crassostrea virginica, were obtained from the Cowart Seafood Corp., Lottsburg, VA. All the oyster meats (weighing 8.02 ± 0.82 g) were autoclaved (Steris S1-120, Isothermal Sterilizer, liquid cycle at 121°C for 10 min) to neutralize common microbial flora. The oyster meats were then subdivided into six groups of three oyster meats and placed in a vacuum-sealed bag. The virus treatments consisted of a saline control, 5.0 × 103 MNV-1 per ml, and 1.0 × 104 MNV-1 per ml. Two bags of each oyster meat were individually injected with 1 ml of either saline or virus (at 5.0 × 105/ml or 1.0 × 106/ml) in Dulbecco’s minimal essential medium. The oysters from each treatment were then further subdivided into two HPP groups. One set of oysters (n = 3 per virus treatment per HPP group) was vacuum sealed and subjected to a 400-MPa (58,016-psi), 5-min HPP treatment at an initial temperature of 7.8°C using a Quintus 35 L food press (Avure Technologies, Kent, WA). Ramp-up time to 400 MPa was 92 s, with an adiabatic temperature rise of 12.7°C observed during pressurization. A second set of oysters (n = 3 per virus treatment per non-HPP group) was vacuum sealed only but not HPP treated and served as positive controls. Following the treatment process, the three oyster meats from each treatment were blended in 30 ml of PBS. Ten milliliters of the resulting mixture was then centrifuged at 800 × g, and the supernatant was recovered. Oyster homogenate supernatants were assayed as previously described, and each mouse received by oral gavage 0.2 ml of either 0 (sterile PBS, vehicle,) 100, or 200 PFU of MNV-1.

Experimental design. Mice were randomly assigned to 6 groups (n = 4 per treatment) and orally gavaged with oyster...
supernatants (Table 1). Body weight data were collected at 0, 2, 5, 6, and 9 days postinfection. At the termination of the study, liver, spleen, and brain from each mouse were collected, sectioned, and stored in either RNAlater (Qiagen, Valencia, CA) for RT-PCR or 10% buffered formalin (Fisher, Suwanee, GA) for histopathology.

**PCR analysis.** Each mouse tissue section (~30 g) stored in RNAlater was transferred into a 2-ml sterile RNase- and DNase-free conical tube containing 100 μl of autoclaved 0.5-mm-diameter glass beads (Next-Advance, Averill Park, NY) in 600 μl of RT buffer and homogenized in a bullet blender blue (Next-Advance) according to the manufacturer’s protocol for 5 min at setting 8. RNA was then purified from the resulting supernatant by use of an RNA-Easy Mini kit (Qiagen) according to the manufacturer’s protocol. RNA was quantified on a Nanodrop spectrophotometer (Nanodrop, Wilmington, DE), and the concentrations were standardized at 1.0 μg/μl in molecular-grade water (Mediatech). To generate cDNA, each sample was reverse transcribed by using an Omni-script RT kit (Qiagen) per the manufacturer’s protocol. Briefly, 2 μl of sample RNA was added to 18 μl of RT mastermix (2 μl of 10× RT buffer, 1 μl of Omniscript RT, 2 μl of deoxyribonucleoside triphosphate mix, 2 μl of 0.4-μg/ml oligo-RT primers and/or random hexamers, 1 μl of RNase inhibitor, and 10 μl of RNase-free water) in a sterile, RNase- and DNase-free 1.5-ml microcentrifuge tube and vortexed gently for 5 s. Tubes were then centrifuged briefly to collect the liquid and incubated at 37°C for 60 min. A real-time PCR was performed with 1 μl (1 μg) of the resulting cDNA by two methods, A and B.

For method A, 1 μl of cDNA was added to 24 μl of a prepared SYBR Green master mix (Bio-Rad, Hercules, CA) with 0.2 μM forward primer, TCC TGA CTG GCT CTA ATG (location, 3,494 to 3,476) (Invitrogen, Carlsbad, CA), specific to MNV-1, designed by using Beacon Designer, and placed in a reaction vessel in an IQ-5 real-time PCR analyzer (Bio-Rad). Samples underwent 1 cycle at 95°C for 10 min and 40 cycles ramping from 60°C for 1 min to 95°C for 15 s, measuring SYBR Green fluorescence at 60°C. To ensure the quality of the primers, ROX dye was used and a melt curve was calculated from 60 to 90°C in 0.5°C increments for each sample.

For method B, 1 μl of cDNA was tested using the primers and MGB-Taqman probe set as originally described by Baert et al. (J): Fw-ORF1/ORF2, CAC GCC ACC GAT CTG TTC TG; Rv-ORF1/ORF2, GCG CTG GCG CAT CAC TC (Integrated DNA Technologies); and MGB-ORF1/ORF2, 6FAM-CGC TTT GGA ACA ATG-MGBNFQ (Applied Biosystems). Reactions were performed using the OneStep RT-PCR Kit (Qiagen) in accordance with the manufacturer’s recommended procedures in 25-μl reaction mixtures containing 200 nM each primer, 200 nM probe, 5 μl of RNase inhibitor (Invitrogen), and 1 μl of cDNA. Real-time PCR assays were performed in a Smart Cycler (Cepheid). The amplification profile included 10 min at 95°C and 50 cycles of 15 s at 95°C, 1 min at 60°C, and 1 min at 72°C. The fluorescence was measured during the annealing step of each cycle. The cycle threshold (Ct) was defined as the cycle number at which the fluorescence of each sample crossed the default threshold value of 30.

**Histopathology.** Formalin-fixed sections from brain, liver, and spleen were trimmed and placed in plastic cassettes according to tissue and treatment, paraffin embedded, and stained with hematoxylin and eosin. The slides were coded and submitted to a board-certified pathologist for analysis and scoring. All tissues were evaluated for stages of inflammation, degree of lymphoid involvement, and evidence of cell death. Tissues that appeared normal received a score of 0 (zero). Liver and brain scores of 1, 2, and 3 were classified as mild, moderate, and severe inflammation, respectively. Spleen scores of 1, 2, and 3 corresponded to mild, moderate, and severe lymphoid depletion and necrosis, respectively.

**Statistical analysis.** Results were tabulated in MS Excel (Microsoft, Redmond, WA) and statistically analyzed via the Dunnett’s test of significance with an α of P ≤ 0.05 in Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL).

**RESULTS**

**Non–HPP-treated MNV-1 homogenate alters weight gain.** Six-week-old STAT-1−/−–deficient female mice were fed either HPP-treated (5 min, 400 MPa at 7.8°C) oyster or extracts of non–HPP-treated homogenate containing 100 or 200 PFU of MNV-1 per mouse. Since STAT-1−/−–deficient mice lack a competent immune system, oysters were sterilized prior to injection of MNV-1 to neutralize common microbial flora and to ensure that only the effects of MNV-1 were being tracked in this study. Non–HPP-treated MNV-1–infected mice showed clinical signs of disease by day 5 after oral exposure with significant body weight loss, while mice fed HPP-treated homogenate containing either 100 or 200 PFU of MNV-1 were comparable to controls (Table 2). Based on recorded weight loss in excess of 10%, as well as increasing morbidity (i.e., ocular swelling and general lethargy), all mice receiving non–HPP-treated MNV-1 were euthanized on day 6 postexposure.

Real-time PCR detection of MNV-1 in the liver, spleen, and brain of non–HPP-treated mice. RT-PCR analysis showed that liver, spleen, and brain from mice that were fed oyster supernatant were positive for the presence of MNV-1 RNA (data not shown). RT-PCR analysis showed that tissues from mice fed oyster homogenate extracts containing MNV-1 that were HPP treated were negative for the presence of viral RNA, as were control mice gavaged with vehicle.

**Non–HPP-treated MNV-1–induced lesions in the murine liver and spleen.** Livers from mice that received 100 or 200 PFU of non–HPP-treated MNV-1 oyster homogenates had mild (100-PFU livers) to moderate (200-PFU livers), random supplicative inflammation and scattered individual hepatocyte necrosis (Fig. 1). Livers from mice gavaged with HPP-treated MNV-1–infected homogenates were histologically normal and similar to saline control mice (Fig. 1). Spleens from mice gavaged with sham HPP-treated MNV-1 homogenate had moderate (100-PFU spleens) to
severe (200-PFU spleens) lymphoid depletion. Additionally, spleens from mice given 200 PFU of non–HPP-treated MNV-1 homogenate displayed necrosis of the red pulp and moderate individual lymphocyte necrosis. Spleens from mice that received HPP-treated MNV-1–infected homogenates and spleens from saline control mice were histologically normal (Fig. 2). Brains collected from mice in all six treatment groups were histologically normal (data not shown).

**DISCUSSION**

Consumption of raw shellfish is a major risk factor for acquisition of a norovirus infection. While preventing contamination of shellfish in their natural habitats is of paramount importance, one cannot be certain that shellfish from even the most pristine waters are virus-free. Cooking shellfish appears to be generally effective, but many consumers prefer eating oysters and clams raw. Consequently, a postharvest method for eliminating enteric viruses from raw shellfish would have positive commercial and health-related applications. Within the last decade, HPP, a nonthermal process for pasteurizing food without loss of food taste or texture, has emerged as a potential intervention technique for shellfish potentially contaminated with viruses (3, 18, 19).

The focus of this study was to determine the efficacy of HPP for inactivation of MNV-1 in an oyster medium via an acute oral challenge. This was achieved by orally gavaging STAT-1⁻/⁻–immunodeficient mice, an animal model known to be susceptible to this viral strain (12, 25), with a virus-oyster homogenate from oysters that were either HPP treated or not. We determined that a 5-min, 400-MPa treatment was sufficient to inactivate, 3 log PFU of virus per ml. Analysis of weight gain data showed that these mice, at both MNV-1 doses, had significant (P ≤ 0.05) weight loss compared with controls. Analysis of the liver, spleen, and brain by real-time RT-PCR showed that the mice gavaged with either of the two MNV-1 doses collected from non–HPP-treated oysters displayed significant (P ≤ 0.05) levels of MNV-1 compared with either the controls or the

**TABLE 2. Percent body weight gain or loss**

<table>
<thead>
<tr>
<th>Day</th>
<th>Saline</th>
<th>100 PFU of MNV-1</th>
<th>200 PFU of MNV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non–HPP treated</td>
<td>3.60 ± 1.12</td>
<td>3.20 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>HPP treated</td>
<td>3.44 ± 0.81</td>
<td>1.78 ± 0.72</td>
</tr>
<tr>
<td>Day 5</td>
<td>Non–HPP treated</td>
<td>6.51 ± 0.54</td>
<td>-5.04 ± 1.75</td>
</tr>
<tr>
<td></td>
<td>HPP treated</td>
<td>3.77 ± 0.81</td>
<td>2.66 ± 2.36</td>
</tr>
<tr>
<td>Day 6</td>
<td>Non–HPP treated</td>
<td>7.23 ± 1.14</td>
<td>-10.77 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>HPP treated</td>
<td>7.34 ± 1.24</td>
<td>3.99 ± 1.51</td>
</tr>
<tr>
<td>Day 9</td>
<td>Non–HPP treated</td>
<td>9.42 ± 3.28</td>
<td>Euthanized on day 6</td>
</tr>
<tr>
<td></td>
<td>HPP treated</td>
<td>10.73 ± 2.23</td>
<td>7.43 ± 1.61</td>
</tr>
</tbody>
</table>

Values are means ± standard errors of the mean, in grams. A positive number denotes weight gain, and a negative number (−) denotes weight loss. Values in boldface indicate significance at P values of ≤0.05 (Dunnett’s test, n ~ 4 mice per treatment).

**FIGURE 1. Histopathology of the liver.** Representative images showing the livers of mice that were orally gavaged with oyster homogenate containing saline, 100 PFU, or 200 PFU of MNV-1 that either was not HPP treated or was HPP treated. The arrows denote cell clusters indicative of random nonsuppurative inflammation (magnification, ×100).
HPP-treated virus-challenged mice. Based on these results, it was determined that MNV-1 was both viable and virulent when non–HPP-treated MNV-1 was administered to the mice.

MNV-1 has a single positive-stranded RNA genome, which by definition is potentially infectious when transfected within cells. In an aqueous environment, high pressure is known to inactivate pathogens via effects exerted on cell membranes and via disruption of hydrogen bonding resulting in alteration of the tertiary and quaternary protein structures. It is generally accepted that HPP does not disrupt covalent bonds and, therefore, does not damage single-stranded RNA molecules. For HAV, it was shown that HPP did not rupture the virion, since viral RNA was not digested by RNase after pressure treatment (19), and recently HPP’s mechanism of action against MNV has been shown to be directed against the capsid protein, blocking the virus’s ability to interact with its target receptor, thus blocking infection of RAW cells (29). Knowing that the MNV RNA genome is capable of initiating an infection if it enters the cell cytosol, it is conceivable that HPP might block in vitro infection while not necessarily blocking infection in a more natural in vivo context. For example, if a strong affinity for a cellular receptor is not a prerequisite for initiation of a norovirus infection within the digestive tract, or if MNV-1’s reported tropism (35) for macrophages and dendritic cells results in direct uptake of HPP-treated virions, one could conceive of an infection scenario wherein virus could penetrate target cells in vivo, resulting in initiation of an infection that would not be observed in an in vitro assay. We confirm here that HPP does block infection as assessed by an in vivo assay, thus preventing infection of immunocompromised mice, as well as judged by in vitro tissue culture assays.

Since pathologic changes within the liver and spleen have been reported with MNV-1 infections in STAT-1−/− mice (25, 33), we examined these tissues and the brain as a means to track the disease progression. Although virus was detected by real-time RT-PCR in the 6-day postinfected brains of the non–HPP-treated MNV-1–infected mice, histopathologic analysis of the brains yielded no significant lesions among these mice or in any of the other treatment groups. We theorize that this is a reflection of the stage of infection relative to the duration of exposure at the time the mice were euthanized. Another potential explanation for the lack of brain lesions is that the blood-brain barrier may have retarded entry of MNV-1 and attenuated the inflammation process at the time these tissues were analyzed. Examination of the liver and spleens of these same mice showed pronounced MNV-1–related lesions, supported by C1 values indicating high viral levels within these tissues. This also supports work by Wobus et al. (35), who showed that macrophages and dendritic cells, which are prevalent in these tissues, are primary cell targets of the virus (35). In the liver, viral hepatitis and hepatic necrosis were evident in both of the non–HPP-treated virus-challenged groups with severity increasing relative to the initial dose administered, which is a common clinical disease indicator with this virus (35). The spleens of these mice had moderate to severe lymphoid depletion that also increased relative to dose, suggesting the likely loss of these leukocytes through cell death, which was confirmed by our pathologist as increased necrosis. In a study conducted by Mumphrey et al. (25) using the same strain of mice, the investigators evaluated multiple time points over 72 h and also showed increased depletion of white pulp via apoptosis (25). Thus, it is possible that the difference in modes of cell death is reflective of the time of evaluation and the stage of the disease. As expected, these lesions were absent in the HPP-treated, MNV-1–challenged mice as well as the two unchallenged control groups.

To summarize, we have shown that HPP is an effective food pasteurization procedure that can be utilized to inactivate MNV-1 in an oyster medium. These in vivo results, reported here, compare well with our previous in vitro observations. Further, through the combined analysis of real-time RT-PCR and histopathology, we were able to follow MNV-1 into three principal organs and correlate it
with pathology. We confirmed the previously reported pathologic changes in the conventional target organs and viral penetration into the central nervous system without inflammatory changes 6 days after oral exposure. These findings support the prospect of HPP’s use as an intervention strategy for shellfish potentially contaminated with HuNV.

ACKNOWLEDGMENTS

We thank Dixon Smiley and Dee Snyder (VA Polytechnic and State University/Virginia-Maryland Regional College of Veterinary Medicine), animal care technicians of the Teaching & Research Animal Care Support Service at the Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, for the care and oversight of the mice. We also thank Ms. Diana Hartle, Reference Librarian, University of Georgia, for her assistance with the formatting of the manuscript. We thank Dr. Gary P. Richards and Dr. Brendon Niemira, Agricultural Research Services, U.S. Department of Agriculture, for critical review of the manuscript. This publication is a result of research sponsored by U.S. Department of Agriculture/Cooperative State Research, Education, and Extension Services grant no. VA-428240.

REFERENCES